## PV17 - **EFFECTS OF TRICOSTATIN A AND TUBASTATIN A, TWO DEACETYLASE INHIBITORS, ON *ANGOMONAS DEANEI*: THE SYMBIONT DIVISION DEPENDS ON THE MICROTUBULE DYNAMISM**

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*Angomonas deanei* is a monoxenic trypanosomatid that harbors a symbiotic bacterium in its cytoplasm. In this mutualistic association, the division of the prokaryote is controlled by the host during its cell cycle and is dependent of the microtubule cytoskeleton, in a way that each daughter cell inherits only a single bacterium. Histone Deacetylases (HDACs) are enzymes that catalyze the removal of acetyl radicals (deacetylation) from proteins, such as histones and tubulin, influencing on gene expression and cytoskeleton dynamism of the trypanosomatid. Tricostatin A (TSA) and Tubastatin A (TST) are two HDACs inhibitors used in studies as antitumour and antiparasitic agents, and more recently, as chemotherapeutic agent against Trypanosoma cruzi. In this context, the objective of this work was to analyze the effects of TSA and TST on *Angomonas deanei*, especially to investigate if the symbiont synchronic division with other host cell structures is affected. Thus, *A. deanei* was treated with different concentrations of the inhibitors for up to 60 hours and then processed for Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Immunofluorescence assays. In this last case, anti-acetylated tubulin and antiporin antibodies were used to verify the compounds' effects over the host cytoskeleton and the endosymbiont division. Additionally, the relation between cell proliferation and cell viability was also analyzed. Preliminary results show a dose-dependence inhibition of cell proliferation, but not of cell viability. Ultrastructural alterations on treated protozoans were also observed, like changes in kDNA topology and symbiont filamentation, indicating that the bacterium division depends on microtubule dynamism. Our next steps are to quantify the tubulin acetylation by fluorimetric assays and to employ the negative stain technique to verify possible changes in the microtubule cytoskeleton arrangement.**Supported by:**CNPq/ FAPERJ **Keywords:** Angomonas deanei; cytoskeleton; deacetylase inhibitor

## PV18 - **TCFR1 FERRIC IRON REDUCTASE OF TRYPANOSOMA CRUZI IS INVOLVED IN FE HOMEOSTASIS**

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Introduction: *Trypanosoma cruzi*, the etiologic agent of Chagas' Disease, is a parasite that infects mainly humans. *T. cruzi* presents high requirement for iron (Fe), mobilizing heminic and nonheminic iron. Fe-reductase is an enzyme located in cellular membrane of some organisms and it is involved on $Fe^{3+}$ to $Fe^{2+}$ reduction. In some parasites, like *Leishmania amazonensis*, this enzyme has been already identified. A NADPH-dependent activity and an activity responsive to iron depletion are important features of this enzyme. However, no study has showed the presence of a Fe-reductase in *T. cruzi* or how the uptake process of this micronutrient occurs. Objective: This work identified, for the first time, the Fe-reductase in *T. cruzi* (TcFR) and demonstrated the importance of a Fe ion transport system in this parasite, which is coupled with the Fe-reductase. Material and Methods: Epimastigotes of *T. cruzi* (DM28c strain) were maintained in Brain Heart Infusion medium (BHI), 30μM hemin and 10% Fetal Bovine Serum (FBS) or in BHI without hemin and iron depleted. The ability of parasites to reduce extracellular ferric iron to ferrous iron was measured with the cell-impermeable compound potassium hexacyanoferrate ($K_3Fe(CN)_6$). Results and Discussion: We found that *T. cruzi* presents a putative Fe-reductase, TcFR, which is homologous to Fe-reductase of *L. amazonensis*, LFR1. The growth rate of DM28c maintained at heme and Fe-depleted medium is lower than cells maintained in a complete medium, but the TcFR expression and Fe-reductase activity increase in parasites maintained at heme-iron depleted medium. Conclusion: These results showed that TcFR may be involved in reduction of $Fe^{3+}$ in $Fe^{2+}$, a crucial step for this ion can be incorporated by these cells by specific transporters. Therefore, the development of mutants overexpressing the TcFR protein is fundamental for the elucidation of this phenomenon.**Supported by:**CNPq, FAPERJ e CAPES **Keywords:** Fe-reductase; trypanosoma cruzi,; iron metabolism

PV19 - **INTERACTION OF LEISHMANIA SPP. WITH IXODID TICK CELL LINES**

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Leishmaniasis is a group of neglected diseases caused by protozoa of the genus Leishmania. Canine leishmaniasis is a dangerous disease, since dogs are the main reservoir of the parasite. Most infected dogs come from poor areas, where there are ectoparasites, such as the tick *Rhipicephalus sanguineus*. Its presence raised the hypothesis that it may be a vector of the parasite. This hypothesis is based on PCR analysis of macerated ticks and/or tissues extracted from infected dogs. The aim of this study was to evaluate the interaction capacity of promastigote forms of *Leishmania amazonensis* and *Leishmania infantum* with embryo-derived cell lines of *Ixodes scapularis* (IDE8) and *R. sanguineu*s (RML-RSE) *in vitro*. For this analysis, tick cells were incubated with promastigotes of *L. amazonensis* or *L. infantum* with a ratio of 5 parasites per cell for 2, 24 and 48 h at 34°C. Preliminary results showed that promastigote forms bind and are endocytosed by tick cells as evidenced by electron microscopy. Endocytic index showed no significant difference at the different times evaluated between RML-RSE or IDE8 and *L. amazonensis*, however, an increase of 15 fold in the endocytic index was observed for RML-RSE and *L. infantum* at 24 h compared to 2 h. The parasite load in tick cells was evaluated after 48 h of infection by counting. The data showed that the parasite load of *L. infantum* was 6 fold higher than that of *L. amazonensis*. The viability of tick cells after 48 h of infection by *Leishmania spp*. was analyzed by lactate dehydrogenase (LDH) assay. Results demonstrated no difference in LDH compared to control uninfected cells. These preliminary data indicated the ability of *Leishmania spp*. to interact with tick cells *in vitro* and suggest a species-specific relationship between *L. infantum* and *R. sanguineus*. In vivo infection needs to be developed in order to verify the ability of the tick to maintain the *Leishmania spp*.**Supported by:**faperj **Keywords:** Cell interaction; canine leishmaniasis; tick cells

PV20 - **DIFFERENTIAL EXPRESSION OF A TRYPANOSOMA CRUZI RNA BINDING PROTEIN POSSIBLY INVOLVED WITH THE CONTROL OF VIRULENCE GENES**

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Trypanosoma cruzi, the causative agent of Chagas disease, is characterized by a highly heterogenous population with strains and clones presenting different biological, biochemical and molecular characteristics. The CL-Brener clone is highly virulent and causes parasitemia in different animal models of infection. In contrast, the CL-14 clone is non-virulent, even when inoculated in immune deficient mice, in which it causes no parasitemia. This parasite has its genome organized in polycistronic transcription units, resulting in gene expression regulation that relies on post-transcriptional mechanisms exerted by RNA binding proteins (RBPs). These proteins control stability, translatability and localization of the mRNA population within the cell. RNA-Seq analysis comparing gene expression in the various life cycle stages of the CL Brener and CL-14 cloned strains revealed that the gene TcCLB.507611.300 encodes an RBP whose mRNA is three times more abundant in trypomastigotes of CL-14 than CL-Brener trypomastigotes. Quantitative PCR experiments confirmed this difference, which suggests that this RBP might have a regulatory role related to the non-virulent phenotype of CL-14 trypomastigotes. In silico analyses were used to predict the physicochemical properties, subcellular localization, and the 3D structure of this RBP, which contains a RRM motif. Using the pROCK expression vector, CL-Brener trypomastigotes overexpressing this gene were generated. Analyses of possible changes in gene expression as well as in the infection capacity of the transfected cell lines are underway. To identify the targets of this RBP in CL-14, small guide RNAs as well as donor sequences constructs necessary for the generation of knockout CL-14 trypomastigotes using the CRISPR/Cas9 technology have been generated. **Supported by:**CNPq **Keywords:** Trypanosoma cruzi; rna binding protein; gene expression regulation

PV21 - **MOLECULAR CHARACTERIZATION BY PCR-RFLP OF *LEISHMANIA* SP. ACTING AS ETIOLOGICAL AGENTS OF AMERICAN CUTANEOUS LEISHMANIASIS IN THE METROPOLITAN REGION OF BELéM, PARá STATE, BRAZIL**

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In Amazonian Brazil, American cutaneous leishmaniasis (ACL) behaves as a primary zoonosis of wild mammals and the transmission of the different *Leishmania* sp. that act as etiological agents of the disease is made by the female bite of several species of phlebotomine sand flies (Diptera: Psychodidae). In the metropolitan region of Belém, Pará state, Brazil, whose vegetation cover is represented by secondary residual forest, the ACL has shown a rare, but regular, endemic character. In recent years, there has been an accelerated growth of the municipalities that comprise the metropolitan region of Belém, especially with the beginning of constructions and the displacement of the dwelling of the population to areas neighboring secondary points of native forest. By means of the PCR-RFLP analysis, there were characterized thirty (30) samples of *Leishmania* sp. isolated from ACL patients that contracted the disease within the metropolitan region of Belém, in the period from 1995 to 2016. DNA samples were amplified by using the primers RPOR2/RPOF2 (Simon et al., 2010), then the PCR products were subjected to enzymatic digestion by the enzymes *Tsp*RI and *Hga*I. After analyzing the electrophoretic patterns obtained, it was observed that *Leishmania* (*Viannia*) *lindenbergi* represented 40% of all parasites characterized (12/30), followed by the species *Leishmania* (*Viannia*) *lainsoni* with 36.6% (11/30), *Leishmania* (*Leishmania*) *amazonensis* with 13.4% (04/30), and *Leishmania* (*Viannia*) *braziliensis* with 10% (03/30). With basis on these results, it should be emphasized that even thought the metropolitan region of Belém has suffered strong ecological transformation in the last fifteen years, this change has not been a barrier for the circulation of the enzootic cycles of the identified *Leishmania* species, and the presence of these active *Leishmania* sp. enzootic cycles indicates that the phlebotomine vectors of these parasites have also adapted to these forest fragments.**Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq **Keywords:** Pcr-rflp; cutaneous leishmaniasis; leishmania

PV22 - **MOLECULAR CHARACTERIZATION OF IRON TRANSPORT IN *TRYPANOSOMA CRUZI* AND ITS POTENTIAL ROLE IN PARASITE VIRULENCE**

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Chagas' disease is caused by the parasite protozoan *Trypanosoma cruzi*. Regarding the protozoan *T. cruzi*, it requires both a heme and a nonheme iron source for an optimal growth rate. Iron is a micronutrient that plays an essential role in almost all living organisms, being an essential cofactor in many metabolic pathways and enzymes. Fe is also harmful due to its capability in catalyzing the formation of reactive $O_2$ species (ROS) and, for this reason, all living systems have developed tight mechanisms for the control of uptake, metabolism and storage of Fe. However, there is very limited information available on iron uptake and sources by *T. cruzi*.

Here, we identify a putative iron transporter in *T. cruzi* genome, TcIT, homologous to the newly described iron transporter in Leishmania amazonensis, LIT, and to the Arabdopsis thaliana iron transporter IRT1. TcIT is localized on epimastigotes plasma membrane. Epimastigotes grown in iron-depleted medium presents a *TcIT* transcript increased when compared to epimastigotes grown in control medium. As expected, intracellular Fe concentration in cells maintained at iron-depleted medium is lower than those control condition. This result is reflected in a high $O_2$ consuption, high intracellular ATP concentration and $H_2O_2$ production by cells maintained at iron-depleted medium.

The investigation of mechanisms of non-heme iron transport at the cellular and molecular level will allow elucidating initial points of iron metabolism in *T. cruzi* and the involvement of iron transport in virulence and parasite proliferation. **Supported by:**FAPERJ, CNPq, Capes **Keywords:** Iron transport; trypanosoma cruzi; iron metabolism

PV23 - **RHODNIUS PROLIXUS AND TRIATOMA INFESTANS AS BIOLOGICAL FILTERS FOR DIFFERENT TRYPANOSOMA CRUZI STRAINS**

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Several reports showed that depending on the triatomine species, not all T. cruzi strains are able to sustain infection inside the vector. This suggests that the digestive tract of these insects may act as a biological filter. For example, Y strain (TcII) does not infect Rhodnius prolixus, while Ds28c and CL-Brener strains do. To evaluate this phenomemon, R. prolixus and Triatoma infestans were artificially fed with epimastigotes from different strains (DM28c-TcI, YUYU-TcII, Bug2145cl10-TcV and Brener-TcVI CL - 1x107 parasites/mL). DM28c was isolated from Didelphis marsupialis whereas CL-Brener, YuYu and Bug2145cl10 were isolated from T. infestans. Twenty nymphs had their posterior midgut and rectal ampoule dissected at times of 49 to 50 days after infective feeding. After 28 and 49 days of infection, triatomine were fed again with rabbit inactivated blood for diuresis. Slide smears were analysed to investigate the proportion of epimastigotes/metacyclic forms (15 triatomines at each stage). All strains were able to accomplish metaciclogenesis in both species of vectors.However, the intensity of infection was higher in Bug2145cl10 (TcV) strain for both triatomine species. In conclusion, using the above mentioned strains, T.infestans and R. prolixus were not able to act as a biological filter. All strains were able to sustain infection up to 50 days and accomplish metacyclogenesis. However, the intensity of the infection varied where Bug2145cl10 (TcV) was able to exhibit a higher infection compared to other strains.**Supported by:**FAPEMIG, CNPQ **Keywords:** Trypanosoma cruzi; rhodnius prolixus; triatoma infestans

PV24 - **ENDOSYMBIOSIS IN TRYPANOSOMATIDS: THE SYMBIONT INFLUENCES THE CONSUMPTION OF CARBON SOURCES AND ENERGY PRODUCTION OF THE HOST TRYPANOSOMATID**

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Some trypanosomatids co-evolves with a symbiotic bacterium in a mutualist relationship that involves intense metabolic exchanges. In this study we verified the growth of *Angomonas deanei*, WT and aposymbiotic (APO) strains, in medium containing different carbon sources (glucose or L-proline, 8 mM), as well as complex and chemically defined mediums. We also tested the proliferation of this protist in culture medium containing dichloroacetate (DCA; 20, 50 and 100 µM), a metabolic inhibitor that has been used in cancer treatment since it blocks the aerobic glycolytic pathway and recovering mitochondrial oxidative phosphorylation, thus restoring apoptosis in cells that are refractory to anticancer agents. Furthermore, we performed viability tests and labeled cells with Mitotracker® or anti-aldolase antibody, followed by flow citometry and optical fluorescence microscopy analyses. Results showed that protist presents higher proliferation taxes when grown in medium containing glucose when compared to proline Viability tests revealed that 85% cells were viable when gown in the presence of glucose, whereas this value was only 26% when protist were cultivated with proline or in medium with no carbon source. Considering the growth in the presence of 20 µM or 50 µM DCA for 24h, the number of WT cells increased in 165%, 150%, respectively, when compared to the control. For APO cells an increase in cell proliferation was only observed after 60h: 90, 43 and 82% after using 20, 50 and 100 µM DCA, respectively in Taken together, our result showed that differently from glucose, proline cannot be considered as an adequate carbon source to fuel the *A. deanei* energy metabolism. Furthermore data obtained with DCA reinforces the idea that symbiont-containing trypanosomatids presents a higher phosphorylating activity than the APO cells that are more fermentative. **Supported by:**CNPq e FAPERJ **Keywords:** Angomonas deanei; dichloroacetate; glucose

PV25 - **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 to date, the fifth histone (H1 or linker histone), has not been identified. In other eukaryotes, H1 links nucleosomes and its absence could interfere with the chromatin condensation. We identified a small and basic protein in *Toxoplasma*, similar to H1-like of *Kinetoplastidae* 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. By Western blot, TgH1-like showed twice the expected size (20 kDa), which could be explained by the presence of ubiquitination, as described in the literature. 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, which may be related to an alteration in 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 knockout parasites 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, ICC - FIOCRUZ/PR, Fundação Araucária **Keywords:** Histone; chromatin; toxoplasma gondii

PV26 - **INFLUENCE OF DIFFERENT AXENIC CULTURE MEDIA ON GROWTH, VIABILITY AND MORFOLOGY OF LEISHMANIA INFANTUM**

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In vitro culture of parasites of the genus Leishmania plays an important role in diagnostic studies, vaccines and treatment of leishmaniasis. The use of different culture media seems to influence the growth and viability of these parasites. Thus, the culture media are formulated in order to mimic the main physicochemical characteristics of the insect vector body fluid. Thus, the objective was to evaluate the influence of modifications on the composition of standard culture media (LIT, Schneider's® and Grace's®) on the growth, morphology and viability of Leishmania infantum parasites. For this study, we used the OP46 strain of Leishmania infantum isolated from a naturally infected dog and kept in vivo in hamster Mesocricetus auratus in our laboratory. The parasites were grown in the culture media mentioned above with the following modifications: without supplementation, supplemented with albumin and supplemented with fetal bovine serum (FBS) (Vitrocell®). From the initial inoculum of 1 x 107, the parasites were counted in the Neubauer chamber for 10 consecutive days. Mortality of the parasites using Propidium Iodide was also quantified. The morphology of the parasite was verified through the preparation of slides with smears of the culture and later stained with a Panótico Rápido®. It was observed that the parasites grew distinctly in all media, supplemented or not. In media without supplementation, there was a higher mortality of the parasites. In media supplemented with albumin, a lower mortality occurred in relation to media supplemented with SFB or not. However, the LIT culture medium plus FBS showed a higher parasite growth peak in relation to the others. In view of this, it was possible to observe that the culture medium changes the growth and viability of the parasites of the Leishmania infantum species.**Supported by:**Capes **Keywords:** L. infantum; culture medium; biology

PV27 - **CHARACTERIZATION OF THE RHS PROTEIN FAMILY ("RETROTRANSPOSON HOT SPOT") IN *TRYPANOSOMA CRUZI***

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RHS (Retrotransposon Hot Spot) gene family encodes a trypanosome-specific protein whose functions remain unknown. RHS carries a hot spot site for insertion of retrotransposon which gives rise to a pseudogene composed of one or more retroelements flanked by two separate halves of RHS. *T. cruzi* genome has 195 genes and 557 RHS pseudogenes. We identified RHS conserved domains (pfam07999, PTZ00209 and TIGRO1631) in all *T. cruzi* lineages and other trypanosomes. To characterize the structure of RHS family, 139 transcribed genes containing the RHS domain were classified by phylogenetic analysis into 10 groups. Sixty-five RHS sequences with low bootstraps were included in the unclassified groups and about 60% of them were generated by recombination resulting in a mosaic gene structure formed by fragments of different RHS genes. Retrotransposons may also have mediated RHS gene transposition to another genomic site. The mosaic nature of RHS family may reflect the plasticity and capacity of rearrangements of the *T. cruzi* genome. Several factors contribute to produce inconsistent phylogenetic affinities, and therefore to identify a common ancestor for RHS genes in *T.cruzi.* Among these factors are the expansion of this family by gene duplication, recombination between RHS sequences and the large number of pseudogenes generated by retrotransposon insertion. About 30% of RHS sequences are located in the subtelomeres, a region very susceptible to recombination, including ectopic homologous recombination. Expression of RHS was analyzed using monospecific polyclonal antibodies raised in mice against RHS recombinant protein. Analysis of cellular localization by immunofluorescence demonstrated that RHS proteins are evenly distributed in the nuclear region of *T. cruzi* replicative forms (amastigote and epimastigote). The nuclear localization of RHS in *T. cruzi* suggests that it may be involved in the control of chromatin structure and gene expression as proposed for *T. brucei* **Supported by:**FAPESP, CNPq and CAPES **Keywords:** Rhs; t. cruzi; characterization

PV28 - **AMPK PROTEIN KINASE IS INVOLVED IN *TRYPANOSOMA CRUZI* SURVIVAL UNDER NUTRIENT STRESS**

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Trypanosoma cruzi presents a high capacity for environmental adjustment but the mechanisms that signal this adaptation is largely unknown. The control of energetic state of eukaryotic cells occurs through a protein kinase activated by as a consequence of the increase in AMP (AMPK) relative to ATP levels, which coordinates cell growth, autophagy, lipid and glucose metabolism.AMPK is formed by 3 subunits(α, β and γ) and free AMP binds to the γ regulatory subunit, causing a conformational change of the α subunit, modifying the exposure of T172 that can be phosphorylated by other serine / threonine kinases. In trypanosomatids t was shown that in T. brucei α1 phosphorylation related to kinase activation induced differentiation via inhibition of the mTOR complex but this is not related to autophagy induction. Here we start to characterize the AMPK of T. cruzi, which is also formed by two α, two β and two γ subunits. We verified that when epimastigotes are incubated in nutrient depleted medium, dorsormorphin, an inhibitor of AMPK phosphorylation, caused parasite death. Death was related to a decrease in the ATP levels and could be inhibited addition of energy sources, as proline. At the same time, we observed that the phosphorylation levels of AMPK α1 also decreased. We also found that AMPK phosphorylation occurs when the epimastigotes from the Y and CL Brener, but not DM28C strains are incubated in starved medium to induce metacyclogenesis. These data suggest that the stress required for parasite differentiation activates AMPK and the contribution of this enzyme may be variable between different parasite strains.**Supported by:**CAPES **Keywords:** Ampk; energy metabolism; kinase

PV29 - **FREE LIVING AMOEBA DIVERSITY ON MONJOLINHO RIVER AT STATE OF SãO PAULO: A MORPHOLOGY AND MOLECULAR APPROACH**

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Naegleria, Acanthamoeba, Sappinia and Balamuthia comprises the main genres of the free living amoeba (FLA) group whose protists have a worldwide distribution being recovered either from soil or freshwater samples. They are capable of causing infections affecting humans, for instance the Primary Amoebic Meningoencephalitis (PAM) evoked by Naegleria fowleri leading to death in less than a week since the appearance of first symptoms. To date, in Brazil and the other South American countries there is a lack of FLA's occurrence reports linked with an underestimation in the number of PAM cases reported, due to the difficulty in obtaining an accurate diagnosis. Thereby, this research aims to identify the environmental occurrence of FLA in five sites of a public freshwater called Monjolinho river at São Carlos - SP. The methodology adopted to address this goal includes a limnological water characterization, supported by the Limnology Laboratory of the Federal University of São Carlos, besides the morphological and molecular analysis. The latter has been performed using a set of 18S rDNA primers for global (FLA group) and genre specific sequencing. Hitherto, Naegleria gruberi has been identified in one of the five collection sites and we have been working on DNA extraction of the organisms from the remaining four sites. Regarding the in vivo assays, 500 ml of the water obtained in each collection site was filtered through a 0.45µm membrane and transferred onto non-nutrient agar plates cultivated at 26, 37 and 44º C aiming identification of thermotolerant species. Then, the isolated trophozoites have been observed with scanning electron microscopy, besides four different staining methods for optical microscopy which morphological identification has been confirmed in the Page's key. The expected outcomes of this investigation will be of great importance since they have the potential to reveal pathogenic species encountered in freshwater in the urban environment.**Supported by:**CAPES **Keywords:** Fla; freshwater; monjolinho river

PV30 - **GLYCEROL AS A NOVEL ALTERNATIVE CARBON SOURCE FOR TRYPANOSOMA BRUCEI BLOODSTREAM FORMS.**

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The bloodstream forms of Trypanosoma brucei (TbBSF) have been recently found in skin and adipose tissue of their mammalian host. Glucose was the only carbon source known to be used by TbBSF to feed their central metabolism, however, the metabolic behavior of extravascular tissue-adapted parasites has not been addressed yet. Since the production of glycerol is an important primary function of adipocytes, we have adapted TbBSF to a glucose-depleted but glycerol-rich culture medium (CMM_Gly/GlcNAc) and compared their metabolism and proteome to those of parasites grown in standard glucose-rich conditions (CMM_Glu). According with our results, BSF consumed 1.7 folds more glycerol than glucose, required 2 folds more O2 per consumed carbon and were 11 times more sensitive to SHAM, a specific inhibitor of alternative oxidase (TAO). This is in agreement with the 1.8-fold increase of the TAO expression level compared to the CMM_Glu conditions. When the parasites were in the presence of equimolar concentrations of glucose or glycerol, they consumed both, although glucose was preferred. Metabolomic analyses by mass spectrometry showed that 13C-labeled glycerol was incorporated into hexose phosphates through gluconeogenesis in the absence of glucose. The RNAi-mediated downregulation of glycerol kinase abolished glycerol metabolism and was lethal for the parasites in CMM_Gly/GlcNac medium. Interestingly, TbBSF adapted to growth in CMM_Gly/GlcNac modified their metabolism by increasing glycerol consumption and decreasing that of glucose, however, glycerol kinase content decreased 7.8 folds in this condition. This suggests that glycerol kinase is in a huge excess in glucose rich conditions and may have an alternative unknow function. Altogether, these data demonstrate that TbBSF are well-adapted to glycerol-rich conditions that could be encountered by the parasite in extravascular niches, such as the skin and adipose tissues.**Supported by: Keywords:** Metabolism; glycerol ; gluconeogenesis

## PV31 - **DELTA AND EPSILON TUBULIN IN *TRYPANOSOMA BRUCEI***

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The trypanosomes cytoskeleton is primarily made of microtubules, which presents important functions for cell division and flagellar movements. These microtubules are composed by a polymer of alpha and beta tubulin dimers, formed by the gamma tubulin in association with other proteins, in a process called microtubule nucleation. These tubulins have been well characterized and are part of a superfamily of six proteins, alongside delta, epsilon and zeta tubulins. Despite all the information available for alpha, beta and gamma, little is known about the localization and molecular function of the other tubulins in trypanosomatids. Therefore, studies that would assess the localization of delta and epsilon tubulins would be of great importance to the understanding of their characterization and the understanding of their role in the cell. In this study, we are investigating the role of delta and epsilon tubulins in *Trypanosoma brucei* cells. Cell lines were constructed expressing a SAS-6 gene (protein located in the basal body cartwheel) with mScarlet from the endogenous locus. Following the SAS-6 tagging, plasmids were constructed with the delta and epsilon tubulin genes fused with 10 X TY-1 epitope tag and transfected in the SAS-6:mScarlet line. Immunofluorescence assays using an anti-Ty-1 epitope showed the SAS-6-mScarlet to the pro basal body and mature basal body, which was used as reference localization when compared to the delta and epsilon tubulins. The delta and epsilon localizations showed specific localization to the basal body region, but only during the early stages of basal body duplication. **Supported by:**MRC - Medical Research Council **Keywords:** Tubulin; cellular localization; trypanosoma brucei

## PV32 - ***TRYPANOSOMA CRUZI* EPIMASTIGOTES RNASEQ SUGGESTS METABOLIC ADAPTATION TO HEME TREATMENT**

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Chagas disease is a neglected illness caused by *Trypanosoma cruzi*. The parasite epimastigotes forms inhabit triatomine midgut, an environment rich in products of blood digestion such as heme. Once heme molecule showed a role in parasite proliferation, we analyzed heme effect in gene transcription of epimastigotes. Hence, we studied epimastigotes transcriptomic using *deep sequencing* analyses of poliA mRNA in an Illumina NextSeq500. RNAseq analyses identified a total of 19607 genes and 1370 of them were regulated by heme. Altogether 30 differentially expressed genes involved in energetic metabolism were validated by qRT-PCR. About 60% of glycolysis genes were upregulated. Only cytosolic glyceraldehyde-3-phosphate dehydrogenase and glycosomal phosphoglycerate kinase (PGK) were downregulated. Cytosolic PGK were upregulated by heme, thus glycolysis ATP production may occur in cytosol and NADH in glycosome. Heme molecule also increased 66,7% of genes transcription involved with fermentation process, highlighting upregulated glycosomal NADH-dependent fumarate reductase. Cytosolic enzymes showed higher values, mostly malic enzyme, enolase and aspartate aminotransferase. Mitochondrial enzymes genes did not present the same induction and the mainly upregulated enzyme is involved in glycine metabolism, that implies the parasite uses fermentation to produce ATP. The NADH levels were decreased in heme treated-parasite, which reaffirms more NADH being oxidized by fermentation. Therefore, transcriptomic analyses suggest that heme modulate *Trypanosoma cruzi* gene expression and promotes epimastigotes adaptation that allows parasite to progress in its life cycle. **Supported by:**FAPERJ, CNPq and INCT-EM **Keywords:** Trypanosoma cruzi; heme ; deep sequencing

PV33 - **GROWTH AND CELLULAR DIFFERENTIATION PATTERNS OF *TRYPANOSOMA CRUZI* AND *T. RANGELI* IN AXENIC CULTURES DISPLAY USEFUL MARKERS FOR THEIR DISTINCTION**

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Introduction: *Trypanosoma cruzi* and *T. rangeli* can share several vertebrate and invertebrate hosts. Accordingly, their differential diagnosis is of utmost importance, and these species can also be distinguished by their biological and morphological features. Methods: Fifteen reference strains of *T. cruzi* (8) and *T. rangeli* (7) belonging to different genotypes were grown in LIT medium with 10 or 20% fetal calf serum (LIT-10 or LIT-20, respectively). At some days (10th-20th), Giemsa-stained smears of all stocks were examined under light microscopy (x1,000) for determining the % of each developmental stage. Results: *T. cruzi* strains were able to grow in LIT-10, but *T. rangeli* only grew in LIT-20, the most fastidious being the KP1(-) strains. *T. cruzi* strains showed epimastigotes (predominantly), trypomastigotes, dividing stages, besides a low % of not-well defined forms. Their rates of metacyclic trypomastigotes ranged from 0.1% (Dm28c) to 26.0% (MR). Otherwise, *T. rangeli* strains were more polymorphic than those of T. cruzi, displaying tadpole-like forms, besides typical spheromastigotes at rates from 6.4% (H14) to 41.4% (R1625). Metacyclic trypomastigotes were not seen in *T. rangeli* cultures. Discussion: Striking differences in the growth rates and cellular differentiation patterns were found between these species, some stages being suitable diagnosis markers, mainly the spheromastigotes of *T. rangeli*. This species presents a complex life cycle in triatomine bugs; tadpole-like forms and spheromastigotes have been described within cells of their hemolymph, and they probably are precursors of metacyclics. Based in the present study, we are considering the possibility that the spheromastigotes described by Brack (1968) in *Rhodnius prolixus* experimentally infected with *T. cruzi* could be stages of *T. rangeli* in a mixed infection. Then, the putative double developmental cycle proposed for *T. cruzi* by Brack remains doubtful.**Supported by:**FIOCRUZ, UFF **Keywords:** Trypanosoma cruzi; trypanosoma rangeli; spheromastigotes

PV34 - **THREE-DIMENSIONAL ANALYSIS OF HEMOGLOBIN UPTAKE AT LATE STAGES OF *P. CHABAUDI* ASEXUAL DEVELOPMENT**

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Malaria is a disease caused by protozoan parasites from the genus *Plasmodium* with the highest impact on public health in endemic areas. Morbidity and mortality of malaria results from the asexual replication of *Plasmodium* in the erythrocyte of the mammalian host. In the course of its intraerythrocytic development, malaria parasites incorporate massive amounts of the host cell cytoplasm. Hemoglobin uptake occurs through cytostomes, a structure that originates by the invagination of the parasite plasma membrane and the parasitophorous vacuole membrane. Internalized hemoglobin is digested in a compartment with acidic pH named food vacuole, releasing aminoacids and others by products, namely heme. Due to its toxic effects, free heme is immobilized and stored in a crystal form known as hemozoin. This mechanism is essential to parasite development and represents a physiological step used as target for many antimalarial drugs. So far, most of the studies about the mechanisms of hemoglobin uptake on malaria parasites focus on the trophozoite stage, as it is described as the stage were the majority of hemoglobin catabolism occurs. Recently it was demonstrated that hemoglobin catabolism occurs at *P. falciparum* early ring stage, however little is known about hemoglobin uptake at late stages of development. In this work, we observed *P. chabaudi* late development stages by serial section electron tomography. Three-dimensional analysis of parasites that were undergoing nuclear division (early schizont) revealed small cytostomes and multiple hemoglobin vesicles in the parasite cytoplasm. Hemoglobin uptake structures were also observed in late schizont parasites, however fewer hemoglobin vesicles were seen in the parasite cytoplasm, which might indicate a decrease in the hemoglobin uptake process. Taken together, these results provide new insights about hemoglobin uptake mechanism in *P. chabaudi* late stages of development.**Supported by:**CNPq, CAPES, FAPERJ, FINEP **Keywords:** Malaria; hemoglobina; microscopia eletrônica

PV35 - **NEW ISOLATES OF SYMBIONT-HARBORING TRYPANOSOMATID: STRUCTURAL AND PHYLOGENETIC CHARACTERIZATION**

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The symbiosis in trypanosomatids has been used to better understand the origin of organelles, since in this model two primitive organisms co-evolve in an obligatory association. The relationship between the protozoan and the symbiont is mutualistic and characterized by intense metabolic exchanges and structural changes in the protozoan. Among the four genera of trypanosomatids that contain a symbiotic bacterium in the cytoplasm, systematic investigations were so far limited to *Angomonas* and *Strigomonas*. Recently, two new genera of insect trypanosomatids carrying endosymbionts were created and named as *Novymonas* and *Kentomonas*. The main objective of this work is to characterize, from an ultrastructural and phylogenetic point of view, new isolates of symbiont-bearing trypanosomatids, especially those from the *Kentomonas* genus. Scanning electron microscopy analyzes showed that both isolates present a ring shape structure at the exit of the flagellar pocket and dimensional differences: one of the species presents twice the length (cell body and flagellum) and width of the other. Transmission electron microscopy showed the proximity of the bacterium in relation to the mitochondrial branches. Fluorescence microscopy revealed that the symbiont in mainly located at the anterior region and also suggested that protozoa of *Kentomonas* genus can differentiate, since the kinetoplast moves to the posterior region of the protozoan. The generation time corresponds to 4h to one of the isolates, while in others this value varies from 5 to 6 h. The inferred phylogenetic tree, based on SSU rRNA and gGAPDH gene sequences of 257 trypanosomatids, revealed a new genus and two new species (not yet named) that nest among *Strigomonas* and *Angomonas*. Other new species cluster in genus *Kentomonas*. Symbiont of this new isolates were classified as β-Proteobacteria of the Algaligenaceae family, related to the genus *Taylorella*.**Supported by:**FAPERJ e CNPq **Keywords:** Endosymbiosis; trypanosomatids; new isolates

PV36 - **EFFECT OF LYSOPHOSPHATIDYLCHOLINE ON THE MODULATION OF GROWTH, DIFFERENTIATION AND INFECTIVITY OF LEISHMANIA MEXICANA.**

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Leishmaniasis is among the major emerging parasitic diseases that affect humans. These infections are caused by different species of the genus *Leishmania*. Among these, *Leishmania mexicana* causes cutaneous and eventually diffuse (anergic)lesions, occurring especially in Mexico and Central America. Lipid mediators, including lysophosphatidylcholine (LPC) and platelet activation (PAF), play an important role in cell differentiation and infectivity of some trypanosomatids. PAF triggers a signal transduction cascade that activates protein kinase CK2, through protein kinase C (PKC), in *Herpetomonas muscarum muscarum*, and stimulates the activity and expression of CK2 in *Leishmania tropica*. Recently, our group showed that *Trypanosoma cruzi* synthesizes a bioactive LPC (C18:1LPC), which aggregates platelets and induces cell differentiation of the parasite. Based on these studies, the hypothesis formulated for this project was that C18:1 LPC would present a modulatory effect on several aspects of the physiology of *L. mexicana.* We analyzed the proliferationof promastigotes for seven days in the presence and in the absence of LPC anddetected that the LPC-treated parasites have grown three times more than the untreated ones. We have also tested if C18:1 LPC would trigger the differentiation of the parasites from promastigotes to amastigotes. The parasites were then cultivated for up to 30 days in the presence and in the absence of LPC and we observed that the LPC-treated parasites presented 40% more amastigote forms than the untreated ones. Preliminary results from the interaction of *L. mexicana* with BALB/c mice peritoneal macrophages showed an increase of about 10% in the association index of LPC-treated macrophages with *L. mexicana*, within 24 hours after the infection. Therefore, our results suggest a modulation of several important features of the life cycle of *L. mexicana* by C18:1 LPC.**Supported by:**CNPq, FAPERJ, INCT-EM, CAPES **Keywords:** Trypanosomatids; leishmania mexicana ; lysophosphatidylcholine

PV37 - **A TARGET-BASED APPROACH TO IDENTIFY BIOACTIVE COMPOUNDS AGAINST *T. CRUZI* TCK2, A PROTEIN KINASE INVOLVED IN PARASITE DIFFERENTIATION AND SURVIVAL IN THE MAMMALIAN HOST**

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Eukaryotic translation initiation factor 2-alpha kinases (EIF2AK) are protein kinases that phosphorylate eIF2α under stress situations, a key event to generate remedial responses in cells. We found that eIF2α phosphorylation occurs in *Trypanosoma cruzi* parasites induced to differentiate from replicative to non-replicative and infective forms. *T. cruzi* has three EIF2AK related kinases. One of them, TcK2, is topologically related to mammalian PERK and was found relevant for infection and parasite growth. Here we identified small molecules that can interact with TcK2 and are active against the amastigote form. First, we utilized a structural genomic approach to identify TcK2 constructs that could be produced in a soluble form using a heterologous *E. coli* expression system. Then we showed that the purified recombinant TcK2 kinase domain was active using both auto- and trans-phosphorylation *in vitro* assays. To find ligands for TcK2, we screened a commercial library (~400 compounds) of biologically active human kinase inhibitors using a thermal shift assay - Differential Scanning Fluorimetry (DSF). Phenotypic assays revealed that amongst the identified hit compounds GSK416A inhibited *T. cruzi* Y strain amastigotes proliferation in mammalian cells with a selectivity index of ~10 (EC50 of 1.43 vs 13.69 µM for *T. cruzi* and mammalian cells, respectively). We have also obtained co-crystals of TcK2 kinase domain in complex with PLX-4720, one of the hits identified by DSF, but these so far diffracted X-rays poorly. We are currently working on improving the X-ray diffraction quality of these co-crystals. We will also evaluate the potency of the identified hit compounds in an *in vitro* enzymatic assay using mouse eIF2α as a substrate for TcK2. We expect our results will help us identify small molecules that can be used to illuminate TcK2 role during parasite differentiation and host infection, and to chemically validate this kinase as a novel therapeutic target for Chagas' disease. **Supported by:**FAPESP and CNPq **Keywords:** Tck2; drug treatment; crystallography

PV38 - **LYSOPHOSPHATIDYLCHOLINE (LPC) AS MODULATOR OF THE SURVIVAL PROCESSES OF DIFFERENT SPECIES OF THE GENUS *LEISHMANIA***

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*Leishmania* spp are responsible for several forms of leishmaniasis. Lipid mediators, including LPC and platelet-activating factor(PAF) have been described as presenting a key role in the infection of some parasitic protozoa. Our group demonstrated that PAF modulates the infection of mouse peritoneal macrophages by *L. amazonensis* and that *T. cruzi* synthesizes a C18:1-LPC, with the ability of aggregating platelets, similarly to PAF. In the present study, we demonstrate the effects of LPC on *L. i. chagasi*(LC), *L. amazonensis*(LA) and *L. mexicana*(LM) proliferation and differentiation, as well as in the interaction of these parasites with mouse peritoneal macrophages. We observed a 32% increase in proliferation of LC, LA and LM on the 5th day of growth, when the parasites were treated with C18:1-LPC, as compared to the control. Pre-treatment of the parasites with WEB 2086(PAF receptor antagonist) reversed the LPC effects. Also, the number of differentiated forms exceeded the number of promastigotes on the 8th day after induction of differentiation by LPC, as compared to the control parasites, which present the phenomena on the 13th and 16th day for LA and LC, respectively. We also tested the effects LPC on the infection of mouse peritoneal macrophages when parasites were pre-treated for 4 hours with this lipid. Our results indicate an enhancement of the infection when LPC-treated parasites were used in the interactions. We have also built a 3D model of a putative *Leishmania* PAF receptor(PAFR) based upon the amino acid sequence submitted to the Phyre2 server and validated using the PROCHECK. A molecular docking study was performed to predict the interactions between the PAFR model and PAF or LPC using Molegro Virtual Docker. The docking data suggested that LPC is predicted to interact with the PAFR model in a fashion similar to PAF. These results suggest that LPC modulates the growth, differentiation and infectivity of *Leishmania* spp via a putative PAFR in these parasites.**Supported by:**CNPq, CAPES, FAPERJ and INCT-EM **Keywords:** Leishmania spp; lysophosphatidylcholine (lpc); platelet-activating factor (paf)

PV39 - **AUTOPHAGIC INDUCTION IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES UNDER NUTRITIONAL AND PH STRESS**

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*Trypanosoma cruzi* is the causative agent of Chagas disease, a neglected illness that affects millions of people in Latin America. This protozoan parasite has a complex life cycle, alternating between replicative and infective stages comprising triatomine bugs and mammals. Autophagy is a constitutive recycling process required for the removal of aged organelles and cytosolic components through lysosomal degradation. Environmental changes inside the hosts, such as nutritional and pH stress could trigger autophagy inducing parasite differentiation. The present work evaluated autophagy occurrence in epimastigotes cultivated under different nutritional and pH conditions by fluorescence microscopy and western blotting. Both Atg8 expression and the percentage of monodansylcadaverine labelled parasites under nutritional deprivation and pH stress. Moreover, a band migrating between 15 and 20 kDa was observed after western blotting analysis suggesting that the antibody can specifically detect Atg8 protein. We also analyzed the correlation between autophagy and oxidative stress by amplex red reaction. ROS production was higher in epimastigotes cultivated under nutritional deprivation and basic pH but, surprisingly, autophagy inhibition by 3-MA has not increased ROS levels. Thus, our data show that autophagic activity increases during stress conditions, which is evidenced by a higher number of autophagossomes and acidic compartments. However, further experiments are necessary to correlate autophagy and oxidative stress in *T. cruzi*, contributing to increase the knowledge of the mechanisms that regulate autophagy in this parasite.**Supported by:**Faperj, CNPq and FIOCRUZ **Keywords:** Trypanosoma cruzi; autophagy; ros

PV40 - **CHARACTERIZATION OF THE PROTEIN KINASE OF TRANSLATION INITIATION FACTOR 2 OF *TRYPANOSOMA CRUZI***

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Trypanosomes have to adapt to diverse environmental conditions. Most of these modifications occur through changes in RNA processing, RNA stability and protein synthesis, which leads to the preferential expression of proteins that act in the stress recovery. In eukaryotes, this control occurs partially at protein synthesis initiation through the availability of eukaryotic initiation factor 2 (eIF2) complex with GTP and the initiator tRNA. eIF2-GTP is regenerated after each round of initiation in a reaction which is inhibited by the phosphorylation of the alpha subunit of eIF2 (eIF2a) by specific protein kinases activated by different cellular stresses. Trypanosomes present three protein kinases (K1, K2 and K3) with characteristics of eIF2-kinases. We previously found that growth and dealing with oxidative agents are dependent of the Trypanosoma cruzi K2 (TcK2). This enzyme has a similar topology to the mammalian kinases know as PERK, for which several inhibitors have been developed. Therefore, the goal of our work was to test these inhibitors against TcK2 as a possible target to treat Chagas' disease. We obtained several recombinant proteins harboring the kinase domain of TcK2 using Escherichia coli. We found that some of these recombinants were soluble and displayed kinase activity against mammalian eIF2a, detected by specific monoclonal antibodies. We then optimized a screening assay to test the available and other compounds as potential enzymatic inhibitors, which could be used against proliferative or intracellular forms of T. cruzi. With this assay we found that specific PERK inhibitors were not able to inhibit TcK2, a result that opens possibilities to develop specific inhibitors for the T. cruzi enzyme.**Supported by:**FAPESP **Keywords:** Protein kinase; inhibitors ; assay plataform

PV41 - **BASALIN IS ESSENTIAL FOR BASAL PLATE AND CENTRAL PAIR FORMATION IN TRYPANOSOMATIDS.**

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Trypanosomes and *Leishmania* have a single motile flagellum whose beating is mediated by a canonical microtubule axoneme. While motile cilia/flagella have '9+2' axonemes – with nine outer doublets and a central pair of microtubules - '9+0' axonemes lack a central pair of microtubules and are typically immotile. Axonemal doublets elongate from the basal body/centriole, but the central pair of microtubules is nucleated independently, at or very near an electron dense 'basal plate', which remains a poorly characterized component of the canonical eukaryotic flagellum. We identified two proteins – TZP103.8 and basalin – which are important for central pair formation in trypanosomes and *Leishmania*. Ablation of TZP103.8 and basalin (by RNAi and knockout strategies) led to a strong reduction in cell proliferation and generated immotile cells. Transmission electron microscopy (TEM) analysis showed a prevalence of 9+0 axonemal profiles in immotile populations, which would explain the motility defect. Interestingly, TEM also showed that the basal plate disappeared after basalin ablation (hence the name), indicating that basalin is essential for basal plate formation. By light microscopy analysis, both TZP103.8 and basalin localized to the transition zone (TZ) area from which the axoneme elongates, and basalin was required for TZP103.8 localization to this region, suggesting that it is incorporated in the growing axoneme before TZP103.8. While the *Trypanosoma brucei* basalin was identified by proteomic analysis of the TZ, its *Leishmania mexicana* counterpart was identified by synteny analysis and is highly divergent compared with the *T. brucei* orthologue. The striking similarities in ablation phenotypes between the *T. brucei* and *L. mexicana* basalins revealed the potential of synteny analysis in the identification of cryptic orthologues in trypanosomatids. **Supported by:**Medical Research Council (UK), Wellcome Trust (UK) **Keywords:** Flagellum; basal plate; synteny analysis

PV42 - **IMMUNOLOCALIZATION OF COSTA PROTEINS FROM *TRITRICHOMONAS FOETUS***

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Apart from its veterinary interest, *Tritrichomonas foetus* is also important for its evolutionary and cellular aspects. Its complex cytoskeleton, still not fully characterized and understood, is composed of a variety of protein structures, in which we highlight the costa. This peculiar structure differs in many aspects from cytoskeleton filaments found in the muscle, cilia, flagella or other structure containing microtubules. The costa presents itself as a prominent striated fiber, composed of a complex filament ensemble and globular structures that is connected to the recurrent flagellum through a filament network. Although previous studies identified that *T. foetus*' costa shares some proteins with *Trichomonas vaginalis*, none of them were able to acknowledge which specific proteins belong to this structure. In a recent study from our group a proteomic analysis of an enriched costa fraction revealed the presence of 44 hypothetic proteins without conserved domains. Several alignments were made between these proteins and others, but no significant similarity was found, suggesting that *T. foetus*' costa may contain a new class of proteins. Thus, this study aimed at locating some of these proteins to confirm their presence in the costa. To achieve this, the most promising proteins were selected through bioinformatics analysis to produce specific polyclonal antibodies and to make endogenous tag through molecular cloning to proceed with their immunolocalization. As result, it was obtained a successfully *T. foetus* mutant using the plasmid pMASTER-NEO fused with the selected protein 13283, which allows the tagging with an HA epitope, enabling its further detection. Also, the specific polyclonal antibody against protein 11810 was produced. Both proteins, through immunofluorescence techniques, such as conventional and super-resolution fluorescence microscopy, were localized, with promising results. More studies to characterize these and the other proteins are still in process. **Supported by:**CNPq **Keywords:** Tritrichomonas foetus; costa; immunofluorescence

PV43 - **LYSINE K157 ACETYLATION REGULATES TRYPANOSOMA BRUCEI ALDOLASE ACTIVITY**

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Recently, in our laboratory, we described the acetylome of the protozoan parasite that causes African Trypanosomiasis, Trypanosoma brucei, by comparing the procyclic form, present in the invertebrate host, and the bloostream form, present in the mammalian host. We found 288 lysine-acetylated in 210 proteins in the procyclic form (PCF), and 385 lysine-acetylated sites in 285 proteins in the bloodstream form (BSF). Notably, only 27 proteins were common among the forms, reflecting differences in the mechanisms of adaptation of T. brucei during its life cycle. Interesting, the differences in the acetylation was more evident in the glycolytic enzymes, where lower levels were detected in BSF, which use glycolysis as main energy source, compared to PCF, which relies on oxidative phosphorylation for ATP production. This found suggested that acetylation could play a role in regulating the activity of glycolytic enzymes in T. brucei, as previously observed for aldolase and glycerol-3-phosphate dehydrogenase in mammals. To investigate that, first we compared the 3D structure of fructose 1,6-bisphosphate aldolase of T. brucei with human protein and found that residue K157, homologue to K147 that negatively regulates human aldolase activity when acetylated is conserved in parasite protein. Also, residues K117 and K163, which we detected acetylated in our analysis, are located close to the catalytic site of the enzyme. To verify if these residues play a role in enzyme activity, we generated mutated versions of parasite proteins where we replaced the lysine residues by glutamine that mimics an acetylated lysine, heterologous expressed and purified the proteins and used them for in vitro activity assays. We found that mutation at K157 completely abolished enzyme activity compared to wild-type version, while substitution of K117 and K163 had no effect on enzyme activity. These results demonstrate that acetylation could be another way to regulates glycolytic enzymes in T. brucei. **Supported by:**CNPq and FAPESP **Keywords:** Trypanosoma brucei; acetylation; aldolase

PV44 - **CRISPR/CAS9-MEDIATED MYC TAGGING OF TRYPANOSOMA CRUZI HISTONE H2BV**

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Trypanosoma cruzi, the etiologic agent of Chagas disease, is responsible for significant cause of death from the South of US to many countries in Latin America. The parasite alternates between replicative non-infective forms (epimastigotes and amastigotes) and non-replicative infective forms (cellular and metacyclics trypomastigotes). It was previously observed that differentiation between these life forms follows changes at the overall levels of transcription as well as at the nuclear/chromatin structure. Recently, our group identified that histone variant H2Bv is presented in high abundance at non-replicative forms. Here, we aim to better understand the role of H2Bv in T.cruzi chromatin by generating parasites expressing this variant tagged by CRISPR/Cas9 systems using a PCR-based method. Adding a tag to a particular protein allows us to better determine its location possibly shedding light into their cellular function. Thus, sgDNA targeting sequences were designed using the Eukaryotic Pathogen CRISPR guide RNA/DNA Design Tool. Donor DNA was amplified from the pMOtag 23 M (Myc)/ puromicine plasmid using primers against the 3´ UTR of H2Bv. SgDNA along with donor DNA were transfected into CL-Brener parasites expressing the Cas9 protein. The transfected parasites were selected with Puromicine for two weeks. Myc-tagged parasites were confirmed by Western Blot and DNA sequencing. Currently, we are optimizing chromatin immunoprecipitation (ChIP) assays in order to evaluate H2Bv genomic location. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; crispr/cas 9; h2bv

PV45 - **THE INVOLVEMENT OF THE PARAFLAGELLAR ROD PROTEIN (PFR) IN CELL SIGNALING DURING AMASTIGOGENESIS**

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The amastigogenesis of *T. cruzi* occurs *in vivo* inside the cells of mammalian hosts and needs an incubation period in the acid phagolysosome. This differentiation process can be induced *in vitro* by subjecting the tissue-culture-derived trypomastigotes (Ty) to an acidic DMEM medium. During this process, the parasite undergoes a progressive internalization of the flagellum, in which it changes from the trypomastigote form, characterized by a thick and elongated shape with a prominent kinetoplast at the terminal region, a long flagellum and an undulating membrane to a rounded shape with longitudinal fissures without flagellum (amastigote). In all kinetoplastids there is a protein in the structure of the flagellum, the paraflagellar rod (PFR), forming a highly organized complex with the filaments of the cytoskeleton that surround the structure of the axoneme microtubules. In addition to physically support the flagellum, PFR is required to assist in mobility, allowing its movement in viscous media such as blood and lymph. The flagellum is known to be an environmental sensor and to participate in some signaling pathways. The involvement of PFR in the signaling process during amastigogenesis was followed by Western blot and immunofluorescence. Our results suggest that there are changes at the phosphorylation levels during this process. In agreement with this data, the intracellular cAMP/cGMP concentrations quantified during the differentiation process showed differences between the pH 6 medium (pro-amastigogenesis) and the pH 7 medium (control) at different incubation times. The localization of PFR in fractions from the medium during the differentiation process indicates that this protein is not only being internalized but is also it is released to the medium. In conclusion, our data suggest that the PFR protein is participating on the signaling process that drives the amastigogenesis.**Supported by:**CNPQ **Keywords:** Paraflagellar rod protein; amastigogenesis; phosphorylation

PV46 - **NEW TECHNOLOGIES FOR *TRYPANOSOMA CRUZI* TYPING: A NEXT GENERATION SEQUENCING PIPELINE BASED ON HYPERVARIABLE REGIONS OF KDNA MINICIRCLES**

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The kinetoplastic DNA of *Trypanosoma cruz*i consists of dozens of maxicicles and about 30,000 minicircles. Each minicircle (≈ 1.4 kb) is organized into four conserved regions (≈ 120 bp) and intercalated by an equal number of hypervariable regions (≈ 240 bp) known as mHVRs (minicircle hypervariable regions). There are few known mHVR sequences, possibly due to technical difficulties for sequencing this kind of DNA regions in the past. Nowadays, the Next Generation Sequencing (NGS) techniques open the possibility of a deeper knowledge of the mHVR sequence diversity. In the present work, we have optimized a mHVR targeted sequencing pipeline based on the Illumina MiSeq technology for the analysis of different strains of the main *T. cruzi* lineages (TcI-TcVI). Approximately 1.5 million reads were analyzed for each of the following strains: Tev55cl1 (TcI), PalDa20cl3 (TcI), Tu18 (TcII), Esmeraldo (TcII), X109 (TcIII), CANIII (TcIV), LL014R1 (TcV), MNcl2 (TcV) and LL015P68R0cl4 (TcVI). Sequences were clustered according to different identity thresholds (85%, 90% and 95%). In addition, different measures of alpha and beta diversity were calculated. Results show that sequencing at low depth (10.000 reads) is enough to put in evidence the main body of sequence diversity. It was also observed that strains of the same lineage clustered together in principal component analysis and UPGMA graphs. The number of clusters of sequences was variable among strains and some clusters were strain-specific or lineage-specific. The results of this work indicate that this reproducible NGS pipeline could be applied for genetic typing of *T. cruzi*. These are the first results in the framework of a broader work that aims to study the mHVR diversity and its evolution, as well as searching for lineage- specific sequences for typing. **Supported by:**CONICET-Consejo Nacional de Investigaciones Científicas y Técnicas **Keywords:** T. cruzi; mhvr; ngs

PV47 - **DELETION OF HISTONE DEACETILASE 2 AFFECTS REPLICATION OF TOXOPLASMA GONDII**

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Histone deacetylation is a form of epigenetic regulation that is associated with gene silencing, since the removal of acetyl groups by histone deacetylases (HDACs) results in a more condensed chromatin. In Toxoplasma gondii little is known about these enzymes, but it is believed they are an essential part of gene expression regulation. T. gondii has 7 HDACs, including HDAC2 (TgHDAC2), the aim of our study. Although it is considered a classic HDAC of class I very similar to other eukaryotes, the TgHDAC2 has 2 amino acid insertions inside the HDAC domain, which is unique to Toxoplasma, and whose function remains unknown. In addition, RNA-seq data from T. gondii database showed an increase of tghdac2 expression during S phase of the cell cycle. Due to its particularity, our goal is to characterize HDAC2 of T. gondii (TgHDAC2). To this end, we have obtained the knockout of tghdac2, which was replaced by homologous recombination, by hxgprt gene. So far, we observed a lower infectivity and proliferation rate of tghdac2 knockout parasites, suggesting a role during cell cycle progression, possibly during the S phase. Proliferation assays with EdU incorporation indicated that the lack of tghdac2 leads to a delay during the S phase of the cell cycle, which may indicate that these parasites have fewer replication forks or the replication is slower. Besides the knockout we also obtained the overexpression of the protein, which was located in the nucleus of the parasite, again indicating a nuclear function. Furthermore, circular dichroism analyzes had shown that recombinant HDAC2 is a very stable and enveloped protein, and in this way we are trying to obtain its crystal to analyze its three-dimensional structure by X-ray crystallography.**Supported by:**Fiocruz; Capes; CNPq **Keywords:** Toxoplasma gondii; epigenetics; histone deacetylase

PV48 - **GLUTAMINE PARTICIPATES IN THE BIOENERGETICS OF *TRYPANOSOMA CRUZI***

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*Trypanosoma cruzi* is the etiologic agent of Chagas disease. During the life cycle the parasite alternates between two different hosts: mammals and reduviid insects. In addition, inside both hosts, *T. cruzi* differentiate into different stages and colonizes various environments, such as, the host-cells cytoplasm and the bloodstream (in the mammalian host), and the intermediate and final portion of intestine (in the insect vector). These environments offer different nutrient compositions, including variations in the available carbon sources. We have shown that, in this context, amino acids play an important role in the parasite survival. The aim of this work was to evaluate the participation of glutamine (Gln) in the bioenergetics of *T. cruzi*. We have previously shown that *T. cruzi* can obtain Gln by uptake and biosynthesis. Also we demonstrated that Gln supports metacyclogenesis and is able to keep the viability of the parasites during the differentiation process in comparable levels than the standard metacyclogenesis medium (TAU 3AAG). Moreover, parasites maintained in the presence of Gln as the solely carbon and energy source maintained comparable intracellular ATP levels and viability to parasites kept in the presence of proline, an already described as energy source. These data led us to hypothesize the participation of Gln of the bioenergetics of the cell. This hypothesis implies the conversion of Gln must into glutamate and subsequently into alfa-ketoglutarate which can be oxidized through the TCA cycle, triggering the production of $CO_2$. Thus, the parasites were incubated in the presence of radiolabeled Gln (L-[$^{14}$C(U)]-Gln) which allowed us to trap and quantify the radiolabeled $^{14}CO_2$. We detected a linear increase of $^{14}CO_2$ overtime, showing that Gln is oxidized to $CO_2$. We are currently obtaining more data on the ability of Gln to fuel OxPhos, in order to better characterize the role of Gln as an important metabolite for the energy metabolism of the *T. cruzi*. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; glutamine; bioenergetics

PV49 - **TGHDAC4: AN APICOPLAST HISTONE DEACETYLASE OF *TOXOPLASMA GONDII***

FRAGOSO, M.S.I.*[1]; DE SIQUEIRA, C.M.[1]; SEVERO, V.R.[1]; MARTINS-DUARTE, E.S.[2]; AVILA, A.R.[1]; NARDELLI, S.C.[1]
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Epigenetic regulation play an important role controlling gene expression and other DNA-related processes in *Toxoplasma gondii*. One category of enzymes that act as epigenetic regulators is histone deacetylases (HDACs), which has a well-known 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 studied class of deacetylases. Protein alignments showed that the only region similar to other organisms is 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-localize with TgHU, a histone-like protein. Preliminary data of transmission electron microscopy suggest the presence of TgHDAC4 in the apicoplast, at the periphery of the organelle. Western blot assays identified a protein smaller than expected, a common characteristic of apicoplast proteins due to cleavage signal and transit peptides. We aim to verify if these signals are responsible for directing the protein to the apicoplast by adding an YFP tag. On the other hand, the deletion of *tghdac4* was lethal, despite several attempts. We are currently focusing on the construction of conditional knockouts lines using Auxin-inducible Degron system. In addition, we are performing immunoprecipitation assay followed by mass spectrometry to identify the proteins that interact with TgHDAC4. 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 e ICC/Fiocruz-PR **Keywords:** Toxoplasma; apicoplast; histone deacetylase

PV50 - **GENE EXPRESSION REGULATION OF A HYPOTHETICAL PROTEIN AND A RNA BINDING PROTEIN DEPENDENT OF ARGINASE ACTIVITY IN *LEISHMANIA AMAZONENSIS***

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Arginase is an enzyme that uses L-arginine to produce urea and ornithine, being this last one a precursor of polyamines pathway, important to parasite replication. Besides this physiological role, our research group is also exploring the role of L-arginine in NO production as an immunoregulatory factor of the host immune response. The activation of one pathway or other will define the fate of infection, leading to parasite death or parasite replication and resistance to host´s defense mechanisms. Using RNA-seq approach that describe how stability and processing of mRNA can occur during the mechanisms of gene expression regulation, we described the existence of differential gene expression regulation in the comparison of promastigotes from *L. amazonensis* wild type (La-WT) to *L. amazonensis* arginase knockout (La-arg-) (Aoki et al., 2017). Among the differentially expressed genes, two transcripts appeared as most regulated: a hypothetical protein (LmxM.15.1520) presenting 3.1-fold change; and a RNA binding protein (RBP) (LmxM.07.0990) presenting 0.37-fold change. According to in silico analysis, the hypothetical protein contains a signal peptide in the N-terminal region, no conserved domains, no transmembrane domain and is conserved among Leishmania spp. RBP presents no transmembrane domain, no signal peptide and is also conserved among Leishmania spp. In addition, RBP presents a conserved core domain for a nucleoplasmin family of histone chaperone proteins. Both genes were cloned into pSNBR vector, transfected into La-WT and selected mutants overexpressing those genes by neomycin selection. The overexpression/selection method has been used for identification of drug targets and potential drug resistant genes. The data obtained in the characterization of those mutants will provide important knowledge on how Leishmania is able to modulate the mechanisms of gene expression regulation to allow the parasite survival.**Supported by:**FAPESP (#2014/50717-1 and #2016/03273-6), CAPES, CNPq **Keywords:** Rna-seq, transcriptome,; differentially expressed genes; polyamines pathway

PV51 - **ESTABLISHMENT OF MOLECULAR KARYOTYPE AND SYNTENIC ASSOCIATIONS AMONG ISOLATES OF *TRYPANOSOMA RANGELI***

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*T. rangeli* comprises a pool of heterogeneous strains which have been classified into two main genetic lineages KP1(+) and KP1(−), based on the presence or absence of KP1 kDNA minicircles. Intra-strain karyotype heterogeneities have been reported in *T. rangeli*, suggesting that chromosomal rearrangements occurred during the evolution of this parasite. Comparison of karyotypes of related species and even among isolates from the same species is an interesting strategy to identify chromosomal variations involved in the speciation process, as well as in chromosomal evolution. The objective of our work is to study the chromosome variability and evolution in *T. rangeli* by molecular karyotyping and aCGH. The karyotypes of *T. rangeli* SC-58 and Choachí isolates had already been established. In this work we defined the karyotype of other isolates of *T. rangeli*: C23 (KP1-) from *Aotus* sp; R1625 (KP1+) and H14 (KP1+) from *H. sapiens* e PIT10 (KP1-) from *Panstrongylus megistus*. The karyotypes of C23 and R1625 comprise 14 chromosome bands ranging from 0.31-3.20 Mb, while the isolates H14 and PIT10 have, respectively, the highest and lowest number of bands. There are 21 bands ranging from 0.33-3.98 Mb in H14; and 16 bands ranging from 0.29-3.17Mb in PIT10. Although some isolates have the same number of bands, they differ from each other by the size of chromosomal bands. Preliminary analysis by aCGH (comparative genomic hybridization) confirmed these results, for instance, isolate H14 showed more events of DNA gain when compared with other isolates. Although the DNA content seems to be constant between strains of two genetic groups *T. rangeli* (Naves et al, PLoS One 2017, 12(12):e0189907), several karyotypic changes are revealed by molecular karyotyping and aCGH. The synteny among these isolates will be analyzed by hybridization of chromosomal bands with genetic markers from three large syntenic blocks conserved among *T. rangeli.***Supported by:**FAPESP, CNPq e CAPES **Keywords:** Trypanosoma rangeli; karyotype; synteny

PV52 - **EFFECTS OF THE KNOCKOUT OF ZINC FINGER PROTEINS TCZC3H39, ZFP29 AND ZFPTTP IN TRYPANOSOMA CRUZI.**

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Gene expression regulation is essential for Trypanosoma cruzi to adapt to the different conditions the parasite faces during its life cycle. In trypanosomatids, this control is mainly mediated by post-transcriptional mechanisms which act on the metabolism of mRNAs and proteins. In this context, RNA binding proteins (RBPs) are key players due to their interaction with the mRNA by coordinating its fate in the cell. The RBPs repertoire of T. cruzi comprises different groups of proteins, including those with the C3H zinc finger domain. This investigation aims to study the zinc finger proteins, TcZC3H39, ZFP29 and ZFPTTP in T. cruzi by investigating their expression and localization throughout parasite's life cycle as well as the associated mRNAs targets in both epimastigotes and parasites under nutritional stress. Recently, the CRISPR/Cas9 system has been described as an efficient method for gene knockout, which is being applied in several organisms, including T. cruzi. Therefore, in order to determine the role of TcZC3H39, ZFP29 and ZFPTTP in the RNA metabolism of T. cruzi, we used this gene editing system to disrupt these genes and evaluate the respective associated phenotypes. After knockout with specific guide RNAs for each target gene, major changes were observed in the morphology and cell cycle of the parasites. In general, cells presented larger size, loss of their original shape and also G2/M phase arrest in the cell cycle. So far, we were able to confirm the knockout of ZFPTTP while the confirmation of TcZC3H39 and ZFP29 disruption is currently in progress. Furthermore, after three independent assays targeting these genes, the results consistently showed that the absence of TcZC3H39, ZFP29 or ZFPTTP compromises the viability of the cells. This suggests that TcZC3H39, ZFP29 and ZFPTTP are essential for the parasite survival and play an important role in its biology.**Supported by:**PAPES-Fiocruz, CAPES, CNPq, Fundação Araucária. **Keywords:** Trypanosoma cruzi; zinc finger protein; gene knockout

PV53 - **COMPARATIVE GENOMIC HYBRIDIZATION (ACGH) ANALYSIS OF *TRYPANOSOMA CRUZI*, *TRYPANOSOMA RANGELI* AND *TRYPANOSOMA CONORHINI* GENOMES.**

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*T. cruzi* and *T. rangeli* are sympatric species that share vectors and mammalian hosts, including humans. *T. conorhini* is a parasite of *Rattus* spp. transmitted by *Triatoma rubrofasciata*. It has been suggested that *T. rangeli* is phylogenetically closer to *T. conorhini* than *T. cruzi*. Despite the divergence between trypanosoma species, the gene colinearity observed in their genomes may reflect the evolution from a common ancestor through duplication and/or horizontal gene transfer. Using phylogenetically close species or different isolates is a good strategy for comparative genomic studies.
The aCGH (Comparative Genomic Hybridization) technique allows a comprehensive chromosome tracking, capable of accurately determining chromosomal aneuploidies. In this work we compared by aCGH the genomes of *T. cruzi* (CLB), *T. rangeli* SC-58 (KP1-) and Choachí (KP1 +) and *T. conorhini*. We found 244 chromosomal abnormalities in *T. rangeli* and *T. conorhini*, 90.2% of them were related to loss/deletions. The most of chromosomal abnormalities varied from 100-500 kb in size, the smallest one 2.7 kb and the largest 1.95 Mb. The majority of changes were found in *T. conorhini* followed by *T. rangeli* SC-58 and Choachí. Analysis of genes involved in the chromosomal aberrations showed an increase of the proportion of multigenic gene families such as trans-sialities, Mucin Associated Surface Protein (TcMUC), GP63, DGF1 (Dispersed genes Family -1) and RHS (Hot-Spot Retrotrasposon). These data are in agreement with previous estimates showing that the genome size of *T. cruzi* was significantly higher than that of *T. rangeli*.
DNA sequencing analysis of the isolate SC-58 allowed the design of new array using this genome as a reference and inclusion of other isolates in the study: *T. rangeli* C23 (KP1 (-)from Aotus sp; R1625 (KP1 (+) from H. sapiens); *T. rangeli* H14 (KP1 (+) from H. sapiens) and *T. rangeli* PIT10 (KP1 (-) from P. megistus. The analysis with this array is ongoing. **Supported by:**CNPq, FAPESP, CAPES **Keywords:** Acgh; trypanosoma rangeli; genome

PV54 - **TRYPANOSOMA CRUZI SIRTUINS ARE IMPORTANT FOR OXIDATIVE STRESS RESPONSE**

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Acetylation is a post-translational modification that happens mainly at residues of lysine, which can affects protein-protein interactions and enzyme activities. Recently, we described the acetylome of Trypanosoma cruzi epimastigote form and found 389 lysine-acetylated sites in 235 proteins involved in several biological processes, including some involved in oxidative stress. In T. cruzi the main route of defense to oxidative stress is related to the synthesis of trypanothione, a pathway that comprises several steps and enzymes. Among these enzymes, we detected trypanothione synthase, trypanothione reductase and mitochondrial superoxide dismutase A acetylated. Superoxide dismutases are enzymes that catalyze the conversion of the anion superoxide into hydrogen peroxide, and were demonstrated to be negatively regulated by acetylation in human. Acetylated human superoxide dismutase is activated when deacetylated by the sirtuin SIRT3, a NAD+-dependent lysine deacetylase. To investigate if acetylation is important in regulating oxidative stress response in T. cruzi, we used parasites overexpressing the both cytoplasmic and mitochondrial sirtuins, TcSir2rp1 and TcSir2rp3, respectively. First, we evaluated the resistance of these parasites under different oxidant drugs, hydrogen peroxide and menadione, and found that TcSir2rp3 overexpressors (TcSir2rp3-ox) were more resistant compared to wild-type parasites. The same was observed when we used benznidazole and nifurtimox drugs used for treatment of Chagas disease that acts causing oxidative stress in the parasite. Since these parasites are more resistant to different oxidant agents, we measured the levels of reactive oxygen species (ROS), after exposure to these drugs and found that TcSir2rp3-ox cells generate less ROS compared to wild-type cells. These results suggested that acetylation could play a role in the in oxidative stress response in T. cruzi.**Supported by:**FAPESP and CNPq **Keywords:** Trypanosoma cruzi; oxidative stress; sirtuins

PV55 - **DIVERGENT COMPONENTS FOR TRYPANOSOMATID MRNA EXPORT PATHWAY**

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Protozoan parasites adapt to changing conditions during host transitions and life cycle progression, mainly achieved through altered gene expression. Nuclear mRNA export is an important component of this process, but poorly understood in trypanosomes. Orthologs of only few proteins involved in mRNA export in higher eukaryotes are detectable. We previously described conserved components of the mRNA export pathway in T. cruzi, including TcSub2, a component of the TREX complex and Hel45, a shuttling RNA helicase. The analyses of the interactomes of TcSub2 and Hel45 uncovered additional components of this system. Some TcSub2/Hel45-associated proteins are specific to trypanosomatids, named as TcFOP-like, TcAPI5-like and TcNTF2-like. The kinetoplastid-specific NTF2 isoform is essential for mRNA export and for parasite survival. We are currently investigating the phenotypic effects of knockdown of homolog genes by RNAi in T. brucei. We suggest that these lineage-specific innovations are likely part of the evolutionary adaptation to polycistronic transcription/trans-splicing. Furthermore, we intend to investigate the structure of those specific proteins by crystallography in order to provide the basis for studies on structure-based drug design.**Supported by:**CAPES, CNPq, FUNDAÇÃO ARAUCÁRIA, FIOCRUZ **Keywords:** Mrna export; tcsub2; tchel45

PV56 - **IDENTIFICATION OF GENES INVOLVED IN SYNTHESIS OF BIOACTIVE PHOPHOLIPIDS IN *TRYPANOSOMA CRUZI***

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Lysophosphatidylcholine (LPC) and platelet activating factor (PAF) are phospholipids that act in various biological processes. Our laboratory has gathered biochemical evi-dence that LPC and PAF trigger cell differentiation and infectivity of *Trypanosoma cruzi* towards mouse macrophages. The present study aims to identify genes coding for key enzymes involved in the synthesis of PAF and LPC in *T. cruzi*, using in silico analy-sis tools. Enzymes related to the synthesis of PAF and LPC were selected from the KEGG pathways database, and a search for their gene sequences was performed on genomic data bank TritrypDB. The genes that were not found in the genomes of trypanosomatids were then sought in other species present in the Excavata group, and their sequences were used for processing a *blastp* against the genome of *T. cruzi*, aiming at finding protein coding regions homologous to those expressed by this protozoan. To confirm the existence of these genes, polymerase chain reactions (PCR) were performed and the amplified regions were sequenced. Only two out of ten genes involved in the biosynthesis of PAF were found in *T. cruzi*; five were present in other organism and three other genes were not found in the databases used. Twenty-three out of 46 genes involved in the biosynthesis of LPC were found in *T. cruzi* genomes, 19 were found in other organisms and four were not found in the databases used. Taking into account the above results, it was possible to construct maps indicating the possible biosynthetic pathways for both PAF and LPC in *T. cruzi*. Eight out of ten genes that code for enzymes of the LPC biosynthetic pathways in *T. cruzi* were amplified using PCR; the products of these amplifications were then sequenced. Ongoing experiments are aimed at modeling three-dimensional enzyme structures for applications in drug design, which may serve as targets for new drugs to treat Chagas disease.**Supported by:**CNPq, CAPES, FAPERJ, INCT-EM **Keywords:** Trypanosoma cruzi; platelet activating factor; lysophosphatidylcholine

## PV57 - *LEISHAMANIA MEXICANA* CYCLIN A INDUCIBLE OVEREXPRESSION REVEALS A DRUG TARGET CANDIDATE AND ITS ROLE IN CELL CYCLE CONTROL

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Treatment available for leishmaniasis have low efficacy and severe side effects, and identification of new chemotherapeutic agents is a high priority. Drug discovery campaigns use target deconvolution as a strategy to elucidate drug targets and improve chemical probes in terms of toxicity and specificity. Acquired resistance to compounds was observed in organisms overexpressing potential drug target proteins. Here, we used, as proof of concept for our model, an inducible system to overexpress Cyclin A (CYCA), a protein involved in *Leishmania mexicana* cell cycle and known to be a partner for the cdc2-related protein kinase 3 (CRK3). The cell line constitutively expressing T7 RNA polymerase (T7) and tetracycline repressor (TR) genes, both integrated at the ribosomal small subunit (SSU rRNA) locus, maintained virulence and can be used for infections in immune competent models as BALB/c mice. Parasites were transfected and correct antisense integration of c-terminus eGFP tagged protein was confirmed, followed by western blot and downstream analysis. We analysed two independent clones and overexpression of CYCA was always observed in a tetracycline concentration-dependent manner, which allows for controlled overexpression of potentially toxic proteins. Phenotypic alterations were observed in *L. mexicana* promastigotes as growth defects, loss of fitness in amastigogenesis and cell cycle arrest in G2/M phase. Unfortunately, this T7TR cell line is not suitable for overexpression in amastigotes since genes controlling expression of T7 and TR are promastigote specific, and our attempts to use stronger promoters as the SSU rRNA locus failed to produce tetracycline dependent overexpression cell lines. To our knowledge, this is the first report describing CYCA function in *L. mexicana* and the resources generated can be extremely useful for the understanding of cyclin functions and its potential as a target in drug screenings.**Supported by:**Conselho Nacional de Desenvolvimento Científico (CNPq) **Keywords:** Leishmania; overexpression; target deconvolution

## PV58 - ARTISTIC SCIENCE: MOLECULAR CRAFTS ON THE BENCH

DEL CAMPO AVILA, C.C.[*1]; MARÍN, P.A.[1]; CALDERANO, S.G.[1]; ARAUJO, C.B.[1]; ELIAS, M.C.[1]
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*Trypanosoma brucei* has a sophisticated replication control to keep the stability of the genome under stress conditions. When compared with other eukaryotes it is possible to identify important differences that turn this parasite unique in its replication process. The dynamics of this process is still unclear. Thus, further analysis of a single DNA molecule is important for understanding the changes in the genetic structure and DNA replication. Characterization of genes at the level of kilobases over large genomic regions is possible with DNA combing technology, which consists in the stretching of single DNA molecules by their extremities to a silanised glass surface. The molecular events, such as replication, density and direction of the forks, replication origins and velocity, fork distance and stalling, can be followed up by fluorescent hybridisation or through fluorescent detection of incorporated nucleotide analogues on the combed DNA molecule. The DNA molecules are isolated from the cell and trapped in agarose plugs that can be subjected to pulsed-field gel electrophoresis (PFGE) for the analysis of a specific fragment, or can be directly stretched (combed) on the slides for a whole genome analysis. In our laboratory we have improved this technology in order to optimize the assay for trypanosomatids replication analysis. Our optimization allowed us to quadruplicate the number of molecules per analysed microscopic field, to decrease the number of breaks of the molecules allowing a significant increase in the size of molecules in the order of 1 megabase, to improve the resolution of the events of incorporation, and to prevent DNA degradation during the PFGE analysis. Our optimization will allow the researchers in the field to advance in the replication studies in trypanosomatids and go further with the studies of the dynamics of replication of the minichromosomes in *T. brucei*, enabling new findings that were previously limited by the technique. **Supported by:**Processo Fapesp: 2013/07467-1 e 2016/50050-2 **Keywords:** T. brucei; replication; dna combing

PV59 - **ATR AND ATM SELECTIVE INHIBITORS SENSITIZE LEISHMANIA MAJOR PROMASTIGOTES TO OXIDATIVE DAMAGE**

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Protozoan parasites of the genus Leishmania are the causative agents of a group of tropical diseases collectively called leishmaniasis. Like other organisms, control of cell cycle and DNA damage repair is supposed to be coordinated by central proteins from signaling pathways namely the phosphatidylinositol 3-kinase related kinases, among them the ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3 related (ATR). The aim of this study was to evaluate the use of specific inhibitors for human ATR (VE-821) and ATM (KU-55933) in Leishmania major. We have identified putative homologues of LmATM and Lm ATR and from the molecular docking analysis, it was possible to infer that VE-821 and KU-55933 have binding affinity for the catalytic sites of ATR and ATM, respectively. L. major promastigotes incubated with these inhibitors show slight growth impairment and minor changes in cell cycle and morphology. It was noteworthy that the treatment with VE-821 and KU-55933 increases susceptibility of L. major promastigotes the oxidative damage caused by hydrogen peroxide. These inhibitors could significantly reduce the number of surviving L. major cells after H2O2 exposure in a dose-dependent manner. These results suggest that the use of specific inhibitors for ATR and ATM in Leishmania interferes in the signaling pathways of this parasite, which can impair its tolerance to DNA damage, probably affecting its genome integrity. The use of these inhibitors may represent a novel alternative for therapies against leishmaniases.**Supported by:**Fapemig and CNPq **Keywords:** Leishmania; atr; oxidative damage

PV60 - **A NEW SOURCE OF REACTIVE OXYGEN SPECIES IN *LEISHMANIA AMAZONENSIS*: CHARACTERIZATION OF A HEME-ACTIVATED NADPH OXIDASE-LIKE**

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*Leishmania amazonensis* is one of leishmaniasis' causative agent, a disease that has no cure and leads the appearance of cutaneous lesions. Recently, our group showed that heme activates a $Na^+/K^+$ ATPase in these parasites through a signaling cascade involving hydrogen peroxide ($H_2O_2$) generation. Heme has a pro-oxidant activity and signaling capacity, but the mechanism by which this molecule increases $H_2O_2$ levels in *L. amazonensis* has not been elucidated yet. In the present work, we investigated the source of $H_2O_2$ stimulated by heme, ruling out the participation of mitochondria and raising the possibility of a NADPH oxidase (Nox) instead. Although the absence of a Nox sequence in trypanosomatids' genome, it was described a ferric iron reductase (LFR1) in *L. amazonensis*, an enzyme with a very similar Nox structure which reduces iron instead of oxygen. Based on our experiments, we believe that LFR1 is a bifunctional enzyme, reducing oxygen or iron, and both activities are modulated by heme, whose extracellular concentration varies according to the parasite life cycle. Comparing *L. amazonensis* WT $H_2O_2$ production to LFR1 overexpressing (OE) mutant we verified that OE cells produce more $H_2O_2$ than WT cells. In addition, heterologous expression of LFR1 in mammalian HEK293 cells showed higher $H_2O_2$ production when compared to cells transfected only with plasmid containing GFP. This is the first time that a Nox activity is described in trypanosomatids. This finding could help to better understand the parasite life cycle and may also clarify the action mechanism of some antileishmanial drugs that increase the level of ROS. Besides, LFR1 is the only protein, so far, that has the ability to reduce iron and to generate $H_2O_2$, suggesting a common ancestor of NADPH oxidase and iron reductase families.**Supported by:**CNPq **Keywords:** Ros; heme; nadph oxidase

PV61 - **INORGANIC PHOSPHATE INFLUX IS IMPORTANT FOR AEROBIC METABOLISM AND GROWTH OF FREE-LIVING AMOEBA *ACANTHAMOEBA CASTELLANII***

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Acanthamoeba castellanii is a free-living amoeba and the etiological agent of granulomatous amoebic encephalitis and amoebic keratitis. A. castellanii can be present as trophozoites or cysts form. The trophozoite is the vegetative form of the cell and has a great infective capacity compared to the cysts from which is the dormant form that protects the cell from environmental changes. The phosphate transporters are group of proteins able to internalize phosphate from the extracellular medium to intracellular medium. The transporters are responsible for maintaining the phosphate homeostasis and in some organism, they regulate the cellular growth. The aim of this work was to biochemically characterize the phosphate transport in A. castellanii and its role on cell growth and metabolism. To measure inorganic phosphate (Pi) uptake, cells were grown in liquid PYG medium, at 28oC for 2 days. The activity was measured by rapid filtration of intact cells incubated with 0,5 µCi $^{32}$Pi for 1 hour. Control activity was 257 ± 2 pmol Pi/h$^{-1}$ x 10$^{6}$ cells. A. castellani presented a linear activity up to 1 hour with a cell density ranging from 105 to 2 x 106 amoeba/ml. The uptake was higher in acid pH range.. The phosphate uptake rate was higher in the initial days of the culture when compared to logarithm phase. The enzyme kinetics results showed that the phosphate transporter has high affinity for phosphate ($K_{0,5}$ and Vmáx of 88 +- 10 µM and 547 +- 17 pmolPi x h-1 x 10$^{-6}$ cells, respectively). The oxygen consumption of intact trophozoites increases according to Pi addition treated with FCCP no effect of Pi on oxygen flow was observed. The addition of increase Pi concentration not only increase the oxygen utilization but also increases intracellular ATP pool, theses fenomen was abolished when cells were treated with FCCP or are exposed to hypoxia condition. All together these results reinforce the hypothesis of Pi is a key nutrient for acanthamoeba castellanii metabolism.**Supported by:**CNPq FAPERJ CAPES **Keywords:** Oxidative metabolism; phosphate; acanthamoeba

PV62 - **UNRAVELLING THE ROLE OF PROTEIN KINASES IN LESIHMANIA DIFFERENTIATION**

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Leishmania encounter a multitude of environmental stresses during transmission between the extracellular promastigote stages in the sandfly and intracellular amastigote stages in mammalian macrophage phagolysosomes. Differentiation between these stages is thought to be tightly regulated via phosphorylation. Leishmania mexicana has 194 protein kinases and 11 PIKK kinases, each playing a role in the tightly regulated processes involved in cell division and differentiation between life cycle stages. 30 of these kinases are absent across the entire Trypanosoma genus indicating roles unique to Leishmania survival. We have employed CRISPR-Cas9 editing techniques to both endogenously tag and knockout each protein kinase gene individually, in order to investigate their roles. Live cell imaging of promastigote stage cells revealed 10 % of the kinases to be associated with the flagellum, 8% nucleus, 8 % basel body and 5% endosome. We were able to produce null mutants, each containing a unique barcode, for 79% of the protein kinases with 21% being essential for proliferation. The null mutants have been pooled and assessed for their ability to differentiate in vivo and in vitro and to respond to specific stresses such as pH and nutrient changes. BarSeq next generation sequence has been used to identify mutants with loss of fitness under these conditions. Data from a pilot screen with 26 protein kinase null mutants, including 5 LUKS, 6 CMGCs and RDK1 (Repressor of Differentiation Kinases) validates our approach. Individual phenotyping of RDK1 and selected Leishmania unique kinases confirmed the phenotypes identified in the BarSeq screen. By compiling these kinome-wide data we provide new insights into how stress responses and differentiation signalling pathways interface during the life cycle.**Supported by:**Wellcome Trust **Keywords:** Kinase; differentiation ; leishmania mexicana

PV63 - **CHARACTERIZATION OF AMINO ACID PERMEASE 3 GENE ORGANIZATION IN *LEISHMANIA* SPP. BY LONG AMPLICON PACBIO SEQUENCING**

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Arginine is a key amino acid for macrophage metabolism and it plays an important role in pathogens elimination by oxidative damage. On the other hand, arginine is also an important substrate used by arginase to produce urea and ornithine, this last one being part of polyamine pathway implicated in *Leishmania* replication and establishment of the infection inside macrophages. The arginine uptake is mediated by cationic amino acid transporters (CATs) in mammalian cells, and by an amino acid permease (AAP3) in *Leishmania*, which is different from the host. *aap3* was first characterized in *L. donovani*, and later in *L. amazonensis*. In these species the gene exists in two copies organized in tandem. However, relatively little is known about the gene organization in other species. The goal of this study was to establish whether the gene exists in two or more copies in several species of both subgenus *L. (Leishmania)* and *L. (Viannia)*. We also improved the information about the UTR and intergenic regions and the inter- and intraspecies variability of the sequences. Furthermore, as we have already shown that *aap3* can be a sensitive and specific target for genus detection and species differentiation, sequence data is essential to improve that diagnostic assays. We therefore isolated total RNA and genomic DNA from 16 *Leishmania* strains belonging to 7 species. Based on the sequence of *L. amazonensis* we designed primers for reverse transcription and Sanger sequencing of the 5´ and 3´UTR regions. Using these sequences, which comprises the two *aap3*-ORF copies, we designed primers for long amplicon PacBio sequencing. The PacBio sequencing revealed that for all species the gene was present in two copies in tandem, with relatively conserved ORFs and 5´-UTR, while highly variable 3´-UTR. Since the two copies are differentially expressed during *Leishmania* infection, the mapping of UTRs and the inter- and intraspecies variability can be useful for understanding the gene regulation.**Supported by:**UiB, FAPESP and CNPq **Keywords:** Aap3; pacbio; large amplicon sequencing

PV64 - ***TRYPANOSOMA CRUZI* INOSITOL PHOSPHORYLCERAMIDE SYNTHASE AS A POTENTIAL DRUG TARGET FOR CHAGAS DISEASE**

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Despite more than a hundred years of research, a vaccine to prevent Chagas disease (CD) is not available. Therefore, there is a need for the identification of new therapeutic targets to develop an effective treatment for CD, caused by the protozoan parasite *Trypanosoma cruzi.* Sphingolipids (SLs) are an essential part of all eukaryotic cellular membranes. The primary SL in numerous protozoa is inositol phosphorylceramide (IPC), which is absent in mammals. Thus, the IPC synthase apparently constitutes an ideal target for the development of new and harmless drugs to treat CD. Bioinformatics analyses showed that IPC synthase is an integral membrane protein that remains highly conserved between the kinetoplastids *T. cruzi* and *Leishmania major*. Because inhibitors of the *L. major* IPC synthase were recently identified, the objective of this study is to evaluate their effect on the *T. cruzi* enzyme, named *TcIPCS*, using different approaches: by *in silico* evaluating of the structural conservation between *L. major* and *T. cruzi* IPCS, by generating knockout (KO) lineages using the CRISPR-Cas9 as well as parasites over-expressing the *TcIPCS* gene and by performing in vitro enzymatic assays using recombinant TcIPCS. An epimastigote cell line with one *TcIPCS* allele deleted was generated using regular gene KO protocols. To obtain a *TcIPCS* null mutant, in vitro synthesized small guide RNAs will be used to transfect epimastigotes together with Cas9 and a DNA construct with the neomycin resistance gene flanked by the *TcIPCS* gene. To generate *TcIPCS* over-expressing parasites, a pROCK expression vector containing the *TcIPCS* coding region with an HA tag was prepared and transfected into epimastigotes. Moreover, a plasmid to express the recombinant TcIPCS in *E. coli*, to be used in enzymatic assays, has been generated. Finally, the structures of *L. major* and *T. cruzi* IPCS were modeled by threading using I-tasser to be evaluated using structural and conservation/coevolution analysis.**Supported by:**CNPq, FAPEMIG **Keywords:** Chagas disease; trypanosoma cruzi; ipc synthase

## PV65 - **DISSECTING THE ATR PATHWAY OF *LEISHMANIA MAJOR*: CHARACTERIZATION OF CHROMATIN BINDING PROFILE OF RPA1 AND HUS1 IN RESPONSE TO REPLICATION STRESS**

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The eukaryotic ATR pathway orchestrates the cellular response to a wide variety of DNA injuries including double strand breaks and replicative stress. The detection of accumulated single-stranded DNA (ssDNA), which is a common effect of genome injuries, sets the stage for ATR recruitment and activation leading to the stalling of cell cycle progression, protection of DNA replication forks and recruitment of DNA repair activities. The tripartite complexes RPA (RPA1-RPA2-RPA3) and 9-1-1 (RAD9-RAD1-HUS1) have a central role in ATR pathway. In *Leishmania major* 9-1-1 complex and RPA-1 participate in the response to replication stress, suggesting a conserved ATR pathway in these deep-rooting eukaryotes. To further characterize this pathway in *Leishmania* and identify RPA and/or 9-1-1-enriched loci in response to replication stress, we used chromatin immunoprecipitation (ChIP) assay of *L. major* RPA1- or HUS1-associated loci. ChIP assays were carried out in endogenous-tagged RPA1 and HUS1 cell lines (3xMyc-RPA1 and HUS1-12xMyc). The growth pattern and cell cycle progression profile of these cell lines were comparable to that of wild-type cells. The ChIP protocol was fully optimized and both RPA1 and HUS1 were efficiently immunoprecipitated from samples submitted or not to replication stress. The multi-copy spliced leader gene locus, a ~40 kb tandem array of ~100 copies of the mini-exon gene in the *L. major* genome, was probed to validate the ChIP assays. Next-generation sequencing of ChIP products is being analysed to identify specific profiles of the parasite's response to different genotoxic agents and conditions. In addition, such characterization of the replication stress response is being carried out in an ATR-deficient cell line.**Supported by:**FAPESP (16/18191-5; 16/50500-2) **Keywords:** Atr pathway; chip; hydroxyurea

## PV66 - **TARTRATE-RESISTANT ACID PHOSPHATASE TYPE 5 OF *TRYPANOSOMA CRUZI* IS INVOLVED IN PARASITE PROTECTION AGAINST MACROPHAGE OXIDATIVE METABOLITES**

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Chagas disease is a major health problem in Central and South America and, after recent cases in North America and Europe, has become a global disease problem. *Trypanosoma cruzi* (the causative agent of Chagas disease) presents a complex life cycle that involves adaptations in vertebrate and invertebrate hosts. As a protozoan parasite of hematophagous insects and mammalian hosts, *T. cruzi* is exposed to $O_2$ reactive species. In silico analysis of the *T. cruzi* genome demonstrated an acid phosphatase type 5 (TcACP5) with high homology to the human ACP5, which was further cloned, superexpressed using a heterologous expression system and purified to homogeneity. It exhibited a molecular mass that matched the predicted amino acid sequence. $V_{max}$ and apparent $K_m$ for *p*NPP hydrolysis were 7.7 ± 0.2 nmol pNPP × $\mu g^{-1}$ × $h^{-1}$ and 169.3 ± 22.6 µM, respectively. The pH dependence was characterized by sharp maximal activity at pH 5.0, and inhibition assays demonstrated its sensitivity to acid phosphatase inhibitors. Similar high activities were obtained by saturating P-Ser and P-Thr as substrates. Purified TcACP5 metabolizes hydrogen peroxide ($H_2O_2$) in vitro and decreases LPS-stimulated $H_2O_2$ generation by macrophages; this increases the infectivity of the parasite by 80%, as revealed by the association index. Taken together, these results suggest that TcACP5 plays a central role in phosphoryl transfer and redox reactions, which appears to be crucial for *T. cruzi* survival and evolution in the mammalian host cells. **Supported by:**FAPERJ; CNPq; CAPES **Keywords:** Trypanosoma cruzi; tcacp5; reative oxigen species

PV67 - **NUTRITIONAL AND PH STRESS INDUCE CHANGES IN THE MITOCHONDRIAL FUNCTIONALITY OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES**

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a neglected illness that affects millions of people in Latin America, being an emergent illness in non-endemic countries due to the triatomine-independent transmission routes and the immigration globalization. *T. cruzi* has a single mitochondrion, an organelle responsible for ATP production and the main site for formation of reactive oxygen species. During the parasite life cycle, stress conditions such as pH and nutritional changes induce protozoan differentiation: epimastigotes to metacyclic trypomastigotes in insect, and trypomastigotes to amastigotes in mammals. These stress conditions induce physiological and morphological changes in several organelles, such as mitochondria. The main goal in this work is to evaluate mitochondrion remodeling in *T. cruzi* epimastigotes submitted to nutritional stress and pH variation. After 24 and 96h, only nutritional and alkaline stress induced an important reduction in respiratory rates. On the other hand, when cultivated under nutritional deprivation and pH stress, parasites decreased complex II-III and increased complex IV activities in both times. Regarding citrate synthase, only parasites cultivated under nutritional and acid stress for 24h had a significant reduction in this enzyme activity. In addition, the production of oxygen reactive species was higher in parasites submitted to nutritional and alkaline stress. Our data suggest that reactive oxygen species may alter mitochondrial enzymatic complexes activities reducing respiratory rates in epimastigotes cultivated under stress conditions. The knowledge on mitochondrial functionality in different stress conditions is critical for understanding the molecular mechanisms that occur during the biological cycle of *T. cruzi*.
**Supported by:**FAPERJ, CNPq and FIOCRUZ **Keywords:** T. cruzi; mitochondrion; ros

PV68 - **LOCALIZATION OF TCRAB5 PROTEIN IN EPIMASTIGOTES OF TRYPANOSOMA CRUZI BY ENDOGENOUS TAGGING**

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Trypanosoma cruzi epimastigotes that live in the insect's gut are capable of uptaking macromolecules from the extracellular medium for nutrition. Due to the close spacing of the subpelicullar microtubules associated to the plasma membrane of the parasite, endocytosis is restricted to the regions where they are absent, as the flagellar pocket and the cytostome. Rab5 is a small endocytosis-related GTPase associated with the correct docking and fusion of vesicles and a molecular marker of early endosomes in several eukaryotic cells. T. cruzi Dm28c genome has two isoforms of Rab5, known as TcRab5a and TcRab5b, but the localization and function of the correspondent proteins are still unknown. Our goal in this work is to characterize TcRab5 isoforms and check if they could be established as early endosome markers, as their homologous proteins in other eukaryotes. By using the CRISPR-Cas9 method, we have generated epimastigote mutants expressing TcRab5b tagged with mNeonGreen and c-myc. Western blotting analyses confirmed the tagged protein expression in these cells. By fluorescence microscopy, we observed vesicular compartments containing TcRab5b at the perinuclear region, as expected for to observe ultrastructural localization of this protein using anti c-myc antibody and its presence in BSA-gold-containing compartments. Both TcRab5a and TcRab5b null mutants did not survive, suggesting that these proteins might be essential for the parasite, as described in other trypanosomatids. early endosomes. After ten minutes of uptake, these fluorescent compartments partially colocalized with the endocytic tracer, reinforcing this compartment as an early endosome. Electron microscopy will be performed Together these results suggest TcRab5b as an early endosome marker in Trypanosoma cruzi epimastigotes. **Supported by:**CNPq **Keywords:** Tcrab5; crispr-cas9; early endosomes

PV69 - **PHYLOGENETIC RELATIONSHIP OF SUBFAMILY LEISHMANIINAE**

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Nowadays, trypanosomatids classification is also based on molecular taxonomy. The molecular phylogeny allowed the classification of genus into subfamilies. Thus, analyzes inferred by gGAPDH and SSU rRNA genes revealed that the heteroxenic genus: *Leishmania* and *Endotrypanum* and the monoxenic genus: *Crithidia, Lotmaria, Leptomonas, Novymonas* and *Zelonia* compose a large monophyletic clade encompassed by the subfamily Leishmaniinae. However, the selection of DNA sequences to be analyzed is still a point of debate. The glycosomal glyceraldehyde 3-phosphate dehydrogenase (gGAPDH gene) and the small subunit region of ribosomal RNA (18S or SSU rRNA) are widely used in the classification of monoxenic trypanosomatids, while the database for Leishmania spp. is mainly based on 70kDa heat shock protein (Hsp70) and other markers such as internal transcripts of the rDNA gene (ITS 1 and ITS 2). The lack of overlapping between these targets is a problem for the inference of more robust phylogenetic relationships, particularly in the comparison between distinct clades. This study aims to infer the phylogenetic relationships of members from Leishmaniinae subfamily based on gGAPDH, SSU rRNA and ITS targets. In addition, new isolates collected from Amazon and Atlantic Forest were identified. So far, it was confirmed the taxonomic status of 17 species of *Leishmania*. Four species of *Crithidia*, two of *Leptomonas*, one of *Leishmania* and one of *Herpetomonas* are amenable to taxonomic revision. Ten isolates belonging to genus *Crithidia* have been identified at species level. The genetic relationship between several genus of Leishmaniinae subfamily is being reanalyzed based on gGAPDH and V7V8 gene. **Supported by:**CAPES, CNPQ, FAPERJ and FIOCRUZ **Keywords:** Trypanosomatids; leishmaniinae subfamily; molecular phylogeny

PV70 - **AN INTRODUCTION TO TCORC1B**

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*T. cruzi* is a protozoan which belongs to the Trypanosomatidae family and it is the etiological agent of Chagas disease, a neglected disease that affects millions of people worldwide. This parasite alternates between replicative and non-replicative forms in its hosts, thus the need for intense control of DNA replication, which involves coordinated action of several proteins. DNA replication starts with the assembly of the pre-replication complex component ORC into replication origins followed by the recruitment of other proteins from pre-RC, including Orc1b, whose function is not yet clear. It is known that in *T. brucei*, TbOrc1b has an important role in DNA replication and it interacts with Orc1/Cdc6 and MCM3. To better understand the role of TcOrc1b protein in the DNA replication dynamics of *T. cruzi*, the recombinant protein was expressed in E. coli BL21, purified and utilized to antibody production in HIII mice. A non-reduced sample of the protein presents a higher molecular weight than the reduced one in an acrylamide gel, and after a mass-spectrometry analysis, TcOrc1b appears to form aggregates with itself, suggesting the formation of a complex structure in its native form. A preliminary analysis of this protein has identified a Cdc6 and a ATPase domain. To evaluate the functionality of the Cdc6 domain, we are performing a yeast complementation assay, to see if a *S. cerevisiae* thermosensitive mutant of the Cdc6 gene can be complemented with the TcOrc1b. We also tested the thermosensitive mutant of Orc1 and saw no function complementation, indicating that TcOrc1b does not have the same function as yeast Orc1 protein. In addition, we tested the protein for ATPase activity, and despite the presence of a N-terminal AAA+ domain, it presented no activity. A functional ATPase domain is necessary to modulate ORC complex activity in its association to replication origins and in the interaction of Cdc6 to Cdk1, suggesting that TcOrc1b does not participate in this process. **Supported by:**FAPESP (2017/07693-2), CeTICS FAPESP (2013/07467-1), CNPq (870219/1997-9). **Keywords:** T. cruzi; dna replication; tcorc1b

PV71 - **A TALE OF THREE PARASITES: DIFFERENTIAL SUSCEPTIBILITY TO ANEUPLOIDIES IN *TRYPANOSOMA CRUZI*, *TRYPANOSOMA BRUCEI* AND *LEISHMANIA* MAY BE RELATED TO GENOME REPLICATION STRATEGIES**

CUNHA, J.L.R.*[1]; ALMEIDA, L.V.[1]; COQUEIRO-DOS-SANTOS, A.[1]; BAPTISTA, R.P.[2]; RODRIGUES-LUIZ, G.F.[3]; VALDIVIA, H.O.[4]; CARDOSO, M.S.[1]; CERQUEIRA, G.C.[5]; MCCULLOCH, R.[6]; BARTHOLOMEU, D.C.[1]

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Aneuploidy, the presence of an aberrant number of chromosomes in a cell, usually result in severe abnormalities in multicellular eukaryotes. However, unicellular eukaryotes rely on aneuploidy as a mechanism to allow rapid adaptation to changing environments, having a positive fitness in stress conditions and promoting drug resistance. Aneuploidies have been described in yeast and parasites of the *Leishmania* genus, showing the importance of this biologic process to eukaryote adaptations. In the present work, we have employed whole genome sequencing followed by read depth coverage and allele frequency analyses to evaluate the presence and estimate the levels of aneuploidies in *T. cruzi* and *T. brucei*. We have evaluated 19 *T. cruzi* isolates, including strains from the TcI, TcII, TcIII, TcV and TcVI DTUs, as well as 26 *T. brucei* isolates, encompassing the three subspecies, *T. b. brucei*, *T. b. gambiense* and *T. brucei rhodesiense*. We found a complex pattern of chromosomal duplication/loss among *T. cruzi* DTUs, which is not in agreement with the phylogeny, suggesting that aneuploidy generation is a common occurrence in the parasite evolution. Aneuploidies were also observed in recent-isolated *T. cruzi* TcII field isolates, showing that they are not long-term culture artifacts. TcChr31, the only supernumerary chromosome in the majority of the *T. cruzi* isolates, is enriched with genes related to glycosylation pathways, an important biological process to the parasite survival. Differently to what was observed in *T. cruzi* and *Leishmania*, none of the *T. brucei* subspecies presents aneuploidies. As *T. brucei* has several origins of replication in each chromosome, while *Leishmania* present a highly preferred origin per chromosome, a clash between the transcription and replication machineries in *Leishmania* may result in duplication failure of a given chromosome. Hence, the retention of extra chromosomal copies in *Leishmania* could mitigate against eventual chromosomal loss. **Supported by:**CNPq, FAPEMIG, CAPES **Keywords:** Aneuploidy; copy number variation; trypanosomatids

PV72 - **COMPARATIVE GENOMICS OF *LEISHMANIA MAJOR*-LIKE ISOLATES FROM BRAZIL**

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Leishmaniasis is a complex of diseases caused by parasites belonging to the *Leishmania* genus. *Leishmania major* is an important agent of cutaneous leishmaniasis in the Old World, while in the New World other species are commonly associated to cutaneous leishmaniasis, such as *Leishmania braziliensis* and *Leishmania panamensis*. However, in some countries from South America, parasites similar to *L. major* have been isolated from patients that have never traveled abroad. Several studies have been carried out to characterize these isolates, but it is still unknown whether they were recently imported from the Old World or if they were new species originated in the Americas. Besides, there are no genome-wide studies involving these isolates, hampering our understanding of their phylogeny, biology and the mechanisms underlying the differences observed in their infectivity. Therefore, in the present study, we sequenced and characterized the genomes of three *L. major*-like isolates, named BH49, BH121 and BH129. First, we performed phylogenetic analysis based on whole genome comparisons using these three isolates, and New and Old World species of Leishmania in order to better understand the taxonomic classification of these isolates. We observed that these isolates grouped together and interspersed with *L. major* strains, suggesting that they are, in fact, strains from *L. major*. This result indicates that there are *L. major* strains currently circulating in South America. Although phylogenetically similar, each isolate presented a different pattern of aneuploidies and gene duplication/deletions, where only the BH121 strain presented large segmental duplications. Next, we analyzed the gain or loss of pseudogenes, showing that the isolate BH129 was the one that presented more gains and losses of stop codons in the coding regions and pseudogenes, respectively. Finally, we are looking for biomarkers in their genomes that could allow the genotyping and serotyping of these isolates. **Supported by:**CNPq, CAPES, FAPEMIG **Keywords:** Comparative genomics; phylogeny; aneuploidies

PV73 - **IONIZING RADIATION EFFECTS ON ORGANIZATION OF SPLICED-LEADER (SL) GENE OF *TRYPANOSOMA CRUZI***

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The SL (Spliced Leader) RNA is a key molecule in the processing of polycistronic pre-mRNA of trypanosomatids. Previous studies in Leishmania showed that transfectants overexpressing the SL gene lose virulence in vivo (Toledo et al, 2009, 58:45). In this work, we characterized the SL gene in the mutants obtained by ionizing radiation. Epimastigotes of clone CL Brener (CLB) were exposed to gamma-radiation doses of 100-500 Gy and cloned by serial dilution. Eighteen irradiated clones were recovered and analyzed. The chromosomal bands were separated by PGFE and hybridized with SL gene as a probe. We observed differences in the chromosome profile of 9 clones indicating the occurrence of large chromosomal rearrangements in the SL loci. Three SL-mutants were chosen for enzyme restriction and sequencing analyses. SL genes are arranged in tandem repeats separated from each one by an intergenic spacer (IGS). Digestion with restriction endonucleases that cut within the SL gene or IGS revealed differences between the SL mutants and control parasites, for instance, the size of the SL monomer (~600 bp) is slightly higher in the mutants. Sequencing of SL repeats of mutants detected addition of nucleotides in the SL coding region. Analysis of the copy number of SL repeats showed 2.5-6.8-fold increased in the mutants.
To evaluate whether the irradiated parasites maintained their infective capacity, Balb/C mice were inoculated with trypomastigotes of SL-mutants, and the parasitemia monitored every two days. Parasitemia was significantly lower in SL-mutants when compared to that of non-irradiated control, and there was also a delay in the peak of the parasitemia in the mutants.
We detected chromosomal alterations in the irradiated clones using SL markers which may reflect some recombination event (deletion, translocation, duplication) during the DNA repair process. These recombination events could affect some biological traits of the parasite, for instance, the virulence. **Supported by:**FAPESP, CAPES e CNPq **Keywords:** Spliced leader; ionizing radiation; recombination

PV74 - **LYSOPHOSPHATIDYLCHOLINE (LPC) MODULATES CELL DIFFERENTIATION OF *LEISHMANIA AMAZONENSIS***

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*Leishmania amazonensis* is one of the etiological agents of American tegumentary leishmaniasis (LTA) in the New World. The phospholipid lysophosphatidylcholine (LPC) is an important bioactive component of membrane lipoproteins and is involved in diseases such as atherosclerosis and inflammatory diseases, being produced under physiological and pathological conditions. LPC is present in the saliva of *Rhodnius prolixus*, acting as a hemostatic molecule. Also, LPC modulates the infection of *Trypanosoma cruzi* in mouse peritoneal macrophages. Our group has shown that *T. cruzi* synthesizes C18:1 LPC, which is similar to platelet-activating factor (PAF), as it aggregates platelets and triggers cell differentiation of this parasite. Our aim here is to demonstrate the effects of C18:1 LPC on the proliferation, differentiation and infection of mouse peritoneal macrophages by *L. amazonensis.* We demonstrated an increase (32.3%) of *L. amazonensis* proliferation on the 5th day of culture, when treated with C18:1 LPC, as compared to the control. In experiments of differentiation from promastigotes to amastigotes, we observed that the percentage of differentiated (intermediate) forms exceeded the percentage of promastigote forms on the 8th day of experiment in the presence of LPC; on the other hand, this increase was observed only on the 16th day of culture in untreated parasites (control). We have also observed an increase in the percentage of differentiated forms when the parasites were treated with LPC in relation to the control on the 15th day (50%) and on the 28th day (29%) of culture. We have also tested the effects of LPC on the infection of peritoneal macrophages of BALB/c mice by *L. amazonensis*. Preliminary results indicate an increase in infection when the parasites were treated for 24 hours with C18:1 LPC, as compared to the control. Altogether, these results suggest a modulation of cell growth, differentiation and infectivity of LPC C18:1-treated *L. amazonensis*.**Supported by:**CNPq, CAPES, FAPERJ, INCT-EM **Keywords:** Leishmania amazonensis; lysophosphatidylcholine; platelet-activating factor

PV75 - **WING MORPHOMETRY AS A TOOL IN THE PHYLOGENY OF MOSQUITOES *CULEX* SUBGENUS *CULEX***

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*Culex* is the largest subgenus within the genus *Culex*, with 198 described species with cosmopolitan distribution. Adult females of many species feed on humans and/or animals and can be important vectors of arboviruses, avian malaria and filarial worms. Furthermore, they are often the most common mosquitoes feeding on humans in urban areas. However, the internal classification of subgenus *Culex* is in a chaotic condition. The aim of this study was to establish phylogenetic hypotheses among species of the subgenus *Culex*, and to test the phylogenetic position of species from complexes. Of the 28 *Culex* (*Cx*.) spp described in Brazil, 10 were used in this study. Adult mosquitoes were collected in São Paulo with CDC traps from Zoo (*Cx. ameliae*, *Cx. bidens*, *Cx. coronator*, *Cx. declarator* and *Cx. habilitator*) and from different urban parks (*Cx. chidesteri*, *Cx. dolosus*, *Cx. eduardoi*, *Cx. nigripalpus* and *Cx. quinquefasciatus*). Mosquitoes were classified morphologically with the use of identification keys. Mosquitoes were analyzed by wing geometric morphometrics, including tests of the canonical variables and a phylogenetic tree was constructed by the Neighbor-Joining method, illustrating the patterns of species segregation. Analyzes were performed and the images generated with TpsUtil 1.29, TpsRelw 1.39, MorphoJ 1.02 and Past 2.17c. Results showed that the wing geometric morphometrics was efficient to distinguish with high levels of confidence the species of *Culex* by the variation in shape of wings, resulting in the significant segregation of species (Procrustes values ranged from 1.3970 to 9.1715, $P < 0.0001$ for 89% of values). However, the species were grouped accordingly to their geographic locations rather than their traditional phylogenetic structure. In fact, these categories are often based on superficial similarities and did not reflect natural relationships. Additional experiments using DNA barcoding will be performed to compare the topologies of the phylogenetic trees. **Supported by:**FAPESP **Keywords:** Culex; mosquitoes; phylogeny

PV76 - **DISSECTING THE ATR PATHWAY OF LEISHMANIA MAJOR: USE OF CRISPR/CAS9 GENOME ENGINEERING FOR ENDOGENOUS TAGGING OF RPA, 9-1-1 AND ATR COMPLEXES.**

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The eukaryotic Replication Protein A (RPA) complex is composed by three distinct subunits and constitutes a key factor in many processes of the DNA metabolism. RPA1 is the largest subunit of the complex and bears most of the domains involved in the RPA-DNA interaction. Upon DNA replication stress, RPA-coated ssDNA and the independent binding of the 9-1-1 complex, participate in the recruitment of the ATR-ATRIP complex, a central step in the ATR pathway. ATR kinase activation leads to cell cycle arrest, stabilization of replication forks and recruitment of adequate repair activities. To better understand the function of these central elements of the ATR pathway in Leishmania, we used the CRISPR/cas9 genome editing to tag the endogenous RPA1 locus to mediate the expression of a N-terminal tagged version of the protein. The 3xMyc-RPA1 cell line has both alleles of the RPA1 locus replaced and the expressed 3xMyc-RPA1 is localized exclusively in the nuclear compartment. The pattern of response to the replication stress, as well as the binding to the chromatin fraction in response to Hydroxyurea (HU), was comparable between 3xMyc-RPA1 and wild type cells. These findings suggested that endogenous tagging did not affect RPA1 function. Alternatively, a cell line expressing a functional NeonGreen N-terminal-tagged RPA1 was also generated and could be used in live cells studies. Also, the RPA1 was tagged in an ATR-deficient cell line and the same genome editing strategy was used to generate cells expressing endogenous-tagged 9-1-1 subunits or the ATR kinase. These cell line are currently being used to characterize Leishmania response to replication stress. Supported by FAPESP (16/18192-1; 16/50500-2). **Keywords:** Replication protein a; atr; crispr/cas9

## PV77 - **BIOCHEMICAL CHARACTERIZATION OF AN ECTO-PHOSPHATASE IN FREE-LIVING AMOEBA *ACANTHAMOEBA CASTELLANII***

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*Acanthamoeba castellanii* is a free-living amoeba belonging to the family Acanthamoebidae distributed worldwide and found in several environments such as drinking water and soil. In its life cycle, *Acanthamoeba castellanii* presents two forms: the trophozoite and the cyst, the trophozoite being the proliferative form of the parasite and the cyst the dormant form. Ecto-enzymes are enzymes with catalytic site directed towards the extracellular medium. Because of that, these enzymes can participate in biological processes such as adhesion, cell differentiation and proliferation. Ecto-phosphatases are ecto-enzymes capable of hydrolyzing phosphorylated substrates present in the extracellular medium. In microorganisms this group of enzymes is responsible for processes such as adhesion, proliferation and interaction with host cells. This work aims to characterize an ecto-phosphatase activity in *A. castellanii* trophozoites. The activity has an acidic character, with a peak pH of 5.5. The ecto-phosphatase exhibits Michaelis-Menten kinetics with values of $K_m$ = 2.517 ± 0.5365 mM p-NPP and $V_{max}$ = 26.32 ± 2331 nmol p-NP x h -1 x $10^{-6}$ cells. Activity was modulated by classical inhibitors of phosphatase – sodium orthovanadate, ammonium molybdate, sodium fluoride - and inorganic phosphate. Ecto-phosphatase hydrolyzed phosphothreonine and phosphotyrosine in the same ratio however phosphoserine hydrolysis occurred at a lower rate, thus being classified as a dual specificity phosphatase. The divalent metals $Mg^{+2}$, $Ni^{+2}$ and $Co^{+2}$ increased the ecto-phosphatase activity. **Supported by:**CAPES, FAPERJ, CNPq **Keywords:** Acanthamoeba castellanii; ecto-phosphatase; dual specificity phosphatase

## PV78 - **DETERMINING ORTHOLOGUES EPITOPES IN *EIMERIA* SPP., THE CAUSATIVE AGENTS OF AVIARY COCCIDIOSIS: AN *IN SILICO* STRATEGY TO DETERMINE MOLECULAR TARGETS.**

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Aviary coccidiosis is a disease that affects chickens (*Gallus gallus domesticus*) and is caused by parasites from the genus *Eimeria* (*E. maxima, E. acervulina, E. necatrix, E. brunetti, E. mitis, E. praecox* and *E. tenella*), mainly associated with economic losses in the poultry industry. Due to vaccination or a precise early diagnosis are needed to improve host survivability. Therefore, *in silico* identification of pathogen exclusive epitopes, which could be useful for diagnosis or vaccines, will contribute to guide the in vivo tests using these molecular targets. Thus, an orthology analysis was performed in silico to search for epitopes shared among these species. Proteome sequences from the seven species, available on GenBank, were used to create the orthologous groups by OrthoMCL software. This analysis determined 7,710 groups of orthologue proteins, of which 1,411 contain at least one protein of each studied species. Then, for each group, the epitopes were predicted using Bepipred. A total of 15,145 epitopes were established, all being conserved (>90%) within their respective groups. 10,801 of these didn't have a corresponding protein in the chicken host. Considering only the most pathogenic species (*E. necatrix* and *E. tenella*) 5,338 epitopes were exclusively shared among them and these could be the first molecular targets to be considered for *in vivo* trials. It's worth noting that 361 of these epitopes are shared among all the *Eimeria* species analyzed and 28 of these were found in all the models investigated. These results show that this in silico method decreases 99.82% of the putative epitopes shared by all *Eimeria* spp.. This approach could be helpful to drive the selection of epitopes to be used as molecular targets for coccidiosis diagnosis or vaccines, however additional analysis ought to be performed to determine the location and molecular characteristics of these epitopes.**Supported by:**CNPq/PIBIC **Keywords:** Chicken; bioinformatics; homology

PV79 - **EFFECTS OF MUTATIONS IN THE C-TERMINAL REGION ON THE OLIGOMERIZATION STATE OF THE MACROPHAGE MIGRATION INHIBITORY FACTOR 2 OF *LEISHMANIA MAJOR* (*LM*MIF2)**

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Migration Inhibitory Factor (MIF) was the first cytokine to be identified from the human T cells and participates in innate and adaptive immune response. MIF has been considered an important factor in the control of parasites infections, presenting a beneficial or a detrimental role, depending on the pathogen. MIF deficient mice are susceptible to the infection by *L. major* and *T. cruzi*. Interestingly, homologues of mammalian MIF have been isolated from parasites species, including Leishmania : LmMIF1 and LmMIF2. The LmMIF1 acts by inhibiting apoptosis, contributing to the survival of the infected macrophage and the LmMIF2 is more expressed than LmMIF1 in L. major. Here we investigated the effect of site-directed mutations of the carboxy terminal residues of LmMIF2 on the oligomerization state and structural stability. The rLmMIF2 and the C-terminal mutants were expressed as in E. coli, purified and the oligomeric state of MIF and mutants was evaluated by gel filtration using pH 4 and pH7. The profiles of the gel filtration, using three different protein concentrations, rLmMIF2 and WtDel10 and W66LDel10W103 mutants presented a dimeric state at pH7. For the mutants W66Ldel5 and W108FDel5, it was observed the presence of dimers and trimers, depending on the concentration tested. The results obtained for pH4 show that there was a difference in the chromatographic profile of the samples with the pH reduction. rLmMIF2 and WtDel10 and W66LDel10W103 were observed only in trimeric form, independent of the concentration tested, whereas W66LDel5 and W108FDel5 were observed as trimers, tetramers and pentamers, depending on the concentration tested. The C-terminal portion is essential to maintain stable dimer conformations at pH7.0 and trimer at pH4.0. **Supported by:**FAPESP **Keywords:** Leishmania; oligomerization; mutation

PV80 - **FAZ10 LOCALIZES TO THE INTERMEMBRANE STAPLES IN THE FAZ INTRACELLULAR DOMAIN AND IS REQUIRED FOR CLEAVAGE FURROW POSITIONING IN TRYPANOSOMA BRUCEI**

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Trypanosoma brucei, the causative agent of sleeping sickness, has been used as an experimental model for cellular, biochemical and molecular studies. The flagellum, which is the most prominent organelle, harbors most of the cytoskeletal content of the cell is linked to the cell body via the Flagellar Attachment Zone (FAZ). The FAZ is a large and complex interconnected set of fibers, filaments and junctional complexes composed of several cytoskeletal proteins. In all genera of the Trypanosomatidae family, we have described a novel class of High Molecular Weight Proteins (HMWPs: 500-3500 kDa), which may play a role in the organization and regulation of the cytoskeleton. Trypanosomatid cytoskeletons contain abundant HMWPs, but many of their biological functions are still unclear. Here we report the characterization of the giant FAZ protein, FAZ10 in T. brucei. Detergent-extracted cytoskeletons revealed by gradient SDS-PAGE that FAZ10 is an essential giant protein (2000 kDa) found in both procyclic and bloodstream forms of T. brucei. Immunolocalization using an antibody against FAZ10 demonstrated that FAZ10 localizes to the intermembrane staples in the Flagellum Attachment Zone -FAZ intracellular domain. We generated RNAi 2913 T. brucei cell line to knockdown FAZ10 and its depletion led to defects in cell morphogenesis, flagellum attachment and kinetoplast and nucleus positioning. Moreover, ablation of FAZ10 impaired the timing and placement of the cleavage furrow during cytokinesis, resulting in premature or asymmetrical cell division. Additionally, the lack of FAZ10 seems to cause a noticeable reduction in ClpGM6 (FAZ flagellum domain) protein levels, but do not affect its distribution in the cell. Together, our data support the critical role of FAZ10 in modulating the FAZ architecture with direct implications in the T. brucei biology. **Supported by:**FAPESP, CAPES-PDSE and CNPq **Keywords:** Cytoskeleton; flagellum attachment zone; trypanosoma brucei

PV81 - **FUNCTIONAL CHARACTERIZATION OF HISTONE H2B VARIANT IN DIFFERENT LIFE FORMS OF TRYPANOSOMA CRUZI**

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The differentiation of Trypanosoma cruzi is followed by changes in parasite morphology followed by a complete reorganization of nuclei and chromatin structure. Studies indicate that the deposition of histones variants changes chromatin structure possibly affecting gene regulation. Recently, we found that histone H2Bv previously shown to be associated to transcription start sites in T. brucei, is enriched at trypomastigote's chromatin of T. cruzi and here, the aim of our study is to shed lights into the role of this variant in T.cruzi. To this end, we have generated H2Bv heterozigotous knockout (HtzKo) parasites by homologous recombination, once homozygous mutant parasites were inviable. In general, HtzKo clones proliferate and differentiate into metacyclics more efficiently than wild type (wt) - parasites. By LC-MS/MS, we confirmed that H2Bv is decreased at HtzKo chromatin, and strikingly, we found that other histones variants (namely, H2AZ and H3v) are also less abundant. Importantly, total extracts analysis of HtzKo and wt indicate that histones variants (except H2Bv) are equally expressed, suggesting that H2Bv may guide the chromatin deposition of other histones variants. To have better insights into H2Bv role, we have also performed pulldown assays using both recombinant H2Bv and canonical H2B (as a control) to identify their specific interaction partners at epimastigotes or trypomastigotes. Many interesting proteins were identified, but here we highlight the identification of a bromodomain factor-2 (BDF2) exclusively at trypomastigotes extracts. BDF2 is known to recognize histone acetylation and, in accordance, we found H2Bv acetylated at K97. We envisage that a complex epigenetic mechanism of regulation involving BDF2, histone acetylation and H2Bv may drive the establishment of phenotypic differences observed between T.cruzi life forms. **Supported by:**CAPES, FAPESP **Keywords:** Histone; epigenetic; differentiation

PV82 - **THE TRIATOMA SORDIDA SALIVARY PROTEOME: A COCKTAIL OF PHARMACOLOGIC REAGENTS**

PRAÇA, Y.R.*[1]; ARAÚJO, C.N.[1]; SILVA, S.M.M.[1]; BENTES, K.L.S.[1]; CHARNEAU, S.[1]; MANDACARU, S.[1]; BASTOS, I.M.D.[1]; SANTANA, J.M.[1]
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The hematophagous arthropod saliva is the major invertebrate fluid that acts as the medi the crosstalk with its prey, in which a plethora of biological reactions take place to f of blood and to modulate the vertebrate immune responses. These include i or, anticoagulant, anti-inflammatory and immunosupressive acti is highlighted by the fact that the salivary gland prot s disease vector recurrently found in perido and Uruguay. Here, we perform ida. We identified 58 prot were revealed, including lip ases, hemolysin-domain pr evealed a high similarity among T. d in the literature. This study highlights the me characterization and improves our knowledge of ti he **by:**FAPDF **Keywords:** Triatominae; triatomine saliva;

PV83 - **IDENTIFICATION OF FUNCTIONAL PARTNERS AND EFFECT OF MUTAGENESIS ON THE EIF4E5 TRANSLATION INITIATION FACTOR HOMOLOGUE FROM LEISHMANIA INFANTUM**

CAVALCANTI, T.Y.V.L.[*1]; DE LIMA, G.B.[1]; MALVEZZI, A.M.[1]; DE MELO NETO, O.P.[1]
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Gene expression in trypanosomatids is regulated mainly at the post-transcriptional level with translation being a likely target for regulatory events. In eukaryotic translation, the initiation stage is the most regulated stage and requires the cooperation of several eukaryotic Initiation Factors (eIFs). The eIF4F complex, formed by three subunits (eIF4A, eIF4E and eIF4G), stands out among these and in trypanosomatids multiple eIF4F complexes have been indentified based on different eIF4E (four) and eIF4G (five) subunits. The EIF4E5 based complex has not been formally described in Leishmania infantum, only in Trypanosoma brucei. This work aimed to study EIF4E5 in L. infantum in order to identify functional partners and compare with those found in T. brucei. Since likely binding sites for eIF4G and other protein partners were found within EIF4E5, based on conserved tryptophan residues (W45 and W53), the effects of mutagenesis on these residues was also investigated. Wild type and mutant DNA fragments were cloned and transfected into L. infantum, generating recombinant proteins with a C-terminal HA tag. After cell lysis by cavitation, whole cytoplasmic extracts from the transfectants were assayed by Western blot to confirm expression with the HA tag. This was followed by immunoprecipitation experiments with anti-HA agarose beads. After mass spectrometry identification of the immunoprecipitated polypeptides, a comparison was carried out between those specifically bound to the wild type and mutant proteins. The results revealed that EIF4E5 interacts specifically with EIF4G1, an eIF4G homolog, but not with EIF4G2, known to bind T. brucei EIF4E5. Other EIF4E5 partners from T. brucei were also found, however several proteins linked to signaling pathways were identified only in Leishmania. Several of these interactions were specifically abolished when the W45 residue was mutated revealing unique aspects related to the function of this protein in trypanosomatids.**Supported by:**Capes, CNPq, FACEPE **Keywords:** Cap binding protein; translation initiation factor; protein synthesis

PV84 - **NUCLEOSOME OCCUPANCY ANALYSIS HIGHLIGHT DIFFERENCES AND INTERESTING FEATURES PRESENT AT GENOME STRUCTURE OF TRYPANOSOMA CRUZI LIFE FORMS.**

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Trypanosomatids present biological particularities, such as the absence of canonical promoters for RNA polymerase II and polycistronic transcription of genes. The physical packaging of DNA in structures called nucleosomes is considered the central determinant of DNA accessibility, and characterizing differences on nucleosome occupancy in different cells may reveal important regulatory characteristics of the organism's biology. Therefore, the aim of this project is to determine the occupation of the nucleosomes throughout the genome of T. cruzi identifying possible differences between replicative and non-replicative forms. Initially, we generated genome-wide maps of nucleosome positioning by MNase digestion coupled to next-generation sequencing (MNase-seq). We found that non-replicative forms contains more regions classified as high nucleosome occupancy. Preliminary data show that the intergenic regions appear to be depleted in nucleosomes at both life forms. Moreover, there is a marked tendency of nucleosome enrichment at the ends of the chromosome, which would correspond to the subtelomeric regions. Generally, subtelomeric regions are formed by heterochromatin, which would justify the concentration of nucleosomes in their peripheries. Analyzing the global enrichment of nucleosomes in relation to the beginning and end of genes (start codon and termination codon) we observed that in replicative forms, this distribution appears throughout of gene, whereas in the non-replicative form, a marked tendency toward nucleosomal enrichment both at the start and end codons. Additional analyzes are being carried out in order to add shed lights into nucleosome organization of T.cruzi aiming to find interesting differences between replicative and non-replicative forms.Supported by FAPESP and Instituto Serrapilheira **Keywords:** Mnase-seq; nucleosomes; trypanosoma cruzi

PV85 - **STRUCTURAL CHARACTERIZATION OF KINETOPLASTID KINETOCHORE (KKT) PROTEINS OF *TRYPANOSOMA CRUZI***

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The correct transmission of the genetic material is essential to the survival of living beings. The molecules involved in the chromosome segregation have greater divergence among the eukaryotes. The identification of centromeric and kinetochore proteins in *T. cruzi* has been hampered by the divergence between the primary sequence of these proteins with other eukaryotes. Recently, 19 kinetochore proteins (named KKT- Kinetoplastid Kinetochore protein) were identified in *T. brucei* (Akiyoshi & Gull 2014, Cell 156:1247). In our study, we investigated whether the conserved protein domains identified in *T. brucei* KKT are conserved in *T. cruzi* orthologs and also if other domains not found in *T. brucei* would be present in *T. cruzi.* KKT proteins of *T. cruzi* were compared to sequences deposited in the Conserved Domain Database (CDD), NCBI. The Pfam, Interpro and Expasy platforms were used to complement this analysis. Kinase domains (STKc_PLK, S_TKc, PKc_CLK, PLK, SPS, JNK-SapK), abundant in cell cycle control proteins, are distributed over several KKTs. The presence of kinase domains in KKT is expected, since they play an important role in the cell cycle progression by different phosphorylated substrates. Others domains are found in KKTs such as SMC domain present in proteins involved in chromosomal segregation, SOG2 involved cytokinesis in yeast and FHA, a cell signaling domain. The conserved domains found in KKTs suggest that these proteins may be involved in the cell cycle control and chromosomal segregation, suggesting that orthologs found in T. cruzi exhibit functions similar to those found in *T. brucei*.
KKT9 has expressed in bacteria in order to generate specific antibodies which can help us to elucidate the role of this protein. The expression of native KKT9 protein during the T. cruz*i* cell cycle will be analyzed by western blot and confocal microscopy using polyclonal antibodies. **Supported by:**FAPESP, CNPq, CAPES **Keywords:** Chromosome segregation; kinetochore proteins; bioinformatic

PV86 - **CHARACTERIZATION OF A RNA BINDING PROTEIN THAT CONTROLS GENE EXPRESSION IN EPIMASTIGOTES AND AFFECTS METACYCLOGENESIS IN *TRYPANOSOMA CRUZI***

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In Trypanosoma cruzi, unlike other eukaryotes, protein-coding genes are transcribed into polycistronic pre-mRNAs and, because of that, regulation of gene expression depends essentially on post-transcriptional mechanisms to respond to changes in the environments between insect vectors and vertebrate hosts. Binding of RNA binding proteins (RBPs) to mature mRNAs canresult in modulation of RNA stability, translation or cellular localization. Transcriptome analysis comparing in vitro cultured epimastigotes, trypomastigotes and intracellular amastigotes revealed changes in gene expression that reflect the parasite adaptation to changes in energy sources, oxidative stress responses, cell cycle control, cell surface components. Significant changes in the expression of genes encoding RBPs were also detected, including a gene encoding a RBP containing a "zinc finger" motif named TcZC3H99. Transcript levels of this protein are 25-fold up-regulated in epimastigotes when compared to trypomastigotes and amastigotes. RNA-seq analyses of epimastigote cell line in which the TcZC3H99 gene was disrupted revealed the role of this RBP in controlling the expression of genes involved with parasite proliferation and differentiation. TcZC3H99-/- mutants grew slowly and presented increased capacity to differentiate into metacyclic trypomastigotes compared to wild-type (WT) parasites. These mutants also showed reduced expression levels of genes that are up-regulated in wild type epimastigotes. Co-immunoprecipitation assays confirmed the mRNA binding capacity of TcZC3H99 to transcripts encoding proteins associated with parasite differentiation. We suggest that this RBP not only positively regulates the expression of genes involved with epimastigote survival and proliferation in the insect vector, but also acts as a negative regulator of parasite metacyclogenesis. **Supported by:**FAPEMIG, CNPq and CAPES **Keywords:** Gene expression; rna binding protein; trypanosoma cruzi

PV87 - **MOLECULAR CHARACTERIZATION OF THE *TRYPANOSOMA CRUZI* FLA1-BINDING PROTEIN**

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The flagellum of trypanosomatids is laterally attached to the cell membrane by a protein complex named Flagellar Attachment Zone (FAZ). Crucial for cellular morphology, motility and division, the FAZ composition is stage-specific in *Trypanosoma brucei*. While procyclic forms express the Flagellum Adhesion Glycoprotein 1 (FLA1) and the FLA1-Binding Protein (FLA1BP), bloodstream forms express the Flagellum Adhesion Glycoprotein 2 (FLA2) and the Flagellum Adhesion Glycoprotein 3 (FLA3). In *T. cruzi* a FLA1/FLA2 ortholog (Gp72) has been described but, so far, there are not reports of a FLA1BP/FLA3 ortholog for this taxon. In this study we described and performed a preliminary characterization of a FLA1BP ortholog in *T. cruzi* (*Tc*FLA1BP). *Tc*Fla1BP is a transmembrane protein and it is 37.42% identical to *Tb*FLA1BP and 36.44% identical to *Tb*FLA3. A fragment of ~584 bp close to the C-terminal portion of the *Tc*FLA1BP was cloned, heterologously expressed and used to generate an anti-*Tc*FLA1BP polyclonal antiserum. Western blot assays using extracts from *T. cruzi* (Y strain) epimastigotes (EPI) and trypomastigotes (TRYPO) and the anti-*Tc*FLA1BP recognized proteins of ~100 kDa in both forms, which is higher than the predicted 79.25 kDa of the *Tc*FLA1BP and can be due post-translational modifications. Although RNAseq data analysis from other groups seems to indicate an upregulation of *Tc*FLA1BP transcription in TRYPO, no difference was observed on the expression levels between EPI/TRYPO. IFA assays using anti-*Tc*FLA1BP revealed a weak signal throughout the cell membrane of EPI and TRYPO but stronger on the FAZ of both forms, is in accordance to previous reports for *Tb*FLA1BP and *Tb*FLA3. Our results indicate that *Tc*FLA1BP retains both *Tb*FLA1BP and *Tb*FLA3 characteristics and is expressed by *T. cruzi* EPI and TRYPO as also observed for *T. rangeli*.**Supported by:**CAPES, CNPq and UFSC
**Keywords:** Flagellum; faz; fla1bp

PV88 - **RECOMBINANT RHODNIUS NEGLECTUS SALIVARY TRIABIN PRODUCED BY BACTERIAL EXPRESSION SYSTEM IS IMMUNOGENIC IN MICE**

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Rhodnius neglectus (Hemiptera: Reduviidae) is a potential vector of Trypanosoma cruzi, etiological agent of Chagas disease. This kissing bug presents in its salivary glands important molecules to counteract hemostatic mechanisms and useful for the transmission of the parasite to the vertebrate host. Among them, members from the triabin protein family have been described. This family encompasses a wide variety of proteins, including proteins that act as carriers of small [illegible], protease inhibitors, ligands of specific cell-surface receptors and [illegible] other macromolecules. In this study, the expressi[illegible] glands in Escherichia coli is [illegible] polyclon[illegible] [illegible] and p[illegible] used to raise antibodies ir[illegible] combinant R. neglectus salivary triabin led to o[illegible] The polyclonal antibodies raised against the recombinant salivary tri[illegible] reacted with antigens from the saliva of R. prolixus, Triatoma infestans and Dipetalogaster maxima species. Results suggest that species from both triatomine tribes Rhodniini and Triatomini share triabin members with similar epitopes. As a perspective, this recombinant protein may aid in the understanding of the dynamics host-vector interactions. **Supported by:**FAPDF, CAPES **Keywords:** Triatomine; saliva; triabin

PV89 - **EDUCATION FOR HEALTH PROMOTION VIA LEISHMANIASIS VECTOR CONTROL**

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Leishmaniasis is a stigmatizing/potentially fatal disease of great importance to public health, affecting 98 countries, causing circa 20,000 deaths annually and about 1 billion people in the world are at risk of infection. The disease is transmitted during the sand flies bloodmeal. These insects are found in areas of forests and places with abundant organic matter. With the advancement of deforestation and inappropriate garbage disposal, insects perform peridomiciliary colonization, leading to increased leishmaniasis transmission. The study aims at the implementation of health promotion educative activities for vector control, emphasizing proper garbage disposal backyard cleaning by the population, also highlighting the consequences of unregulated deforestation. In order to do this, we use the expertise of the project "Science on the Road: Education and Citizenship" (https://www.youtube.com/user/MarcosVannier/featured), via its itinerancy, producing and employing educational, ludic and interactive materials used in health fairs and events for health education, implementing strategies to control vector-borne diseases, as well as different infectious and parasitic diseases, using games, videos, games, resin-made parasites and vectors in makets of houses, communities landscapes with deforestation and accumulation of garbage in interactive models, allowing the critical development and empowerment of the population in these areas, thus promoting the prophylaxis of these diseases. These socio-educational interventions can change habits and practices through the knowledge of the population, reducing the risk of transmission of leishmaniasis. **Supported by:**CNPq, FAPESB and Fiocruz **Keywords:** Leishmaniasis; prophylaxis; health promotion

PV90 - **PROSPECTING INSECTS TRYPANOSOMATIDS FROM BRAZILIAN BIOMES**

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The Trypanosomatidae family is a protozoa group of medical, veterinary and agricultural importance, as they cause diseases in humans, domestic animals and plants. Despite being a relatively well-studied group, the taxon's biodiversity is still poor known. In the last decade there has been numerous studies aiming isolation and characterization of non-pathogenic trypanosomatids, however there are many gaps to be filled. Present work aimed the isolation and molecular identification of trypanosomatids found colonizing diptera, hemiptera and lepidoptera insects, which were sampled within the Atlantic Forest and Amazonian domains. During the years 2016, and 2017 insects were collected in both biomes in question. Insects were photo-documented and dissected to search for protozoa colonizing internal organs and positive samples were inoculated in NNN / LIT medium supplemented with penicillin and inactivated fetal bovine serum. Axenized cultures were identified by sanger sequencing of gGAPDH and SSU targets, and were deposited in the Protozoa's Collection of Fiocruz (COLPROT). To date, molecular identification of 58 of the 100 isolates obtained has been performed, revealing that 32 isolates represent new taxa (probably 4 species of Crithidia and 3 new genera), and another 26 correspond to previously described species. The identification of the remaining isolates and the morphological characterization of the new taxa are being conducted. Our results show that there is an immense biodiversity of trypanosomatids to be described and new samplings covering other orders of insects and biomes should be performed. **Supported by:**CAPES, FAPERJ **Keywords:** Hidden biodiversity; molecular taxonomy; trypanosomatids

PV91 - **CREDITING: A POWERFUL TOOL FOR GENE TUNING IN *TRYPANOSOMA CRUZI.***

PACHECO-LUGO, L.A.*[1]; SAENZ GARCIA, J.L.[1]; CALLISAYA, A.M.[1]; DIAZ, Y.[2]; NETTO COSTA, R.[3]; DAROCHA, W.D.[1]

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*Trypanosoma cruzi* is the etiological agent of Chagas disease, a neglected tropical disease that affects around 7 million people worldwide, majority of the cases are reported in Latin America. The scarcity of molecular tools for genetic manipulation is a critical step for functional genomics studies in *T. cruzi.* The CRE-lox recombination system from the bacteriophage P1 is a widely used method to achieve conditional targeted deletions, inversion, insertions, gene activation, translocation, and other modifications in chromosomal or episomal DNA. In this work we have adapted the Cre-lox system in *T. cruzi* to expand the toolbox for this parasite hard-to-manipulate. First, we tested an inducible system where a split version of CRE recombinase is activated by a GFP-dependent mechanism (CRE-DOG system). As proof-of-concept, an inverted sequence of tdTomato flanked by head-to-head oriented LoxP sites as a reporter cassette for CRE activation was tested. The CRE-DOG approach, however, rendered very low recombination efficiencies as shown by confocal microscopy. To improve recombination, we decide to express in prokaryotic system and purify the CRE recombinase fused to the C-terminus of the nuclear localization signal histone H2B to transfect it directly into epimastigotes (CREditing system). The protein transduction by electroporation resulted in high expression levels of the gene reporter Tdtomato, reaching up to 85% of recombination detected both by confocal microscopy and flow cytometry. Also, the CREditing system shown be functional to turn-off the GFP in an inducible way, resulting in high levels of GFP decay as early as 24 hours post-transfection. Finally, the CREditing approach also worked for removal of floxed selectable markers integrated in the Tubulin locus of *T. cruzi.* Taken together, the CREditing approach reported here is an efficient method to regulate finely the gene expression in epimastigote forms of *T. cruzi.* **Supported by:**CAPES, CNPq, Fundação Araucária **Keywords:** Cre recombinase; gene tuning; gene expression

PV92 - **INFLUENCE OF *SERRATIA MARCESCENS* AND *RHODOCOCCUS RHODNII* ON PHYSIOLOGY OF *RHODNIUS PROLIXUS***

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*Rhodnius prolixus* is an important vector *Trypanosoma cruzi,* etiologic agent of Chagas disease, considered a public health problem mainly in Latin America. The transmission of the parasite to vertebrate hosts is correlated with the vector competence of the insect. In this context, *T. cruzi*, which develops in the digestive tract of triatomines, faces lithic factors of the digestion process, interacts with the intestinal microbiota and immune responses, among other obstacles to complete its cycle. On its microbiota, the Gram-positive bacteria *Rhodococcus rhodnii* is known as its simbiont, and a it is common to fing colonizing the digestive tract of *R. prolixus* the Gram negative bacteria, *Serratia marcescens*. Some studies in our group have investigated the role of *S. marcescens* in insect physiology. To date, it has been speculated that *S. marcescens* collaborates with blood digestion and insect protection against parasites due to their proven in vitro and in vitro tripolytic activities. However, there is still little knowledge of the role of these bacteria, not only for insect development but also for *T. cruzi* infection via modulation of immune responses. Therefore, in the present work different concentrations of antibiotics Ampicilli, Penicillin and hygromicin were tested in order to decrease the intestinal microbiota of the insects in the fourth stage and to perform oral infection of *R. rhodnii* and *S. marcescens i*n the fifth stage. Different concentrations of the bacteria were tested for infection of the insects, observing the longevity, moult, excretion and amount of bacterial colony forming units in the intestinal tract. The optimal concentration of antibiotics and bacteria for infection were standardized and at first it was observed that the bacteria did not modify the excretory profile of the insect. In the future, it is intended to observe the effects of these bacteria on the humoral immune system of the digestive tract and influence on the development of *T. cruzi.* **Supported by:**CAPES, INCTEM **Keywords:** Rhodnius prolixus; serratia marcescens; rhodococcus rhodnii

PV93 - **PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SUGARY FEEDING EFFECTS IN RHODNIUS PROLIXUS**

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Chagas disease affects 8 million people, being a chronic and severe disease caused by the parasite Trypanosoma cruzi. The triatomines, the main vectors of this disease, make up a large and diverse group of insects that have colonized temperate, subtropical and tropical ecotopes. The development of vector control strategies has resulted in a dramatic reduction in new cases of vector-borne transmission in endemic countries, however in the last decade there has been a series of re-infestation events in houses treated with insecticides by populations of wild triatomines. There were also outbreaks attributed to the oral infection associated with the consumption of contaminated foods, mainly sugar cane and fruits, such as guava and açaí. Triatomines have adapted to a variety of natural habitats, including some genera that show strong association with particular vegetation. The association of triatomines with plants has always been considered as derived from the habitat preference of their vertebrate hosts. Triatomines collected in the field are usually found in a very poor nutritional state. Our group described the first report of sugar feeding and phytophagy in one species (Rhodnius prolixus) which was considered to be a blood-restricted feeder for over a century. Our findings suggest that local plants may play a nutritional role in the maintenance of triatomines. We recorded the ingestion of the sugar solution by adding bromophenol blue in commercial sucrose 10% soaked in cotton. The results of the ingestion in nymphs and adults of Rhodnius prolixus show that the smaller instars have a pronounced avidity by sugar when compared to insects of 5th stage and adults. A 60% mortality rate was observed in first-stage insects, according to stage 26.6%, third stage 23.3%, fourth stage 10%, and in the fifth stage no deaths were observed. Observing these data, we can see that the sucrose showed to be quite toxic and that the physiological impact is higher in the first instar.**Supported by:**FIOCRUZ, FAPERJ, CNPq e CAPES **Keywords:** Rhodnius prolixus; sugar; insects

PV94 - **IMMUNE RESPONSE TO ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* INFECTION IN *RHODNIUS PROLIXUS.* (HEMIPTERA: REDUVIIDAE)**

GUIMARÃES, M.Q.*[1]; SILVA, C.A.O.[1]; DE PAULA, A.R.[2]; SAMUELS, R.I.[2]; VIONETTE DO AMARAL, R.J.[1]; SORGINE, M.H.F.[1]; DA SILVA, J.R.[1]; DA FONSECA, R.N.[1]; MURY, F.B.[1]
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The *Rhodnius prolixus* is a hematophagous insect and vector of *Trypanosoma cruzi*, a protozoan parasite that causes Chagas disease. Insects are exposed to a wide range of microorganisms and have interconnected powerful immune reactions. Innate immunity is the first line of defense being divided into humoral response that is related to antimicrobial peptides (AMP). The *Metarhizium anisopliae* is an entomopathogenic fungus used as biological control agents and start the infection process mainly by penetration through the insect cuticle. Here we have investigated the effect of *M. anisopliae* infection on the modulation immune response and to linking with embryogenesis process in *Rhodnius*. For this adults females of *R. prolixus*, in starvation or rabbit blood feeding, were challenged by conidial suspensions of *M. anisopliae* ($1\times10^7$ conidia/mL) using a Potter tower and in both conditions after 24 and 72h, midguts and fat body were dissected. The modulation of immune responses was studied by analysis of the abundance of mRNAs encoding Dorsal and Cactus (Toll pathway), Relish (IMD pathway), Eiger (TNF ortholog), STAT and SOCS (Jack-STAT pathway) and Defensin and Lysozymes (LysA, LysB) (AMPs) by qPCR. The Toll pathway was activated in fat body of the blood meal insects 72h after infection. Up-regulation of the Relish was also observed in the midgut blood feeding, 72h after infection. However, the transcripts level of LysA, LysB were down-regulated in insects of this condition. We did not observe Eiger and STAT/SOCS expression significant at any time. Furthermore, we demonstrate that fungus infection did not interfere with insect survival and DAPI staining analysis don't exhibited specific morphological defects. In this current study, we have implicated that the Toll and IMD pathways are involved in immunity against *M. anisopliae*. **Supported by:**CNPq / INCT-EM **Keywords:** Rhodnius prolixus; immune response; metarhizium anisopliae

PV95 - **INHIBITION OF HEMOGLOBIN PROTEOLYSIS BY FREE HEME IN THE MIDGUT OF RHODNIUS PROLIXUS (HEMIPTERA: REDUVIIDAE)**

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Rhodnius prolixus is a hematophagous hemiptera of great medical importance because it is the vector of the protozoan parasite Trypanosoma cruzi, the etiologic agent of Chagas' disease. Studies to better understand the physiological and biochemical mechanisms of the digestive system of this vector are important for the development of strategies for vector control. In order to gain new knowledge about digestive enzymes in this insect, we studied the role of heme on hemoglobin proteolysis in the posterior midgut during blood digestion. We incubated the midgut extracts of adult females 4 and 6 days after a blood meal either in the presence and absence of free heme to detect heme-binding proteins by means of native PAGE (12%) followed by heme staining protocol. Midgut extracts were used as source of enzymes for hemoglobin proteolysis assays with and without heme. Hemoglobin content was accessed by SDS-PAGE (15%). We showed that free heme is able to associate to a protein in the midgut extract. In vitro assays, followed by resolution of proteins through SDS-PAGE, revealed that free heme led to a partial inhibition of hemoglobin proteolysis by midgut extract in vitro. The same in vitro inhibitory effect was not evident in the presence of quinine, a drug recognized for inhibiting heme aggregation into hemozoin. Feeding insects with blood containing 130 µM quinine revealed that the hemoglobin digestion was seriously delayed, since a higher content of this protein was left in the midgut of R. prolixus. The hemozoin is neither able to associate to the same protein that heme does in the midgut extract nor has any inhibitory effect on hemoglobin proteolysis. Our data allow us to conclude that free heme is able to inhibit hemoglobin proteolysis, what may represent a strategy to set the pace of hemoglobin digestion, avoiding generation of high levels of free heme in the midgut lumen that is toxic for the insect. **Supported by: Keywords:** Rhodnius prolixus; digestion; heme

PV96 - **CHARACTERIZATION OF *LPG2* GENE DUPLICATION IN *LEISHMANIA INFANTUM* AND THE USE OF CRISPR/CAS9 FOR GENOME EDITING**

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Introduction: The *lpg2* gene encodes a Golgi GDP-mannose transporter enzyme, important in the process of synthesizing LPG and other PG-containing molecules in its structure. We have previously attempted to generate a *lpg2 Leishmania infantum* knockout parasite exploring the homologous recombination procedure. However, after obtaining the resistant parasites (Neo and Hyg), we were still able to amplify *lpg2* gene by PCR. These data raised two hypotheses: that the gene disruption did not occur as expected; alternatively, that *lpg2* is present as a multi-copy gene in *L. infantum* (although evidence for this in the available genome assembly was lacking). Herein, we describe the characterization of the *lpg2* gene structure in *L. infantum* MCAN/BR/89/BA262 genome and set the stage for CRISPR/Cas9 system to obtain the *lpg2* knockout parasites. Methods: Double resistance parasites had their genomic DNA extracted for homologous recombination confirmation by PCR and by sequencing with MinIon (Oxford Nanopore Technologies) using different sets of primers targeting the *lpg2* region. Results: The results demonstrated the occurrence of homologous recombination and the integration of the markers (Neo and Hyg) as expected in the *lpg2* locus. However, during mapping of the sequences against the *L. infantum* JPCM5 genome (GenBank assembly GCA_003020905.1) we could identify a second copy for the *lpg2* gene in a tandem array. This result led us to set the CRISPR/Cas9 system for *lpg2* disruption; two gRNAs were designed and cloned in pLdCN plasmid. Four rounds of electroporation were performed with oligo donors as a stop codon source. Conclusion: In contrast to other *Leishmania* species, the *lpg2* gene is present in two copies in the *L. infantum*. This supports the use of the CRISPR/Cas9 system for *lpg2* gene disruption; experiments are under way to characterize knockout parasites. Supported - CAPES, IGM, FAPESB. **Keywords:** Leishmania; crispr/cas9; phosphoglycan

PV97 - **CELLULAR ULTRASTRUCTURE HAEMOGREGARINA CYRILIA LIGNIERESI**

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The structure of the trophozoite of Cyrilia lignieresi (CL) has the usual structures seen in members of the Apicomplexa phylum, but the macrogamethocyte ultrastructure is not well characterized. Objective: To describe the macrogamethocyte cell ultrastructure of the haemogregarine CL through tomographic series obtained in Transmission Electron Microscopy (TEM) reconstructed in three-dimensional structure. Method: Blood samples of Synbranchus marmoratus infected with haemogregarine Cyrilia lignieresi were captured in 1997 in the periphery of Belém of Pará, northern Brazil, and processed by the routine technique for TEM. The processed samples were sectioned in ultramicrotome (200nm thickness), contrasted with uranyl acetate and lead citrate, and examined in a microscope TECNAI G20 FEG 200kv for acquisition of the tomographic series. The alignment of tomographic series and the design of structures and organelles of the parasite in a three-dimensional model was realized in the program IMOD. Results: CL macrogamethocytes appear to be larger in size than parasitophorous vacuole (PV) within the parasitized erythocyte due to folding of the posterior region of the parasite. It has distinct groups of micronemes that are easily dispersed with each other and with an anterior region. In the interior and along the PV, between the membranes of the parasite observed, notice the presence of spherical bodies with different diameters. In this case, the graph three-dimensional, is presented in tubular format, which is not considered a great indicator in the literature in two-dimensional images. It was also possible the presence of vesicles, which appear to sprout from the PV membrane into the red blood cell. Conclusions: Macrogamethocytes of CL besides the usual plants characteristic of the phylum Apicomplexa, presents tubular forms in inner membrane, previously present as a spherical species. Structures should be further studied to improve their importance for the parasite. **Keywords:** Celular ultraestruture; haemogregarine; cyrillia lignieresi

PV98 - **MINICIRCLES SEQUENCE (KDNA) ANALYSIS FOR LEISHMANIA SPECIE IDENTIFICATION IN CATS.**

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Visceral leishmaniasis (VL) in felines is increasingly frequent in endemic areas in Brazil. The domestic dog is considered the main reservoir of the disease, although the epidemiological importance of cats has been increasingly investigated. Cats with VL frequently present cutaneous alterations and no severe visceral changes are observed, different from what occurs in dogs. Therefore, there is a need to investigate the genetic characteristics of the parasite in felines. In order to identify the species of Leishmania that infects cats in Teresina-PI, Brazil, we isolated the parasite from cats and sequenced a conserved 120 bp kDNA region, of 6 animals. For the phylogenetic analyzes we included a filtered popset containing sequences from the same region studied, in all 6 species of leishmania were used to perform the analyzes. The distances were then used to generate a neighbour- joining tree, in order to provide an alternative visualization of the data graphically, we constructed a network of haplotypes from highly conserved regions. The aligned sequences point to the isolates of cats are Leishmania infantum, and the grouping of all of them in a single clade suggests peculiar characteristics of the strain of the region and require genomic analysis to confirm such characteristics, this will be the second step of this work.**Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq **Keywords:** Visceral leishmaniasis; minicircles (kdna); cats

PV99 - **MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF A PLATELET ACTIVATING FACTOR ACETYHYDROLASE (PAF-AH) OF *TRYPANOSOMA CRUZI***

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Chagas disease affects 8 million people. In the host vertebrate, the parasite invades many cells, finding a favorable environment to continue its life cycle. Previous reports have linked phospholipase A$_2$ (PLA$_2$) activity to cell invasion events by several parasitical protozoa. PLA2 constitute a famil s that contains various subgroups, including platelet activating factor acetylhydrola ious studies suggest that *T. cruzi* parasites have PLA$_2$, but no enzymatic act this parasite. The goals of the present work were to construct a th *T. cruzi*, to document the presence of PLA$_2$ in this para its enzymatic activity. The 3D model was constr e Phyre2 server. The validation was protein encoded by the *T. cruzi* P of *T. cruzi* and comparing with h me a very similar tertiary structure sites. The immunofluorescence and th *T. cruzi* (Dm28c) epimastigotes. The lized to the cytoplasm, surrounding the nucleus, in the endoplasmic reticulum; a faint label was also observed pimastigotes. The PAF-AH activity assay, which showed that the e ted an activity of 0.1 mmol/min/ml, which was abolished by specific PAF-AH in ary, this is the first study to elucidate the 3D structure and enzimatic activity of a PAF-AH *cruzi*. Ongoing investigations in our laboratory aim to lead the way for the use of this *T. cruzi* PAF-AH as a target for new chemotherapeutics against Chagas disease. **Supported by:**CNPq, CAPES, FAPERJ and INCT-EM **Keywords:** Modelling; lipase; phospholipids

PV100 - **ENDOSYMBIOSIS IN TRYPANOSOMATIDS: THE INFLUENCE OF THE SYMBIOTIC BACTERIUM ON THE HOST TRYPANOSOMATID METABOLISM**

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*Angomonas deanei* has been used to investigate metabolic co-evolution, since the trypanosomatid host and the symbiotic bacterium maintains a mutualistic association. We analyzed the influ f the symbiont on the host metabolism, comparing the wild type (WT) and the aposymbiotic ( When glucose is available as the single carbon source on a define medium (SDM ains presented a generation time close to 6h. But, when proline was pres vas no carbon source, the trypanosomatid growth was slowe he protist ultrastructure was affected, with cells preser ar ATP level was quantified at different nutri ter oxidative phosphorylation inhibi WT protists presented 66% less int der OPI, APO cells maintained the . The cellular amount of ATP decrea the single carbon source. Conversely, hen different conditions were tested. Oxygen or cells cultivated in complex medium containing serum nger buffer, compared to PBS solution. When cells were grown i ce or containing proline, the oxygen consumption rates were lower when c in the presence of glucose. Considering the A. deanei glucose consumption, WT ce to consume 4 mM glucose after 6h, whereas in APO cells 45% of the initial glucose ren ed in the medium. So, *A. deanei* metabolism decays in glucose-starving condition and proline can not be considered as a good carbon source to fuel this protist **Supported by:**CNPq, FAPERJ **Keywords:** Angomonas deanei; metabolism; carbon source