PV-001 - **A comparative study demonstrates vertical inheritance and maintenance of arsenic resistance metabolism in eukaryotes**

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Arsenic has been a ubiquitous component, usually in low concentrations, in the environment since primitive Earth. Because arsenic compounds present a certain level of toxicity to all living organisms, it represents a constant environmental pressure on life. Bacteria already have their mechanism of resistance to arsenic thoroughly described. However, these mechanisms are still somewhat obscure for eukaryotes though we know arsenic-resistant lineages in the group. While we currently attribute the origin of the arsenic resistance machinery in eukaryotes to lateral gene transfer (LGT) from bacteria, only a handful of eukaryotes were deeply studied. In this work, we investigate the origin and evolution of resistance to arsenic in eukaryotes using a broader phylogenomic framework. We hypothesize that, since arsenic pressure is constant throughout Earth's history, resistance mechanisms should be present in some way in eukaryotic ancestors. We identified homologs for each of the arsenic resistance genes in eukaryotes and traced their possible origin through phylogenetic reconstructions. We reveal that: i. an important component of arsenic-resistance machinery must have originated before the last common eukaryotic ancestor (LECA); ii. The late events of gene duplication and LGT generated new homologs that, in many cases, replaced the ancestors. Thus, we accepted our hypothesis of ancestral presence, although LGT is an important component in the evolutionary process of these resistance mechanisms. The environment of origin and evolution of the first eukaryotes is still the subject of intense debate. Thus, the selective pressure imposed by arsenic on these organisms is still uncertain. However, as arsenic is constantly present in the environment and we found evidence of ancestral eukaryotic homologs to these metabolisms, we infer that basic resistance machinery must be ancestral and changed throughout the eukaryotic diversification.
Supported by: Fundação de amparo à pesquisa do Estado de São Paulo (FAPESP), processo 2019/22109-0 **Keywords:**Environmental adaptation.Resistance to toxic metalloids.Functional phylogenomics

PV-002 - **Identification of components of a divergent mRNA export pathway in trypanosomes**

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mRNA export is central for gene regulation and expression. In trypanosomes, transcription is polycistronic and all mRNAs are processed by *trans*-splicing, export is mediated by non-canonical mechanisms. We have described several conserved mRNA export pathway components in *Trypanosoma cruzi*, including orthologs of Sub2, a component of the TREX complex, and eIF4AIII, a core component of the exon junction complex (EJC). Few orthologs encoding proteins involved in mRNA export in higher eukaryotes are detectable in trypanosome genomes and examples of mechanistic divergence well known. To uncover additional components of the trypanosome mRNA maturation and export system we undertook an unbiased search for protein interactors of TcSub2 and TceIF4AIII. Significant overlap between TcSub2 and TceIF4AIII interacting protein cohorts suggests that both proteins associate with similar machinery. We confirmed interactions of mRNA export proteins with conserved core components of the EJC and multiple additional complexes together with divergent proteins specific to trypanosomatids. The highly interactive super-interactome uncovered here, capable of supporting RNA processing from splicing through to nuclear export, highlights kinetoplastid-specific and conserved components creating an amalgamation supporting unique mRNA maturation mechanisms in trypanosomes.
Supported by:CNPq 421990/2017 **Keywords:**RNA export.Trypanosomes.divergent proteins

PV-003 - **Phylogenetic reconstruction and evolution of the Rab GTPase gene family in Amoebozoa**

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Rab GTPase is a paralog-rich gene family that controls the maintenance of the eukaryotic cell compartmentalization system. Diverse eukaryotes have varying numbers of Rab paralogs. Currently, little is known about the evolutionary pattern of Rab GTPase in most major eukaryotic 'supergroups'. Here, we present a comprehensive phylogenetic reconstruction of the Rab GTPase gene family in the eukaryotic 'supergroup' Amoebozoa, a diverse lineage represented by unicellular and multicellular organisms. We demonstrate that Amoebozoa conserved 20 of the 23 ancestral Rab GTPases predicted to be present in the last eukaryotic common ancestor and massively expanded several 'novel' in-paralogs. Due to these 'novel' in-paralogs, the Rab family composition dramatically varies between the members of Amoebozoa; as a consequence, 'super group'-based studies may significantly change our current understanding of the evolution and diversity of this gene family. The high diversity of the Rab GTPase gene family in Amoebozoa makes this 'supergroup' a key lineage to study and advance our knowledge of the evolution of Rab in Eukaryotes. **Supported by:**FAPESP: 2017/19388-0, 2019/22692-8 e 2016/14317-4 **Keywords:**Phylogenomics.Eukaryotic evolution.Rab therapeutic intervention

PV-004 - **Characterization of a Fatty-Acid Binding Protein-like in *Leishmania amazonensis***

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Leishmania parasites are the causative agents of a group of diseases collectively known as leishmaniases. These neglected tropical diseases are endemic in Africa, Southeast Asia, and South America, where more than 1 billion people are at risk of infection. There are no vaccines and therapy remains limited due to drugs toxicity and resistance. During their life cycle, *Leishmania* alternate between invertebrate and vertebrate hosts. Hence, they must adapt to different environments and compete with their hosts for several essential nutrients, such as lipids and fatty acids (FAs). Considering the crucial role of Fatty-Acid Binding Proteins (FABPs) in lipid metabolism, we looked for proteins containing FABP-like domains in the *Leishmania* genome. We identified a putative *L. mexicana* gene (*LeiFABP*) encoding a conserved hypothetical FABP-like domain. Therefore, our goal is to functionally characterize LeiFABP in *L. amazonensis*. At first, we designed the primers and optimized the PCR conditions to amplify the open reading frame of *L. amazonensis LeiFABP*. At the moment, we are generating the constructs for expression of LeiFABP fused to GFP using the *Leishmania* expression plasmids pXG-GFP+ and pXG-GFP2+. With this, we will confirm the subcellular localization of LeiFABP in *L. amazonensis* and characterize the overexpressing parasites regarding replication and virulence. LeiFABP characterization may help elucidate the distinctive features of *Leishmania*' FAs metabolism and trafficking, indicating new targets for the development of better therapeutic strategies against leishmaniases.
Supported by: Fundação Amparo a Pesquisa do Estado São Paulo - Processo 2020/07465-2
Keywords: Leishmaniasis.lipid metabolism.neglected tropical diseases

PV-005 - **Could inositol pyrophosphates have been influencing parasitism within kinetoplastids?**

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Inositol pyrophosphates (e.g.: IP_7 and IP_8) are highly energetic metabolites involved in a wide range of cellular processes. IP_7 and IP_8 are synthesized through complementary pathways that involve IP6K and PP-IP5K kinases, respectively. Trypanosomatids parasites (e.g.: *Trypanosoma cruzi* and *Leishmania* spp.) have orthologous genes for IP6K, but the genes encoding to PP-IP5K are apparently absent. Curiously, *Bodo saltans* (a free-living kinetoplastid organism) own a PP-IP5K homolog with 42% identity relative to human PP-IP5K. The goal of this study is to investigate whether the absence of PP-IP5K (and consequently IP_8) is mutually exclusive relative to parasitism within kinetoplastids. Thereunto, we carried out evolutionary analyzes using IP6K and PP-IP5K kinases to confirm the alleged loss of PP-IP5K. Using deep analyzes with HMMER, MUSCLE, TRIMAL, and PHYML, we reconstructed phylogenetic trees and get robust evidence that confirms the absence of PP-IP5K in all trypanosomatids but *Paratrypanosoma confusum*. Predictions of the tertiary structures pointing that the catalytic domain of *P. confusum* PP-IP5K, although it has 44% identity to human PP-IP5K, is naturally unstructured, which puts its function in suspicion. Our findings, although preliminary, suggest that the transition from a free-living to a parasitic lifestyle has resulted in the loss of PP-IP5K. Our next steps include performing PP-IP5K knock-in (from *Bodo saltans*) in *T. cruzi* using CRISPR/Cas9 system. We intend to perform a phenotypic characterization of the generated KI lineage, in addition to conducting infection assays. Also, we intend to perform knock-out (KO) of PP-IP5K in Vero cells (used as a host cell) to evaluate infection alterations. This work will clarify if the PP-IPs have any relation with the parasitism developed within the kinetoplastids, contributing to unveil new routes for the developing antitrypanosomal therapies. **Supported by:**CAPES, FAPESP (2019/10753-2; 2020/10277-3) **Keywords:**inositol pyrophosphates.knock-in.parasitism

PV-006 - **Effect of the calcium binding protein TcCAL1 on the host-cell invasion by Trypanosoma cruzi**

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Calcium (Ca^{2+}) plays crucial role in the life cycle of *T. cruzi*. The parasite genome encodes for several Ca^{2+}-binding proteins, although most of them remain uncharacterized. TcCAL1 is a hypothetical Ca^{2+}-binding protein with EF-Hand motifs identified through proteomic analysis of *T. cruzi*. Our work aims to unravel the role of TcCAL1 in the *T. cruzi* life cycle. Previously, we found that the overexpression of TcCAL1 decreased the metacyclogenesis rates of epimastigotes *in vitro*. Now, we evaluated the virulence of TcCAL1-overexpressing parasites in mammalian cell invasion assays. To study this, the gene encoding tccal1 was fussed to a 6x histidine tag and cloned into the pTREX expression vector. Epimastigotes from strain Y were transfected with the plasmid pTREX/TcCAL1x6His or the empty vector pTREX (controls). After selection, recombinant epimastigotes were differentiated into metacyclic trypomastigotes in blood-agar/LIT medium and then incubated for 24h with Vero cells using a 300:1 parasite:host cell ratio. At 48h post infection, samples were Giemsa-stained to calculate the percentage of infected cells and the number of intracellular amastigotes by counting three hundred Vero cells per sample in randomly selected fields; results were compared by using the Student´s t-test. Preliminary data showed that the percentage of infection significantly increased in host cells incubated with TcCAL1x6His-overexpressing parasites in comparison to controls (41.2% versus 18.9%, respectively; P = 0.0092), as well as the number of internalized amastigotes (1398 versus 926 parasites/100 cells, respectively; P = 0.024). Based on this finding, we hypothesize that high levels of TcCAL1 protein disrupt the intracellular Ca^{2+} concentration interfering with *T. cruzi* differentiation and increasing host cell invasion. Future experiments will be focused on the validation of the effects of TcCAL1 on cell invasion and the experimental verification of Ca^{2+} binding by TcCAL1. **Supported by:**PICT 2016 NUMBER 1028 INGEBI FOUNDATION **Keywords:**Trypanosoma cruzi., Ca2+-binding protein.host-cell invasion

PV-007 – **Investigating the Kennedy Pathway:phosphatidylcholine and phosphatidylethanolamine synthesis in *Trypanosoma cruzi***

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The Kennedy pathway is the main biosynthetic route for ph and phosphatidylethanolamine (PE) formation. The importance of P sitic protozoa has been well demonstrated. Disruption of PC um *falciparum* and *Leishmania donovani;* disruption of PE h nosoma *brucei*. However, information is severely lackin etabolism in *Trypanosoma cruzi*.
Although bioinformatic analysis of the T ngle bifunctional choline and ethanolamine kinase at the s investigation revealed two functional kinases. These pu pressed in *Escherichia coli*, purified and characterised. Kine 35.190 (*Tc*E/CK), is bifunctional, with a higher affinity for ontrastingly, *Tc*CLB.487739.20 (*Tc*C/EK), although also choline than ethanolamine.
*Tc*E/CK ' he subcellular localisation of *Tc*E/CK was determined to n alleles of *Tc*E/CK was only possible in the presence of an e g essentiality. At present, metabolomic phenotyping is underway for lines as well as in vivo characterisation of *Tc*C/EK.
The nes for compensatory PE/ PC biosynthesis suggests *T. cruzi*, like *T. brucei*, relies the Kennedy pathway. This will allow drug selectivity, as humans have additional, alterna ates for PE/PC biosynthesis. In conclusion, the enzymes of the Kennedy pathway in *T. cruzi* ar appealing drug targets with a fundamental role in parasite survival and warrant further investigation. **Supported by:**EPSRC (UKRI) and University of St. Andrews **Keywords:**Metabolism.Phospholipid.Trypanosoma cruzi

PV-008 - **An unconventional RNA-binding protein defining the mRNA fate of the major variant surface antigen.**

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The variant surface glycoprotein (VSG) enables salivarian trypanosomes to evade host's adaptive immunity via antigenic variation. VSG mRNA comprises 10% of total cell protein and the high stability of VSG mRNA is essential for VSG expression, and so trypanosome survival. To determine how VSG mRNA stability is maintained, we used mRNA affinity purification to identify all its associated proteins. CFB2 (Cyclin F-box protein 2), an unconventional RBP, was specifically enriched with VSG mRNA. We demonstrate that CFB2 is essential for VSG mRNA stability, describe cis acting elements within the VSG 3'-untranslated region that regulate the interaction, identify trans-acting factors that are present in the VSG messenger ribonucleoprotein particle, and mechanistically explain how CFB2 stabilizes the mRNA of this key pathogenicity factor.
CFB2 has an N-terminal cyclin-F-box domain. F-box proteins are found in SCF complexes (SKP1-Cullin-F-Box), which are E3 ligase components of the ubiquitination machinery. We have demonstrated that the interaction between CFB2 and SKP1 is involved in autoregulation of CFB2 abundance. Ongoing biochemical studies aim to scrutinize the novel RNA binding domain and find out whether there is a link between SCF-complex formation and nucleic acid binding activity. **Supported by:**H2020 European Research Council (649019) /core funding from the University of Heidelberg/ **Keywords:**Trypanosoma brucei.VSG.RNA binding protein

PV-009 - **How much does it cost to build a trypanosome? Estimating the amount of ATP necessary for maintaining and duplicating a bloodstream-form Trypanosoma brucei cell**

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ATP is the major energy currency of cells since its hydrolysis generates free energy which is used to power biological processes. ATP hydrolysis is required for synthesis, transport and polymerization of monomers for macromolecules as well as for the assembly of the latter into cellular structures. ATP is also necessary for cellular processes not directly related to the synthesis of biomass, such as maintenance of membrane potential and cellular shape. *Trypanosoma brucei* is a unicellular flagellated parasite with a complex life cycle occurring between an insect vector and a mammalian host. Glucose, abundantly available in the bloodstream of the mammalian host, is used as the main energy source by the bloodstream form (BSF) of this parasite. BSF *T. brucei* has been shown to use very little or no carbon from glucose for the synthesis of its biomass, but to depend on the extracellular availability of other essential nutrients as carbon sources for the biosynthesis of precursors. Approximately 97% of the glucose consumed by the BSF is directed to ATP synthesis. Here, we made a detailed estimation of the energy budget during the BSF cell cycle, with the energy costs of the cell's two main commitments: to stay alive and to duplicate. As glycolysis is the only source of the produced ATP, we calculated that a single parasite produces $5.9x10^{11}$ molecules of ATP/cell cycle. We found that biomass production accounts for 43% of the total energy budget, with translation being the most expensive process. Interestingly, the budget for flagellar motility, VSG recycling, maintenance of transmembrane ion and proton gradients and ATP-dependent transport processes accounts for 32% of the total ATP. Finally, there is still 25% available in the energy budget that is being used for other cellular processes of which the relative costs remain to be elucidated. These data put a new perspective to the assumptions about the relative weight of cellular processes BSF undergo during its cell cycle. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo **Keywords:**Trypanosoma brucei.Metabolism.ATP

PV-010 - **Biological evaluation of (E)-N'-(thiophen-2-yl methylene)benzohydrazide as an antileishmanial agent**

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Leishmaniasis, caused by protozoa *Leishmania sp*, is a neglected tropical disease that affects between 12 and 15 million people in the world. Currently, the drugs available are limited, toxic and with low effectiveness. Thus, given the difficulties involved in its treatment, the search for new efficient and less toxic drugs is a priority in the medical field. This study evaluated the in vitro activity of (E)-N'-(thiophen-2-yl methylene)benzohydrazide (BH6), a new n-acylhydrazone, against promastigotes of *L. amazonensis*. The antiproliferative activity was determined by XTT method and IC_{50} and IC_{90} were calculated by linear regression. Further, ultrastructural changes were observed by transmission electron microscopy (JEOL JM1400) after 72 h of treatment. Fluorimetric analyses were performed in fluorescence microplate reader (VICTOR X3, PerkinElmer) to investigate ROS generation using H2DCFDA and lipid inclusions using Nile Red and by flow cytometry (BD FACSCalibur) to evaluate mitochondrial membrane potential with Rhodamine 123 and membrane integrity with propidium iodide (PI). The results showed an antiproliferative activity, with an IC_{50} and IC_{90} of 7.18 and 19.66 µM, respectively. Promastigotes treated with BH6 revealed important ultrastructural changes, such as mitochondrial swelling, concentric membranes inside the mitochondria and cytoplasm, presence of autophagosomes and lipidic inclusions. Moreover, BH6 induced an increase in intracellular ROS levels after 24 h treatment, and a decrease in mitochondrial membrane potential. There was no increase in PI-positive cells, indicating that the plasma membrane appears to be intact. In addition, there was an augmentation in lipid inclusions, corroborating with TEM observations. These findings suggest that BH6 induced oxidative damages, probably leading to cell death. Some studies are still needed to define the mechanism of action of this compound; however it has shown a potential activity against *L. amazonensis*. **Supported by:**Capes, CNPq, FINEP and Pronex/Fundação, COMCAP-UEM **Keywords:**antiproliferative activity.Leishmania amazonensis.oxidative stress

PV-011 - **The Fantastic Four: identification of four cruzipains subtypes in *T. cruzi***

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Cruzipain is the main cysteine protease of *Trypanosoma cruzi,* associated with metacyclogenesis, host cell infection, and modulation of the immune response. Initial works from the ’90s describe dozens of cruzipain gene copies in *the T. cruzi* genome. However, genome data obtained with Sanger or Second Generation Sequencing did not allow determining the number of cruzipain copies. In the past few years, *T. cruzi* genomes were sequenced with Third Generation Sequencing. These new technologies generate longer reads, having better performance in repetitive regions than the previous ones. Thus, we investigated the genomic localization, the number of copies, and the phylogenetic relationship of cruzipain genes in three *T. cruzi* genomes sequenced with PacBio and Nanopore. We searched for cruzipain sequences in Dm28c (TcI), YC6 (TcII), and CL Brener (TcVI) with Blast. The gene sequences were aligned with Clustal Omega, and phylogenetic trees were constructed with MEGA-X. We also evaluated the expression of cruzipains copies with RNA-Seq data available from the CL Brener strain. The new subtypes identified by us had their 3D structure predicted by homology modeling. Based on the phylogenetic reconstructions and analysis of the sequences, we subdivided the cruzipains into two Families and four subtypes. Family I comprises czp1, the first described in the literature and targeted in drug design efforts for Chagas disease. Family II comprises czp2, which also has been previously described, and the novel czp3 and czp4. The four of them have significant differences in the active site residues, which likely impact their substrate specificity and interactions with small molecules. Family I sequences are mostly expressed in epimastigotes, while the Family II ones in the mammalian forms. Our results suggest the importance of the biological and biochemical characterization of all cruzipain subtypes to better understand *T. cruzi* biology and improve drug design for Chagas disease.
Supported by:CAPES, CNPq, FAPEMIG, FAPERJ, and FAPESP **Keywords:**cruzipain,Trypanosoma cruzi, cysteine protease

PV-012 - **Investigation of kDNA minicircles copy number in *Trypanosoma cruzi* genetic groups by duplex qPCR**

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Chagas disease is caused by *Trypanosoma cruzi*, a protozoan belonging to the order Kinetoplastida. Organisms of this order possess DNA in the nucleus and in a single mitochondrion whose DNA is organized the kinetoplast, an organelle composed of minicircles and maxicircles of kinetoplast DNA (kDNA). T. cruzi population can be divided in six genetic groups (TcI - TcVI) and the contribution DNA in present in kinetoplast and nucleus for total DNA the parasites remain elusive. The main aim of this work is to investigate kDNA minicircles copy number in strains and clones of different T. cruzi genetic groups. Genetic characterization by PCR-RFLP of nine *T. cruzi* strains/clones associated them to TcI (n=2), TcII (n=3), TcIII (n=1), TcV (n=1), and TcVI (n=2) genetic groups. In duplex qPCR, the single copy gene DHFRTS (dihydrofolate reductase - thymidylate synthase) was used as a reference gene for quantification of kDNA minicircles. No significant difference among normalized Cts was observed in four different DNA concentrations (0.005 to 5 ng of DNA) tested. We used the Pfaffl method to correct relative efficiencies. Lin’s coefficient was used to predict the number of parasites according to the estimated values of normalized Cts determined by duplex qPCR. *T. cruzi* JG strain (TcII) was the one that presented the highest kDNA minicircles copy number (1284 copies per copy of DHFRTS) and the CL Brener clone (TcVI) had the lowest kDNA minicircles copy number (7.49 copies per copy of DHFRTS). These results revealed a great variation in kDNA minicircles copy number in *T. cruzi* strains analyzed. These differences can be due to real variations in minicircles copy number, but we cannot exclude the possibility that polymorphisms in the annealing sites of primers and/or probes may have influenced quantifications. Further studies using different primers or probes can reveal the complete extension of differences of kDNA in the genetic groups of *T. cruzi*. **Supported by:**CAPES,CNPQ, FAPEMIG **Keywords:**trypanosoma cruzi.kDNA.DNA content

PV-013 - **Role of histone-fold domain containing Oocyst Rupture Proteins during oocyst and sporozoite stages**

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

PV-014 - **Could the roles played by inositol pyrophosphates (PP-IPs) in trypanosomatids be related to DNA metabolism and virulence?**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of processes, such as telomere homeostasis and homologous recombination. However, the mechanism of action of these metabolites is not fully understood. The PP-IPs (IP_7 and IP_8) are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. *Trypanosoma cruzi* and *Leishmania* spp., etiological agents of neglected diseases, apparently do not present homologs for PP-IP5K. Also, DNA metabolism in these organisms presents peculiarities that seem to be exclusive to this family. The goal of this study is to elucidate the roles of PP-IPs in DNA metabolism, and parasitism developed by trypanosomatids. For this, we first carried out in silico analysis. We confirmed the absence of PP-IP5K in all trypanosomatids but *Paratrypanosoma confusum*. Predictions of the tertiary structures pointing that the catalytic domain of *P. confusum* PP-IP5K, although it has 44% identity to human PP-IP5K, is naturally unstructured. Curiously, *Bodo saltans* (a free-living kinetoplastid) own a PP-IP5K homolog with 42% identity relative to human PP-IP5K. This finding suggests that the transition from a free-living to a parasitic lifestyle has resulted in the loss of PP-IP5K, which makes us wonder if the absence of PP-IP5K (and consequently IP_8) is mutually exclusive relative to parasitism within kinetoplastids. To investigate that in deep, we are performing knock-in (KI) of PP-IP5K from *B. saltans* into *T. cruzi* and *L. braziliensis*. Next, we intend to evaluate the virulence of the KI strains generated. We are also performing assays to deplete the IP6K gene in *T. cruzi* and *L. braziliensis* to investigate the participation of IP_7 in DNA repair, and infection capacity. Finally, using a groundbreaking approach, we intend to track the peptides pyrophosphorylated by IP7. The participation of PP-IPs in these pathways could provide new routes for developing antiparasitic therapies. **Supported by:**São Paulo Research Foundation (FAPESP) 2019/10753-2; 2020/10277-3 **Keywords:**Inositol pyrophosphates,DNA repair,virulence

PV-015 - **Characterization of *Blastocystis* subtypes isolated Minas Gerais State, Brazil**

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Blastocystis is an intestinal protozoan that has gained relevance in recent years owing to its possible role in the pathogenicity of the human hosts. Although underestimated, the prevalence of *Blastocystis* spp. in human hosts ranges from 17.8% to 86.63% in Brazil. It is known that humans are mainly infected by ST1–ST4 and rarely by ST5–ST9. In Brazil, the subtypes ST1 - ST4 and ST6 - ST8 have already been demonstrated. However, the molecular epidemiological profile of *Blastocystis* has not been established in several cities and regions of Brazil. The main aim of this study is to genetically characterize the isolates of *Blastocystis* species obtained from human and animal fecal samples previously positive for the parasite and investigate the presence of Blastocystis in the water supply of a city in the interior of Minas Gerais. SSU rDNA gene PCR amplicons were generated from 21 *Blastocystis* isolates obtained from humans, one bovine isolate, four pig isolates and from 18 water samples. Amplicon sizes obtained varied (0.9 to 1.3 kb) from the expected (1.1 kb) in 18/21 isolates from humans and 17/18 water isolates. PCR-RFLP demonstrated the presence of the subtypes ST1 (53.3%), ST3 (40.0%) and ST2 (6.7%) for human isolates; ST10 (100%) for bovine isolate; and ST5 (50.0%), ST1 (25%), and ST3 (25%) for pig isolates. Sequencing of PCR products showed 98–99% identity to the *Blastocystis* sequences deposited in Genbank, except for sequences from water samples that showed the highest identity with algae rDNA. **Conclusion**: Main *Blastocystis* subtypes circulating in the Triângulo Mineiro, Brazil, are ST1-ST3, ST5, and ST10. *Blastocystis* ST1 and ST3 found in humans and pigs reinforce the zoonotic transmission potential of the parasite. *Blastocystis* specific primers should be designed to investigate the parasite in environmental samples.
Supported by:CAPES **Keywords:**Blastocystis.Subtypes.Genetic characterization

PV-016 - **Chemical genetic approaches to study the role of protein kinases in *Leishmania* cell cycle regulation.**

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Cell division is a core biological process during the development of both multicellular and unicellular organisms. It is a conserved process throughout eukaryotes, which has diverse evolutionary roots, resulting in a unique repertoire of protein component in Trypanosomatids, including *Leishmania*. Some of these components has been identified in *Leishmania*, but the extension of its repertoire and their role in the coordination of the cell cycle remains unclear. Here, we used genetic and chemical approaches to explore the role of some essential protein kinases in cell cycle progression. We used CRISPR-Cas9 to perform precision editing of the *L. mexicana* genome to generate analogue sensitive mutants suitable for chemical genetic inhibition. For the kinetochore protein kinase KKT2, CRK9 and CMGCa replacement of the bulky gatekeeper methionine residue with a glycine in the ATP-binding site makes the enzymes sensitive to the bulky inhibitor 1NM-PP1. For the CLK1 and CLK2 replacement of a cysteine near to the ATP-binding domain prevents binding of the inhibitor AB1, validating the specificity of this compound against CLK1/CLK2. The specific inhibition of CLK1, CLK2, KKT2 caused a cell cycle arrest in G2/M stage of the promastigote. A further investigation, by fluorescence microscopy labelling the mitotic spindle, revealed that KKT2 inhibition is followed by a significant accumulation of cells in early mitosis, where mitotic spindle coordination in the nucleus failed. Moreover, it was observed that CMGCa inhibition also impaired chromosome segregation, but the cell body development reaches a more advanced stage, suggesting CMGCa activity is required later in mitosis than KKT2. In addition, CLK1/CLK2 inhibition doesn't affect the coordination of the mitotic spindle, but it blocks cell cycle progression in cytokinesis. These studies bring new insights into the essential biological process of cell division in *Leishmania* and provide a source of new potential therapeutic targets. **Supported by:**GCRF **Keywords:**L. mexicana, Chemical genetic approaches,Cell Cycle

PV-017 - **Could the kinase IP6K influence telomere dynamics in *Leishmania braziliensis*? Preliminary analyses**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of cellular processes, such as telomere length regulation and homologous recombination. However, the target proteins of PP-IPs, as well as their mechanism of action, are still not well established. The main PP-IPs involved in these processes are the so-called IP_7 and IP_8, which are synthesized by pathways involving the kinases IP6K and PP-IP5K, respectively. Trypanosomatid parasites, such as *Leishmania* spp., have homologs genes for IP6K but do not have homologs for PP-IP5K, making them excellent models study of IP_7. This work aims to generate a lineage KO for IP6K ($IP6K^{-/-}$ or $IP6K^{-/+}$) in *Leishmania braziliensis*, using the CRISPR/Cas9 approach. Then, we intend to evaluate the telomere homeostasis relative to wild type (WT) after shortening the telomeres of IP6K KO lineage with H_2O_2. Currently, we are carrying out the transfections using an *L. braziliensis* lineage expressing T7-Cas9 and two DNA donors containing a different resistance marker each (puromycin and blasticidin). After performing clone selection through serial dilutions, we will confirm the depletion of IP6K (generating lineages $IP6K^{-/-}$ or $IP6K^{-/+}$) by PCRs and qPCR. Episomal IP6K add-back lineages will be generated to demonstrate the specificity of the assay and eliminate bias due to off-targets. Next, using the IP6K KO lineages ($IP6K^{-/-}$ or $IP6K^{-/+}$) and controls (WT and add-back), we will perform growth curves, FACS analysis, and we will standardize the STEX (single telomere extension) assay to evaluate the telomere dynamics. It is worth mentioning that this work will help us to answers questions of fundamental importance, such as: Could the *Leishmania* telomeres be used as a new route for the development of antiparasitic drugs? The kinase IP6K (or the inositol pyrophosphate IP_7) could contribute to that? This project will be of great relevance to answer these questions. **Supported by:**FAPESP - 2020/16481-1 **Keywords:**CRISPR/Cas9, inositol pyrophosphates, telomere dynamics

PV-018 - **Genomic comparison of *Trypanosoma cruzi* to trypanosomes of the clade *T. cruzi* isolated from bats**

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Bat trypanosomes have been implicated in the evolutionary history of *T. cruzi* clade. It has been suggested that *T. cruzi* and *T. rangeli* evolved from a lineage of bat trypanosome that later adapted to terrestrial mammals. Here we study the karyotype organization and syntenic associations between the species of clade *T. cruzi*, especially those infecting bats (*T.cruzi bat*- Tc bat, *T. cruzi marinkellei*- Tcm and *T. dionisii*- Tdio). Tc bat and Tcm karyotypes are quite similar in number and size of chromosomal bands to the *T. cruzi* lineage TcI. While, the number and size of bands of Tdio were lower than those of *T. cruzi*. Chromosome synteny across bat trypanosomes was demonstrated by chromoblot hybridization with specific markers of syntenic blocks conserved in *T. cruzi* lineages. We performed comparative genomic hybridization (aCGH) to identify chromosome regions harboring copy number variations (CNV) in bat trypanosomes using the genome of *T. cruzi*- CL Brener as a reference. We found 410 CNVs distributed as follows: 295 in Tcbat, 93 in Tcm and 104 in Tdio. Most commonly observed aberrations were deletions and duplications of small chromosomal segments (<50kb) which may be due to homologous recombination. Alterations comprising the entire length of chromosome have also been identified, featuring a chromosomal aneuploidy event. Our data indicates that these changes have occurred in chromosome counterparts in Tcm and Tcbat. Aneuploidy events can be explained by non-disjunction of counterparts in mitosis or by overreplication of one homologue and/or under-replication events. Other chromosomal aneuploidy events have been identified in the homologues of in Tcm, Tcbat and Tdio. We note that changes in isolates Tcm, Tcbat and Tdio were flanked by members of the multigene families suggesting that these genes play an important role in recombination events. **Supported by:** CNPq -Número de processo:134397/2015-0 **Keywords:**Trypanosoma cruzi, karyotype, Genomic comparison

PV-019 - **The knockout of a noncoding RNA differentially expressed in Leishmania braziliensis emerges from the 3'UTR of the FtsX-like gene, regulates its expression and promotes phenotypical changes**

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Leishmania braziliensis (Lbr) is the main etiological agent of cutaneous leishmaniasis in Brazil. Such parasites have an unusual mode of gene expression control and mRNAs processing, compared with other eukaryotes. We have previously shown the presence of stage-preferentially expressed noncoding RNAs (ncRNA) during the life cycle of *Lbr*. Herein, we selected some of these differentially expressed ncRNAs to evaluate putative functional roles and phenotypical changes, based on the individual knockout (KO) in promastigotes expressing the CRISPR/Cas9 system. Four ncRNAs were successfully knocked out in *Lbr* and here, we highlight the 20.1_ncRNA97 KO, which showed a remarkable decline in the log phase progression of promastigotes, lower metacyclogenesis rate and increased sensitivity response to nutritional and oxidative stresses in promastigote and axenic amastigote stages, respectively. According to three different *in silico* ncRNA predictors, the 20.1_ncRNA97 was identified as a ncRNA. A RNAseq analysis indicates that such ncRNA might be part of the 3'UTR of the upstream gene LBRM2903_200026000 (FtsX-like protein), but by circular RT-PCR assay and sequencing of the 20.1_ncRNA97 transcript, a 147 bp-long polyadenylated transcript was confirmed. In fact, the expression of the upstream coding gene was negatively affected in the KO parasites. Little is known about FtsX protein in *Leishmania*, but in other organisms it is reported as a transmembrane protein, vital to the final step of the cell division. Consequently, KO parasites with lower FtsX gene expression had their cell cycle recovery slower after the hydroxyurea synchronisation, suggesting the relevance of 20.1_ncRNA97 as regulator of FtsX gene expression, either as a cis- or a trans-element. Northern blotting, comparative transcriptomics and pull-down assays will give us general and robust information to unveil the real function of this ncRNA for the parasite development. **Supported by:**Capes, CNpq, FAPESP (18/14398-0; 2020/00088-9)
Keywords: Leishmania, ncRNAs, Gene expression

PV-020 - **What would be the target proteins of inositol pyrophosphates (PP-IPs) in the human pathogen Leishmania braziliensis? A preliminary approach**

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In model eukaryotes, inositol pyrophosphates (PP-IPs) – mainly IP_7 and IP_8 – are involved in a wide range of cellular processes. However, target proteins of PP-IPs, as well as their mechanisms of action, are still poorly understood. IP_7 and IP_8 are synthesized through complementary pathways that require the kinases IP6K and PP-IP5K, respectively. Trypanosomatids, single-celled eukaryotes parasites (e.g., *Leishmania* spp.), have an ortholog of IP6K, but lack orthologs of PP-IP5K, meaning they are unable to synthesize IP_8. This feature makes trypanosomatids good models to study IP_7. Thus, the goal of this study is to track and identify the main target proteins of IP_7. To pursue this goal, we performed the cloning of recombinant IP6K (as well as its catalytic domain), and IP5K (which will be used as control) using the pET28 system. Preliminary results showed that these kinases are expressed after induction of 1mM IPTG. We intend to purify these proteins and use them to synthesize *in vitro* IP_7 labeled with γ-(Propargil)-imido on the β-phosphate moiety (IP_7-labeled). As a control, IP5K recombinant will be used to synthesize IP_6-labeled (which is a non-pyrophosphate). This approach will allow us to selectively conjugate biotin, via a click chemistry reaction, to *L. braziliensis* proteins that have received the β-phosphate from IP_7-labeled. We will then isolate biotin-labeled proteins using strepdavidin immunoprecipitation and identify them using mass spectrometry. Given pyrophosphorylation by PP-IPs is not enzymatic and still a poorly understood mechanism, our work may uncover new routes for drug development and, therefore, the effective treatment of leishmaniasis. **Supported by:**FAPESP 2020/16465-6 **Keywords:**Inositol pyrophosphates, Recombinant proteins, Mass spectometry

PV-021 - **Attempts of generation of Leishmania infantum deficient in ascorbate peroxidase using CRISPR/Cas9**

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Ascorbate peroxidase (APX) is a redox enzyme of the trypanothione pathway that converts hydrogen peroxide into water molecules regulating the oxidative stress in *Leishmania* and avoiding cell damage. The aim of this study was to delete the APX gene in *L. infantum* to investigate the role of this enzyme in this parasite. The APX (LINF_340005600) knockout attempt was performed using CRISPR/Cas9 approach according to the protocol described by Beneke et al. (2017). The pTB007 plasmid, which has hygromycin as a resistance marker, was used to express *Sp*Cas9 and T7RNAP. *L. infantum* RPV (MHOM/BR/2002/LPC-RPV) promastigotes parasites carrying this plasmid and successfully expressing Cas9 were transfected with donor DNAs and guide RNAs chosen using the LeishGEdit tool. The selection of *Leishmania* clones was done by plating the parasites in semi-solid M-199 medium containing the selective drugs. Deletion assessment was performed by PCR to evaluate the presence of the APX coding sequence, and also the presence of the resistance marker sequences. The results showed the presence of a 1077 bp fragment which corresponds to neomycin resistance marker, indicating the correct integration of donor DNA in the transfected parasites. However, PCR results also demonstrated the amplification of a 912 bp fragment in the mutant parasites, showing that copies of the APX gene were retained by aneuploidy or gene amplification. RT-qPCR and Western blot assays revealed that APX transcript and protein levels in APX *L. infantum* mutant clones were similar to wild-type parasites. The growth of promastigote forms of WT and APX mutant clones and the susceptibility of these parasites to trivalent antimony were also evaluated, but no significant difference was observed between the wild-type line and the mutants. The unsuccessful attempts using CRISPR/Cas9 method to delete APX suggest that this gene is essential in *L. infantum*. **Supported by:**FAPEMIG (FAPEMIG - CBB-PPM and APQ-02816-21); CNPq (CNPq 304158/2019-4); CAPES (001) and Programa Inova FIOCRUZ (VPPCB-007-FIO-18-2-94) **Keywords:**Leishmania infantum, ascorbate peroxidase, CRISPR/Cas9

PV-022 - **Investigation of the role of the ABC proteins (subfamily F) in paromomycin resistance in *Leishmania amazonensis***

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Leishmaniasis is a parasitic disease with wide geographical distribution in tropical and subtropical areas, including Brazil. There are no vaccines available to control the disease and chemotherapy is restricted to some toxic drugs. Although not approved for the treatment of leishmaniasis in Brazil, paromomycin has been used in the treatment of visceral leishmaniasis in the South from Asia, with good clinical efficacy. Studies in other organisms have shown that the proteins of the subfamily ABC-F (ATP Binding Cassette) affect the susceptibility to paromomycin, such as, for example, the elongation factor 3 (EF-3) involved in the translation process of *Saccharomyces cerevisiae*. The search for homologues of these proteins in the databases of the *Leishmania amazonensis* genome identified three genes in the genome this species: *ABCF1*, *ABCF2* and *ABCF3*, as well as in other *Leishmania* species. In this work, the possible involvement of these proteins in susceptibility and resistance to paromomycin was investigated, using molecular genetic techniques such as overexpression and gene inactivation of *ABCFs* genes of *L. amazonensis*. Once the respective transgenic lines were selected, it was found that the promastigote forms of the transgenic lines did not have their *in vitro* growth altered significantly in relation to the wild-type. The overexpressing line of the *ABCF1* gene showed an EC_{50} value of about 1.7 times greater than the wild-type, indicating that overexpression of this gene confers resistance to paromomycin. It was only possible to generate null mutants for the *ABCF2* gene, indicating that the *ABCF1* and *ABCF3* genes are essential for the parasite in the promastigote stage.The null mutants for the *ABCF2* gene showed an EC_{50} value that corresponded to half the EC_{50} value of the wild-type strain, suggesting a possible involvement in the susceptibility to paromomycin due to the *ABCF2* protein. **Supported by:**Fundo de Apoio ao Ensino à Pesquisa e à Extensão (FAEPEX) no processo nº2608/19 **Keywords:**Leishmania, ABC-F.paromomycin

PV-023 - **Identification of aneuploidy events in *Trypanosoma cruzi* by FISH assay**

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T. cruzi shows remarkable genetic variability traits which may play an important role in its adaptative response in the hosts. Even though *T. cruzi* is considered a diploid organism, whole genome sequencing analysis has been shown the presence of aneuploidy in this parasite which may be an important factor in generating genetic variability. We investigated the presence of aneuploidy in *T.* cruzi by fluorescence *in situ* hybridization (FISH) that allows the evaluation of ploidy at the individual cell level. We used single-copy genes (H49 and JL8) as specific-markers of chromosome TcChr39 of CL Brener. They encode repetitive antigens carrying a large tandem array of amino acid motifs. The central region of genes H49 and JL8 comprises an uninterrupted large array of tandem repeats which is a good target to DNA hybridization. The repeats were labeled with Digoxigenin-11-dUTP, cleaved with Dnase I into ≤ 100 bp fragments and incubated with permeabilized epimastigotes. Hybridization signals were revealed with anti-Digoxigenin and Alexa 488 plus; and nucleus and kinetoplast were labeled with DAPI. Mitotic cells were excluded from the analysis. The ploidy was estimated on a mean of 600 labelled cells for each probe by 3 independent observers. FISH assay showed that the copy number of H49 and JL8 genes varied in the cell population. We found two copies of H49 in 42.2% of the cells, followed by cells with one (28.9%), three (14.8%) and more than three copies (3.8%). While, the proportion of cells with one or two copies of JL8 was very similar (40.8% and 41.9%). Using confocal microscopy, we confirmed the existence of aneuploidy events. The variable copy number of H49 and JL8 genes among individual cells may generate intra-strain heterogeneity and chromosomal mosaicism. Our results could be explained by segmental duplication, but we could not rule out the occurrence of whole-chromosome aneuploidy. We carried out dual-colour FISH on the chromosome TcCh39 using H49 and JL8 probes. **Supported by:**FAPESP, CAPES e CNPq
Keywords: Trypanosoma cruzi, aneuploidy, FISH

PV-024 - **DNA replication in the binucleated protozoan parasite Giardia lamblia**

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Giardia lamblia is a binucleated protozoan containing complete copies of the genome in both nuclei. However, little is known on how this organism handles with DNA replication in each nucleus, which challenges the synchronization of replication during the cell cycle. In this study, we used modern molecular biology techniques to investigate DNA replication in the binucleate trophozoite. Firstly, we pulsed cells with CldU and chased it with IdU, isolated and combed DNA, and visualized the thymidine analogs with green and red antibodies, respectively. Our analysis of replication kinetics shows that the mean replication rate in Giardia was 3.64 ± 1.32 kb.min-1 and higher than in *Trypanosoma cruzi* (2.05 kb.min-1) and *Leishmania donovani* (2.37 kb.min-1). Similar, the mean inter-origin distance was slightly higher in Giardia (IOD = 335.7 ±36.49 kb) compared to other unicellular protozoa parasites. We also estimated the minimum number of origins (MO) required to duplicate an entire chromosome within the S-phase duration. We observed that trophozoites use, on average, 1.55 times more origins than the minimum needed indicating that Giardia activates few backup origins during DNA replication. Further we analyzed the replication patterns in the two nuclei after consecutive dual-pulse labeling with CldU and IdU. As trophozoites attach to surfaces with the ventral disc facing down, this allowed us to establish a basis for nuclear positioning as right or left considering the dorsal view of attached parasites. Replication occurred concerted in 78.5% of cells (N = 388) meaning that both nuclei replicate concomitantly (62.5% showing both nuclei yellow, 7% red, and 9% green), while the remaining cells (21.5%) exhibit variable patterns. Curiously, in this last group 7.5% of cells had at least one nucleus without replication activity. In conclusion, our findings evidence a stunning replication synchronicity between both nuclei in Giardia lamblia, consequence of a smoothie DNA replication **Supported by:**CNPq, and FAPESP **Keywords:**genome duplication, two nuclei, intestinal parasit

PV-025 - ***In vitro* activity of crystal violet structural analogues for drug repositioning against trypanosomatid parasites**

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Trypanosoma cruzi, the causative agent of Chagas disease, uses proline (Pro) as main carbon source when glucose is not available. Pro is also involved in many essential biological processes, such as differentiation and cellular invasion. We have reported that the permease TcAAAP069 mediates Pro uptake and it is involved in *T. cruzi* survival. AAAP transporters family is present in all trypanosomatid parasites and is low represented in mammals. Recently, we have validated TcAAAP069 as one of the targets of crystal violet (CV), a compound used for several years in blood banks to eliminate *T. cruzi*, and then we applied a virtual screening approach in order to identify drugs approved for clinical use that might have similar effects to CV. The antihistamines loratadine and cyproheptadine; and the antibiotic clofazimine inhibited Pro transport and had trypanocidal effect with IC_{50} values between 1 and 13 µM in trypomastigotes and amastigotes.
We also evaluated the antiparasitic effects of the drugs in *Trypanosoma brucei* and *Leishmania donovani*, the causative agents of human African trypanosomiasis and visceral leishmaniasis, respectively. All mentioned CV analogues presented activity against *T. brucei* procyclic trypomastigotes and *L. donovani* promastigotes, with IC_{50} values between 1 and 44 µM. Moreover, preliminary tests on *L. donovani* with cationic liposomal formulations of the drugs showed that drug-loaded liposomes had a higher leishmanicidal activity than free compounds. LdAAP24 and TbAAT6 are the orthologous genes of TcAAAP069 in *Leishmania* and *T. brucei*, therefore the Pro transport inhibition of CV analogues will be evaluated as possible mechanism of action of such drugs.
In conclusion, CV structural analogues are promising anti-trypanosomatid compounds, in addition to being approved drugs used in humans, known as repurposed drugs, which could significantly reduce the requirements for its possible application in the treatment of neglected diseases. **Supported by:**Global Challenges Research Fund (GCRF; MR/P027989/1), Agencia Nacional de Promoción Científica y Tecnológica (FONCyT PICT 2015–0539 and 2018-1801 and 2018-1871), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) **Keywords:**Trypanosomatid parasites.drug repurposing.proline transport

PV-026 - **Identification of a mitochondrion-cell membrane tethering complex in *Toxoplasma gondii***

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Toxoplasma gondii possess a single and dynamic plant-like mitochondrion which is validated as a drug target. This organelle changes its shape during the lytic cycle of the parasite, being predominantly in a peripheral distributed lasso-shape in intracellular parasites and collapsed in extracellular ones. This dynamic is poorly understood since Toxoplasma lacks most of the canonical homologues for the mitochondrial fission and fusion pathways. Our laboratory described a unique outer mitochondrial membrane protein named **LMF1** (***L***asso ***M***aintenance ***F***actor ***1***) that is an interactor of the parasite's homologue for the fission related protein Fis1. Intracellular parasites lacking LMF1 fail to form the lasso-shaped mitochondrion. These mutant parasites also present defects in mitochondrial division and a proliferation phenotype, showing that the correct positioning of the mitochondrion is important for intracellular growth. As LMF1 has no critical domains, we are interested in understanding the proteins that helps LMF1 in the process of tethering the parasite's mitochondrion to the parasite's periphery. Using a yeast two-hybrid screen we were able to narrow down LMF1 putative interactors by generating dual tagged cell lines for confirmatory co-immunoprecipitation assays from parasites. Among these proteins we found IMC10 (TGGT1_230210) as one of the LMF1 interactors. IMC10 is a component of the inner membrane complex, an organelle that lies underneath the plasma membrane and it is a possible link of the mitochondrion with the parasite's pellicle. Using a tetracycline-inducible system we were able to knockdown IMC10 and by immunofluorescence microscopy we observed that, without IMC10, the parasite presents abnormal mitochondrial shape and distribution to the daughter cells during division. Given that, we are showing for the first time that LMF1 mediates the contacts with the mitochondrion and parasite pellicle by protein-protein interaction with IMC10. **Supported by:**NIH/NIAID Project # 5R01AI149766-02
Keywords: Protein-protein interactions.tethering complex.mitochondrion

PV-027 - **Generation of *Trypanosoma brucei* cell lines expressing *Trypanosoma cruzi*'s histidine degradation pathway**

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Trypanosoma cruzi is the etiologic agent of Chagas disease, which is transmitted by triatomine insects. As the life cycle of *T. cruzi* needs an invertebrate vector and a vertebrate host to completion, this parasite is highly adapted to different environments. For example, when glucose is limited in the insect vector's gut, *T. cruzi* can degrade amino acids producing ammonia. Histidine (His) is present in high concentrations in the insect's gut and the parasite can use it to generate ATP through its conversion in glutamate. The coding sequences for the first two enzymes and putative coding sequences for the last two enzymes are present in *T. cruzi*'s genome. However, they are absent in *Trypanosoma brucei*, which makes this parasite a useful model to investigate the biological role of this pathway. We transfected *T. brucei* procyclic form with two plasmids that allow constitutive expression of histidine ammonia-lyase (*Tc*HAL) and urocanate hydratase (*Tc*UH). We confirmed the presence of the enzymes' coding sequences by polymerase chain reaction (PCR), the expression of the enzymes by Western blotting and that the enzymes were expressed in their active forms by enzymatic activity assays. Simultaneous expression of TcHAL and TcUH in procyclic *T. brucei* affects the parasite proliferation rate while their individual expression does not affect parasite proliferation. Also, parasites expressing TcHAL and TcUH simultaneously are not able to use His to maintain cell viability during severe nutritional stress and the presence of urocanate, the product of *Tc*HAL, drastically reduces cell viability when compared to the negative control. Analysis of the complete His degradation pathway in *T. brucei* can contribute to elucidate which evolutionary advantages it brings to *T. cruzi´*s life cycle and provide valuable insights to understanding the parasite metabolism.
Supported by:CAPES **Keywords:**cruzi, brucei, histidine metabolism

PV-028 - **Is the transmembrane glycoprotein p67 a functional lysosomal hydrolase in *Trypanosoma brucei*?**

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p67 is a type I transmembrane glycoprotein of the terminal lysosome of African trypanosomes. p67 is synthetized as initial gp100 glycoprotein that in the lysosome is cleavage to N-terminal (gp32) and C-terminal (gp42) subunits that remain noncovalently associated. Cleavage is blocked by either inhibition or RNAi silencing of the lysosomal protease TbCatL. Other than death, the only phenotype of p67 knockdown is a grossly enlarged lysosome. p67's exact function is unknown, but recent bioinformatic surveys indicate it is the founding member of a phospholipase B-like (PLBL) subgroup of the NTN hydrolase superfamily. PLBLs are found in Eukaryota and p67 models precisely on the crystal structure of the murine orthologue. NTNs autoactivate by internal cleavage at a nucleophilic residue to generate alpha (gp32) /beta (gp42) subunits, which remain non-covalently associated. The N-terminal residue of the beta subunit (Cys, Ser or Thr) then serves as the catalytic nucleophile for hydrolysis reactions. The conserved alpha/ beta cleavage site in p67 is C241/S242. Using a combination of RNAi^{R} p67 constructs in p67 RNAi cell line background it is observed that wildtype (CS) RNAi^{R} p67, but not mutant (AA), is able to rescue cell growth under p67 RNAi induction. The AA mutant is still cleaved in a TbCatL-dependent manner to generate gp32/gp42 subunits, but the gp42 polypeptide is slightly longer at the N-terminus. Thus, p67 activation involves upstream trans-proteolytic cleavage within the gp32/gp42 linker region followed by downstream auto-activation at the CS junction. The precise hydrolytic activity of the PLBL family is under debate. However, preliminary 'omic' data in knockdown cells suggest p67 has amidase activity, either ceramidase or peptidase. Multiple p67 orthologues are found in other parasitic protozoa including *Entameoba*, *Giardia* and *Trichomonas*. Thus it is likely that these hydrolases function as virulence factors in many parasitic diseases. **Supported by:**NIH/NIAID (US) AND UB JSMB (US) **Keywords:**transmembrane glycoprotein p67.phospholipase B-like.NTN hydrolase superfamily

PV-029 - **Delineation and titulation of synthetic peptide as a target for diagnosis of Trypanosoma evansi.**

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Trypanosoma evansi has an essential internal membrane system for removing and adding membrane proteins. In parallel, the recycling system ensures that proteins, including Surface Variant Glycoprotein (VSG) and Surface Invariant Glycoprotein (ISG), return to the surface to maintain the coating. The main evasion strategy of the immune system is antigenic variation, where VSGs undergo frequent remodeling. Unlike these, the ISGs do not show this variation, making them potential targets for diagnosis. The objective was to evaluate the production of polyclonal antibodies against the synthetic peptide of ISG, testing its use as a target for specific diagnostic tests for *T. evansi*. For this, the coupling reaction of KLH to the synthetic peptide was performed. The peptide/KLH conjugate was used in the production of polyclonal anti-peptide antibodies. Two Wistar rats (*Rattus norvegicus*) were used. The immunization protocol involved 5 intraperitoneal injections, FAC and FAI with an interval of 21 days. At the end, whole blood was collected to obtain serum. Antibody detection was performed by indirect immunofluorescence test. Positive control serum (*T. evansi* in formaldehyde 4%) and negative control serum (PBS) were diluted 1:80. The anti-peptide serum was tested at dilutions from 1:40 to 1:1280. The reading of the slides was performed under an epi-fluorescence microscope. The fluorescence emitted by the slide with fixed antigen was identified in titrations from 1:40 to 1:320. Antibody production depends on the animal's immune response. Despite decades of trying to create a comparable protein detection method, no other system (phage display, aptamers, etc.) has achieved the specificity of antibodies created from an animal's immune system. The results suggest that further studies should be carried out to confirm the immunogenicity of the peptide. **Keywords:**epitope, ISG, Surra

PV-030 - **MOLECULAR SCREENING OF LEISHMANIINAE CLINICAL ISOLATES OBTAINED FROM HUMAN CASES OF VISCERAL LEISHMANIASIS TO PERFORM COMPARATIVE GENOMICS**

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Visceral Leishmaniasis (VL) is a vector-borne dise[illegible]. Brazil. It is a neglected disease, largely associated with lack [illegible]oreover, occurrence of species other than *Leishmania* (e.g. m[illegible]*eptomonas*) in cases of leishmaniasis has been reported be[illegible].y that such parasites may be involved with disease develop[illegible]al isolates obtained from patients diagnosed with VL in Ser[illegible]o be analyzed through Whole-Genome Sequencing (WGS) i[illegible]s had been previously screened by means of molecular char[illegible]quencing, morphological analysis and molecular phylogene[illegible]s displayed morphological and phylogenetic differences when[illegible]nstead, they were phylogenetically more closely related to the [illegible]on-infective to humans, suggesting the presence of still unidentified [illegible]mples were further evaluated and screened to meet criteria required for V[illegible]s polyclonal samples from clinical isolates as well as 28 obtained through si[illegible]inical isolate cultures. Forty two samples were genotyped as *Crithidia*-like, while 9 wer[illegible]belonging to genus *Leishmania*. DNA extraction was performed and DNA integrity was asse[illegible]y agarose gel electrophoresis. Lastly, quantification with Qubit fluorometer was employed. Following next-generation sequencing of the samples, results shall be further analyzed through comparative genomics as to characterize this new, potentially emerging *Crithidia-like* parasite. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 2016/20258-0, scholarship 2019/24764-6)**Keywords:**visceral leishmaniasis, next-generation sequencing, crithidia-like

PV-031 - **Distribution of active chromatin profile in *Trypanosoma cruzi* epimastigotes and metacyclics forms using FAIRE-seq**

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Trypanosomes' protein-coding genes lacks dedicated promoters and are organized into co-directional gene clusters with unrelated function, resulting in a polycistronic gene transcription unit (PTU). Transcription start and termination sites are believed to be in the intergenic regions between two divergent (dSSR) or convergent (cSSR) PTU, respectively, presenting several epigenetic alterations that regulates transcription. Ribosome profile analysis of *Trypanosoma cruzi* showed more genes being translated in epimastigotes (EPI) than in metacyclics (META). Furthermore, alterations in nucleus and chromatin structures are observed during its life cycle, with EPI showing higher amount of euchromatin. To explore the chromatin changes along *T. cruzi* cycle, FAIRE-seq (Formaldehyde-Assisted Isolation of Regulatory Elements) assays were performed. Reads were preprocessed and mapped to Dm28c strain genome using GATK Best Practices *pipeline*. Alignment ambiguities were removed using Q10 score. Custom scripts and UTRme were used to obtain additional genomic annotation; deepTools 3.3.0 was used for sample normalization and make plots. In general, EPI forms presented higher euchromatin content (expressed as RPGC) than META forms. Combining the FAIRE data with gene expression level (TPM) revealed a positive correlation. At dSSR and cSSR, FAIRE enrichment was observed on both life forms, being higher compared to the whole PTU, and significantly augmented on EPI. Noteworthy, the multigenic families MASP, GP63, mucin and trans-sialidase exhibited distinct FAIRE landscapes in contrast to non-multifamily genes. Additionally, the open chromatin profile marks the difference between core and disruptive genomic compartments. Interestingly, the loci of tRNA genes are greatly enriched by active chromatin mainly at EPI forms. FAIRE data highlights important structural chromatin changes during *T. cruzi* differentiation cycle, reflecting the overall transcriptional state of each analyzed life form. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo; 2019/19690-3 **Keywords:**Epigenetics, Bioinformatics, tRNA

PV-032- **Effect of ionizing radiation exposure in the cell cycle, morphology and growth of *Trypanosoma cruzi***

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Ionizing radiation induces DNA damage and chromosomal rearrangements in T. cruzi. To investigate the gamma radiation effects on the organization of T. cruzi genome, epimastigotes of CL Brener were exposed to doses of 100-500 Gy of gamma-radiation and then cloned by serial dilution. Eighteen clones were recovered and analyzed for growth in culture and the karyotype by pulsed-field gel electrophoresis. Clone (F9) was isolated from a cell population exposed to a radiation dose of 500 Gy and showed changes in morphology and growth, affecting the clone expansion and culture maintenance. In addition to slow growth, clone F9 showed morphological changes in cell body and flagellum which seems to be thinner and shorter, affecting the motility of parasite. Using Giemsa staining and anti-flagellin antibody, we observed a partial detachment of the flagellum from the cell body and presence of free flagella in the medium. Transmission electron microscopy analysis revealed that in both clone F9 and wild-type (WT) parasites, the flagellum emerges from the flagellar pocket and the axoneme structure remains unaltered. However, the flagellum of clone F9 is detached from cell body after emerging from the flagellar pocket that affected the mobility of parasite. In WT epimastigotes the kinetoplast is located between the nucleus and the flagellum emergence site, while in clone F9 the kinetoplast and flagellar pocket are laterally arranged in relation to the nucleus. The parasite's nucleus-kinetoplast disposition could explain the less wider cell body of F9 cells. To understand the growth deficit, we analyzed the cell cycle of cells arrested in the G1 phase using hydroxyurea-induced cell-cycle synchronization. The G1-phase duration was significantly reduced in F9 cells. Taken together, our results suggest the presence of mutations in the protein genes of flagellum attachment zone (FAZ). This region controls cell morphology by regulating cell length, organelle position and cell division. **Supported by:**FAPESP. N 2017/23312-9 **Keywords:**Ionizing radiation, flagellum, morphology

PV-033 - **Standardization of cutting-edge techniques for the study of T. cruzi replication dynamics**

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DNA replication is a process that ensures accurate DNA duplication and timely genetic material inheritance. In the case of *Trypanosoma cruzi*, Chagas's disease etiological agent, two very distinct life forms are present in each host, presenting a different replication and infection pattern, indicating that DNA replication dynamics play a significant role in the infection evolution. In order to better understand DNA replication in this parasite, two different techniques are under standardization in the laboratory: chromatin immunoprecipitation followed by Illumina sequencing (ChIP-seq), which allows identifying DNA sites bound by proteins of interest, and DNAscent, that is based on BrdU incorporation into *T. cruzi*'s DNA followed by Nanopore sequencing, allowing the detection on BrdU enriched regions in the genome and then the identification of replication fork direction and potential origins of replication sites, where DNA replication is initiated. Among the components of the pre-replication machinery in this parasite are Orc1Cdc6 and Orc1b, and, aiming to better understand the DNA replication event, these proteins were individually tagged, using a 3xTY1 tag in Cl Brener cell lineages, and submitted to a ChIP-seq experiment. The protein-DNA binding intensity profile of the two proteins was generated for triplicates using two different *T. cruzi* genomes for comparison (Cl Brener and TCC) and is currently under analysis. For the DNAscent technique, synchronized and non-synchronized cultures of Cl Brener were incorporated with BrdU during early-S phase and the extracted DNA was submitted to single cell sequencing, using the Oxford Nanopore Minion sequencer. BrdU enriched regions were detected and are now under evaluation. After all protein binding regions and BrdU enriched regions are processed, it will be possible to validate the establishment of both techniques and better understand the replication dynamics in *T. cruzi*. **Supported by:**Projetos FAPESP 17/07693-2, 20/00694-6, 13/07467-1 **Keywords:**T. cruzi,ChIP-seq,DNAscent

PV-034 - **Histone H2B.V demarcates strategic regions in the Trypanosoma cruzi genome**

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Trypanosoma cruzi is a flagellate *Trypanosomatid* that causes Chagas disease. It transits between its vertebrate and invertebrate hosts, constantly alternating its replicative and infective forms. Among life forms, studies on global transcription showed different transcript levels, which is accompanied by changes in chromatin conformation. Recent studies have elucidated epigenetic components associated with gene regulation present in the chromatin of trypanosomatids that change throughout the life and cell cycle of these organisms. Our group identified, by quantitative proteomics, that several proteins associated with chromatin (including a variant of histone H2B- H2B.V) are differently abundant in *T. cruzi* life forms. These results, however, neither allowed to specifically explore the RNA Polymerase II location in the genome, nor its relationship with histone variants deposition. We performed H2BV and H3 ChIP-seq and observed that 91.3% of H2B.V-enriched peaks were located at dSSR, and only 8.7% were found at non-SSR in the CL Brener assembly; however, not all dSSRs contained H2B.V enrichment. These latter usually flank monocistrons and small polycistrons. Among the H2B.V peaks located at non-SSRs, those were found mainly close to tDNAs and between regions of conserved (mostly protein-coding genes) and disrupted (non-syntenic regions, mostly virulence factors) genome compartments. Currently, we obtained parasites expressing RPB9 (an RNA Pol II subunit) and H4.V tagged, by CRISPR Cas9, to investigate their genomic location by ChIP-seq assays, in order to map the RNA Pol II start and ending regions. These results will be compared to global run-on assays to elucidate the T. cruzi transcription start and terminal regions. **Supported by:**CAPES e FAPESP (17/06104-3 ; 2018/15553-9) **Keywords:**Trypanosoma cruzi, Variant histone, ChIP-seq

PV-035 - **Using CRISPR-dCas9 system as tool for epigenetic studies in *Trypanosoma cruzi***

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In the CRISPR-Cas9 system, the Cas9 is guided by an sgRNA to the target DNA, where it is able to generate a double strand break. The dead Cas9 (dCas9) can bind DNA but is unable to cleave it. So, dCas9 has been used as tool in epigenetic studies. Some aspects of gene expression are unclear in *Trypanosoma cruzi*, which causes Chagas disease. To improve the knowledge about proteins associated with specific chromatin regions in *T. cruzi*, this work aimed to generate a parasite expressing dCas9. To do that, pLEW13-Cas9, a vector carrying Cas9 gene, was used as substrate in mutagenesis reactions generating a vector carrying dCas9 gene (pLEW13-dCas9). The pLEW13-dCas9 was confirmed by Sanger sequencing and used to transfect wild type (WT) epimastigotes of the CL Brener strain. After six weeks of selection, the DNA of WT and transfected ($dCas9^{FLAG}$) parasites was extracted and used to amplify the dCas9 sequence. It showed that only transfected culture amplifies the entire dCas9 gene. To verify if dCas9 was being expressed in the $dCas9^{FLAG}$ parasites, a Western blot was performed. A band for dCas9 was observed only for $dCas9^{FLAG}$ parasites. Since these results suggested that transfected culture was expressing dCas9, these parasites were serial diluted in order to obtain clones. After 20 days, 11 clones were selected and tested by PCR and Western blot. As expected, all clones were carrying dCas9 gene and expressing the protein. Hereafter, it was analyzed the growth profile and cell cycle progression of $dCas9^{FLAG}$ parasites. For most of the clones, the presence of dCas9 gene did not compromise the basic functions of the cell, once WT and $dCas9^{FLAG}$ parasites have the same growth rate and cell cycle progression under normal culture conditions. Ongoing trials aim to use $dCas9^{FLAG}$ parasites in locus-specific chromatin immunoprecipitation which may contribute for a better understanding of factors involved on the establishment and maintenance of gene silencing and activation in *T. cruzi*. **Supported by:**FAPESP 2019/19834-5 e FAPESP 2018/15553-9 **Keywords:**dCas9, Trypanosoma cruzi, epigenetic

PV-036 - **Investigating the transcription and regulatory machineries associated to tRNA genes (tDNAs) in *Trypanosoma cruzi***

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The machinery involved with tDNA transcription in eukaryotes is dependent of the complexes TFIIIC, TFIIIB and RNA polymerase III (RNAP III). In *Saccharomyces cerevisiae*, six proteins form the TFIIIC complex, namely TFC1, TFC4, TFC7, TFC3, TFC6 and TFC8, while the proteins constituents of the TFIIIB complex are BRF1, BDP1 and TBP. The RNAP III contains 17 subunits, namely RPC1, RPC2, RPC40, RPC19, RPB6, RPB5, RPB8, RPB10, RPB12, RPC11, RPC17, RPC25, RPC82, RPC53, RPC37, RPC34, and RPC31. The TFIIIC complex and RPC17/RPC31 subunits of RNAP III have not been identified in *T. cruzi* and little is known about the expression regulation of tDNA in these parasites. Recently, our group observed, by FAIRE-seq technique, that the regions of active chromatin (euchromatin) associated with these genes are more enriched in epimastigote (EPI) than in metacyclic trypomastigote (MT) forms. To better explore these findings and understand how the tDNA transcription occurs in *T. cruzi,* we identified the proteins associated with tDNAs expression as well as evaluated their expression levels at EPI and MT forms. Thus, different in silico approaches (BLAST, DELTA-BLAST and HMMER) were used to identify protein homologs of the tDNA machinery and the translatome dataset on TritrypDB was used to evaluate their expression levels in parasite life cycle. We found all 17 subunits of RNAP III, including RPC17 and RPC31, which are annotated as hypothetical protein. Only two (TFC1 and TFC4) out of the six proteins from the TFIIIC complex were identified suggesting that either this complex works without some members or that it is composed of evolutionary distant proteins not yet identified. All components of TFIIIC, TFIIIB and RNAP III identified are up regulated in EPI than MT. Furthermore, we generated parasites expressing tagged TFC1 by CRISPR-Cas9 to study the proteome around tDNAs using immunoprecipitation assays to better understand the tDNA transcription and their regulatory process in *T. cruzi*. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo - Processo nº 20/02708-4 **Keywords:**Trypanossoma cruzi, tDNA, transcription

PV-037 - **Evaluation of Trypanosoma cruzi infection in the central nervous system by qPCR and bioluminescent imaging**

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In Chagas disease, cardiac and digestive disorders are the most investigated, however, the damage to the central nervous system and the migration of *T. cruzi* through the brain are less explored. The aim of this study was to evaluate in BALB/c mice, the progression and distribution of *T. cruzi* infection through different regions of the brain. Mice were inoculated with 10^5 trypomastigotes of Dm28c-wild type (WT).The anterior and posterior region of the encephalic cortex, cerebellum and encephalic bulb were collected at the 3, 5, 10 and 15 days after infection (DPI). To quantify *T. cruzi* in the tissues, genomic DNA was purified to perform the quantitative PCR (qPCR), using primers directed to the satellite DNA. Parasites were not detected in most tissues at the 3 DPI; at the 5 DPI, the average of all the brain regions displayed 1.1 equivalent parasites (par. eq.)/µg DNA; at the 10th and 15th DPI, displayed 207.7 and 8910.6 par. eq/ µg DNA, respectively. The most infected regions were: (i) posterior cortex, (ii) frontal cortex, (iii) encephalic bulb and (iv) cerebellum. To perform the *in vivo* bioluminescent imaging, the infection rate of Dm28c WT and Dm28c expressing luciferase (Dm28c-Luc) was evaluated in LLC-MK2 infected cells. The cells were fixed and stained with Giemsa at 24, 48 and 72 hours after infection. The infection rate of the Dm28c-Luc and Dm28c-WT were similar. Thus, mice inoculated with 10^6 trypomastigotes of Dm28c-Luc were evaluated at 1, 7 or 14 DPI by the *in vivo* imaging system. Intense bioluminescent/infection foci was observed in the abdomen at the 7 DPI. Small foci were observed in the head at the 14 DPI. *Ex vivo* evaluation showed infection/bioluminescence in 60% of the brains, at the 14 DPI. The qPCR data suggest that *T. cruzi* was able to disrupt the blood-brain barrier early in the infection, increasing the parasite load in the brain at the 15 DPI, corroborated by the bioluminescent imaging. **Supported by:**PIBIC-Fiocruz-CNPq **Keywords:**Trypanosoma cruzi, Brain, mice

PV-038 - ***Cytochrome b* phylogeny suggests the occurrence of a new species of *Triatoma brasiliensis* complex (Hemiptera, Reduviidae, Triatominae) in State of Rio Grande do Norte, Brazil.**

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Recently, in the state of Rio Grande do Norte (RN), specimens of triatomines of the genus *Triatoma* were captured in the wild environment cohabitating with *Triatoma brasiliensis* in rocky outcrops. These samples presented morphological characteristics similar to *T. brasiliensis* and *T. petrocchiae*. In this work, we evaluated the phylogenetic relationships of this n. sp. with the members of the *T. brasiliensis* species complex by genetic parameters. We also comparatively analyzed sequences of the mitochondrial *cytochrome b* gene from *T. brasiliensis* and specimens of *Triatoma* n. sp with sequences from members of *T. brasiliensis* species complex, in addition to some taxa of other groups deposited in GenBank. Phylogenetic reconstruction was inferred by the maximum likelihood method, targeting a fragment of cytochrome b gene (432 pb). Node support was estimated using 1,000 bootstrap pseudo-replicates (PR-B). The phylogenetic reconstruction showed branches supported by PR-B values >70. We observed a well-supported clade (PR-B = 89) referring to species of *T. brasiliensis* species complex, including *Triatoma* n. sp. as a member of this monophyletic group (PR-B = 100). All six specimens of *Triatoma* n. sp. were grouped into a single clade as a sister species of *T. petrocchiae* (PR-B = 77). The values of genetic distance between *T. petrocchiae* and *Triatoma* n. sp were estimated by the Kimura method 2-Parameter (K2-P), having been higher (all K2-P > 0.15) than the ones for well-recognized species, such as *R. robustus* and *R barretti* (all < 0.11). Our results demonstrate, therefore, the emergence of an independent evolutionary unit within the *T. brasiliensis* species complex. **Supported by:** MCTI/CNPq/MS-SCTIE-Decit 404056/2012- 1; MCTI/CNPq/Universal 475572/2013-0; CAPES (Scholarships) **Keywords:**Triatoma brasiliensis, Triatoma petrocchiae, Cytochrome b

PV-039 - **Disruption of active trans-sialidase genes impairs the egress from mammalian host cells and generates highly attenuated *Trypanosoma cruzi* parasites**

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Trans-sialidases (TS) are unusual enzymes present on the surface of *Trypanosoma cruzi*, the causative agent of Chagas disease. Encoded by the largest gene family in the *T. cruzi* genome, only few members of the TS family have catalytic activity. Active trans-sialidases (aTS) are responsible for transferring sialic acid from host glycoconjugates to mucins, also present on the parasite surface. The existence of several copies of TS genes has impaired the use of reverse genetics to study this highly polymorphic gene family. Using CRISPR-Cas9, we generated aTS knockout cell lines displaying undetectable levels of TS activity as shown by sialylation assays and labelling with antibodies that recognize sialic acid-containing mucins. In vitro infection assays showed that disruption of aTS genes does not affect the parasite capacity to invade cells or to escape from the parasitophorous vacuole but resulted in impaired differentiation of amastigotes into trypomastigotes and parasite egress from the cell. When inoculated in mice, aTS mutants were unable to establish infection even in the highly susceptible IFN-γ knockout mice. Mice immunized with aTS mutants were fully protected against a challenge infection with the virulent *T. cruzi* Y strain. Altogether, our results confirmed the role of aTS as a *T. cruzi* virulence factor and indicated that aTS play a major role during the late stages of intracellular development and parasite egress. Notably, mutants lacking TS activity are completely avirulent in animal models of infection and may be used as a live attenuated vaccine against Chagas disease. **Supported by:**CNPQ, FAPEMIG, INCTV **Keywords:**Trypanosoma cruzi, Trans-sialidase, CRISPR-Cas9

PV-040 - **Comparison of lipid droplet formation and eicosanoid metabolism in Brazilian *Leishmania* spp.**

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Introduction: Lipid droplets (LD) are cytoplasmic organelles responsible for the storage of neutral lipids and for the metabolism of eicosanoids in animal eukaryotic cells. Recent data have shown that LDs can be markers of virulence of intracellular pathogens such as ***Leishmania infantum***. In addition, LDs have been described as organelles responsible for the production of prostaglandin F2α in ***L. infantum,*** a molecule capable of modulating the immune response of macrophages during the parasite-host interaction. Knowledge about the lipid mediators produced by parasites is still quite limited and new approaches are needed to identify which mediators are produced and the effects of these mediators on the regulation of cellular processes. Methods: Herein, we used optical microscopy techniques to evaluate the effect of polyunsaturated fatty acids on the formation of LDs and lipid mediators on the production of brazilian ***Leishmania*** species. In addition, we study in silico and in vitro enzymes involved in the eicosanoid metabolism in those parasites. Results: We found that the LDs of ***L. infantum*** and ***L. braziliensis*** are susceptible to change in the presence of polyunsaturated fatty acids, with the clear effect being that induced by arachidonic acid (AA). However, the presence of polyunsaturated fatty acids did not affect the formation of LDs in ***L. amazonensis***. ***In silico*** analysis indicates COX-2 and PGFS from parasites are very similar according clinical form-related. Regarding the production of lipid mediators, we evaluated the presence of 41 different mediators in parasites treated with AA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in cell extracts and in the parasite supernatant by LC-MS. We identified the presence of 14 bioactive lipids, especially when the parasites were stimulated with AA. Conclusions: Altogether, our data opens new perspectives for understanding ***Leishmania*** biology and for the development of drugs with antiparasitic activity. **Supported by:**Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB). BOL0171/2019 **Keywords:**Lipid droplet, Parasites, Lipid metabolism

PV-041 - **Chasing for chromatin-associated proteins and histone PTMs patterns associated to cell cycle stages in *Trypanosoma cruzi***

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Epigenetic marks such as histone post-translational modifications (PTMs) can alter the chromatin compaction and interfere in the gene expression, DNA replication and repair and cell cycle control. For trypanosomatids, these modifications are associated to the transcription start and ending regions as well as associated to cell cycle phases and DNA damage. The insertion or removal of histones PTMs happens through chromatin modifying enzymes, that depends on cell metabolic products, such as S-adenosyl methionine (SAM) and Acetyl-CoA. Therefore, metabolic alterations and, consequently, the availability of these products that act as substrates and/or cofactors of these enzymes, may be related with the chromatin modulation structure and function through changes in histone PTMs patterns. The aim of the present study is to evaluate quantitatively the global pattern of histone PTMs and the expression of nuclear proteins in different cell cycle phases of *T. cruzi*. Epimastigote cultures were cell cycle synchronized with hydroxyurea (HU) to obtain parasites in the G1/S, S and G2/M transition. Histones from parasites at different cell cycle stages were extracted, propionylated and processed for LC-MS/MS analysis using data independent acquisition (DIA). Our findings showed that, for the 0 h time (G1), there was an increase in histone PTMs for the H4 N-terminal peptides $^{16}QK_{me1}KILR^{21}$, $^{16}QK_{me1}K_{me1}ILR^{21}$ and $^{1}AKGKKSGEAKGTQK_{ac}R^{15}$, compared to unsynchronized parasites. Further analyzes will be realized to evaluate global changes in histone PTMs along the cell cycle. In addition, we are currently evaluating proteomic changes on parasite nuclei along cell cycle. We aim to correlate the levels of metabolic enzymes, especially those producing substrates such as SAM and acetyl-CoA, with the levels of histone PTMs throughout the cell cycle. **Supported by:**Fapesp - 2018/14432-3 **Keywords:**Trypanosoma cruzi, Epigenetic, Cell cycle

PV-042 - **Protein partners and post-translational modifications directly associated with two *Leishmania* homologues of eIF2γ, the core subunit of the translation initiation factor eIF2.**

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The regulation of gene expression in trypanosomatids is mostly mediated by post-transcriptional events, with many of those believed to be involved in the initiation stage of mRNA translation. In eukaryotes, this requires the activity of the eIF2 translation initiation complex. eIF2 functions by mediating the recruitment of the small ribosomal subunit bound to Met-tRNAi. eIF2 is formed by the eIF2α, eIF2β, and eIF2γ subunits, with eIF2γ being the core subunit. Herein, the discovery of two eIF2γ homologues in *Leishmania* sp. is under investigation to define their likely functional distinctions. First, we accessed the expression profile by Western blotting with polyclonal serum able to recognize both homologues. eIF2γ-1, the more conserved in sequence with the mammal counterpart, is approximately 30-fold more abundant than eIF2γ-2 in *L. infantum* promastigotes cells. eIF2γ-1 was also shown to be represented by multiple isoforms in exponential growth phase suggesting changes by post-translational modifications. Next, we performed immunoprecipitation assays, followed mass-spectrometry, using the two ectopically expressed homologues, tagged with an HA epitope. The eIF2γ-1 was found to co-precipitate with the other eIF2 subunits as well as with the EIF2B complex, a classical eIF2 partner. As for eIF2γ-2, neither eIF2β nor the subunits of the EIF2B subunits were found to be efficiently co-precipitated. Other known eIF2 partners, such as eIF5 and D123, were found to co-precipitate with both homologues. They also co-precipitated with protein kinases, including the cell-cycle regulated CRK3. Later, Site-directed mutagenesis studies were performed using the HA-tagged protein, aiming to identify possible phosphorylation sites. Our results are consistent with one or both eIF2γ homologues being involved in novel regulatory events which might be linked with the post translations modifications encountered and, indeed with the newly identified interactions with protein kinases. **Supported by:**FACEPE IBPG-1437-2.02/18 **Keywords:**Gene expression regulation, protein synthesis, eukaryotic Initiation Factor

PV-043 - **Analysis of nascent transcripts in Trypanosoma cruzi**

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Trypanosomatids have a genomic structure organized in polycistronic regions. Specific promoter for each gene have not been described thus, post-transcriptional mechanisms are mainly responsible for gene expression regulation. Nascent transcript could elucidate the real impact of transcription regulation on the expression levels of different CDS, between different polycistronic regions, as well as among transcripts from different RNA classes (rRNA, snoRNA, snRNA and mRNA) in *T. cruzi*. To evaluate that, we obtained nascent transcripts using a Global Run-on (GRO) assay by Br-UTP incorporation in epimastigotes. Transcripts were captured by immunoprecipitation and sequenced using a kit that captures small RNAs by adding a polyA tail, in the Illumina NextSeq assuming 1 x 75 bp. Because most small RNAs ware less than 75 bp, there was a dip in quality towards the end of the reads. Reads processing included: trimming for low quality; universal Illumina adapters and polyA tail removal as well as the first four nucleotides from 5' and short reads (<25 nt). Reads were mapped to *T. cruzi* Dm28c indexed genome (release 46) previously masked for the rRNAs, using Bowtie2. We evaluated transcription at specific genomic regions (CDS, polycistron, tRNAs, snoRNAs and snRNAs) by performing analysis on deepTools and compared it in parallel with transcripts publicly available, *T. cruzi* RNA-seq datasets. Although GRO-seq replicates differ between each other regarding their expression profile, it differ from RNA-seq datasets too, mainly at 5' and 3' UTR from CDSs and at polycistronic start sites. GRO-seq data seems to have mRNA processing. To faithfully evaluate that, we are removing transcripts that harbor spliced leader sequence at their 5' UTR. In summary, we obtained a pipeline to process and analyze nascent transcripts, which allow us to conclude that our dataset is reliable. Future analysis will be carried out to compare among CDS and polycistronic regions, in TPM, compared to RNA-seq. **Supported by:**PROEX (Programa de Excelência Acadêmica) CAPES , 88887.569623/2020-00 **Keywords:**Gro-seq, Trypanosoma cruzi, Nascent Transcripts

PV-044 - **Freeze tag: generation of PF16 tagged and knocked out mutants in *Leishmania (Viannia) braziliensis* stably expressing Cas9 nuclease.**

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Since the genome of *Leishmania* was sequenced, in 2005, the use of gene editing techniques has increased, as part of the effort to understand the biology of these complex organisms. Loss-of-function studies using homologous recombination helped to elucidate the role of different proteins in *Leishmania*. This parasite has a very plastic genome in which re-arrangements and aneuploidy are commonly observed, making homologous recombination even more challenging. In 2017, Beneke et al, generated LeishGEdit, a PCR-based tool for knocking out and tagging lines using CRISPR/Cas9. In this system, a plasmid containing the T7 RNA polymerase and Cas9 coding sequences (pT007) is delivered to *Leishmania* for episomal expression or integration in the β-tubulin locus. *L. braziliensis*, the main causative agent of tegumentary leishmaniasis in Brazil, presents a sequence divergence at the β-tubulin locus which impedes the use of the same vector for integration of Cas9 in the genome. To overcome this limitation, the *L. major* β-tubulin sequences, present in the pT007, were replaced by a *L. (Viannia)* β-tubulin conserved sequence. The integration of this cassette in *L. braziliensis* M2903 genome and the expression of Cas9 and T7 in these parasites was successfully obtained. To test Cas9 activity paralyzed flagella protein 16 (PF16) was successfully knocked out in *L. braziliensis* resulting in a phenotype of immobility in these transfectants. The add-back of this gene was generated using an in-locus add-back strategy in which a cassette containing a C-terminal tagged PF16 coding sequence and a puromycin resistance gene was amplified from tagged mutants' genomic DNA and used to return this gene to the original locus. Phenotypic analysis revealed that add-back parasites recovered their swimming capacity. In conclusion, we successfully generated an *L. braziliensis* line stably expressing functional Cas9 and T7 proteins, a new tool for the CRISPR/Cas9-mediated genome editing in this specie. **Supported by:**Capes, CNPq e FAPESP (2016/23405-4, 2020/00087-2 e 2018/14398-0) **Keywords:**Leishmania Braziliensis, CRISPR/Cas9, Flagella

PV-045 - **A conserved Zinc Finger RNA binding protein is implicated in the control of differentiation of both *Trypanosoma cruzi* and *T. brucei***

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Control of gene expression in Trypanosomatids relies mainly on post-transcriptional mechanisms exerted by RNA binding proteins (RBPs) that control stability, translatability, and localization of the mRNA population within the cell. In *Trypanosoma cruzi,* RNA-Seq analysis comparing gene expression in different life cycle stages showed that the gene TcCLB.506739.99 (TcZC3H12), which encodes a RBP containing a zinc finger motif, is upregulated in epimastigotes in relation to trypomastigotes and amastigotes. Functional characterization using gene knockouts identified a role for this RBP in repressing the differentiation from replicative epimastigotes (the insect stage) to infectious metacyclic trypomastigotes. To further characterize the mechanism of gene expression control by TcZC3H12, yeast-2-hybrid and RNA immunoprecipitation experiments were done. Like other zinc finger proteins from *Trypanosoma*, TcZC3H12 interacts with the MKT1-PBP1 complex. Analysis showed that this RBP is bound to, for example, transcripts that can affect differentiation and multiple metabolic pathways. In *T. brucei*, previous studies with monomorphic parasites showed that the knockdown of ZC3H12 ortholog (Tb927.5.1570, TbZC3H12), did not impact parasite growth. To investigate a role for TbZC3H12 in differentiation, polymorphic parasites, that maintain differentiation capacity, were now used. CRISPR/Cas9 knockout in procyclics (PCs, the insect stage) reduced the parasite growth rate and increased social motility on semi-solid medium. Direct knockout in blood stream forms (BSF) was not possible, but RBP10 overexpression was able to promote differentiation in specific conditions, resulting in BSF that also showed a slower growth rate. To identify the targets of this RBP in *T. brucei*, RNA-Seq of knockout PCs and BSF, and RNA immunoprecipitation analysis of tagged TbZC3H12 are underway. **Supported by:**DAAD (German Academic Exchange Service), DFG (German Research Foundation) **Keywords:**Trypanosoma, RNA binding proteins, parasite differentiation

PV-046 - **Analysis of duplicated Ribosomal Proteins RPS16 and RPL13 in *Leishmania***

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In *Leishmania* parasites most genes encoding ribosomal proteins (RPs) are present as two or more copies in the genome. Some of the duplicated genes code for identical proteins, some for distinct, but all of them carry a divergent untranslated regions (UTRs). These differences at the UTRs or protein coding sequences (CDS) might confer loss or gain of function to the proteins. In this context, our aim was to study RPs S16 and L13 expressed from duplicated genes in *Leishmania major*. RPs S16 transcripts have divergent UTRs and identical CDSs, and L13 copies have, in addition to divergent UTRs, non-identical CDSs. We generated knockout transfectants (KO) of each one of the four genes and transfectants with tagged versions (*myc tag*) of each CDS using the CRISPR/Cas9 system for genome editing. Distinct levels of expression of each copy of S16 (RPS16_90 and RPS16_80) and L13 (RPL13_15 and RPL13_34) were observed; RPS16_90 and RPL13_34 protein copies were always lower than the counterpart. Moreover, the levels of expression of one of the S16 paralogs would go up when the second copy was knocked out, suggesting a paralog compensation to maintain the levels of the protein. Curiously, we failed to obtain RPL13_15 KO when RPL13_34 were *myc* tagged, in both N and C terminus, suggesting that the tag affects RPL13_34 function. Furthermore, KO transfectants were subjected to nutrient deprivation conditions and a recovery period in fresh medium. Cell viability analysis revealed that KO parasites for all the four genes showed increased cell viability after the nutritional stress and recovery period. Immunofluorescence analysis, using the four *myc* tagged transfectants, revealed a similar distribution of the protein throughout the cytoplasm. We are currently analyzing the subcellular distribution of these proteins after stress. **Supported by:**FAPESP (2019/05257-6 and 2018/14398-0) **Keywords:**Leishmania, Ribosomal Protein, Extra-ribosomal function

PV-047 - **Distribution of *Trypanosoma cruzi* and its vectors in a semi-arid area of northeastern Brazil**

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Trypanosoma cruzi is genetically divided into six discrete typing units (DTUs), TcI – TcVI. In the state of Rio Grande do Norte (RN), Brazil, TcI, TcII and TcIII have been found in several vertebrate reservoir and triatomine species. However, this occurrence and distribution within vectors may be underestimated in this area. Therefore, we investigated *T. cruzi* DTUs associated with triatomines and identified bloodmeal sources, in anthropic and sylvatic environments in the state of RN. The genotyping of *T. cruzi* isolates was performed using the mitochondrial cytochrome c oxidase subunit 2 gene, the D7 region of the 24Sα rDNA and the spliced leader intergenic region. The bloodmeal sources of triatomines were identified amplifying the 12S rRNA locus. Five triatomine species were captured, and the most frequent was *Triatoma brasiliensis* (84.3%; 916/1086), which was found in 16 of the 23 municipalities surveyed, and infested all types of environment investigated. The TcI was found in all mesoregions surveyed in 51.5% (17/33) of the culture-positive samples. In contrast, TcII (9.1%; 3/33) was detected in the Central mesoregion, while TcIII (27.3%; 9/33) was found in all mesoregions. The geographic distribution using ecological niche distribution modelling revealed an overlap of different DTUs. *Triatoma brasiliensis* was found infected in all mesoregions and with all three *T. cruzi* DTUs, including mixed infections. The DNA of rodents was found in triatomines infected with either TcI or TcIII, while that of domestic animals and humans was associated with both single and mixed infections. Our findings demonstrate that different DTUs of *T. cruzi* are widely dispersed among triatomines in our study area. The association of *T. brasiliensis* with several different mammalian hosts, as well as overlapping areas with different DTUs, suggests that this triatomine species may have an important role as a vector in both anthropic and sylvatic environments. **Supported by:**CNPq, CAPES **Keywords:**Triatoma brasiliensis, Peridomicile, Blood-feeding