PV017 - **ENDOSYMBIOSIS IN TRYPANOSOMATIDS: IS THE GLYCOSOME DISTRIBUTION AND CONTENT INFLUENCED BY THE SYMBIOTIC BACTERIUM?**

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A hallmark of trypanosomatids is the compartmentalisation of the major part of the glycolytic pathway in glycosomes, peroxisome-like organelles. Glycosomes has been especially characterized in T. brucei and represent a selective advantage for the protist metabolic flexibility and to its efficiency of adaptation to different environmental conditions. Some non-pathogenic trypanosomatids, as Angomonas deanei, co-evolves in a mutualistic relationship with an endosymbiotic bacterium. Intense metabolic exchanges occur between the associated partners and the symbiont may influences the host cell metabolism. In this work, our main goals were to verify if the symbiont influences glycossome distribution and enzyme content by comparing the wild strain of A. deanei with the aposymbiotic (APO) one. Thus, we performed ultrastructural approaches, cell fractioning and proteomic analyses. Data obtained by transmission electron microscopy, as well as by electron tomography and FIB-SEM, showed that in symbiont-harboring protists the glycosomes are always surrounding the symbiont, while in APO cells this organelle is homogeneously distributed in the cytoplasm. Regarding cell fractioning, cells were physical disrupted with silicon carbide beads and submitted to Optiprep gradient following a protocol adapted from the one established to T. brucei. Unexpectedly, we obtained a better efficiency on cell fractioning after using the APO strain. This result may be related to the fact that symbionts maintains a close association with glycossomes, which interfere with the organelle isolation. Our proteomic analysis of the glycossome fraction obtained from APO protits revealed great quantities of typical glycosomal proteins as peroxin-2 and metabolic enzymes as hexokinase, glucose-6-phosphate isomerase and glycerol-3-phosphate dehydrogenase. Now we are improving our cell fractioning protocol in order to verify if the presence of symbiont influences the trypanosomatid glycossomal content. **Supported by:**CNPq
Keywords: Endosymbiosis; angomonas deanei; glycosome

PV018 - **HOW TRYPANOSOMA BRUCEI HANDLES WITH DNA STRAND BREAK PROMOTED BY PHLEOMYCIN AND IONIZING RADIATION.**

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Trypanosomatids are ancient eukaryotes used as a good model for investigating aspects associated with genomic maintenance. In response to genome instability the cells have evolved a complex set of surveillance systems and DNA repair mechanisms that may result in DNA damage repair, inhibition of the cell cycle progression and DNA replication. During its lifecycle Trypanosoma brucei switches between replicative and no replicative forms being the non replicative forms associated with the infection. Studying the switch between activated or blocked DNA replication is important to understand the molecular basis of this fundamental biological process in this pathogen. In this study, we show the response of T.brucei against DNA strand breaks generated by phleomycin and Ionizing Radiation treatments. We characterized the kinetics of DNA damage response after damage and analyzed the possible effects on the DNA replication dynamics. Using Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and replication protein A (RPA) profile assays we observed no DNA strand break two hour after treatment and a differential RPA positive profile at one hour compare to the control. These results lead us to propose that a mechanism for DNA repair is activated after damage and the process occur at two hours after damage. However, despite the process of repair occurred two hours after treatment, EdU incorporation was completely recovered at five hours. This DNA replication block was not observed by adding caffeine during the treatments, strongly suggesting that an intra S signaling pathway is activated inhibiting DNA replication acting probably in a dependent protein kinases activity manner. We are now investigating whether this signaling pathway is controlling DNA replication origin activation and/or replication fork elongation. We also intend to evaluate if the ATM protein (Ataxia Telangiectasia Mutated) and ATR (ATM-and Rad3-related) are involved in this signaling pathway.
Keywords: Check point; dna damage repair; dna replication arrest

PV019 - **THE HEXOSAMINE BIOSYNTHETIC PATHWAY: A WAY FOR *TRYPANOSOMA CRUZI* SURVIVAL**

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Trypanosoma cruzi uses amino acids and carbohydrates as carbon and energy sources. L-glutamine (Gln) is critical for the biosynthesis of UDP-GlcNAc, the glycosil donor for N and O-glycosilation. UDP-GlcNAc in trypanosomatids is obtained from Hexosamine Biosynthetic Pathway (HBP). There are three HBP key steps under investigation in this work: (i) the conversion of D-fructose-6-P into D-glucosamine-6-P, catalysed by GF6PA (EC 2.6.1.16); (ii) the conversion of D-glucosamine-6-P into N-Acetyl-D-glucosamine-6-P, catalysed by GNA (EC 2.3.1.4); (iii) the incorporation of extracellular D-glucosamine (GlcN) or N-Acetyl-D-glucosamine (GlcNAc) that can be phosphorylated by the *T. cruzi* hexokinase. Hence we investigated the role of free GlcN and GlcNAc as well as HBP inhibitors in different cellular processes of *T. cruzi* life cycle. Epimastigotes incubated only with GlcN or GlcNAc maintain their viability, proliferation, stimulate the O_2 consumption and ATP production, and support metacyclogenesis (GlcN = 24,6 ± 2 %; GlcNAc = 28,1 ± 0,7 %) to the same extend than TAU3AAG (28,7 ± 1,7%). In addition, we obtained the kinetic parameters for the recombinant GF6PA (K_m for F-6-P = 0,561 ± 0,13 mM ; K_m for Gln = 1,11 ± 0,12 mM; V_{max} for F-6-P= 1,35 ± 0,08 µmol glutamate.min^{-1}.mg^{-1}; V_{max} for Gln = 1,39 µmol ± 0,04 glutamate.min^{-1}.mg^{-1}). We identified 2 inhibitors for GF6PA (FDI and GAGMH) which reduced the epimastigote proliferation and triggered membrane damage, at high concentrations. Moreover these molecules diminished the trypomastigote bursting in CHO-K1 infections (EC_{50} FDI = 149,8 ± 8,4 µM; EC_{50} GAGMH = 29,4 ± 3,1 µM, non-toxic up to 500 µM in CHO-K_1). These results show that, in addition to their obvious participation in the formation of glycoconjugates, GlcN and GlcNAc participate of the cell bioenergetics, differentiation and resistance to metabolic stress. The HBP as a whole is also involved in the parasite proliferation and in the progression of the intracellular infection. **Supported by:**CAPES; FAPESP; CNPQ **Keywords:**Trypanosoma; glucosamine; hexose

PV020 - **WHAT ARE THE BASES OF DIFFERENTIAL SUSCEPTIBILITY TO MILTEFOSINE IN *L. (V.) BRAZILIENSIS* CLINICAL ISOLATES?**

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In 2014 approximately 21,000 cases of tegumentary leishmaniasis were notified in Brazil, where *L. (V.) braziliensis* is considered the most important etiological agent. The arsenal available for treatment is limited and challenging due to parenteral administration, toxicity, high cost, and emergence of resistance. Miltefosine is an oral drug, already in use for visceral leishmaniasis in India and cutaneous leishmaniasis in Colombia. Although it has been as effective as antimony in clinical trials, miltefosine is not yet approved for leishmaniasis treatment in Brazil, and little is known about the susceptibility of *L. (V.) Braziliensis* Brazilian isolates to this drug. We evaluated the susceptibility in vitro to miltefosine of 16 clinical isolates, which had no previous exposition to the drug and found half maximal concentrations that varied by factors of 6 to 15 for promastigotes and amastigotes, respectively. Trying to discover the basis for these differences, we evaluated the miltefosine uptake in promastigotes of these isolates using a fluorescent miltefosine analogue (Miltefosine-BODIPY) and found a positive correlation between susceptibility and uptake. Miltefosine transporter and its Ros3 subunit are described as the main miltefosine entry pathway in Leishmania. We investigated whether polymorphisms in the miltefosine transporter genes could explain the differential susceptibility phenotype. The miltefosine transporter genes (*LbrM 13.1380* and *LbrM 13.1400*) from three isolates and from *L. (V.)* ***Braziliensis*** M2903 reference strain were characterized, and no differences that could explain the differential susceptibility and uptake were found. Sequencing of the *Ros3* gene of these same isolates and efflux analysis are ongoing. Preliminary analysis of miltefosine efflux has not revealed differences in the rate of drug elimination between more and less susceptible isolates. **Supported by:**FAPESP and CNPq **Keywords:**Clinical isolates; susceptibility; miltefosine

PV021 - **LOPINAVIR INDUCES ALTERATION IN STEROL METABOLISM IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTES**

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The search for new treatments against leishmaniasis still remains an urgency due to high frequency of drug resistance registered in endemics areas, side effects of available chemotherapeutic compounds, and complications caused by coinfection with HIV. Lopinavir, a HIV protease inhibitor used clinically, has other pharmacological effects, such as its antileishmanial activity; however its mechanism of action on the parasite has not yet been fully explored. This study aimed to investigate the effects of lopinavir on the sterol metabolism in L. amazonensis promastigotes. Through transmission electron microscopy, we observed an increased amount of lipid inclusions after treatment of promastigotes with lopinavir at the IC50 (15 µM), which is suggestive of altered lipid metabolism. Subsequently, L. amazonensis promastigotes were cultured with increasing concentrations of lopinavir (7.5 µM, 12.5 µM, 15 µM, 25 µM and 30 µM) or 4 µM of miconazole (positive control) or in medium alone for 72 h. Their lipids (sterols) were extracted with methanol/chloroform/water (2:0.5:0.4 v/v) and the sterol composition was analyzed by thin-layer chromatography (TLC). Our results demonstrated that L. amazonensis promastigotes treated with lopinavir presented changes in sterol profile and the increasing inhibitor concentration promoted the accumulation of unidentified sterols. In addition to helping to understand the mode of action of lopinavir in Leishmania, our data contribute to a better understanding of the parasite metabolism. **Supported by:**CAPES-FIOCRUZ
Keywords: Lopinavir; leishmania amazonensis; lipids

PV022 - **IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF ALPHA- AND BETA-AMASTINS FROM *TRYPANOSOMA CRUZI***

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The amastins are known as differentially expressed genes during *T. cruzi* life cycle, and it has been shown in *T. cruzi* and *Leishmania* that they can be associated to invasion, parasite survival or multiplication in their hosts. *In silico* analysis allowed amastin classification in α, β, γ and δ subfamilies in Trypanosomatids, and identified 14 gene copies of amastins in *T. cruzi* (CL Brener clone), among β- and δ-amastins. In this work, we improved amastin characterization by studying β-amastins through gene deletion by homologous recombination and by identifying new amastin members in *T. cruzi*. After searching the unassigned contigs of CL Brener clone for amastins, sequences encoding new *T. cruzi* amastins were found. *In silico* analysis identified the presence of domains, transmembrane regions, conservation sequences and genomic organization. These analysis supports that presence of two α-amastins in *T. cruzi*, similarly to other Trypanosomatids. The expression of alpha-amastins fused to GFP showed distinct cellular distribution for both alpha-amastins, which is a behavior shared with β- and δ-amastins from *T. cruzi*. In parallel, we tried to knock out the β-amastins. Two constructs to delete both alleles were created and sequentially transfected in CL Brener. The integration of the first and the second cassette did not affect the parasite growth. PCR analysis showed the integration of both cassettes at the predicted locus, however, intact copies of the coding sequence of both amastins were detected. These results suggest that β-amastins are essential for epimastigote survival. Despite all efforts described here, the role of these proteins are still unknown and a lot more is needed to understand amastin function.
Supported by:CAPES, CNPQ e FUNDAÇÃO ARAUCÁRIA
Keywords: Amastins; knockout; alpha-amastin

PV023 - **METABOLISM OF SERINE IN TRYPANOSOMA CRUZI: MOLECULAR CHARACTERIZATION OF SERINE HYDROXYMETHYLTRANSFERASE**

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Chagas' disease is a neglected disorder caused by the protozoa parasite Trypanosoma cruzi affecting about 10 million people, mainly in the Americas. T. cruzi is able to use carbohydrates and amino acids as energy sources. In addition, amino acids are involved in other biological processes such as differentiation, resistance to several stress conditions, osmoregulation and are involved in the host-cell invasion. Besides its obvious role as protein components, serine is involved in lipids, nucleotides and other amino acids biosynthesis. For example, one of the relevant functions of serine in the cellular metabolism is the formation of C1 compounds for the biosynthesis of nucleotides. The use of serine for that purpose is initiated by Serine Hydroxymethyltransferase (SHMT). SHMT is a highly conserved PLP-dependent enzyme whose activity was detected in different strain of Leishmania spp and T. cruzi, but the role of SHMT and L-serine in the biology of parasite remains poorly explored. In this work we identified a putative gene encoding SHMT, and we determined that its product is an active SHMT with dual, cytoplasmic and mitochondrial localization. Quantitative PCR revealed that SHMT is less expressed in the intracellular stages of the parasite than in trypomastigotes or epimastigotes. However, Western blot analysis showed SHMT as being equally expressed along the life cycle of the parasite. We generated a single knockout cell line (sKO) by homologous recombination in which one allele of SHMT was replaced by the neomycin phosphotransferase gene. When the phenotype of these cells was analyzed, a slight decrease in growth rate was observed. In in vitro metacyclogenesis no signifficant differences were evidenced. sKO cells are also more sensitive than wt to oxidative stress. Currently we are developing a double knock out for SHMT and we are performing a complete biochemical characterization of the recombinant enzyme. Both approaches will be relevant to unveil new roles for SHMT in the biology of T. cruzi.
Supported by:CNPq e FAPESP
Keywords: Amino acids; gene knockout ; serine metabolism

PV024 - **WOULD A HIGHER EUKARYOTES DNA POLYMERASE THETA SINGLE PROTEIN BE ENCODED IN TRYPANOSOMATIDS BY TWO SEPARATE GENES?**

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DNA polymerase theta (Pol θ) is a member of the A family of DNA polymerases, highly conserved among multicellular eukaryotes. It plays an important role in DNA repair and regulation of the genome integrity. Human Pol θ presents is a single protein with three main domains: a polymerase C-terminal domain; a central domain and a N-terminal helicase domain;. The the helicase activity was has not been detected hitherto in this protein. Genome database analyses of trypanosomatids parasites have shown the presence of Pol θ gene. However, differently from higher eukaryotes, they present individual copies of both polymerase and helicase domains in different loci of their genomes. Comparative bioinformatics analyses demonstrated that both encoded proteins are quite conserved among several eukaryotes that have both domains in one single protein. In order to investigate functional features of these separated genes in trypanosomatids, we cloned, expressed and purified *Trypanosoma cruzi* Pol θ helicase domain (TcCLB.509769.70) in heterologous system. We found that rTcPol θ helicase domain presents ATPase activity that is increased in the presence of ssDNA. Moreover, using dsDNA having each strand labeled with different markers, we were able to detect ATP-dependent helicase activity in rTcPol θ helicase domain. Further experiments are currently being developed performed to understand whether helicase and polymerase domains interact during DNA damage repair, which would evidence a functional merge of two separated genes that are linked in one single copy in other organisms.
Keywords: Dna polymerase theta; helicase; trypanosoma cruzi

PV025 - **CHROMOSOMAL COPY NUMBER VARIATION ANALYSIS REVEALS GENOME STABILITY IN DIFFERENT SUBSPECIES OF *TRYPANOSOMA BRUCEI***

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Trypanosoma brucei is the causative agent of African trypanosomiasis, a disease that endangers millions of people in 36 countries in Africa. Currently, *T. brucei* taxa is divided in three subspecies, named *T. brucei gambiense*, *T. brucei rhodesiense* and *T. brucei brucei.* The first two subspecies are related to human cases of African trypanosomiasis, although sporadic cases of human infections by *T. brucei brucei* have been reported. The genome sequencing and chromosomal level assembly of the Tritryps *Leishmania major*, *T. b. brucei* and *Trypanosoma cruzi* as well as the whole genome sequencing of several strains/isolates from these two genera allowed comparative chromosomal copy number variation (CCNV) analysis to be performed. CCNV, the gain or loss of whole chromosomes, is a mechanism of gene expansion and is usually related to rapid adaptation to new environments or selective pressures driven by the host. CCNV have been reported in different species of *Leishmania* and also in distinct *Trypanosoma cruzi* strains, but so far has not been described in *T. brucei*. To evaluate CCNV events in *T. brucei*, we mapped whole genome sequencing reads from the three *T. brucei* subspecies against the *T. b. brucei* TREU927 reference genome. Read depth coverage and SNP analysis were used to estimate the copy number of all 11 Megabase-sized chromosomes in the three *T. brucei* subspecies. This analysis demonstrated that the 11 chromosomes of all three *T. brucei* subspecies are disomic, in contrast to *T. cruzi* and *Leishmania* genus, in which a great number of polysomy/monosomy were observed. These results suggest that *T. brucei* has lost the ability to sustain chromosomal aneuploidies, as it is present in other close-related parasites. We are currently expanding CCNV analysis to other *T. brucei* samples as well as to other related parasites to have a broader view of these chromosomal expansion/loss events in protozoans.

Supported by:CNPq, FAPEMIG, CAPES
Keywords: Ccnv; genome; t. brucei

PV026 - **IDENTIFICATION OF CALMODULIN-BINDING PROTEINS IN *PLASMODIUM FALCIPARUM***

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Calcium is a ubiquitous second messenger in *Plasmodium falciparum*. Many of the downstream effects of Ca^{2+} in eukaryotes are regulated by calmodulin (CaM). This small sensor protein possesses two globular domains, each containing two EF hands, connected by a region without defined organization. Ca^{2+}-CaM may bind to and modulate the activity of targets as diverse as ionic plasma membrane channels, kinases, adenylate cyclases, plasma membrane Ca^{2+} ATPases, endoplasmic reticulum calcium ATPases and cytoskeleton proteins of various organisms, however few CaM partners are known in *P. falciparum*. Aiming to identify novel CaM-binding proteins in *Plasmodium* we generated a knockin parasite possessing CaM fused to hemagglutinin (HA). By using an anti-HA antibody in western blot and immunofluorescence experiments, we observed that CaM is expressed throughout the erythrocytic development of the parasite in a diffuse pattern in the parasite cytoplasm. We performed immunoprecipitation experiments followed by liquid chromatography and tandem mass spectrometry in order to determine potential CaM partners. Upon treatment of parasites with thapsigargin, an endoplasmic reticulum Ca^{2+} pump inhibitor, in order to raise cytosolic Ca^{2+}, we used a membrane-permeable crosslinker (DSS) to preserve protein interactions. We also used DSS in parasites that were maintained in Ca^{2+}-containing medium or Ca2+-free medium with BAPTA-AM, aiming to chelate intracellular Ca^{2+}. Altogether, we identified 45 proteins involved in ribosomal structure, 6 proteins involved in histone acetylation, 5 proteins involved in glycolysis, 2 proteins involved in polyamine and methionine metabolism,1 protein involved in the linear motor, among others. These results indicate that the CaM of *P. falciparum* regulates diverse cell functions in the parasite.

Supported by:FAPESP
Keywords: Plasmodium; calmodulin; molecular partners

PV027 - **RPA COMPLEX IS EXPORTED FROM THE NUCLEUS IN A LIFE CYCLE DEPENDENT MANNER**

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Trypanosoma cruzi is a protozoan parasite that possesses a complex life cycle that alternates between replicative (Epimastigotes and Amastigotes) and non-replicative (Trypomastigotes). Replication protein A (RPA), the major eukaryotic single-stranded binding protein, is a heterotrimeric complex formed by three subunits RPA-1, RPA-2 and RPA-3 that participates in various vital functions in DNA metabolism, being a fundamental player during replication, repair, recombination and checkpoint signaling.Immunoprecipitation, immunofluorescence and EdU incorporation suggest that TcRPA-1 and 2 form a complex in vivo and participates in RPA canonical functions described for other organisms. Reduction of TcRPA-2 expression by generating heterozygous knockout cells impaired cell growth, slowing down S-phase progression. Moreover, RPA2+/- cells presented a better efficiency in differentiation from epimastigote to metacyclic trypomastigote forms, suggesting that RPA can be involved in differentiation process. Studying RPA in non-replicative forms, we found that RPA complex is exported from the nucleus. While in epimastigotes RPA was found at nuclear space, in metacyclic and bloodstream trypomastigotes, RPA was found in the cytoplasm. In replicative amastigotes, RPA is presented in both compartments. In silico analysis showed a leucine rich nuclear export signal (NES) in TcRPA-2 that is typically recognized by CRM-1 exportin in other organisms. T. cruzi possess a CRM-1 homologue and preliminar results suggest a role for this protein in RPA nuclear export. Moreover, SUMOylation is a post translational modification that is commonly involved in nuclear-cytoplasmic transport regulation. We found a conserved SUMOylation site in TcRPA-1 and a first assay indeed detected sumoylation at RPA-1. The role of this modification in TcRPA shuttling is under investigation. **Supported by:**FAPESP
Keywords: Replication protein a; trypanosoma cruzi; sumoylation

PV028 - **A PLASMID-BASED TOOL FOR CO-EXPRESSING MULTIPLE PROTEINS IN *TRYPANOSOMA CRUZI***

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A powerful tool in understanding *Trypanosoma cruzi* biology and host-parasite interactions is the ability to modify protein expression by knocking out genes of interest or inserting new genes. Among the limitations in the stable expression of multiple proteins of interest by *T. cruzi* is the restricted set of antibiotics available to select for the transgenes and the extended time (often 4-6 weeks) required for this selection. These limitations make the process for generating parasite lines that co-express multiple proteins both time-consuming and inefficient. In this study we have evaluated 3 approaches for co-expressing transgenes in *T. cruzi*, which consist of inserting multiple transgenes separated by: a) 2A intraribosomal peptide skip sequence; b) truncated trypanosome 3' and 5' untranslated regions; and c) full length intergenic regions from several *T. cruzi* genes. Transgenes on separate plasmids under distinct antibiotic resistance genes serve as the standard for comparison. We evaluated the relative ratio of co-expression using fluorescent proteins eGFP and mCherry and quantified protein production from each of the expression sites using luciferase activity. Western blotting was used to validate expression of distinct proteins (in the case of the use of the 2A skip sequences). We have also evaluated this multi-protein expression tool to assess the ability of multiple immune-modifying proteins expressed by *T. cruzi* to alter the mammalian host immune response to infection.
Keywords:2a; multicistronic; utr

PV029 - **EFFECTS OF NALIDIXIC ACID, A PROKARYOTE TOPOISOMERASE II INHIBITOR, ON KDNA ULTRASTRUCTURE**

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The kinetoplast is a hallmark of trypanosomatids and is defined as the enlarged region of the single branched mitochondrion that contains the DNA (kDNA). This structure is unique in nature, since it contains thousands of circular DNA molecules that are topologically relaxed and interlocked, forming an extensive network. The kinetoplast also presents typical mechanisms for RNA editing and DNA replication, thus constituting a potential target for chemotherapeutic agents. On this work, we investigated the effects of nalidixic acid on the kDNA network ultrastructure of Crithidia fasciculata and Trypanosoma cruzi epimastigotes by using Transmission Electron Microscopy (TEM) techniques and Atomic Force Microscopy (AFM). The nalidixic acid is a specific inhibitor of prokaryote topoisomerase II, enzymes that play an essential role on kDNA arrangement and replication. For AFM analysis kDNA networks were isolated from treated and non-treated parasites and during this process, proteins associated to the network were eliminated. Such cells were also observed by TEM after routine processing or after employing the acid phosphotungstic (PTA) technique, which reveals basic proteins. Our results showed that isolated kDNA from non-treated parasites presented an intact and massive network with its typical arrangement that contains fibers homogeneously distributed. The treatment of both trypanosomatid species with 500 µg/ml of nalidixic acid for 48h induced an intense kDNA compaction that generated thicker DNA fibers in relation to control cells. Such ultrastructural alteration was also seen by routine TEM and by the PTA cytochemistry method that intensively stained the condensed kDNA fibers. Taken together our data indicate that nalidixic acid promote an irreversible effect on kDNA arrangement that is observed even after protein removal, as observed by AFM. **Supported by:**FAPERJ and CNPq
Keywords: Nalidixic acid; kinetoplast; trypanosomatids

PV030 - **MODULAR VARIABILITY OF MULTIGENE FAMILIES ENCODING SURFACE PROTEINS UNCOVERS DIFFERENTIAL COMPOSITION OF MOTIFS AMONG *TRYPANOSOMA CRUZI* STRAINS**

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Among the Tritryps, *T. cruzi* owns the largest expansion of multigene families encoding surface proteins. Despite playing crucial role in host-parasite interactions, one third of these gene families were not incorporated into the 41 putative chromosomes in the *T. cruzi* reference strain CL Brener. The large number of members of these families also hinders the assignment of reads to specific gene, as they can map/align with the same reliability to several loci. Although these families are highly polymorphic, they also present motifs shared among distinct members, resulting in a mosaic structure that may favor the generation of sequence variability by rearrangement of defined blocks through recombination. The relative abundance of these conserved motifs can be used to estimate the variability of these regions among *T. cruzi* strains. To this end, we developed a methodology to evaluate the copy number variation of motifs derived from mucin-associated surface protein (MASP), TcMUC mucins and trans-sialidases multigene families. This methodology is assembly independent and only requires next generation sequence reads for a given isolate and a reference genome. The first step of this methodology consists in retrieving all reads that map with all the genes of each family, generating all possible kmers of 30 nucleotides present in these reads. The kmers are then clustered by sequence similarity to generate conserved motifs. Finally, the deep of coverage of each motif is computed and compared among *T. cruzi* strains. Our methodology was used to estimate the relative abundance of all motifs identified in MASP, mucin and trans-sialidase families in different *T. cruzi* DTUs, revealing several differences in their abundance within and among DTUs. Dendrograms based on the abundance of these motifs presented discordances with the phylogeny based on single copy genes, reinforcing the hypothesis that different selective pressures shape the evolution of these two *T. cruzi* genomic regions.
Supported by:CNPq, FAPEMIG, CAPES **Keywords:**Trypanosoma cruzi; copy number variation; surface proteins

PV031 - **IMPLICATIONS OF *STRIGOMONAS CULICIS* OXIDATIVE AND ENERGETIC METABOLISMS DURING THE INTERACTION WITH *AEDES AEGYPTI***

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Strigomonas culicis is a monoxenic protozoan found in the midgut of several mosquitoes, presenting a life cycle restricted to the epimastigote form. Among its peculiarities, there is the presence of an endosymbiotic bacterium, which biological role involves iron metabolism. Despite the colonization of hematophagous insects midgut, reactive oxygen species(ROS)-enriched environment, a detailed evaluation of this protozoa antioxidant mechanisms was not performed yet. In this work, we analyzed *S. culicis* oxidative and energy metabolisms, comparing three different strains: wild type, aposymbiotic and H_2O_2-resistant wild type. Aposymbiotic strain was more susceptible to oxidative stress, being more glycolysis-dependent, with higher glucose consumption and impaired oxidative phosphorylation, as well as the presence of less efficient antioxidant pool. On the other hand, wild type showed higher resistance to oxidative stress, especially H_2O_2 levels, suggesting a mitochondrial dependence. Confirming this hypothesis, H_2O_2-resistant *S. culicis* showed a greater resistance to the oxidative challenge and more dependent to oxidative phosphorylation, demonstrating higher oxygen consumption, an increase in citrate synthase, complexes II and IV activities and high ATP production. Furthermore, this strain produces reduced ROS levels, showing lower lipid peroxidation. Despite physiological changes, no ultrastructural alterations were detected in H_2O_2-resistant strain, even in the mitochondrion. The resistance induction also led to a greater colonization of *Aedes aegypti* midgut *ex vivo* and *in vivo*, reinforcing the hypothesis that the prooxidant environment in the mosquito gut participates in the control of *S. culicis* population.
Supported by:FAPERJ, CNPq and FIOCRUZ **Keywords:**Strigomonas culicis; bioenergetics; oxidative stress

PV032 - **EFFECTS OF TRICHOSTATIN A, AN INHIBITOR OF DEACETYLASES, IN AN ENDOSYMBIONT-BEARING TRYPANOSOMATID**

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In nature many living beings associate through symbioses. These relationships are not only important for cell evolution, since the mitochondrion and the chloroplast have symbiotic origin, but also to the emergence of new species. Trypanosomatids are known to cause disease to men, as well as to plants and animals of economic interest. However, most of these protists is not pathogenic and inhabits only one invertebrate host throughout its life cycle, thus named as monoxenics. Among these species, there are seven co-evolving with an endosymbiotic bacterium in a mutualistic relationship. In such obligatory associations the symbiont divides in perfect synchronization with the host cell nucleus and in a microtubule-dependent manner, so that each trypanosomatid contains only one bacterium. With the goal of studying the coordinated division between *Angomonas deanei a*nd its symbiont, we used in this work Trichostatin A (TSA), an inhibitor of deacetylases which affects the acetylation of microtubules, making the cytoskeleton more stable and impairing cytokinesis. After testing different TSA concentrations in *A. deanei* a reduction on cell proliferation was observed. Data obtained by scanning electron microscopy revealed that treated protozoa presented morphological alterations, as cell body rounding and flagellar shortening. Ultrastructural analyses by transmission electron microscopy, routine mode or negative staining, showed changes in the spacing between subpellicular microtubules and modifications on kinetoplast DNA arrangement. Our next approach is to observe treated parasites by immunofluorescence in order to check for possible alterations in the distribution of acetylated microtubules and on symbiont division pattern. **Supported by:**FAPERJ and CNPq
Keywords: Endosymbiosis; trypanosomatids; trichostatin a

PV033 - **IDENTIFICATION AND CHARACTERIZATION OF RNA BINDING PROTEINS THAT ARE DIFFERENTIALLY EXPRESSED DURING THE TRYPANOSOME CRUZI LIFE CYCLE**

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Trypanosoma cruzi, the causative agent of Chagas disease, has three main forms that are biochemically and morphologically distinct and which are programed to rapidly respond to the drastic environmental changes the parasite faces during its life cycle. Epimastigotes and amastigotes replicate within their invertebrate and vertebrate hosts, respectively, whereas trypomastigotes are programed to invade cells and to travel between hosts. Unlike other eukaryotes, protein-coding genes in this protozoan are transcribed into polycistronic pre-mRNAs that are processed into mature mRNAs through coupled “trans-splicing” and poly-adenylation reactions. Because of this, control of gene expression relies mainly on post-transcriptional mechanisms that must be mediated by RNA binding proteins (RBP) that control steady-state levels and/or translation rates of mRNAs. By searching for motifs known to be present in eukaryotic RBPs, we identified in the T. cruzi CL Brener genome, 170 sequences encoding proteins containing RNA recognition motif, PABP, Alba, Pumillio and Zinc Finger motifs. Using RNA-seq data generated with mRNA from epimastigotes, trypomastigotes and amastigotes extracted at two time points during infection of human fibroblasts, we analyzed the expression of all T. cruzi RBPs throughout the life cycle of this parasite. We found that one gene, TcCLB.506739.99, which encodes a RBP containing a zinc finger motif, is highly up-regulated in epimastigotes, one gene, TcCLB.469784.50, is highly expressed in amastigotes and two genes, TcCLB.511127.10 and TcCLB.511511.6, are highly expressed in trypomastigotes. The importance of the protein encoded by TcCLB.506739.99 was immediately revealed by changes in the growth curves of epimastigote mutants that have one allele of this gene deleted. Its RNA binding capacity as well as the impact of the reduced expression of this potential regulatory T. cruzi RBP on global gene expression of epimastigotes are being currently investigated. **Supported by:**CNPq, FAPEMIG, INCTV
Keywords: Trypanosoma cruzi; rna binding protein; rna-seq

PV034 - ***RHODNIUS PROLIXUS* IMMUNE GENES AND GUT MICROBIOTA ARE MODULATED BY *TRYPANOSOMA CRUZI* INFECTION.**

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Rhodnius prolixus is a major vector of *Trypanosoma cruzi*, the causative agent of Chagas disease in Latin America. In natural habitats, these insects could ingest different microorganisms, triggering the synthesis of immune genes, including antimicrobial peptides (AMPs). The aim of this study was to compare AMPs induction in the midgut and fat body of *R. prolixus* infected with different *T. cruzi* strains. *T. cruzi* Dm 28c clone successfully develops whereas Y strain is rapidly lysed in the *R. prolixus* midgut. The relative expression of the AMPs genes was quantified by RT-qPCR. The AMPs activity against different bacteria was evaluated in vitro using turbidimetric tests with haemolymph and midgut samples. Microbiota were quantified using colony forming units and RT-qPCR. The insects were also fed with blood containing antibiotics and *T. cruzi* Dm 28c epimastigotes. Our results showed the infection by *T. cruzi* Dm 28c induced defC and prol transcripts and a reduction in the midgut bacteria population. In contrast, the T. cruzi Y strain neither induced AMP gene expression nor reduced microbiota. Additionally, the antibiotics treatment reduced the population of *Serratia marcescens*, a tripanolitic bacteria, and increased the number of *T. cruzi* Dm 28c in *R. prolixus* midgut. In insects treated with antibiotics defC expression decreased in the anterior midgut when compared to control non infected insects. However, in insects treated with antibiotics and infected with T. cruzi Dm 28c, *S. marcescens* population was strongly decreased while defC expression increased in comparison to *T. cruzi* infected insects without antibiotics treatment, suggesting a regulatory function of defC on *S. marcescens*, subsequently enhancing parasite development. In conclusion, *R. prolixus* AMP gene expressions and microbiota were modulated in distinct patterns, which depend on the *T. cruzi* strain. **Supported by:**CNPq, FAPERJ, PAPES-FIOCRUZ **Keywords:**Rhodnius prolixus; trypanosoma cruzi; microbiota

PV035 - **TOXOPLASMA GONDII CYTOKINESIS COMPLETION IS DEPENDENT ON APICOPLAST TYPE II FATTY ACID BIOSYNTHESIS**

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The protozoan *Toxoplasma gondii*, the causative agent of toxoplasmosis, harbors a non-photosynthetic secondary plastid (apicoplast). As in plant plastids, the fatty acids synthesis in the apicoplast occurs by the type II fatty acid biosynthesis (FASII) pathway. Although FASII in the apicoplast was shown to be essential for parasite survival, the phenotypic consequences of FASII disruption *for T. gondii* have not been examined in detail. Thus, we performed a detailed light and electron microscopy analysis of intracellular tachyzoites of *T. gondii* after FAS II disruption by treatment with the FASII inhibitor triclosan and after genetic disruption through the indution of the knock down of the FASII component ACP (ΔACP/ACPi line). Our morphological analyses using fluorescence and electron microscopy show that, besides the signature apicoplast loss phenotype, FASII disruption prevented cytokinesis completion in *T. gondii* tachyzoites, leading to the formation of large parasites masses containing several 'tethered' daughter cells. Quantification analysis confirmed the division inhibition was a major phenotype of FASII disruption after triclosan treatment and ACP knock down. Serial sections of a parasite mass acquired with a focused ion-beam scanning electron microscopy (FIB-SEM) showed that tethered daughter cells contained a complete set of organelles and presented a mature basal complex, an indicative of cell maturation, but a defect in cleavage furrow establishment prevented the assembly of a new plasma membrane between daughter cells and complete separation at the end of cytokinesis. Growth medium supplementaion with the exogenous FASII main products (myristic acid and palmitic acid) partially rescued the formation of tethered daughter cells and recovered parasite proliferation. These findings support the notion that FASII is essential to generate lipid substrates required for pellicle formation during T. gondii division.
Supported by:FAPERJ, CAPES and CNPQ **Keywords:**Cytokinesis; cell division; apicomplexa

PV036 - **HISTONE DEACETYLASE INHIBITORS PROMOTE CELL DIVISION IMPAIRMENT AND CYTOSKELETON ALTERATIONS ON *TRYPANOSOMA CRUZI***

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DNA phosphorylation, methytlation and acethylation are important epigenetic events which regulate DNA replication, transcription, repair and gene expression. Histone deacetylases (HDACs) are involved in several biological processes, such as the regulation of chromatin compaction and post-transcriptional modification of cytosolic proteins, like tubulin. The use of HDAC inhibitors (HDACi), such as Trichostatin A (TSA), in cancer treatment arrests cell proliferation, impairs cell cycle progression and promotes intense ultrastructure alterations. Recently, it was shown that HDACi can present antileishmanicidal activity, mainly by targeting the parasite's HDAC6-like enzyme. In this study, we evaluated the effects of TSA and Tubastatin A (TST), a HDAC6-specific inhibitor, over *Trypanosoma cruzi* proliferation, viability, cell cycle and ultrastructure. Our ultrastructural and morphological data, obtained by different microscopy methodologies, suggest that TSA can stabilize the microtubule cytoskeleton, generating trypanosomatids with atypical morphology and sometimes presenting multiple nuclei within the cells. In addition, electron microscopy analysis indicates that, in treated cells, kDNA replication is not followed by kinetoplast segregation. Preliminary investigations by confocal fluorescence microscopy suggest that microtubules of treated cells are further acetylated when compared to control parasites. Treatment with TST also promotes cell morphology alterations and appearance of multinuclear protozoa. Cell cycle analysis of TSA treated cells revealed accumulation of trypanosomatids on G2/M phase. Furthermore, *T. cruzi* proliferation and viability were reduced after 72 h of treatment with 50 and 100 µM of both TSA and TST. In conclusion, our results reinforce the idea that histone deacetylases can not only be explored as chemotherapeutic targets but also to the better understanding of trypanosomatid's cell biology.
Supported by:CNPq and FAPERJ
Keywords: Hdac inhibitor; trichostatin a; trypanosoma cruzi

PV037 - **NUCLEOTIDE EXCISION REPAIR IN TRYPANOSOMATIDS – STREAMLINING AND NEOFUNCTIONALISATION OF THE MACHINERY DUE TO MULTIGENIC TRANSCRIPTION**

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Several aspects of the biology of kinetoplastid parasites differ from what is observed in other eukaryotic organisms, including polycistronic transcription. In this context, the importance of DNA repair pathways can be highlighted. Among them is Nucleotide Excision Repair (NER). DNA lesions that lead to helix distortions or block replication signalise to NER activation. Two sub-pathways act in the first step involving recognition of the DNA lesion: global genomic-NER (GG- NER) and transcription-coupled NER (TC-NER). TC-NER is selective for lesions that blockade RNA polymerase machinery, present in the transcribed strand of expressed genes, whilst the GG-NER acts over the rest of the genome. The way the pathway works in *T. brucei* is still unclear as some components like CSA are missing. With the aim to identify the cellular components involved in TC-NER in *T. brucei*, heterozygous and null mutants for CSB, the protein that recognizes a stalled RNA Polymerase, were generated. CSB knockout cell lines show increased susceptibility to UV treatment. This effect in more pronounced in the presence of Caffeine. The over-expression of CSB results in no difference in the susceptibility or resistance to UV treatment. To better understand the role of each component, C-terminal myc-tagged CSB, XPC, XPG and XPBz variants were used for subcellular localization. They are nuclear localized and no change in the subcellular localization was observed 24 hours after treatment with UV. The identification of the proteins that interact with each of these components by immunoprecipitation is on course. These results together with previous ones suggest a neofunctionalisation of the machinery in trymanosomatids. In a context of polycistronic transcription it can be speculated that TC-NER has a fundamental role in maintaining genetic expression. **Supported by:**CNPq; Wellcome Trust
Keywords: Nucleotide excision repair; trypanosoma brucei; dna repair

PV038 - **CLONING, EXPRESSION AND PARTIAL BIOCHEMICAL CHARACTERIZATION OF AN ACYL-COA DEHYDROGENASE FROM *TRYPANOSOMA CRUZI***

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T. cruzi, the etiological agent of Chagas disease, is a flagellate parasite that alternates between invertebrate and vertebrate hosts (humans among them) during its life cycle. This fact determines that this parasite is submitted to different environments, in which it is submitted to different carbon and energy sources such as carbohydrates, amino acids and lipids. There is a body of evidences showing that beyond their obvious participation in protein synthesis, amino acids participate in others biological processes such as bioenergetics, resistance to different stresses, modulation of differentiation, cell cycle, and infection of the host-cells. The branched-chain amino acids (BCAA – leucine, isoleucine and valine) degradation pathway is complex and it is constituted by more than a dozen of enzymatic steps. The first's ones consist of a deamination performed by a transaminase, which results in branched-chain α-keto acids. Then, an oxidative decarboxylation occurs, that results in acyl-CoA thioesters. These compounds undergo dehydrogenation by enzymes belonging to the acyl-CoA dehydrogenases family. This enzymatic step is usually able to transfer electrons to FAD^+ and further to the ubiquinones pool feeding OxPHOS. We found two *T. cruzi* putative sequences for the medium-chain acyl-CoA dehydrogenase (ACADM), a candidate to participate in both β-oxidation and BCAA degradation, with 70% similarity to the *Homo sapiens* orthologue. The sequence from *T. cruzi* was amplified by PCR, cloned and successfully expressed in *Escherichia coli* BL21 codon plus strain. Kinetic data were obtained from activity measurements in cell-free extracts of epimastigotes (*Km*= 0.291 μM and *Vmax*= 0.036 nmol. min^{-1}. mg^{-1} for Isovaleryl-CoA and *Km*= 3.305 μM, *Vmax*= 0.85 nmol. min^{-1}. mg^{-1} for Isobutiryl-CoA. The kinetic parameters of recombinant protein and the possible participation of this protein and its substrates in the bioenergetics of epimastigotes is being investigated. **Supported by:**CNPq
Keywords: Branched-chain amino acids; acyl-coa; thioesters

PV039 - **COMPARATIVE PROTEOMIC ANALYSIS OF INFECTIVE AND NON-INFECTIVE STAGES OF *TRYPANOSOMA CRUZI* STRAINS FROM MAJOR DISCRETE TYPING UNITS**

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Chagas disease, caused by Trypanosoma cruzi, is a parasitic infection that affects millions of people worldwide. T. cruzi is classified into six different DTUs (discrete typing units), TcI-TcVI, according to specific genetic markers. There is some correlation between the DTU and the epidemiological and clinical manifestations, but the reasons for that remain unclear. The aim of this study is to compare the whole proteome of noninfective (epimastigote) and infective (metacyclic trypomastigote) life-cycle stages of nine different strains or clones, belonging to TcI, TcII, and TcVI, which are the major DTUs causing Chagas disease in Latin America. We conducted in-solution digestion of each sample, followed by LC-MS/MS analysis using a high-resolution Q-Exactive Orbitrap mass spectrometer. The MS/MS spectra were analyzed using Proteome Discoverer and the T. cruzi database available at UniProt. We have identified over 2,000 unique proteins. Some of the identified proteins are exclusively expressed in TcI, TcII, or TcVI. Moreover, we have found clear proteomic differences between the two life-cycle stages. Our comparative proteomic analysis of the three DTUs could eventually explain some epidemiological and clinical manifestations differences observed in T. cruzi infection. Finally, our results indicate the existence of potential markers specific for each DTU and novel targets for new drug and vaccine development. **Supported by:**NIH, CNPq, CAPES, FAPEMIG
Keywords: Trypanosoma cruzi; discrete typing units; proteomics

PV040 - **NUTRITIONAL AND PH STRESS INDUCE CHANGES IN THE MITOCHONDRIAL FUNCTIONALITY OF TRYPANOSOMA CRUZI EPIMASTIGOTES**

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Chagas disease caused by the protozoan *Trypanosoma cruzi* and transmited by the triatomine insect is a serious public health problem in Latin America. This parasite presents a variety of populations with different morphological characteristics, virulence, pathogenicity and drug susceptibility, as well as variations in molecular level. During the parasite life cycle, two differentiation steps occur: epimastigotes to metacyclic trypomastigotes in insect, and trypomastigotes to amastigotes in mammals. Both steps are dependent of stress conditions as nutritional deprivation and acidification. In this work, we evaluate the role of mitochondrion and oxygen reactive species production in *T. cruzi* under nutritional stress and pH variation conditions. We evaluated the mitochondrial oxygen uptake by high performance respirometry, enzymatic activity by biochemical analysis and expression levels of bioenergetic components and antioxidant enzymes by qPCR. After 24 and 96 h, only nutritional stress induces an important reduction in respiratory rates and inhibited citrate synthase activity. qPCR data shows a remarkable increase in citrate synthase, complexes II, III and IV transcripts levels in parasites submitted to pH stress as well as trypanothione reductase and tryparredoxin peroxidase contents. Thus, a better understanding of T. cruzi mitochondrial functionality in different stress conditions can generate knowledge to the development of new anti-parasitic strategies. **Supported by:**Cnpq, capes, faperj, fiocruz
Keywords: Trypanosoma cruzi; nutritional and ph stress; mitochondria

PV041 - **A KNOWLEDGE SHARED DATABASE OF TRYPANOSOMATIDS: INTEGRATING RNAI AND GENOMIC DATA**

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Biology, like most scientific disciplines, is in an era of accelerated information accrual and scientists increasingly depend on the availability of curated information generated from high-throughput genomics data integration. In this work we integrated the information of *Trypanosoma brucei* RNAi knockdown published by Alsford and collaborators in 2011 with *Trypanosoma cruzi* genomic data. The *T. brucei* RNAi target sequencing (RIT-seq), providing information about loss of fitness following RNAi knockdown for more than 99% of the parasite predicted proteome, was integrated with the homologous information obtained from the clustering of *T. brucei* and *T. cruzi* proteomic data in a database driven approach. The designed knowledge database was built using MySQL. Knowledge shared databases could represent an outstanding biological resource with the potential of decrease costs and experimentation time. This is particularly true to closely related organisms like trypanosomatids. The data clusterization was performed by OrthoMCL algorithm with *T. brucei* and *T. cruzi* predicted proteome. Afterwards, from the designed database, a *T. brucei* protein subset was selected with the following characteristics: a) expressed proteins in blood stream forms; and b) RNA inhibition associated with loss of fitness. This subset integrates 765 clusters containing 784 *T. cruzi* and 968 *T. brucei* protein homologs. STRING database was used in order to retrieve all *T. brucei* and *T. cruzi* protein interactions and afterwards a minimum confidence score cutoff of 0.9 was applied in order to build networks for both parasites. The network topology comparisons revealed shared patterns that could be used to disrupt similar pathways in these organisms. Among the potential pathways we found: fatty acids biosynthesis, acid citric cycle, cell duplication, drug metabolism. Our results suggest that some of these shared proteins could be potential new targets for novel drug and vaccine developments.

Supported by:CNPq, Capes, FAPEMIG, Centro de Pesquisas René Rachou, Instituto Oswaldo Cruz **Keywords:**Rnai target sequencing; knowledge shared databases; homology

PV042 - **SYSTEM BIOLOGY OF *T. CRUZI* INFECTIVITY: A TRANSCRIPTOMICS AND SHOTGUN PROTEOMICS STUDY OF INFECTIVE AND NON INFECTIVE STRAINS**

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The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of human Chagas disease. It's estimated that about 6-7 million people, mostly in Latin America, are infected worldwide and approximately 10.000 deaths per year are reported. In order to obtain global insights about the parasite infective process we performed a comparative study of RNA and shotgun proteomics profiles of *T. cruzi* NM1-cl1 and Cl2-cl2 trypomastigotes strains (high and low infectivity respectively). The cutoff applied for the differential expression analysis was a fold change value of at least 0.99 and adjusted p-value below 0.05 in both transcriptomic and proteomic datasets. The functional annotation and the enrichment analysis of this datasets were made using Blast2GO software and metabolic pathways were addressed using KEGG. The analytical pipeline applied allowed the identification of 1076 up regulated and 1.053 down regulated genes in the high infective strain using transcriptomic approach and 307 up regulated and 73 down regulated proteins in the high infective strain using proteomics approach. Our findings suggest that the infectivity phenomena analyzed could be related to multifactorial biological process including, but not restricted to, cell differentiation, host's immune system evasion, genetic variability (diversity), *T. cruzi* specific pathways and glucose metabolism. In addition to the functional biological inferences, a system biology approach was applied (see Guimarães poster and Velloso poster) providing an interesting overview of *T. cruzi* biology

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Keywords: Trypanosoma cruzi; transcriptomics; proteomics

PV043 - **BRINGING TO LIGHT BIOLOGICAL INFORMATION: INTEGRATING MASSIVE RNA AND SHOTGUN PROTEOMICS IN PROTEIN-PROTEIN INTERACTION NETWORKS**

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With the advent of high-throughput genomics, life scientists are facing massive data sets and the biggest challenge is to extract the biological meaning embedded in it. Regarding protein data, a useful approach is building interaction networks that use the same rules of any other complex networks, also known as “graphs”. In a graph, there are elements (nodes) connected by links (edges). In protein-protein interaction networks, nodes are proteins and edges represent the nature of interaction between them. The dataset we are working with came from RNA sequencing (RNASeq) and shotgun proteomics originated from two Mexican strains of *Trypanosoma cruzi* presenting patterns of high and low infectivity. These datasets were connected with the 10,876 predicted genes of *T. cruzi* genome annotation (v.25). In order to select differentially expressed genes (DE) we set a cutoff of fold change at 0.99 and adjusted p-value bellow 0.05. Afterwards, a subset of 233 shared genes was used in the network prediction. Groups of DE shared genes were defined in different pairwise regulatory relationships according to RNA and protein expression rates (up/down): RNA down/protein down (Rd/Pd - 26 genes), RNA down/protein up (Rd/Pu – 8 genes), RNA up/protein down (Ru/Pd – 2 genes) and RNA up/protein up (Ru/Pu – 197 genes). Gene names associated with each gene were used to retrieve *String* v.10 network information, resulting in 7 proteins. A network was built adding the first neighbors of each protein. Functional annotation was performed using *EC number* information. *Cytoscape* software was used to highlight the infectivity biological metabolic pathways ranked in Gonçalves poster. **Supported by:**CNPq / Capes / FAPEMIG **Keywords:**System biology; interaction network; trypanosoma cruzi

PV044 - **PROTEIN KINASE C SIGNALING INTERFERES IN PROLIFERATION AND DIFFERENTIATION OF *LEISHMANIA AMAZONENSIS***

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Protein Kinase C (PKC) comprises a group of multifunctional proteins that catalyze phosphorylation in serine and threonine residues. In mammals, these proteins have a role in development, memory, differentiation and cell proliferation. The PKC was characterized in *Leishmania amazonensis* promastigote, an important specie in **BRA**, related to cutaneous and mucosal leishmaniasis. During the life cycle, the parasite in the invertebrate host oscillates between replicative form and infective form. In vertebrate host, the promastigote differentiates into amastigotes inside the infected macrophage. These morphological and metabolic changes are regulated by protein kinases. Due to this, the goal of this work is to investigate the involvement of PKC of *Leishmania amazonensis* in proliferation and differentiation. To investigate the differentiation, promastigotes were cultivated in Scheneider medium, pH 5.5, with 20% of Fetal Bovine Serum (FBS), in order to induce the differentiation to axenic amastigote, with different concentrations of RO32-0432 (PKC inhibitor) (0.0001 µM-1 µM). The parasites were counted during 144 h in Neubauer chamber to determinate the proportion of differentiated cells. To investigate the proliferation, promastigotes were cultivated in Scheneider medium, pH 7.2, with 10% of FBS with different concentrations of RO32-0432 and PMA (PKC activator) (0.0001 µM-1 µM). The parasites were counted in Neubauer chamber every 24 h for 5 days, to follow cell growth. The RO32-0432 inhibited the differentiation in a dose dependent manner, inhibiting expressively in 1 µM. Both RO32-0432 as PMA inhibited the proliferation of promastigotes in a dose-dependent manner. Furthermore, growth arrest induced by RO32-0432 was observed from 0.01 µM, more potent than PMA. Therefore, it is possible to conclude that PKC signaling of *Leishmania amazonensis* is involved in both promastigote proliferation and the differentiation to amastigotes *in vitro*. **Supported by:**FAPERJ, CNPq, CAPES, PAPES, IOC/FIOCRUZ **Keywords:**Protein kinase c; leishmania amazonensis; differentiation

PV045 - **LEISHMANICIDAL ACTIVITY OF HISTONE MODIFYING ENZYMES INHIBITORS IN *LEISHMANIA BRAZILIENSIS* AND THEIR EFFECT ON HDAC 1 AND 3 GENE EXPRESSION**

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Leishmaniases are neglected tropical diseases caused by protozoan parasites *Leishmania*. The drugs used in the treatment of leishmaniases cause important side effects. This work is part of an effort of several national and international institutions, financed by the European Community via the consortium A-ParaDDisE whose objective is the discovery of new drugs for the treatment of neglected parasitic diseases based on epigenetic targets. Epigenetic modifications in histones tails on DNA are orchestrated by histone modifying enzymes (HMEs) and these modifications determine the activation or gene repression. HMEs inhibitors, such as inhibitors of histone deacetylases (iHDACs) and protein arginine methyltransferases (iPRMTs), have been investigated as potential drugs for cancer therapy and parasitic diseases, since they cause deleterious effects on cells, including cell cycle inhibition. The objectives of this study were to evaluate the action of HMEs inhibitors (iHMEs) in promastigotes and intracellular amastigotes of *L. braziliensis*, the main causative agent of cutaneous Leishmaniasis in New World. Two classes of iHMEs were tested at 10µM: 23 iHDACs and 13 iPRMTs. The effects of compounds were evaluated after 24, 48 and 72 h by the resazurin method, fluorescence and real-time PCR. A total of 3 iPRMTs showed approximately 50% of antileishmanial effect in promastigotes. Compounds that were non toxic to macrophages were tested in intracellular infection assays, 3 iPRMTs and 1 iHDAC showed leishmanicidal effect greater than 50%. One iPRMT presented an IC50 of 0.64µM, which infers a selectivity index (SI) greater than 15. The best iHDAC tested on infection presented an IC50 of 7.20µM. In the real-time PCR these two hot-spot compounds increased LbHDAC1 and LbHDAC3 mRNA expression by at least 2 fold. These data suggest a compensatory effect and reinforce the action of compounds as iHMEs. The SI > 15 validates this compound for testing in an animal model.**Supported by:**UNIÃO EUROPÉIA, UFV, CAPES E CNPQ **Keywords:**Leishmania Braziliensis; histone modifying enzymes; inhibitors

PV046 - **CHARACTERIZATION OF MRNA POPULATIONS ASSOCIATED WITH TWO DISTINCT RNA BINDING PROTEINS IN LEISHMANIA**

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Regulation of gene expression in trypanosomatids occurs mainly at the post-transcriptional level and involves both the control of mRNA stability as well as translation. In both events a critical participant is the Poly-A Binding Protein (PABP), which in Leishmania is represented by three distinct homologues. PABP1 seems to be the most likely candidate performing the functions attributed to PABP during translation in other organisms. The functional roles of PABP2 and 3 remain to be defined, although it is known that they associate with each other and bind to sets of mRNA distinct from those bound by PABP1. As well as the PABPs, a large number of other RNA-binding proteins (RBPs) with typical RNA-binding domains are present in trypanosomatids. This study aimed to identify and compare mRNA targets of RBPs differentially associated with the Leishmania PABPs. First, the three PABPs were submitted to immunoprecipitations (IPs) and mass spectrometry analysis and RBPs differentially associated with PABP1 or PABP2/3 were identified. Among these, RBP23 was found to associate with PABP1 and DRBD2 was found in the PABP2 IPs. To identify mRNAs bound by these two proteins, both were first expressed as HA-tagged fusions in transfected L. infantum cells. Cytoplasmic extracts from these cells were then used in IPs performed with anti-HA beads. mRNAs bound to either RBP23-HA or DRBD2-HA were extracted and used to construct cDNA libraries and next generation sequencing. A large number of reads to ribosomal proteins were found from mRNAs co-precipitated with RBP23, in agreement with previous experiments where such mRNAs were found to preferentially associate with PABP1. Reads derived from the DRBD2 experiment, however, were enriched with histone encoding sequences, again consistent with previous data where histone mRNAs were found in PABP2 IPs. These experiments shed light on the role of the different Leishmania RBPs and their role in the translation of distinct sets of mRNAs. **Supported by:**CAPES **Keywords:**Rna-binding protein; leishmania; rnaseq

PV047 - **MORPHOLOGICAL STUDIES OF THE *TRYPANOSOMA CRUZI* EPIMASTIGOTE EARLY ENDOSOME NETWORK**

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Trypanosoma cruzi epimastigotes take up nutrients by avidly endocytosing macromolecules from the extracellular medium. They exhibit a corset of closely spaced stable microtubules underneath the cell membrane that impairs endocytosis except through the flagellar pocket (FP) and the cytostome, where those microtubules are absent. Although the FP plays a fundamental role in the endocytic process in several trypanosomatids, we have shown the cytostome-cytopharynx complex as the main structure involved in this process in *T. cruzi* epimastigotes. The cytostome opens near the flagellar pocket and invaginates deeply forming the cytopharynx, which is supported by a special set of microtubules, disposed as a gutter. The internalized cargo is subsequently found inside early endosomes, a tubule-vesicular network (TVN), spread from the perinuclear region to the posterior tip of the protozoan. Budding from the network, vesicles fuse with reservosomes, where cargo is stored or degraded. In this work, we used high-resolution electron microscopy techniques, as electron tomography and FIB-SEM, to investigate the interactions between the early endosomes and other endocytic organelles. For this, we incubated epimastigotes with gold-labeled transferrin (Tf) and followed its endocytic traffic. At 4°C, Tf was found exclusively inside the cytopharynx, whose length was dramatically shortened. When the temperature was raised to 12°C, Tf was distributed through the TVN, where it was trapped. No cargo reached the reservosomes even after 2 hours of incubation. Moreover, incubation of epimastigotes with Tf at 28°C for 15 minutes allowed us to investigate transfer from the cytopharynx to the TVN and then to the reservosomes. We concluded, using 3D reconstruction, that the delivery of cargo from the TVN to the reservosomes occurs in a “kiss and run” fashion, with tubular endosomes connecting more than one reservosome at a time.
Supported by:CNPq, FAPERJ
Keywords: Endocytosis; early endosome ; high-resolution electron microscopy

PV048 - **FUNCTIONAL CHARACTERIZATION OF RHODNIUS PROLIXUS CHITINASES**

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Chitinase enzymes are responsible for the hydrolysis of glycosidic linkages within chitin chains. In insects, they act in relocation of chitin during development and molting, digestion in detritivorous insects and predators, and control of thickness of peritrophic membrane.
Rhodnius prolixus is one of the main vectors of Chagas disease, caused by the parasite Trypanosoma cruzi. There are 9 chitinase genes in R. prolixus genome, but the role of the corresponding transcripts has not yet been studied in detail.
Our goal is study the expression of R. prolixus chitinase transcripts, as well as analysis of their physiological role through silencing by RNAi. Knowledge of chitinase roles in insects may reveal new targets for vector control, as well as new aspects of their physiology and interactions between insects and microorganisms.
Through phylogenetic analysis of coding sequences, we found that R. prolixus chitinases belong to different gene groups already described in other insects. We silenced selected chitinase genes to understand their function. We conducted a concentration curve (1µg, 3µg, 6µg and 2 x 3µg) for all R. prolixus chitinase genes in order to define which is the lowest concentration of dsRNA that would allow us to get a higher percentage of silencing. We chose RpCht7 and RpCht3 genes for phenotype analysis, since they showed strong silencing. We observed that RpCht7 silencing increases mortality by 25% compared to GFP controls, with no change in blood intake, diuresis, digestion, time and percentage of molting. However, RpCht7 silencing strongly inhibits oviposition. **Supported by:**FIOCRUZ, FAPERJ, CNPq and CAPES
Keywords: Rhodnius prolixus; chitinases; rnai

PV049 - **CHARACTERIZATION OF A HISTIDINE TRIAD-LIKE 1 (HINT-1) PROTEIN IN PLASMODIUM FALCIPARUM ERYTRHOCITIC STAGES**

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Malaria is a major public health problem and is responsible for thousands of deaths worldwide. It is well known that Plasmodium regulates protein expression tightly in order to successfully survive within the host erythrocyte. Histidine triad (Hint) proteins are described as tumor-suppressing, apoptosis-inducing and transcription factors in eukaryotes. These proteins also have similar properties to calmodulin and other calcium-biding proteins. Plasmodium genome analysis has a predicted Hint; nevertheless studies that support any of its putative functions in the parasite are lacking. In order to shed light on Hint-1 function, we cloned the 3′ region of the genomic sequence of Hint−1, lacking the stop codon, in a knockin-optimized vector containing the hemagglutinin sequence, and transfected the parasite with this construct. After drug cycling, PCR analysis was used to confirm the incorporation of the HA tag to the Hint genomic locus in the P. falciparum, Western blotting assays to verify the expression of Hint−1 in different blood stages of Plasmodium and Immunofluorescence assays to characterize Hint-1 localization. For the imunofluorescence assays we produced policlonal antibodies in mice against BIP, a known reticulum endoplasmic marker. Besides knockin constructs, we also performed the overexpression of Hint-1 in P. falciparum and Hint-1 gene knockout in P. berghei. Hint-1 co-immunoprecipitation assays were conducted so that we could identify potential Hint-1 molecular partners. Immunofluorescence assays with Hint1−HA knockin parasites were performed, and confocal analysis unraveled that the protein is partially co−localized with the ER. Plasmodium berghei Hint1-KO parasites failed to survive, suggesting that Hint-1 gene is essential. Interestingly the co-immunoprecipitation of Hint-1 showed, among others, that a calcium-dependent protease is a possible molecular partner for Hint-1. The current data presents information about uncharacterized Hint−1 in Plasmodium. **Supported by:**fapesp
Keywords: Plasmodium; hint-1; calcium

PV050 - **DESIGN AND CONSTRUCTION OF AN OVEREXPRESSION LIBRARY FOR LARGE-SCALE PHENOTYPING OF *TRYPANOSOMA CRUZI***

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The genome of *Trypanosoma cruzi* was published nearly a decade ago, which allowed to understand many of the aspects related to the basic biology of this eukaryote. T. cruzi is a non-amenable organism for genetic manipulation due to high genetic diversity, gene copy number variation, and the absence of RNAi machinery rules out the use of high throughput screening methods which has been widely used in T. brucei. Then, new approaches are needed to explore gene function in T. cruzi, a parasite where 50% of the genes remain without an assigned function. In this work, we report the construction of an overexpression gene library to do phenotyping in large scale gene function in T. cruzi. Initially, we optimized the electroporation conditions in epimastigote forms of the parasite, which allowed improving the transfection efficiencies, cell viability, and time of selection of stable parasites when compared with standard transfection protocols. ~3400 open reading frames (ORFs) from the Dm28c cloned into Gateway platform were pooled based on ORF size making 8 pools that were transferred to a T. cruzi expression vector (pTREX) modified for Gateway cloning strategy and target sequencing. The pools were transfected into T. cruzi epimastigotes using our optimized electroporation protocol generating T. cruzi overexpression library (TcOvL). The quality of TcOvL carrying the partial ORFeome of Dm28c clone is being analyzed by sequence and will be used to identify genes involved on drug resistance, virulence and differentiation.
Supported by:CAPES and CNPq
Keywords: Large-scale phenotyping; orfeoma; overexpression library

PV051 - **LEISHMANIA SPECIES IDENTIFICATION BY MULTIPLEX QUANTITATIVE REAL TIME PCR**

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On the important relationship between Leishmania species and cutaneous clinical manifestations, the need for a method able to diagnose, quantify and identify the parasite species will be a advance to a better conduct doctor in cutaneous leishmaniasis (CL). This study aims to development of a multiplex quantitative real time PCR able to discriminate between Leishmania spp. We carried out multiple alignment by Mega 6.0 from Leishmania spp. sequences found in GenBank for different targets (HSP70, Citocromo B, ITS1, kDNA, SSU rDNA, G6PD and GP63). Sequence analysis for each target was performed by nucleotide BLAST. The best target was selected and primers and probes were desingned. Assays were carried out per system individually. Efficiency, sensibility and specificity were determined. Therefore, ITS1 sequences from Leishmania **Braziliensis**, Leishmania guyanensis, Leishmania shawi and Leishmania amazonensis were selected. Two sets of primers and probes were created: La System (LaS) (LaF: 5' ATGGCCGATCGACGTTATAG 3'; LaR: 5' TGCGTGTGTGGATAACGGCT 3'; LaP: 5' AATGCCCGTTTCAATACGGCGTT 3') and SV System (SVS) (SVF2: 5' TAGCAAGCCTTTCCCACAG 3'; SVR: 5' CGACGTTAACATATCGCGTA 3'; SVP: 5' CCCACAGATACGCAATACAATCTA 3') able to detect and distinguish between L. amazonensis from L. braziliensis, L. guyanensis and L. shawi. Efficiency: LaS: 97,43%; SVS: 90,57%. Sensibility: LaS: 5fg/uL; SVS: 0,5pg/uL. LaS presented cross-reactivity with Trypanosoma cruzi and Leishmania mexicana, however, it is 105 and 103,5 more specific for L. amazonensis than for T. cruzi and L. mexicana, respectively. SVS was specific to Viannia subgenus species. Combining both (LaS and SVS) a multiplex assays were carried using DNA from L. amazonensis and L. braziliensis, resulting in 77% and 98% efficiency,; 5fg/uL and 0.5pg/uL sensibility. Thus, due to clinical importance of L.amazonensis identification this work can help the medical management, since this species is resistant to antimony, the conventional drug.
Keywords: Leishmania species identification; multiplex-qpcr; american cutaneous leishmaniasis

PV052 - **PROTEIN MODELLING, B-CELL LINEAR EPITOPE PREDICTION AND RNA EXPRESSION PROFILE OF THE VIRULENCE PROTEIN GP63 FROM *LEISHMANIA BRAZILIENSIS***

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In order to survive within the mammalian host, parasites of the genus Leishmania produce proteases that protect them against the innate immune system response. The metalloprotease gp63 is the major *Leishmania* surface antigen which has been found to have multiple functions as part of the parasite's defense mechanisms. This protein is encoded by multiple genes whose numbers vary considerably between different *Leishmania* species and are increased in those belonging to the subgenus *Viannia*, including *L.* ***Braziliensis***. This study sought to evaluate differences and similarities in gp63 sequences from multiple *Leishmania* and *Trypanosoma* species, evaluating intraspecific and interspecific variation. First, a phylogenetic tree was built to evaluate the expansion of gp63 genes within the family *Trypanosomatidae* and to separate subsets of sequences. This phylogenetic assessment demonstrated an independent expansion of the gp63 genes over the years within different *Leishmania* species and was able to separate these genes into well-defined groups. We then chose the most divergent sequences from *L.* ***Braziliensis*** to build 3D models, based on the described gp63 structure. High confidence models were generated from all sequences, confirming a similar three-dimensional structure and with most of the segments which vary in sequence between different gp63 paralogs localizing to the proteins' external regions. A B-cell epitope prediction analysis was then performed and identified a substantial number of these epitopes within the variable regions. Next, RNA sequencing was performed from log phase promastigotes in order to evaluate the expression of these genes. Transcripts from all annotated genes were identified, but these varied considerably in levels, suggesting that some are preferably expressed over the others. These data suggest that the Leishmania gp63 proteins display sequence variations that may help the parasite evade recognition by the host immune system. **Supported by:**CAPES and CNPq **Keywords:**Protein modeling ; antibody epitope prediction ; gp63

PV053 - **EIF2 COMPLEX IN *LEISHMANIA* SPP: MOLECULAR INTERACTIONS AND THE DESCRIPTION OF A NOVEL EIF2γ SUBUNIT**

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Protein synthesis is considered a likely target for more specific and less toxic drugs directed against leishmaniasis and other diseases caused by the trypanosomatid protozoans. A critical step in this process is the initiation stage, in which several eukaryotic initiation factors (eIFs) act and which includes all steps preceding the formation of the first peptide bond. *Leishmania* and other trypanosomatids share the same set of eIFs described in other eukaryotes, however a number of unique features have been described in those factors characterized in more detail, notably the eIF4F complex. The present work aimed to characterize a second initiation complex from *Leishmania*, the heterotrimeric eIF2, formed by the eIF2α, eIF2β and eIF2γ subunits. eIF2 interacts with GTP, the initiator Met-tRNAi and the small ribosomal subunit and is critical for the recognition of the first AUG during translation initiation. Here, we present work on the native eIF2 complex in *L. infantum* promastigotes. Interestingly, two alternative Leishmania eIF2 complexes were indentified, based on two distinct eIF2γ homologues (EIF2γ-1 and EIF2γ-2) interacting with the same eIF2α and eIF2β subunits. These two complexes were studied further and both were seen to bind to a classical eIF2 partner, the GTPase eIF5. Only the second complex, however, was seen to co-precipitate with the guanine exchange factor, eIF2B, indicating functional differences. According to our analysis, EIF2γ-2 emerged after a gene duplication event very early during the evolution of the family Trypanosomatidae but was subsequently lost from Trypanosoma species. Only EIF2γ-1 is required for cell viability in *Leishmania* since a knockout of both gene copies of EIF2γ-2, but not EIF2γ-1, allowed recovery of viable cells. Our results are consistent with yet novel mechanisms acting during translation initiation in *Leishmania* and associated with the second eIF2 complex. **Supported by:**Capes, CNPq **Keywords:**Protein synthesis; translation initiation factor; eif2

PV054 - **RESAZURIN, ONE ALTERNATIVE METHOD FOR ASSESSING THE METABOLISM OF *L. (L.) INFANTUM* PROMASTIGOTES IN CULTURE.**

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In vitro culture system of Leishmania promastigotes are essential for antigen production, studies on parasite metabolism and new leishmanicidal drugs development studies. Morphologic counting of parasites are tedious and troublesome, poor precise and with lack of metabolic activity information. Metabolic methods such as the MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide) have been widely used to assess cell metabolism. Those assays demands formazan extraction for adequate spectrophotometry and there are some reports of toxicity of metabolites. There are others ways to detect cell growth, as fluorescent DAPI or PI staining for DNA as measure of growth and recently some fluorescent markers of mitochondrial activity has been proposed in other systems. We evaluate the use of resazurin (7-hydroxy-3H-phenoxazine-3-one 10-oxide) as an alternative method for assessing the mitochondrial metabolism of promastigotes in culture, based in its conversion to fluorescent compound by oxidative metabolism. We used microplate 26o C 199 medium culture growth of *L.L infantum* promastigotes with growth detection by morphology or PI fluorescence before and after fixation, comparing with MTT or resaruzin oxidation. This approach differentiate metabolically active promastigotes (live) and not metabolically active (dead) due to cell respiratory metabolism. Resazurin fluorescence was the most sensitive assay, detecting 1.5 x 10^5 promastigotes/ml, since the MTT method only detect 1.2 x 10^6 promastigotes/ml. Resazurin assays by colorimetry at 54 nm are worst than MTT, detecting up to 9.6 x 10^6 parasites, but could be an alternative due to its simplicity. By phase constrast microscopy, resazurin treated promastigotes had its morphology and motility preserved at longer incubation times, while MTT treated parasites presented signs of cell death. The results obtained qualify resazurin as a more sensitive and less toxic and less extensive for monitoring promastigotes cultures.
Keywords: Resazurin; mtt; metabolism

PV055 - **KNOCKDOWN OF A HYPOTHETICAL BUT CONSERVED TRYPANOSOMATID PROTEIN AFFECTS CYTOKINESIS IN *TRYPANOSOMA BRUCEI***

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The genome of *Trypanosoma brucei* is completely sequenced. However, approximately 50% of the predicted open reading frames encode hypothetical proteins of yet unknown function. Among them, a considerable number is kinetoplastid-specific and conserved in related species such as *Trypanosoma cruzi*, *Leishmania major* and *Crithidia fasciculata*, but without a counterpart gene in the genome of its mammalian host. Thus, once identified and characterised, proteins encoded by these genes might offer promising new targets for drug development.
Tb427.10.13790 is a prime example for a protein annotated as 'hypothetical conserved'. It has neither domains nor motifs predicted by bioinformatic tools (SMART, Pfam, InterPro) and we have been unable to detect any orthologues in non-kinetoplastid species. Transcriptional silencing using inducible RNA interference (RNAi) led to drastic phenotypic changes in cell morphology of *T. brucei* bloodstream forms. As revealed by light and electron microscopy, Tb427.10.13790 knockdown resulted in an accumulation of 'monster cells' with up to nine nuclei, multiple kinetoplasts and several flagella, strongly implicating a failure in cytokinesis. Subpopulations possessing an enlarged flagellar pocket were observed, concomitant with a ring-like cell shape and increased cell fragility. Growth analyses disclosed a decline in the proliferation rate of about 50% within the first 24h post-induction, which further slowed down over time. Based on these results, we conclude that Tb427.10.13790 plays a crucial role during cell cycle progression in *T. brucei* bloodstream forms. In an attempt to increase our understanding about the protein's precise function and its mode of action, structural investigations, based on *in vivo* crystals, are currently underway. Moreover, experiments on its cellular localisation, as well as the analysis of phenotypic changes associated with overexpression are being performed. **Supported by:**DAAD
Keywords: Rna interference; cytokinesis; phenotypic analysis

PV056 - **EFFECT OF BIOACTIVE LIPIDS: LYSOPHOPHATIDYLCHOLINE (LPC) AND LYSOPHOSPHATIDIC ACID (LPA) ON THE PROLIFERATION AND DIFFERENTIATION OF TRYPANOSSOMA CRUZI**

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Trypanosoma cruzi is etiological Chagas disease agent. This parasites are submitted to morphological and physiological changes during its life cycle. Epimastigote forms replicate and differentiate into infective metacyclic trypomastigotes at the insect vector midgut. Our experimental models are T. cruzi and Rhodnius prolixus, Chagas disease vector. Insect saliva and faeces contains lysophosphatidylcholine (LPC). Other important lysophospholipid is lysophosphatidic acid (LPA) resulted of LPC hydrolysis by lysophospholipase D, Autotaxi (ATX) enzyme. These LPC and LPA are multisignaling molecules, found in human plasma ingested by the vector during blood feeding. The goal of the work is determining the role of LPC and LPA in T. cruzi proliferation and differentiation. We analyzed the effect of LPC or LPA or ATX inhibitor with LPC on parasite proliferation in vitro. We observed growth increasing of LPA and LPC treated parasites and lower growth in parasites treated with ATX inhibitor-LPC. We analyzed parasite proliferantion with differents LPC fatty acid chains in vitro to identify the role of LPC fatty acid chain has in T.cruzi proliferation. We observed growth increasing of 18:0, 16:0 or 18:1 LPC treated parasite. We saw LPC and LPA effect on parasite differentiation by assaying metacyclogenesis in vitro. Metaciclogenesis rate was estimated by stipulating the percentage of metacyclic forms. We observed major percentage of metaciclyc form in in when incubated low concentration of LPC. Both LPC as LPA have effect at T.cruzi proliferation. A number of trypomastigotes increase when culture are treated with LPC, but not increase with LPA. That show LPC is important also proliferation and differentiation of T.cruzi.
Supported by:CNPq, FAPERJ, Capes
Keywords: Trypanosoma cruzi; lysophosphatidylcholine (lpc); lysophosphatidic acid (lpa)

PV057 - **BEHAVIOR OF THE LYSOSOME RELATED ORGANELLE DURING DIFFERENTIATION OF *GIARDIA INTESTINALIS***

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Giardia intestinalis is a parasite that exhibits two forms in its life cycle: flagellated trophozoite and cyst. Although typical lysosomes are not found, the peripheral vesicles (PV) behave as lysosomes. The knowledge of the endomembrane system during *G. intestinalis* encystment is limited. To analyze the behavior of these vesicles during the differentiation process, markers of acidic compartments such as Lucifer yellow (LY) and Acridine orange (AO) were used to track the PVs; cytochemistry for acid phosphatase and a tridimensional reconstruction of these vesicles were performed. Results were observed using fluorescence, transmission electron (TEM) and Focused Ion Bean (FIB) microscopies. Biochemistry analysis of phosphatase activities was done, measuring the rate of p-nitrophenol (p-NP) production. Microvesicular bodies inside PVs were revealed by FIB. LY and AO fluorescence decreased during encystment process. The same results were observed with acid phosphatase technique. It was observed a labeling of acid phosphatase on the plasmatic membrane on 21h-encysted cells. This finding was corroborated with biochemical analysis. The production of p-NP was increased in non-permeabilized cells, showing the presence of phosphatase in the plasma membrane in *Giardia* after 21h of encystment. By TEM we identified PVs with electrondense profile near the wall of cysts induced to excyst. In conclusion, a change in PVs behavior happens during parasite differentiation, with a translocation of phosphatases from PVs to the plasma membrane. Moreover, there is a participation of these organelles during the exit of the parasite from cyst wall. This work was supported by CNPq, FAPERJ, CAPES and INBEB.**Supported by:**CNPq, FAPERJ, CAPES and INBEB
Keywords: Giardia intestinalis; encystation; electron microscopy

PV058 - **ROLE OF THE MICU2 COMPONENT OF THE MCU COMPLEX IN THE MITOCHONDRIAL CALCIUM UPTAKE OF *TRYPANOSOMA CRUZI***

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Calcium signaling in *Trypanosoma cruzi* is important for host cell invasion, differentiation, osmoregulation, cell death and flagellar motility, among other functions. The influx of calcium into the mitochondria occurs through a mitochondrial calcium uniporter complex (MCUC). This complex consists of several components, including two regulatory proteins named mitochondrial calcium uptake 1 and 2 (MICU1 and MICU2). In mammalian cells, these proteins are located in the mitochondrial intermembrane space and play a role in sensing cytosolic calcium levels and regulating the MCU opening. However, in trypanosomes their function remains unknown. In this work we aimed at studying the role of MICU2 in the mitochondrial calcium uptake of *T. cruzi*. The predicted TcMICU2 protein (468 aa) displayed a mitochondrial targeting signal and four EF-hands domains that could be sensitive to changes in cytosolic calcium. We generated a cell line of *T. cruzi* epimastigotes overexpressing TcMICU2 tagged with 3xHA, and we used them to demonstrate by immunofluorescence microscopy the mitochondrial localization of MICU2. Moreover, our results also show that overexpression of MICU2 causes a significant increase in mitochondrial capacity to take up calcium at a free calcium concentration of 100 nM, without affecting the mitochondrial membrane potential. Furthermore, *TcMICU2* knockout cell lines obtained by the CRISPR/Cas9 system are currently under selection. In conclusion our preliminary results suggest that TcMICU2 has a calcium-sensing role in the MCU complex of *T. cruzi*. Further phenotypic analysis of *TcMICU2* overexpressing and knockout cell lines will be performed to complete the functional study of this protein. Work funded by FAPESP N° 2013/50624-0, 2011/50400-0 and 2015/25709-8. **Supported by:**FAPESP
Keywords: Mitochondrial calcium uniporter; calcium signaling ; trypanosoma cruzi

PV059 - **PECULIARITIES OF *TRYPANOSOMA RANGELI* KP1(-) STRAINS ISOLATED FROM THE WILD RODENT *PHYLLOMYS DASYTHRIX* (SANTA CATARINA, BRA), AS COMPARED WITH *T. RANGELI* KP1(+) STRAINS AND *TRYPANOSOMA LEWISI*, AND ANALYZED BY PHENOTYPIC AND GENOTYPIC APPROACHES**

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Trypanosoma rangeli Tejera, 1920 is a non-pathogenic parasite of humans, domestic and sylvatic mammals, being widely distributed in Latin American, and transmitted by triatomine bugs. *T. lewisi* (Kent, 1880) is a non-pathogenic parasite of rats (mainly *Rattus* spp.) throughout the world, being transmitted by fleas. *T. rangeli* shares great similarity with several stages of *T. lewisi*, the type-species of the subgenus *Herpetosoma*. Two main lineages are recognized in *T. rangeli* regarding the presence (+) or absence (−) of KP1 minicircles. In the present study, two *T. rangeli* KP1(−) stocks (SC-58, SC-61) isolated from the rodent *Phyllomys dasythrix* were compared with *T. rangeli* KP1(+) strains and *T. lewisi* by some morphological features, growth in axenic cultures, isoenzyme profiles, and PCR products from kDNA minicircles (primers #121/122). *T. rangeli* blood trypomastigotes averaged 31.3−33.0 µm (total length), being slightly greater than *T. lewisi* (adult forms). In all *T. rangeli* KP1(+) and *T. lewisi*, the nucleus was placed in the anterior portion of the body (nuclear index, NI ≥1.2), a typical feature of *Herpetosoma* spp. However, in most trypomastigotes of *T. rangeli* KP1(−), the nucleus was central or posteriorly positioned (NI ≤1.0). *T. rangeli* KP1(−) were more fastidious in cultures than *T. lewisi* and KP1(+) stocks. Three isoenzymes (MDH, IDH and PGM) clearly distinguished these species, while the strains KP1(+) and KP1(−) of *T. rangeli* could be differentiated at MDH, PGM and GPI loci. All *T. rangeli* strains presented 760 bp amplicon derived from KP2 minicircles. However, the KP3 products were a single large band (~330bp) in the KP1(+) strains, and two distinct bands (350, 300 bp) in the KP1(−). Otherwise, *T. lewisi* displayed 700 and 400 bp amplicons. The present work gives additional markers for distinguishing the main subgroups of *T. rangeli* and *T. lewisi*. The peculiarities of *T. rangeli* isolates from *P. dasythrix* are noteworthy and can be of taxonomic importance. **Supported by:**FIOCRUZ

Keywords: trypanosoma rangeli; kp1 (+) and kp1 (-); trypanosoma lewisi

PV060 - **THE USE OF COMBINED THERAPY WITH A NITROSYL-RUTHENIUM COMPLEX AND BENZNIDAZOLE AS A STRATEGY FOR CHAGAS DISEASE TREATMENT**

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Chagas disease, caused by Trypanosoma cruzi infection, affects approximately 7 million people. Nifurtimox and Benznidazole, the drugs available for Chagas disease, are effective during acute phase but not in the chronic phase, and may cause severe side effects. Antiparasitic combined therapy is an alternative to optimize effectiveness and avoid development of parasite resistance. We have previously studied a class of nitrosyl-ruthenium complexes with potent antiparasitic activity. Here, we studied a combination strategy with one of these compounds, ct-[RuCl(NO)(dppb)(5,5=-mebipy)]$(PF_6)_2$, and benznidazole. For in vitro assay, each drug used alone or in fixed combinations were incubated with Y T. cruzi strain trypomastigotes for 24 h. In vivo drug combination was performed in BALB/c mice infected by intraperitoneal injection of Y T. cruzi strain and orally treated for five consecutive days. Alone, the ruthenium complex presented an EC50 value of 2.1 ± 0.6 µM, while benznidazole presented a higher EC50 value (11.4 ± 1.0 µM). Drug combination reduced both EC50 (0.8 ± 0.02 and 4.1 ± 0.1, respectively). Combination index calculations were used as cutoffs and revealed that this combination has synergistic effects against T. cruzi trypomastigotes. In vivo studies showed that treatment with the ruthenium complex (80 mg/kg) reduced in 50 % the parasitemia, while when administered in combination with benznidazole at suboptimal dose (10 mg/kg), a reduction of 95 % in parasitemia was observed. The group treated in combination had 100 % survival, while the groups treated with each drug alone showed a survival rate of 60 %. In conclusion, we demonstrated that combination of ruthenium-nitrosyl complex and benznidazole presented an anti-T. cruzi synergic effect in both in vitro and in vivo experimental models. Therefore, ruthenium-nitrosyl complex ct-[RuCl(NO)(dppb)(5,5=-mebipy)]$(PF_6)_2$ may represent a suitable partner for further combination therapy studies in Chagas disease. **Supported by:**FAPESB

Keywords: Ruthenium complex; combined therapy; chagas disease

PV061 - **TRANSCRIPTOME PROFILING IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTES ASSOCIATED WITH VIRULENCE ATTENUATION**

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Leishmaniasis is one of the most important neglected tropical diseases an it is known that *in vitro* cultivation of *Leishmania spp.* for long periods results in a progressive loss of virulence. The focus of this work was to integrate -omic data with bioinformatics resources to contribute to a better understanding of an important biological aspect of this parasite: the loss of virulence after successive periods of *in vitro* cultivation. For this purpose, we evaluated by RNA-seq the difference in expression profile of *L. amazonensis* promastigotes freshly isolated from experimentally infected mice (R0) and parasites that were cultured after 30 passages *in vitro* in Schneider's Insect Medium (R30). We have identified 683 genes with significant differential expression, 64.12% of which with decreased expression in R30 compared with R0. This study showed that the loss of virulence in *L. amazonensis* after successive periods of *in vitro* cultivation are likely to be associated with parasite-host interactions mediated by parasite surface proteins, stress tolerance and metabolism of amino acids and fatty acids. Furthermore, we disclosed several other genes that are possibly associated with *Leishmania* virulence and are good candidates for further functional studies. In this study we have also investigate the presence of viral sequences in the *L. amazonensis* RNA-seq reads. To this end, we assembled reads that were not mapped against the *L. amazonensis* reference genome using the Trinity software and performed a Blast search against the NCBI non-redundant database. The results were manually filtered by length and e-value. We identified 35 putative viral unigenes and, based on their sequence similarity, the sequences belong to Picornavirales order and Baculoviridae family. Further studies are necessary to confirm the identity and phylogeny of these putative virus sequences. Once the virus identity is confirmed, the impact of these viruses on the virulence will be investigated. **Supported by:**CAPES, FAPEMIG, CNPq
Keywords: Leishmania; transcriptome; virulence

PV062 - **TRIFLUMURON EFFECTS IN THE PHYSIOLOGY AND REPRODUCTION OF *RHODNIUS PROLIXUS* ADULT FEMALES**

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Studies were carried out to evaluate the efficacy of the growth regulator, triflumuron (TFM, Starycide® sc 480 Bayer), to induce mortality and disrupt both oviposition and egg hatching in Rhodnius prolixus adult females by feeding, topical or continuous treatments. Feeding with TFM resulted in milder biological effects than topical or continuous treatments. One day after treatment, highest mortality levels were observed with topical administration, and 30 days after that both topical and continuous treatments induced higher mortalities than feeding. Oral treatment better inhibited oviposition at lower doses, and hatching of eggs deposited by treated females was similarly affected by the three delivery modes. Topical treatment of eggs deposited by non-treated females significantly reduced hatching. However, treatment per contact of eggs deposited by untreated females was not able to disrupt its eclosion. Moreover, the effects of TFM on insects were often displayed in a dose response manner. Additionally, oral treatment increased the number of immature oocytes per female, and topical treatment reduced the mean size of oocytes. TFM also affected the carcass chitin content, diuresis and innate immunity of treated insects. These results indicate that TFM acts as a potent growth inhibitor of R. prolixus adult females and has the potential to be used in integrated vector control programs against hematophagous triatomine species.
Supported by:FAPERJ, CNPq, CAPES, UFF (PROPPI), and PAPES, PAEF and PROEP
Keywords: Rhodnius prolixus; triflumuron; insect growth regulators

PV063 - **A NEW INSIGHT INTO THE SIALOME OF *TRIATOMA DIMIDIATA*, A VECTOR OF CHAGAS DISEASE**

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Hematophagous vectors produce a sophisticated repertoire of salivary molecules that act on hosts' immune and haemostatic responses. These pharmacological compounds function as vasodilators, antiplatelet, anticoagulant and immunomodulators to assist blood feeding. Although *Triatoma dimidiata* is an important vector of Chagas disease largely distributed in Americas, a limited number of its salivary proteins have been described so far. Here, we provide a deeper insight into the salivary glands molecules that can contribute to the hematophagic habit of *T. dimidiata*. We used RNA-seq as a tool for sequencing the cDNA library salivary glands. From the assembly of >50 million reads, 3,815 coding sequences were disclosed and publicly deposited in GenBank. Lipocalin gene family was the most abundant, comprising more than 89% of the secreted proteins. Other observed secretory members include inositol polyphosphate phosphatase, antigen-5, OBP, 5'nucleotidase, protease inhibitors and proteases. Accordingly, this work greatly expands the previous set of annotated sequences available from *T. dimidiata* salivary glands. Additionally, we provide the salivary proteome analysis, revealing there is a correlation among the most abundant salivary gland transcripts putatively secreted and the most abundant salivary proteins identified. The diversity of *T. dimidiata* salivary proteins secreted during the feeding is imperative to disarranging hosts' inflammation and haemostasis, and neutralizing immune system. **Supported by:**FAPDF; CNPq and CAPES
Keywords: Hematophagy; triatoma dimidiata; sialome

PV064 - **A DEEP INSIGHT INTO THE SIALOME OF *RHODNIUS NEGLECTUS*, A VECTOR OF CHAGAS DISEASE**

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Triatomines are hematophagous insects that act as vectors of Chagas disease. *Rhodnius neglectus* is one of these kissing bugs found, contributing to the transmission of this American trypanosomiasis. The saliva of hematophagous arthropods contains bioactive molecules responsible for counteracting host haemostatic, inflammatory, and immune responses. Next generation sequencing and mass spectrometry-based protein identification were performed to investigate the content of triatomine *R. neglectus* saliva. We deposited 4,230 coding DNA sequences (CDS) in GenBank. A set of 636 CDS of proteins of putative secretory nature was extracted from the assembled reads, 73 of them confirmed by proteomic analysis. The sialome of *R. neglectus* was characterized and serine protease transcripts detected. The presence of ubiquitous protein families was revealed, including lipocalins, serine protease inhibitors, and antigen-5. Metalloproteases, disintegrins, and odorant binding protein families were less abundant. The data presented improve our understanding of hematophagous arthropod sialomes, and aid in understanding hematophagy and the complex interplay among vectors and their vertebrate hosts.
Supported by:FAPDF; CNPq and CAPES
Keywords: Hematophagy; rhodnius neglectus; sialome

PV065 - **IDENTIFICATION OF MOTIFS MEDIATING THE INTERACTION BETWEEN THE TRANSLATION INITIATION FACTOR EIF4E3 AND POLY-A BINDING PROTEIN (PABP) HOMOLOGUES IN LEISHMANIA SP.**

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During the initiation stage of translation, the complex eIF4F (formed by the eIF4E, eIF4G and eIF4A subunits) performs a crucial role by facilitating the recognition of the mRNA by the ribosomes. In trypanosomatids, multiple eIF4E and eIF4G homologues were identified with two eIF4F-like complexes, based on the EIF4E4/EIF4G3 and EIF4E3/EIFG4 subunits, found to be implicated during translation initiation. In Leishmania, the first complex also associates with a Poly-A Binding Protein homologue (PABP1) and a novel EIF4E4/PABP1 interaction has been reported which is not found in other eukaryotes. EIF4E3 and 4 share a conserved eIF4E core and unique N-terminal extensions and all three Leishmania PABPs have the typical N-terminal RNA binding region, linker segment and C-terminal PABC domain. EIF4E3 contains three conserved motifs in its N-terminal extension which are related to motifs found in the EIF4E4 N-terminus and which have been implicated in its interaction with PABP1, but the in vivo data is dubious regarding a EIF4E3/PABP interaction. Here, the existence of interactions between EIF4E3 and the Leishmania PABPs was then investigated in vitro through pull-down assays. First, Glutathione S-Transferase (GST) tagged PABPs expressed in Escherichia coli were tested for their ability to bind to 35S-labelled EIF4E3. All three Leishmania PABPs bound efficiently to EIF4E3 with the binding by PABP1 suggesting a higher affinity, similar to what is seen with EIF4E4. The recombinant EIF4E3 was then tested with the N-terminal motifs mutated, individually or in combination. The interaction with EIF4E3 was disrupted only when all three motifs were mutated, again reminiscent of what is seen with EIF4E4. These results demonstrate a similar mode of interaction between the PABP and eIF4E homologues which are unique to trypanosomatids. However, considering that in vivo EIF4E4, but not EIF4E3, interacts preferentially with PABP1, the basis for this specificity remains to be defined.
Keywords: Trypanosomatids; eif4f complex and pabp; protein interaction

PV066 - **THE ANTIFUNGAL COMPOUND BUTENAFINE ELIMINATES PROMASTIGOTE AND AMASTIGOTE FORMS OF *L. AMAZONENSIS* AND *L. BRAZILIENSIS*.**

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Leishmania sp. produces ergosterol lipid as one of the major sterol of its biological membranes. This biochemical pathway involves different enzymes that present homology with those present in fungi, and because of that, *Leishmania* parasites are sensitive to azoles and allylamines drugs. Considering that, this work aimed to investigate the activity of butenafine against promastigote and intracellular amastigote of *L.* (*L.*) *amazonensis* and *L.* (*V.*) ***Braziliensis***, the most important species causing American tegumentar leishmaniasis in Americas as well as the levels of nitric oxide (NO) and hydrogen peroxide (H_2O_2) produced by treated macrophages. Ultraestructural changes of butenafine-treated promastigotes was also investigated by transmission electron microscopy. Butenafine eliminated promastigote forms of *L. amazonensis* and *L. **Braziliensis*** with efficacy similar to that of miltefosine. In addition, butenafine induced alterations in promastigote forms of *L. amazonensis* that resemble programmed cell death. Butenafine as well as miltefosine presented mild toxicity for peritoneal macrophages, however, butenafine was high effective to eliminate intracellular amastigotes of both *L. amazonensis* and *L. **Braziliensis*** in comparison with miltefosine, and this effect was not associated with elevation in the levels of NO or H_2O_2. Taken together, data presented herein suggest that butenafine can be considered as a prototype drug able to eliminate *L. amazonensis* and *L. **Braziliensis***, etiological agents of anergic diffuse and mucocutaneous leishmaniasis, respectively.
Supported by:FAPESP
Keywords: Butenafine; antileishmanial agent; drug repurposing

PV067 - **DIFFERENCES IN THE SPHINGOLIPID BIOSYNTHESIS AMONG *LEISHMANIA* SPECIES IN THE *LEISHMANIA* AND *VIANNIA* SUBGENERA**

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The Neotropical *Leishmania* species are grouped in two subgenera: *Viannia* and *Leishmania*. Within the *Viannia* species, *L.* ***Braziliensis***, *L. panamensis* and *L. guyanensis* are the most important causal agents of the New World tegumentary leishmaniasis. From the *Leishmania* subgenus, *L. infantum chagasi* is related to visceral leishmaniasis, and *L. amazonesis* and *L. mexicana* may cause mucocutaneous, cutaneous and diffuse leishmaniasis. *Leishmania* expresses a characteristic class of sphingolipid, the inositol phosphoceramide (IPC). The main IPC specie described in *L. major* presents a characteristic mass spectrometry (MS) ion at *m/z* 778, represented by ceramide d36:1 (containing d16:1 sphingoid base and 18:0-fatty acid, Hsu et al. 2008). IPC purified from *L.* ***Braziliensis*** presents the same EIS-MS peak at *m/z* 778 (d36:1), however it contains a d20:1-sphingoid base linked to 14:0-fatty acid (Levatti et al., 2016). In this study IPC was purified from promastigotes of different species of *Viannia* subgenus: *L. guyanensis*, *L. panamensis*, *L. naiffi*, *L. shawi* and *L. lainsoni*; and from species of the *Leishmania* subgenus: *L. amazonensis*, *L. major* and *L. mexicana*. IPC structures were determined by electrospray ionization-MS, and the main ions, at *m/z* 778 and 780, from species of *Viannia* subgenus were characterized as d20:1/14:0 and d20:0/14:0, which clearly differ from the same *m/z* IPC ions from species of the *Leishmania* subgenus - *m/z* 778 (d16:1/18:0) and *m/z* 780 (d16:0/18:0). These results indicate that all *Leishmania Viannia* strains analyzed express IPC with a longer sphingoid base compared to species from Leishmania subgenus, suggesting that the sphingosine synthase specificity between *Leishmania* and *Viannia* subgenera are different, i.e. for parasites of the *Viannia* subgennus it presents an activity of serine stearoyltransferase whereas an activity of serine myristoyltransferase is observed for parasites of the *Leishmania* subgenus. **Supported by:**FAPESP, CAPES, CNPq
Keywords: Sphingolipids; leishmania; inositol phosphoceramide

PV068 - **PHOSPHOLIPASE A2 OF *TRYPANOSOMA CRUZI*: MOLECULAR ANALYSIS AND THREE-DIMENSIONAL STRUCTURE MODELING OF THE PROTEIN**

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Trypanosomatids are similar to superior eukaryotes in several aspects, including the fact that their cellular functions are mediated by signaling pathways triggered by ligands that bind to receptors on the cell surface. Lysophosphatidylcholine (LPC) has biological activity, being produced in physiological and pathological conditions. LPC is produced by the hydrolysis of phosphatidylcholine by phospholipase A2 (PLA2). Recently, our group demonstrated that *T. cruzi* synthesizes a C18:1 LPC, which is able to aggregate platelets. This work intends to use experimental and in silico methods to identifify PLA2 genes in trypanosomatids. Also, we built a three-dimensional structure model for the putative PLA2 of *T. cruzi* using a comparative molecular modeling method. Primers were constructed using the TcPLA2-like from CL Brenner and PAF-acetyhydrolase sequences from *L. mexicana*, available in Trytridb. The construction of the three-dimensional model was carried out using the MHOLLINE program, for modeling from the alignment of primary amino acid sequences. Genes encoding for phospholipase A2 have been identified in *T. cruzi* and other trypanosomatids, like *Leishmania*, *Endotrypanum*, *Crithidia* and *Leptomonas*, only using in silico tools. The construction of the three-dimensional model indicated a human PAF acetylhydrolase as a template. The overlap between the generated structure (*T. cruzi*) and the template structure (human) lead us to conclude that the 3D structures were very similar. The quality of the model was evaluated using the software PROCHECK, which analyzes the models according to their stereochemical parameters. Accordingly, 98.1% of residues falling within favorable and allowed regions of Ramachandran plots indicate a good stereochemical quality of the 3D-model. These results show a molecular modeling that could eventually be useful for the development of more efficient and less toxic chemotherapy against Chagas disease.
Supported by:CNPQ, CAPES, FAPERJ e INCT-EM
Keywords: Bioinformatics; aligment; homology

PV069 - **YEAST TWO-HYBRID SCREENING IDENTIFIED A NEW INTERACTING PARTNER OF EIF4E5 IN *TRYPANOSOMA CRUZI*.**

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In eukaryotes, protein synthesis is a complex process that initiates with the binding of the translation initiation complex eIF4F (eIF4E, eIF4A and eIF4G), to the cap structure present on the 5' end of mRNAs. eIF4E interacts directly with the mRNA cap, eIF4A helicase unwinds the mRNA 5' secondary structure and eIF4G acts as a scaffold for the complex with binding sites for both eIF4E and eIF4A. Trypanosomatids possess six eIF4E and five eIF4G homologs that can form distinct translation initiation complexes, which could contribute to selective translation of transcripts during the protozoan life cycle. Several studies in *Trypanosoma brucei* and *Leishmania* have been developed in order to define the eIF4F complex composition and function. However, nothing has been reported in *T. cruzi*. This study aimed to identify direct interactions between eIF4E1-6, eIF4G1-5 and PABP1-2 factors in *T. cruzi* by yeast two-hybrid assay. For this, all homologues were cloned into AD (prey) and BD (bait) plasmids and transfected in Y and MAV strains. Yeasts were challenged with selective medium in the absence of specifics amino acids and the physical interactions between two distinct translation factors was observed. Our preliminary results showed the presence of strong binding between eIF4E3:eIF4G4, eIF4E4:eIF4G3, eIF4E5:eIF4G1 and eIF4E6:eIF4G5 factors. All these interactions were already described in *T. brucei* and *Leishmania*. However, we identified the interaction between eIF4E5 and eIF4G5, which has not been described in any other trypanosomatid. These results suggest the presence of different translation initiation complexes in *Trypanosoma cruzi* with distinct counterparts, as in the case of eIF4E5 and eIF4G5 factors, whose activity can contribute significantly to the translation regulation in this organism.
Supported by:CAPES, FIOCRUZ, Fundação Araucária
Keywords: Eif4e; yeast two-hybrid; trypanosoma cruzi

PV070 - **MOLECULAR CHARACTERIZATION OF SIX EUKARYOTIC TRANSLATION INITIATION FACTOR EIF4E HOMOLOGS IN *TRYPANOSOMA CRUZI.***

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Posttranscriptional regulation of gene expression is one of the characteristics that make the trypanosomatids interesting objects of study. Translation initiation is a key step for the gene expression control and involves several translation initiation factors (eIFs). Among those factors is eIF4E, a subunit of the eIF4F complex that also includes eIF4G and eIF4A. The eIF4E factor binds to the 5' cap structure of eukaryotic mRNA and recruits the small ribosomal subunit. In trypanosomatids 6 eIF4E and 5 eIF4G homologues were identified, and despite many studies of these multiple homologues have been described in others trypanosomatids as *Leishmania major* and *Trypanosoma brucei*, little is known about these factors in *T. cruzi*. Thus, the aim of our study is to start the molecular characterization of the eIF4E factors in *T. cruzi*. Phylogenetic analysis showed that these factors arose prior to kinetoplastids divergence and are evolutionarily very distant from mammals' eIF4Es, with the TceIF4E5 and TceIF4E6 being the most diverged factors. For the functional molecular characterization, two strategies were chosen: production of polyclonal antibodies against the six TceIF4E homologues and mutant parasites expressing these proteins tagged with GFP. Immunofluorescence assays of these mutants indicated that all eIF4E homologues have a cytoplasmic localization in replicative forms of *T. cruzi* and that TceIF4E5 concentrates around the nucleus. These results were confirmed by immunofluorescence assays in wild type parasites. We are currently performing analysis on the expression levels of TceIF4E1-6 through metacyclogenesis by Western blotting. Furthermore, we will carry out immunoprecipitation assays to identify proteins associated with each TceIF4E homologues using mass spectrometry analysis. Altogether, these approaches will provide new insights into the role of each TceIF4E protein in the translational control of gene expression in *T. cruzi*. **Supported by:**CAPES, FIOCRUZ, FUNDAÇÃO ARAUCÁRIA
Keywords: Trypanosoma cruzi; translation initiation; eif4e

PV071 - **IMPROVING THE GENETIC MANIPULATION TOOLKIT FOR *LEISHMANIA*: AN INDUCIBLE DICRE-BASED SYSTEM FOR REGULATABLE EXPRESSION OF GENES OF INTEREST**

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The current toolkit for genetic manipulation of *Leishmania* is still limited when compared with that available for its related trypanosomatid, *Trypanosoma brucei*. Therefore, the development of new tools is still needed for a robust scrutiny of these protozoa biology. In this way, the dimerizable Cre recombinase (diCre) appeared as a powerful alternative. The diCre recombinase is conditionally activated by rapamycin and mediates the inversion or deletion of any given locus flanked by Lox sequences. In fact, an inducible gene deletion system based on diCre was recently established and proven to be an efficient approach to knockout *Leishmania* essential genes. Here we describe the development of a diCre-based inducible system for protein expression in *Leishmania*. For this, we generated a diCre-expressing cell line, which also encodes a gene of interest (GOI) in anti-sense orientation, flanked by Lox sequences, integrated into the rRNA locus. To validate this system, we used GFP as GOI. We performed PCR and western blotting analysis and confirmed both the inversion of the GFP locus and GFP protein expression upon rapamycin addition. GFP expression was detect using rapamycin concentrations as low as 2nM and as early as 24 hours after induction. The system was also used to monitor protein localization by immunofluorescence microscopy. By this approach we were able to stablish a leaking-free system for expression of GOI. This is a promising inducible system for protein expression in *Leishmania* that can be used, for instance, to study the effect of deleterious mutations on GOI. Also, when compared to the currently available Tetraciclin-inducible system, it uses only two cassettes and, hence, one less selection marker, implying that more genetic alterations could be introduced simultaneously in the same cell line.
Supported by:FAPESP and CAPES
Keywords: Dimerizable cre recombinase; leishmania; genetic manipulation

PV072 - **TGHDAC4: A HISTONE DEACETYLASE UNIQUE TO APICOMPLEXA.**

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Epigenetic regulation can control gene expression without affecting the DNA sequence and reversible histones post-translational modifications (PTMs) has a fundamental role in this process. The focus of our group is Toxoplasma's histones deacetylases (HDACs), since its function is broadly associated with gene silencing in other eukaryotes. *Toxoplasma* has seven HDCAs and we are particularly focused in TgHDAC4, an enzyme unique of Apicomplexa parasites. Database searches showed that the only region shared with other organisms is a portion of the typical HDAC domain. Interestingly, TgHDAC4 has two predicted signals: a nuclear export and a cleavage site, whose functions are unknown. The C-terminal of TgHDAC-4 was missanoted in *Toxoplasma* database; therefore, the first aim was to obtain the complete gene sequence. By sequencing the cDNA with the final portion of tghdac4, we confirmed the presence of additional 677 bp that allowed us to construct the endogenous tag line (TgHDAC4-HA). By immunofluorescence assay, we found that the protein localized near or even colocalized with the apicoplast, a derived plastid found in Apicomplexa parasites. However, the Western blot results showed a protein about 20 kDa smaller than expected, although the corrected insertion of the tag was confirmed by PCR. On the other hand, the deletion of tghdac4 by classical knockout was lethal for the parasite, despite several attempts. Alternatively, we are using CRISPR - Cas9 system. In that case, Cas9 is tagged with GFP, which allows cell sorting of positive parasites and phenotypic analysis in less than 48 hs, assuming that the mutation leads to the parasite´s death. We are currently sequencing the clones to analyze possible mutations caused by Cas9. Finally, antibodies will be produced to confirm the localization and correct size of TgHDAC4 in *Toxoplasma*.
Supported by:CAPES, CNPQ E FIOCRUZ
Keywords: Toxoplasma; histone deacetylase; apicoplast

PV073 - **FIRST DESCRIPTION OF ABCC-LIKE ACTIVITY IN *TRYPANOSOMA CRUZI***

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Chagas disease is caused by the protozoan *T. cruzi*. One of the problems in treating the disease is parasite resistance to benznidazole (Bz), a drug used in chemotherapy. The resistance may be related to the presence of ABC transporters, which are involved in cellular detoxification. The aim of this study was to investigate the presence and activity of subfamily ABCC in *T. cruzi*. The functionality was evaluated by a fluorescent substrate efflux assay in which 2×10^6 epimastigotes were incubated with CFDA in the absence or presence of transporter inhibitors: probenecid, indomethacin and MK571 at 27 or 37 °C. In the assay, CFDA was added to the medium to enter the cell by diffusion then it was excluded to induce transport. The results were analyzed by flow cytometry. The transport activity was confirmed by the addition or non-addition of ATP synthesis inhibitors sodium azide and iodoacetic acid. The CFDA efflux was carried out in CL Brener, Berenice and Colombian strains of *T. cruzi* to analyze and to compare the role of ABCC transporter in the natural resistance of the parasite to Bz. The ABCC activity was assessed by the increase in the median fluorescence intensity (MFI). It was observed that all three inhibitors modulate the ABCC activity in Y strain at 37 °C; however the inhibition was lower at 27 °C; and the MK571 inhibitor was more effective, since it showed a reversal transport index (Ð = MIF with inhibitor/MFI without inhibitor) higher. According, the CFDA efflux was inhibited by ATP depletion. CL Brener and Berenice strains (responsive to Bz) showed greater ABCC activity (Ð > 200) compared to Y strain (moderately resistant) and Colombian strain (resistant) (Ð < 100). Together, these results indicate that ABCC protein does not participate in parasite natural resistance to Bz. However, it cannot rule out the function of this protein in acquired resistance, which is being investigated by our team, using *T. cruzi* strain previously induced to resistance to Bz.
Supported by:CNPq and FAPERJ
Keywords: Chagas disease; abc transporters; multidrug resistance

PV074 - **REPRODUCTIVE ASPECTS OF *ONCOPELTUS FASCIATUS* (LYGAEIDAE) UNDER DIFFERENT CONDITIONS OF INFECTION BY *LEPTOMONAS WALLACEI***

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Oncopeltus fasciatus is an insect of the order Hemiptera, which is often naturally infected with the monoxenic trypanosomatid *Leptomonas wallacei*; this parasite colonizes the digestive tract of the insect. The aim of this study was to observe the reproductive aspects of *O. fasciatus* naturally infected or not by *L. wallacei*, analyzing copulation frequency, number of eggs laid and their viability. Combinations of male and female insects from uninfected and infected colonies were performed (each set of experiment had 6 couples of insects in a plastic pitcher). Adult couples (at day seven after the last molt) were observed for five days after copulation. The number of eggs laid was significantly different between insect groups, being higher in couples formed by uninfected males and females (40.8 ± 8.2, mean and standard deviation) than in infected males x uninfected female (26.1 ± 14.2), uninfected males x infected females (24.8 ± 11.0) and infected males and females (35.1 ± 15.9). The viability of the eggs (laid/hatched) was 81.7% in the couples of uninfected males and females, 57.3% in uninfected males x infected females, 49.9% in infected females x uninfected males and 76.6% in infected males and females (these took about a week more to hatch than in the other groups). The similarities between the results of mixed breeding show that individuals of both sexes seem to have their reproductive capacity hampered by the parasite. Copulation frequency in couples formed by uninfected males and females was 33.3%, 43.4% in uninfected males x infected females, 23.4% in infected males x uninfected females and 50.0% in infected males and females. We conclude that the presence of the parasite adversely affects oviposition and egg viability of these insects. Individuals of *O. fasciatus* in mixed couples seem to suffer more the effects of infection than the naturally infected individuals. **Supported by:**CNPq, FAPERJ, CAPES, INCT-EM
Keywords: Oncopeltus fasciatus; leptomonas wallacei; reproduction

PV075 - ***TRYPANOSOMA CRUZI* I GENOTYPE AMONG STOCKS FROM CHRONIC CHAGASIC PATIENTS UNDER AMBULATORY CARE AT THE EVANDRO CHAGAS NATIONAL INSTITUTE OF INFECTIOUS DISEASES (FIOCRUZ, BRA)**

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Trypanosoma cruzi is the etiologic agent of the Chagas disease in humans, mainly in Latin America, but in the last years became an emerging global health problem. This parasite presents high genetic diversity and can cause different clinical manifestations. In this study, nine trypanosome stocks obtained by hemoculture from chronic chagasic patients were characterized by different approaches. The patients were under ambulatory care at the Evandro Chagas National Institute of Infectious Diseases (Fiocruz), and proceeded from five **Brazilian** States (PE, PB, BA, MG, RS). Materials and Methods. For trypanosome species identification, the isolates were analyzed by classical parasitological approaches and a specific PCR assay. For lineage determination, they were compared by their amplicons of the mini-exon non-transcribed spacer and isoenzymatic patterns. Aiming genotype confirmation, one stock was also analyzed by sequencing of a fragment from TcSC5D gene. Results. All isolates were pure *T. cruzi* cultures, presenting 330 bp products derived from kDNA minicircles. They easily grew in axenic cultures, displaying typical *T. cruzi* stages with large kinetoplast; one stock showed metacyclics only in experimentally infected *Triatoma infestans*. TcI genotype was found in one asymptomatic patient from the State of Paraíba. Six patients were infected with TcII lineage, three individuals presenting clinical symptoms (two with cardiac alterations, and one with megaesophagous), the others being asymptomatic. Two patients infected with TcVI had the disease indeterminate form. Discussion. In Brazil TcII is the main agent of severe chronic infections, whereas TcI is less frequent and usually causes mild chronic disease, unlike that occur in other Latin American countries. Genotyping of *T. cruzi* isolates from patients followed in medical centers is important regarding possible correlations between the parasite lineage and host responses to therapeutic drugs, besides disease prognoses. **Supported by:**FIOCRUZ, UFF, CAPES **Keywords:**Trypanosoma cruzi genotyping; chronic chagas disease; tci

PV076 - **EVIDENCE OF UTR-ASSOCIATED NCRNAS IN *LEISHMANIA***

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It is well established that non-coding RNAs (ncRNAs) regulate a diversified number of cell processes. ncRNAs with independent expression rising from untranslated regions (UTRs) of protein coding genes (so called UTR-associated RNAs, uaRNAs) is a phenomenon conserved in eukaryotes. The discovery of short and unusually AT-rich transcripts in Leishmania major led to a study of putative ncRNAs in the parasite. Three of these odd transcripts were expressed ectopically in L. major in the search for a phenotype, and one of them, named ODD3 that led to a marked phenotype was further investigated. Transcriptomic data in association with RT-PCR and 5'RACE assays suggested that ODD3 is a polyadenylated ncRNA arises from the 3'UTR of one of the copies of the ribosomal protein S16 gene (LmjF.26.0890), a duplicated gene found in tandem on chromosome 26. We analyzed the ODD3 and S16 transcript levels in L. major promastigotes using RT-qPCR. Interestingly, LmjF.26.0890 transcript level does not accompany ODD3 levels; in the stationary phase, LmjF.26.0890 transcript is significantly lower than ODD3 itself. In opposition, LmjF.26.0880 and its 3'UTR are present at equal levels, both higher than LmjF.26.0890 throughout development. We explored the potential of ODD3 as cis or trans-acting element controlling the expression of S16 and other genes. A mutant to overexpress integrated ODD3 into the ribosomal locus was engineered to answer this question. In addition, we generated ODD3 RNA with 4×S1m aptamer tag for the isolation of ODD3 binding proteins. We obtained a list of putative ODD3 binding proteins, 38 proteins in procyclic form and 40 proteins in metacyclic promastigotes. Interestingly, several duplicated ribosomal protein genes in the Leishmania major genome depicted a similar pattern of short transcripts arising from their 3'UTR as shown by RNA-Seq analysis. Therefore, our study indicates that uaRNAs derived from protein coding genes might be a common finding in Leishmania. **Supported by:**FAPESP **Keywords:**Non-coding rna; 3'utr-derived rnas; leishmania

PV077 - **METABOLOMICS ASSOCIATED WITH *IN VITRO* CELL DIFFERENTIATION PROCESS OF *LEISHMANIA AMAZONENSIS***

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Leishmania are heteroxenic parasites with a dynamic life cycle. Their cellular differentiation processes are induced by hosts physical and chemical microenvironmental conditions, culminating in two main phenotypes: amastigote and promastigote forms. Due to their importance, the biochemical and metabolic alterations associated with cell differentiation in *Leishmania* still deserve more investigation. The aim of this study was to identify metabolites and metabolic pathways related to *in vitro* cell differentiation process (IVCDP), using a mass spectrometry-coupled multiplatform analytical systems based on capillary electrophoresis, gas and liquid chromatography. The metabolome of promastigote forms were compared to those at 3, 6, 12, 24, 48 and 96 hours after starting of the IVCDP. Statistical analyzes were run on the identified ions, and those presenting significance were putatively identified, being further confirmed by fragmentation or comparative analysis with analytical standards. A total of 107 statistically significant metabolites were identified (60 were confirmed). A foldchange heatmap was built with values of ion abundance for each compound, showing the individual behavior of each metabolite, during the IVCDP. The metabolites were also mapped to 9 enriched metabolic pathways: metabolism of beta-alanine; sphingolipids; glycine, serine and threonine; arginine and proline; glycerophospholipids; trypanothione; phosphatidylinositol signaling system; biosynthesis of unsaturated fatty acids and ABC transporters. The biological validation of some mapped metabolic pathways are currently underway. These findings indicated significant alterations in metabolic pathways which are, probably, essential for parasite differentiation and may be a significant source of information for virulence factors, drug and vaccine design. In addition, the multiplatform system is an useful tool for investigations on *Leishmania* biology.
Supported by:FAPEMIG, CNPq, MS/DECIT, CAPES, AIRBUS MILITARY
Keywords: Metabolomic; leishmania amazonensis; cell differentiation process

PV078 - **COMPARATIVE *IN SILICO* ANALYSIS OF THE *TRYPANOSOMA BRUCEI*, *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* FLAGELLAR ATTACHMENT ZONE (FAZ) PROTEINS**

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Trypanosomatids contain a single flagellum attached along the cell body through the Flagellar Attachment Zone (FAZ), which is composed by proteins showing distinct cytolocalization: FAZ Flagellum Domain (FFD), FAZ Intracellular Domain (FID) and FAZ Filament Domain (FFID). Differently from *Trypanosoma cruzi* and *Trypanosoma rangeli* where FAZ-related proteins have been poorly studied, 30 proteins have been described for *Trypanosoma brucei*, some revealing to be stage-specific. Blast searches allowed identifying 25 and 26 FAZ orthologs in *T. cruzi* (CL Brener) and *T. rangeli* (SC58), respectively. Among the FFD proteins showing the Calpain domain, 4 genes coding to ClpGM6 were found in *T. cruzi*, 3 *T. cruzi* CL Brener Esmeraldo-Like (65-70% identity) and 1 *T. cruzi* Non-Esmeraldo-Like (42% identity) and a single gene in *T. rangeli* (70% identity). FLAM3 was identified as a single copy gene in both species, however, the *T. rangeli* FLAM3 is truncated and lacks the Clu domain. In *T. brucei*, FID comprises 5 stage-dependent proteins: FLA1BP that binds to FLA1 in procyclic forms and FLA2/FLA3 that interact with FLA3BP in bloodstream forms. In *T. rangeli* and *T. cruzi*, a single ortholog to *T. brucei* FLA1/FLA2/FLA3 and a single ortholog to FLA1BP/FLA3BP were found. All 15 *T. brucei* FFID proteins were identified in *T. rangeli*, not having detected the FAZ14 in *T. cruzi*. In addition, we also identified KMP11 (>90% identity) and other 5 proteins with unknown localization among the *T. cruzi* and *T. rangeli* flagellar proteins, being FAZ10 not identified in *T. rangeli*. These results seem to indicate a reduction of the FAZ complexity in *T. cruzi* and *T. rangeli*. **Supported by:**CNPq, CAPES and FINEP
Keywords: Trypanosoma cruzi; trypanosoma rangeli; flagellar adhesion

PV079 - **CHARACTERIZATION OF A NUCLEOSIDE HYDROLASE ENZYME FROM *TRICHOMONAS VAGINALIS***

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Nucleoside hydrolases (NH) are key enzymes in the purine salvage pathway of protists. NH of the human parasite *Trichomonas vaginalis* are vital and apparently absent in mammals, showing up as excellent target for development of chemotherapeutic agents. This study aims to characterize the substrate preference of a NH enzyme from *T. vaginalis* (*TvNH1*) through molecular docking studies of natural substrates in a 3D model, as well as experimentally cloning, expressing and characterizing its substrate preference. The 3D structure of *TvNH1* was predicted using Swiss-Model Workspace by aligning its primary sequence with the X-ray crystal structure of *Crithidia fasciculata* NH (PDB 2MAS) and with enzymes from other microorganisms. Docking studies were performed with natural substrates in AutoDock Vina program. To perform the cloning, primers were designed based on the NCBI available sequence (XM_001304616) and used to PCR amplify the *TvNH1* from *T. vaginalis* genome (JT strain). The *TvNH1* gene was subcloned into *pMAL-c2* vector and expression was induced in *E. coli* (DH5a strain). Molecular docking studies suggests that *TvNH1* may be of the non-specific type, since adenosine, inosine and uridine present greater number of hydrogen bonds, Van der Waals interactions and shorter distance between the Ca2+ and hydroxyls C2' and C3' from ribose nucleoside. *TvNH1* clones were obtained and sequenced, presenting 100% identity with the database sequence. After subcloning and expressing in the *pMAL-c2* vector, a protein band of expected size for *TvNH1* fused with maltose binding protein (MBP) was observed. The purification of the enzyme will be performed by affinity chromatography using amylose resin. In conclusion, construction of 3D *TvNH1* model was satisfactory, as it allowed us to propose substrate specificity of the enzyme. The recombinant *TvNH1* enzyme will enable us to experimentally access its preference for substrates, allowing us to correlate in silico results with experimental data. **Keywords:**Trichomoniasis; nucleoside hydrolase; molecular docking

PV080 - **FUNCTIONAL ANALYSIS OF THE 9-1-1 CHECKPOINT CLAMP SUBUNITS OF *LEISHMANIA MAJOR*.**

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The 9-1-1 checkpoint clamp participates in the eukaryotic DNA damage response (DDR) and is an important determinant of genome stability. We have recently demonstrated that *Leishmania major* express a functional 9-1-1 complex. The subunits RAD9, RAD1 and HUS1 form a complex *in vivo* and associate to chromatin in response to replication stress. Surprisingly, RAD9 and HUS1 have compartmentalized function within the DDR. This correlates with the fact that some of the cellular pool of RAD9 forms an alternative complex and that some of HUS1 exists as a monomer (Damasceno, et al., 2016; DOI:10.1111/mmi.13441). These findings suggest that the stoichiometry and dynamics of interaction between these subunits is crucial for the role they play. In the present study we did a comparative analysis of the effect of each subunit overexpression on the parasite response to genotoxic stress and on the levels of the partner subunits. We also compared the pattern of subcellular distribution of each subunit in response to genotoxic stress in cell lines that endogenously express myc-tagged versions of RAD9, RAD1 or HUS1. The pattern of expression of each subunit throughout the cell cycle was also comparatively investigated in unperturbed cells and in cells exposed to different genotoxic agents. We found that overexpression of HUS1 confers resistance to hidroxyurea but does not affect the parasite sensitivity to camptothecin, MMS or phleomycin. The immunofluorescence analysis revealed a diverse pattern of subcellular localization. While RAD9 and HUS1 are mostly present in the nuclear compartment, RAD1 is localized both to the nucleus and cytoplasm. A comprehensive comparative analysis of the three subunits will be presented.
Supported by:FAPESP and CAPES
Keywords: Leishmania; 9-1-1 complex; replicative stress

PV081 - **EVALUATION OF *IN VITRO* METACYCLOGENESIS OF *LEISHMANIA TARENTOLAE* AND ITS INFECTIVITY TO HUMAN MACROPHAGES**

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Introduction: *Leishmania tarentolae* is a lizard-infecting species, belonging to the subgenus *Sauroleishmania*, non-pathogenic to humans. *L. tarentolae* is an important model organism for studies on the *Leishmania* biology. Still, many questions about *L. tarentolae* biology remain unanswered, including whether metacyclic promastigotes are produced. Objectives: To evaluate i*n vitro* metacyclogenesis of *L. tarentolae* by morphology, agglutination with lectins and infectivity to THP-1 cells. Methodology: Stationary phase cultures of *L. tarentolae* were centrifuged over Ficoll gradient. Recovered parasites were quantified by hemocytometer counting and evaluated by light microscopy of stained smears. Agglutination tests were performed using different concentrations of *Arachis hypogaea* (PNA) and *Triticum vulgaris* (WGA) lectins with promastigotes obtained from logarithmic phase cultures. Agglutination was assessed, both macroscopically and microscopically, after 1 h of incubation at room temperature. Stationary-phase *L. tarentolae* promastigotes were incubated for 2, 4, 24, 48 and 72 h with THP-1 cells at a parasite/macrophage ratio of 10:1 and infection was monitored. Results: At 10% Ficoll concentration, a small number of cells (0.1-0.2%), resembled typical metacyclic promastigotes were recovered. The mininum concentration required to agglutinate logarithmic phase culture with PNA was 50ug/mL and with WGA, 100ug/mL. Two hours following infection, nearly 9% of the THP-1 cells were found infected with stationary-phase *L. tarentolae* and 72h later, parasites were still detected inside these cells. Conclusions: Although preliminary, our result based on morphology suggests that metacyclics may be produced in low numbers, by L. *tarentolae*. Also, procyclic forms agglutinate with WGA and PNA, which will be further used for evaluation of metacyclics. Additionally, we observed that THP-1 cells actively phagocytise *L. tarentolae* promastigotes. **Supported by:**FAPEMIG, CNPq, MS/DECIT
Keywords:L. tarentolae; in vitro metacyclogenesis; infectivity

PV082 - **IDENTIFICATION AND CHARACTERIZATION OF GLUCOSIDASE GENES IN *LUTZOMYIA LONGIPALPIS***

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Lutzomyia longipalpis is the main vector of *Leishmania infantum* in the Americas and exploits different food sources during developmental stages. The larvae grow on decaying material, adults have a diet rich in sugars, and females also feed on blood. Thus, understanding the molecular, physiological and biochemical role of glucosidases in the development of sandflies is of great importance since the sugar diet is essential for maintaining the longevity, in the process of infection and transmission of Leishmaniasis. Annotation of glucosidase genes was accomplished using the PFAM domain and Vector Base tools. Gene expression in different feeding conditions (water, 1.2 M sucrose, blood) and tissues (gut and rest of the body (RB)) was analyzed by semiquantitative PCR or qPCR. Enzyme activity was determined using as substrates sucrose and methylumbelliferyl-α-glucopyranoside (MUαGlu). We identified 10 genes coding for glucosidases belonging to GH13 family and 6 genes to GH31. Expression analysis showed that genes 2257, 4841 and 8156 are more expressed in the gut than the RB. Genes 2257 and 8156 are induced by blood feeding (qPCR) and their expression is reduced after L. mexicana infection. Gut and RB of L. longipalpis have activities against sucrose and MUαGlu, either in soluble or membrane fractions. Zymography using MUαGli identified one activity in the RB, one soluble activity in the gut and at least three gut membrane activities. L. longipalpis has glucosidase genes belonging to GHF13 and GHF31 with different putative roles in sugar metabolism. These glucosidases have distinctive patterns of expression during the sand fly development. This suggests that some of them are important in the hydrolysis of sugars during the larval development and others might be possibly involved in hydrolysis of sugars or blood glycoproteins in the adult phase, regulating the infection process by Leishmania. **Supported by:**FAPERJ, CAPES, CNPq, FIOCRUZ **Keywords:**Glucosidases; annotation; expression

PV083 - **FUNCTIONAL EVALUATION OF ARGININE METHYLTRANSFERASE 6 OF *LEISHMANIA MAJOR***

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The medical relevance of protozoan parasites of the Leishmania genus and the interest on this ancient eukaryote make it a widely studied organism. Gene expression regulation in these organisms occurs mostly at the posttranscriptional level, and RNA-biding proteins (RBPs) play a central role in it. Recent studies have shown that Arginine-N-methyltransferases (PRMTs), which transfer a methyl group to arginine residues on proteins, play important roles in eukaryotes, and have RBPs as targets. In this work, we developed molecular tools to study the putative PRMT6 of *Leishmania major,* LmjPRMT6. Our results show that (I) parasites overexpressing LmjPRMT6 are less virulent than control parasites, (II) LmjPRMT6 may interact with Tryparedoxin Peroxidase (LmjTryP) *in vivo* and my be involved in oxidative stress pathways, (III) LmjPRMT6 is potentially an essential gene, as suggested by the impossibility to recover PRMT6 knockout parasites. We could not detect PRMT6 methyl transferase activity *in vitro* in spite of independent and complementary assays. We are now investigating presence and activity of different PRMTs, including PRMT6, in *Leishmania braziliensis.*
Supported by:FAPESP E CAPES
Keywords: Leishmania; gene expression ; methylation

PV084 - **EVALUATION OF TGMACK, A NEW SEROLOGICAL AND MOLECULAR TARGET FOR VISCERAL LEISHMANIASIS DIAGNOSIS**

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Leishmaniasis are diseases caused by *Leishmania* genus protozoan and endanger 350 million people worldwide. After genomic sequencing of *Leishmania* spp., molecular studies of their cycle forms (amastigotes and promastigotes) brought important information, which can contribute to the development of new diagnostic approaches and treatments. In this study we evaluated the potencial application of *Leishmania infantum* (syn. *Leishmania chagasi*) gene TGMACK and its encoded protein for development of new diagnostic test. We evaluated the antigenic features of a TGMACK protein fragment using immunoenzimatic assay (ELISA). The results showed specificity of 74% and sensibility of 78% (99% of confidence) and accuracy of 76%, in a randomically chosen population. Additional analysis showed low cross-reaction against *Leptospira interrogans* and *Toxoplasma gondi*, both infective organisms common in dogs. Bioinformatics analysis showed that the TGMACK protein contains one domain found on several organisms, being related to nucleic acid replication, recombination and repair, and might be related to these cross-reactions observed. Alternatively, in order to evaluate the potential use of this gene sequence in molecular assays, we performed PCR analysis using the *L. infantum* specific pair of primers Li_491 and different experimental controls, corresponding to genomic DNAs obtained from *Leishmania amazonensis*, *Leishmania mexicana*, *Trypanossoma cruzi* and *Trypanossoma brucei*. Genomic DNAs of murine macrophage infected and non-infected with *L. infantum* were also used as negative controls. The pair of primers Li_491 proved to be specific as diagnostic target for *L. infantum*, since it amplified a single region of 491 pb. Thus, this target is a promising new alternative to both serological and molecular tests for visceral leishmaniasis.
Supported by:CNPq; FAPESP
Keywords: Visceral leishmaniasis; sorological diagnosis; molecular diagnosis

PV085 - **NOVEL DNA CODING REGIONS AND UNKNOWN PROTEIN MODIFICATIONS REVEAL UNEXPLORED T.CRUZI PROTEOME AND PTMS**

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Chagas disease, also known as American trypanosomiasis, is a neglected tropical disease caused by the Trypanosoma cruzi parasite. In order to develop diagnostic and therapeutic solutions, there was an intense investigation on the parasite biology using omics technologies such as genomics, transcriptomics lipidomics and proteomics. In particular, large scale mass spectrometry-based proteomics studies have allowed the identification and quantification of proteins and their PTMs in different biological conditions. In this study, we questioned the large percentage of unassigned MS/MS spectra commonly observed in large scale bottom up proteomics experiments looking at the T. cruzi (Sylvio X10/1) proteome. A deep proteomics data analysis using proteogenomic and unrestrictive PTMs search approaches allowed us to annotate 30% more MS/MS spectra and identify novel DNA coding regions and unknown PTMs in Trypanosomatids, such as protein arginylation. Overall, this study shows: 1) the importance of assigning protein modifications, analytical artefacts and PTMs, in large-scale mass spectrometry-based proteomics data to deeply profile the trypanosomatids proteome. 2) The need of better elucidation the influence of sample preparation steps on the protein ID and modifications. 3) The identification of novel DNA coding regions in T. cruzi. 4) The discovery of novel PTMs in T.cruzi. **Supported by:**FAPESP
Keywords: Proteomic; ptms; t.cruzi

PV086 - **OVEREXPRESSION OF THE DEUBIQUITINASE OTUBAIN (OTULI) IN *LEISHMANIA INFANTUM* AND CORRELATION BETWEEN ITS ENZYMATIC ACTIVITY AND STRUCTURE CONCERNING STRATEGIC MUTATIONS**

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Deubiquitylating enzymes play an important role in regulating protein degradation and other non-degradative processes, such as immune response modulation and signalling of DNA double strand break, by deconjugating the ubiquitin (Ub) from marker proteins. Exploring the role of deubiquitination in *Leishmania infantum* demonstrates a promising alternative to search for new therapeutic targets for leishmaniasis, since its primary chemotherapy is inefficient. This study aimed to establish two *L. infantum* strains expressing an extra copy of otubain (OtuLi) and a relation between structures obtained from molecular dynamics and enzymatic activity of the recombinant OtuLi in its wild-type and three mutant forms. Regarding the establishment of the strains, the promastigotes of *L. infantum* were transfected with the cassette containing the *otuli* gene and selected with hygromycin. The integration of the cassettes into the 18S rRNA gene locus was confirmed by PCR and the expression of OtuLi fused to the FLAG peptide or to a 6 histidine tail was accessed. The citolocalization of FLAG-OtuLi indicates that the enzyme is in small vesicles in the cytoplasm of the cell with strong markings near the kinetoplast region, confirming the results seen using antibodies specific for OtuLi. The rOtuLi showed activity at acid pH with lysine 48-linked tetra-Ub substrate and the inhibition profile indicates that NEM and Ub-aldehyde are able to inhibit it. Through site-directed mutations in OtuLi, it was observed that the residue near the catalytic site (F82S) or those involved in the interaction with the ubiquitin (L265P and F182S) caused structural changes as shown by molecular dynamics, resulting in a reduction or loss of enzyme activity. The establishment of these strains with the extra OtuLi is the first step for future identification of proteins that interact with OtuLi and thus elucidate its mechanism of action in the parasite. **Supported by:**CAPES, CNPq, COMUE-Sorbonne Université and FAP-DF
Keywords: Deubiquitylation; genetic manipulation; leishmaniasis

PV087 - **TRIMETHYLATION OF THE LYSINE 76 IN HISTONE H3 IS REQUIRED FOR CYTOKINESIS LICENSING AND CELL CYCLE PROGRESSION OF *TRYPANOSOMA CRUZI***

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Dot1 methyltransferase is specific for addition of one, two and three methyl groups in the lysine 79 of the histone H3, modifications required for DNA replication and progression through the cell cycle in most eukaryotes. However, the exact functions of these modifications are still largely unknown. Trypanosomes have two Dot1 homologues, Dot1A and Dot1B. Dot1A is essential for *Trypanosoma brucei* growth and catalyzes the mono and dimethylation of the lysine 76 (H3K76me1 and H3K76me2) during late G2 and M phase of the cell cycle. Dot1B catalyzes the trimethylation of the same lysine (H3K76me3) a modification found in all stages of the cell cycle. Its depletion is tolerated, although the parasites showed defects on cell division. Here we generated *Trypanosoma cruzi* lacking Dot1B by homologous recombination with drug selectable markers. *T. cruzi* Dot1B depletion resulted in parasites lacking H3K76me3 with increased levels of the other methylations and aberrant cell division. The mutated cells also showed a decreased growth phenotype that could be fully rescued by adding caffeine a checkpoint inhibitor of the cell cycle. The cells presented increased levels of a phosphorylated gamma H2A, but did not show increased DNA damage, once Rad9 levels were unaffected, and the chromosomes were intact, indicating that the lack of trimethylation of already mono and dimethylated H3K76 activated a checkpoint. In fact, cell cycle analysis showed that the mutated cells were more arrested in G2/M and this arrest could also be released by caffeine. These results indicated that the main function of the H3K76 trimethylation is to mark mitosis completion allowing the cells to enter cytokinesis and complete the cell division cycle.
Supported by:FAPESP and CNPq
Keywords: Dot1; h3k76 trimethylation; trypanosoma cruzi

PV088 - **THE CHECKPOINT PROTEINS HUS1 AND RAD9 HAVE DISTINTIC EFFECTS ON GENE AND CHROMOSOMAL COPY NUMBER VARIATION IN *LEISHMANIA MAJOR***

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Chromosome and gene copy number variation (CNV) is a common feature of the remarkably plastic genome of Leishmania major and is frequently associated to the parasite resistance to leishmaniasis chemotherapy. In higher eukaryotes, the disturbance in DNA replication and/or DNA damage response (DDR) is a common cause of CNV. In these organisms, the 9-1-1 checkpoint clamp (RAD9, RAD1 and HUS1) is essential not only for the detection and signaling of replication stress, but also for the recruitment of a proper DDR. We have shown that L. major express a functional 9-1-1 homolog. Surprisingly, the subunits RAD9 and HUS1 are also found outside the 9-1-1 context. Here, we report that DNA synthesis is decreased in RAD9-deficient cells (RAD9+/-) but increased in HUS1-deficient cells (HUS1+/-), suggesting the functional compartmentalization of these subunits. Apparently, this effect is not due to a defective activation of early-S origins of replication, as revealed by genome-wide MFAseq prediction of DNA replication initiation. We tested if the effect on replication correlated with CNV of the DHFR-TS locus upon stepwise selection in methotrexate (MTX). Upon replication stress (Hidroxyurea treatment prior to MTX selection) circular amplicons were detected in WT cells from 500µM MTX. In contrast, MTX concentrations as low as 20µM were sufficient to select RAD9+/- and HUS1+/- cells bearing amplicons. It is noteworthy that only linear amplicons were detected in HUS1+/- cells. Whole genome sequencing also revealed distinct pattern of ploidy changes between RAD9+/- and HUS1+/- cells. Altogether, our data indicate that i) altered levels of RAD9 and HUS1 affect the pattern of CNV upon MTX selection; ii) RAD9 and HUS1 act by distinct mechanisms to mediate CNV; iii) the role of RAD9 and HUS1 in CNV possibly involves replication-related processes; iv) RAD9 and HUS1 are required for maintenance and genome stability in Leishmania. **Supported by:**FAPESP, CAPES
Keywords:9-1-1 checkpoint complex ; copy number variation ; genome plasticity

PV089 - **CONTROVERSIES SURROUND THE PHYLOGENETIC STATUS OF *THEILERIA EQUI* BASED ON *18S RDNA* GENE: A STUDY OF SOME GENETIC VARIANTS FROM BRA**

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Theileria equi and *Babesia caballi*, the etiological agents of equine piroplasmosis (EP). The variability of *18S rDNA* gene of *T. equi* has been studied in naturally infected horses from all over the world. The aim of this study was to analyze the heterogeneity of *18S rDNA* gene of *T. equi* in Brazil highlighting aspects of the current taxonomic position of this organism. The DNA was extracted from equine blood samples positives for *T. equi*. The complete *18S rDNA* gene of *T. equi* was amplified following the methodology of Bhoora et al. (2009). The amplification products were then cloned and sequenced. The chromatograms were analyzed, edited and assembled into contigs using DNA Baser3.0. BLASTn was used to identify similar sequences in public databases. Phylogenetic analysis was performed using 12 unique *T. equi 18S rDNA* sequences from Brazil and other sequences from several countries, such as Spain, South Africa, Sudan, USA, Switzerland and Korea. The organisms *T. mutans*, *T. velifera*, *T. annulata, T. parva, Cytauxzoon felis*, *Cytauxzoon sp., B. caballi* and *B. bovis* were also included. ClustalW was used as a multiple alignment method. The final alignment had 1387 bp. The phylogenetic reconstruction method Neighbor-Joining was used, with distances between pairs estimated by Kimura-2-parameter with 1000 replicates. The *18S rDNA* gene of *T. equi* positioned in a different clade than other *Theileria* organisms. The *18S rDNA* gene of *T. equi* grouped into three large well-supported groups, A, B and C. In A, six sequences of **BRA**, RJ1, RJ3, RJ4, RJ5, RJ17 and RJ18, grouped together with sequences from South Africa, Sudan and the USA. In clade B, other six **Brazilian** sequences RJ2, RJ10, RJ12, RJ13, RJ14 and RJ19 grouped with South Africa, USA and Spain sequences. The clade of *T. equi*, based on the *18S rDNA* gene, is well supported and distinct from other *Theleria* or *Babesia* species, being necessary new for better understanding of the correct taxonomic position of this body.
Supported by:FAPERJ **Keywords:**Theileria equi; equine piroplasmosis; 18s rrna gene

PV090 - **EPIDEMIOLOGICAL ASPECTS OF *BABESIA CABALLI* IN HORSES FROM SERRANA AND ITAGUAí MICROREGIONS, STATE OF RIO DE JANEIRO, BRA**

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Babesia caballi is a hemoprotozoa of horses, present in different continents, and is one of the equine piroplasmosis agents. This study aimed to evaluate the epidemiological aspects of infection by *B. caballi* in horses in two microregions of Rio de Janeiro, Brazil. An overall of 516 blood samples were collected from horses of Itaguaí and Serrana microregions, between January and May 2009. The animals were inspected for the presence and level of infestation of ticks. The management conditions for raising of horses were assessed on each property by an epidemiological questionnaire. Ticks collected were properly identified by taxonomic key. The DNA extraction was performed from blood samples of horses for further analysis by polymerase chain reaction (PCR). Of all horses evaluated, 17.2% (n=89/516) had DNA from *B. caballi*. Using a bivariate analysis, it was observed that the highest frequency of positivity occurred in horses from Itaguaí microregion (28.9%, n=70/242). Among the aspects inherent to horses, animals aged below two years were the most affected (33.3%, n=19/57, $p<0.05$). The unsatisfactory condition of the property (25.5%, n=61/239) and the horses bred in close contact with cattle (28.8%, n=28/97) have shown a association with infection. After multivariate analysis, it was observed that the animals aged less than two years (OR=3.33, CI=1.7-6.5), in regions of low altitudes (OR=3.52, CI=1.7-7.3) and animals infested by *Dermacentor nitens* ticks (OR=1.91, CI=1.1-3.4) were factors associated with infection of horses by this hemoparasite. The high degree of infestation by this tick has also been associated with the presence of horses positive for B. caballi (OR=2.11, CI=1.25-3.54). It can be concluded that horses bred in Itaguaí microregion, aged below two years, who have infestations at different levels by *D. nitens* ticks are factors associated the positivity for *B. caballi* in horses at the studied microregions, in Rio de Janeiro, Brazil. **Supported by:**CAPES **Keywords:**Ticks; babesia caballi; equine

PV091 - **COMPARATIVE STUDY OF MORPHOMETRIC DATA OF *HEPATOZOON CANIS* FROM BRA AND *HEPATOZOON* SP. FROM CUBA, COMING FROM NATURALLY INFECTED DOGS**

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Hepatozoonosis is a vector-borne disease with a worldwide distribution and the etiologic agent is a protozoan belonging to Hepatozoon genus. This study aimed to compare morphometrically the gametocytes found in blood of dogs from Brazil and Cuba. Blood samples were collected from cephalic vein of dogs and stored in tubes with EDTA. Total DNA was extracted by commercial kit, according to the manufacturer's recommendations. Blood smears were also performed from peripheral capillary punctures of the auricular pavilions. Smears were fixed in methanol, and colored in Giemsa. Morphometric analysis of gametocytes were realized with optical microscopy and the software Cell^D. The measurements were length, width and area of gametocytes and nuclei. The ratio between length and width of gametocytes were analyzed statistically. All analysis was performed using BioEstat 5.0 software. Mean values were submitted to ANOVA, compared by F test, followed by Student t test in significance level of 5%, with the data presenting normal distribution. DNA samples were submitted to polymerase chain reaction (PCR) to amplification of a fragment (666pb) of 18SrDNA gene specific to Hepatozoon genus. Two samples from Cuba and one sample from Brazil were positive by PCR. Gametocytes were observed within leucocytes in blood smears. The gametocytes of H. canis from Brazil measured 11.82±0.68 ($p<0.001$) x 5.34±0.78 µm, and nucleus measuring 4.10±0.77 x 2.50±0.44 µm ($p=0.017$), with gametocyte area of 59.24±7.76 µm² ($p<0.001$), and nucleus area of 8.87±2.92 µm² ($p=0.013$). The gametocytes of Hepatozoon sp. from Cuba presented rounded morphology, measured 6.43±0.38 x 5.41±0.39 µm, with nucleus measuring 4.14±0.51 x 2.15±0.67 µm, and gametocyte area of 27.39±3.22 µm², and nucleus area of 7.06±2.63 µm². The ratio between length and width of both gametocytes was significant ($p<0.001$). Posterior study related to phylogeny are in progress to define the Hepatozoon species that infect dogs in the Cuba island.**Supported by:**CAPES **Keywords:**Morphometry ; hepatozoon; dogs

PV092 - **NONSPECIFIC ANTIVIRAL RESPONSE OF *LUTZOMYIA LONGIPALPIS* LL5 CELLS: INVESTIGATING THE PUTATIVE ROLE OF SECRETED MOLECULES**

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Phlebotomine sand flies are known as vectors of leishmaniasis, a disease complex caused by Leishmania parasites. Sand flies can also transmit bacteria and viruses. We have been studying immune responses of L. longipalpis, the main vector of visceral leishmaniasis in America. The knowledge of immunological mechanisms involved in the interaction between pathogens and the host vectors is important to control vector transmitted diseases. In previous work our group identified a non-specific antiviral response in the LL5 embryonic cell line from L.longipalpis, caused by non-specific dsRNA. This response is reminiscent of an interferon response in mammals. We are presently trying to identify putative effectors for this response. Secreted molecules have been implicated in cell communication and are involved in immune response, including interferon related responses in mammals.We have conducted a comparative mass spectrographic analysis of conditioned medium from cell cultures 24 and 48 hours after the dsRNA treatment or after mock treatment. We identified a total of 301 peptides, from which 273 were found 24 h and 286 48h post-treatment. The 24h post-dsRNA transfected cells showed 19 peptides with abundance equal or greater than 2 fold change while 17 peptides were 2 or more fold less secreted as compared with mock transfected cells. At the 48h time point these numbers were 33 and 71 respectively. Many of these modulated peptides have a potential role in antiviral or interferon-like responses. Interestingly, the two most abundant secreted peptides at 24h in the dsRNA transfected group were the phospholipid scramblase, an interferon-inducible protein, that mediates antiviral activity against DNA and RNA viruses, and a member of the immunophilin family, which comprises proteins that bind and mediate the effect of immunosuppressive drugs. The transcription profile of some of these candidates was determined and, in most cases, did not follow the pattern of protein abundance.
Supported by:FAPERJ and FIOCRUZ
Keywords: Lutzomyia longipalpis; resposta antiviral; proteomica

PV093 - **MORPHOLOGICAL ASPECTS OF SUBPELICULLAR MICROTUBULES OF TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is a protozoan parasite and exhibits unique features, which differ them significantly from their mammalian host. Among them, the subpelicullar microtubules (SPMT) that follows the helical pattern along the long axis of the cell body organized in a highly ordered array of stable microtubules placed beneath the plasma membrane, absent in flagellar pocket and cytostome-cytopharynx complex. The parasite's life cycle involves symmetrical division and different developmental transitional stages. The maintaining and establishment of cell shape is a fundamental role of cytoskeleton and provides interesting models for cellular biology studies. The morphological knowledge of SPMT during T. cruzi life cycle is limited. To analyze this array of microtubules, tridimensional reconstruction were performed using electron microscopy tomography and focused ion beam-scanning electron microscopy (FIB-SEM). The observations shows that epimastigotes have approximately 60 SPMT, among them, the microtubules next to flagellar pocket are extremely shorter or half-length when compared to others SPMT. Besides this, the helicoidally pattern is held. In conclusion, shorter length microtubules may represent the first evidence that biogenesis of SPMT occurs next to flagellar pocket. At the present time, analysis of SPMT during mitosis and metacyclogenesis are being made and also analysis of the posterior region of cell body to elucidate how SPMT are joined together firmly. These studies can reveal ultrastructural details about the maintenance of cell shape even during its complex life cycle. This work was supported by CNPq, FAPERJ, CAPES and INBEB.
Supported by:CNPq, FAPERJ, CAPES e INBEB.
Keywords: Trypanosoma cruzi; high resolution microscopy; subpelicullar microtubules

PV094 - **GENOMIC DIVERSITY OF *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi has a significant degree of genetic diversity and studying this diversity is of great importance for understanding its biology, patophysiology and ecology. Currently, *T. cruzi* strains are classified in six distinct groups (discrete typing unit, DTU), named TcI to TcVI. Few genomes are available, mainly from DTU I, and the reference genome of CL Brener is DTU VI. We have sequenced the genome of the Dm28c strain, using the SMRT technology, obtaining a significant better representation of *T. cruzi* genome structure. Now, we aim to increase our coverage of *T. cruzi* genomic diversity including a broad number of surveyed genomes. In this sense, we have applied Ion Torrent technology to 25 different strains of *T. cruzi* in order to obtain an overview of sequence diversity, structural insights and copy number variation assessment. We have generated between 500 and 2,000 MB of ~300 nt reads (15 to 70x coverage of a average *T. cruzi* genome) for each strains using Ion PGM sequencer. After mapping the reads against the Dm28c and Sylvio X10 reference genomes, we have calculated the copy number variation (CNV) of genes for each genome, identified SNPs and verified regions of structural variation (insertion, deletion, translocation). Regarding CNV, the majority of *T. cruzi* genes are single copy, and those presenting the higher number of copies (all large protein families and histones, mainly) generally have multiple copies in all genomes, with a few interesting differences. Regarding SNP identification, we have identified more than 500,000 variable positions, representing the broader survey already performed for this organism. We also calculated dN/dS score and ranked the *T. cruzi* genes to identify candidates of host interaction. Most of the hipervariable genes are composed of multi-genic, but new interesting candidates are now being analyzed functionally. All this information is a significant advancement to our understanding of *T. cruzi* biology.
Supported by:CNPq, Fundação Araucária, FIOCRUZ
Keywords: Trypanosoma cruzi; genomics; bioinformatics

PV095 - **CLASSIFYING THE MULTIGENIC PROTEIN FAMILIES OF *T. CRUZI* USING AN INTEGRATION OF LARGE SCALE DATA SETS**

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Trypanosoma cruzi presents in its genome a large portion devoted to codyfing multi-genic families. Trans-sialidases, MASP, DGF-1, RHS, gp63, mucins, among others, consist of thousands of distinct CDS in the genome of this organism. These proteins are also mainly involved with important functional aspects of *T. cruzi* as host-parasite interaction. Here, we have integrated a large spectre of high throughput datasets generated at our group, in the Functional Genomics of Trypanosoma cruzi initiative (TrypanosOmics), abranging genomics (one gold-standard reference genome and ~30 draft genomes of *T. cruzi*), transcriptomics (~900 different samples, abranging a distinct number of biological conditions) and proteomics (~100 different samples) to create a systemic classification of the genetic diversity of these families. We applied these informations to all groups of *T. cruzi* genes comprised of 10 or more copies. Taken together, these results represent a higher level classification of diversity, based not only in sequence, but also in biological function (or expression), representing an atlas of *T. cruzi* molecular anatomy at a functional level.

Supported by:CNPq, Fundação Araucária, FIOCRUZ
Keywords: Trypanosoma cruzi; functional genomics; systems biology

PV096 - **DEVELOPMENT OF AN OPTIMIZED HIGH-THROUGHPUT GENOTYPING METHOD FOR *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the causative agent of Chagas disease, is a major public health problem in Latin America. This protozoan has great genetic diversity, currently being classified into six discrete typing units (DTUs). This genetic diversity implies differences in biological characteristics, clinical manifestations and possibly therapeutic responses. The vast majority of typing methods are based on only a few markers, which prevents further phylogenetic classification. Aiming to solve this restriction, our group developed a high sensitivity genotyping method. This project aims to improve the method developed initially by selecting a new gene panel (n=192) with products around 400 base pairs. This modification is intended to make the laboratoty procedures easier, faster and cheaper. Our genotyping method is based on two complementary PCR protocols as means to simplify the method as well as favoring the correct amplification of the vast majority of the selected targets, generating a robust data on the genomic features and also a correct classification of the strains in a subsequent sequencing by Ion Torrent PGM platform. At present, we applied this method to 20 different strains and we were able to genotype them precisely in the adequate DTUs for all strains.

Supported by:VPEIC- RIO
Keywords: Genotyping; high-throughput ; t. cruzi

PV097 - **HEME CRYSTALLIZATION IN A CHAGAS DISEASE VECTOR DETERMINES INSECT REPRODUCTION AND PARASITE INFECTION**

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Chagas disease is a chronic illness which is not curable, and the most efficient strategy of prevent it is by controlling the population of insect vectors. Hematophagous organisms release large amounts of heme during blood digestion. Free heme is pro-oxidant and cause membrane destabilization but several organisms detoxify heme through its crystallization into hemozoin (Hz). Hz formation is inhibited by quinoline drugs and their potent antimalarial effects are thought to be a consequence of redox imbalance promoted by uncrystallized heme. We hypothesized that farmacological inhibition of Hz formation by quinidine in Rhodnius prolixus, a vector of Trypanosoma cruzi, could lead to several impacts to insect physiology and parasite transmission. Our results demonstrate that quinidine is able to reduce Hz formation in midgut. In the same tissue, reactive species levels were significantly increased and ultrastructural analyses revealed reduced densities of mitochondria and presence of numerous structures similar to autophagosomes. We observed increased heme and lipid peroxidation levels, parallel to reduced urate content in the hemolymph. Protein levels of RHBP in hemolymph were increased by inhibition of Hz formation. Despite there is no increase of a heme oxygenase product (biliverdin) in the heart, free heme levels were increased on this tissue. Besides that, Hz formation impairment leads to a decrease of egg laying which is reversed by the administration of an antioxidant (urate) in the diet. Additionally, quinidine alone is not toxic to Trypanosoma cruzi in vitro, but quinidine reduced parasite burden in vivo on the digestive tract of the insects. Here, we show that impairment of heme crystallization in an insect vector promotes redox imbalance with systemic cellular and physiological consequences and could be thought as a strategy to control vectorial transmission of Chagas disease.
Supported by:CNPq **Keywords:**Heme; hemozoin; rhodnius prolixus

PV098 - **PROTEOLYTIC ACTIVITY OF METASTERNAL GLANDS OF RHODNIUS PROLIXUS INFECTED BY TRYPANOSOMA RANGELI**

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INTRODUCTION: Rhodnius prolixus is a bloodsucking hemipteran, one of the vectors of Trypanosoma cruzi, etiologic agent of Chagas disease and also considered invertebrate host of Trypnosoma rangeli. Adults of R. prolixus have a pair of exocrine glands located in the ventral metathorax, called Metasternal Glands (MGs), which is described to be involved in sexual communication. T. rangeli is a protozoa parasite that invades several organs of triatomines bugs, reducing their survival and development. The literature is scarce on the effect of this parasitism on the sexual pheromones of this insect vector. In this context we seek to verify if T. rangeli infection can affect the proteolytic activity of the MGs. METODOLOGY: The copulation behavior analysis was done in the following groups: infected female and control males; control females and control males in 3 periods of mating behavior: the pre- copulation; copulation and guard. To investigate proteases activities, MGs of R. prolixus females were extracted 7 days after infected blood meal. Then, MGs were centrifuged and the enzymatic profile were determined using specifics peptidic fluorogenic substrates during 60 min at 37 °C. RESULTS AND DISCUSSION: We observed changes of the mating behavior of R. prolixus infected by T. rangeli. The pre-mating and the copulation time have changed in the infected group compared to the control group. Assessing the proteolytic activities we observed none aspartic and cysteine peptidase activities but a significant reduction of trypsin-like serine protease activity in infected MGs compared to control MGs. Our hypothesis is that the biosynthetic pathway of sex pheromones production may have been affected by this proteolytic activity reduction caused by parasitism. More studies of sexual behavior and volatiles analysis of these infected insects should be made in order to know if T. rangeli indeed affects the sexual pheromones production and consequently the mating behavior of R. prolixus. **Supported by:**CAPES **Keywords:**Rhodnius prolixus; metasternal glands; proteases

PV099 - **TYROSINE DETOXIFICATION IS AN ESSENTIAL TRAIT IN THE LIFE HISTORY OF BLOOD-FEEDING ARTHROPODS**

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Blood-feeding arthropods transmit major infectious diseases worldwide and vector control is essential to limit disease spread.
Because these arthropods ingest very large amounts of blood, a protein-rich diet, high concetrations of free amino acids are produced in the insect gut.
Here, we propose that tyrosine detoxification is an essential trait in the life history of all blood-sucking arthropods. Inhibition of tyrosine aminotransferase (TAT) or 4-hydroxyphenylpyruvate dioxygenase (HPPD), the first two enzymes of the phenylalanine/tyrosine degradation pathway, caused the death of insects after a blood meal. Similar results be achieved either by gene silencing or by the use of chemical inhibitors of HPPD, which proved highly effective in killing mosquitoes, kissing bugs, ticks and tse-tse flies, but were not harmful to non-hematophagous insects. . HPPD inhibitors were effectively either topically or through ingestion along with the blood meal.
These findings indicate that HPPD (and TAT) can be a target for the selective control of blood-sucking disease vectors populations. Because HPPD inhibitors are extensively used as herbicides and in medicine, these compounds may provide an alternative less toxic to humans and more environmentally friendly than conventional neurotoxic insecticides that are currently used, with the ability to affect only hematophagous arthropods

Supported by:faperj
Keywords: Hematophagy; tyrosine; vectors

PV100 - **ANALYSIS OF THE RELATIVE EXPRESSION OF GENES RELATED TO MEIOSIS DURING DIFFERENTIATION OF TRICHOMONAS VAGINALIS**

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Meiosis is a particular process of nuclear division responsible for reduction of cell ploidy, promotion of genetic recombination and generation of genetic variability. The parasite of the human urogenital tract Trichomonas vaginalis is the causative agent of trichomoniasis. This parasite is usually found as a trophozoite or as a flagellated amoeboid. However, T. vaginalis trophozoites can differentiate into pseudocysts when subjected to stressful microenvironmental conditions. These pseudocysts present spherical morphology with completely internalized flagella. Although the lack of direct evidence in the literature about the occurrence of meiosis in T. vaginalis, its genome contains some genes whose products are considered exclusive of meiosis process. Our main objective is to investigate the expression of meiosis-specific genes of T. vaginalis during trophozoite to pseudocyst differentiation using the RT-qPCR approach. Differentiation was induced by incubating 107 parasites (JT strain) at 4 ºC during periods of 0, 2, 12h and after recovery of trophozoites at 37 ºC. Total RNA was extracted and cDNA was synthesized. Primer pairs were tested and showed adequate amplification profiles under our experimental conditions. As stablished in previous work, GAPDH was used as the reference gene in the analysis of the relative expression. Expression of five meiosis-specific genes was studied during the process of differentiation: Spo11, Dmc1, Mnd1, Msh1 and Hop2. Our analysis suggests that Dmc1, Hop2, Msh1 and Spo11 appear to be upregulated after 12h of induction, when there is a predominance of pseudocysts, but Mnd1 presented an inconclusive profile, suggesting that these parasites may undergo sexual and/or meiotic process during the pseudocyst stage. In order to get a better understanding on the role of these T. vaginalis meiotic gene orthologues, relative expression analisys of other meiosis-specific genes (Msh4, Hop1, Msh5 and Mre11) is currently under progress.

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Keywords: Trichomonas vaginalis; relative expression; meiosis