PV021 - DYNAMICS OF PROTOZOOLOGY INTO BRAZILIAN SCIENCE

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We used a Brazilian thesis database (CAPES) to search for, in the period from 1987 to 2011, researchers who considerably contributed to protozoology field. Using 60 descriptors, we got 10456 Master or PhD thesis containing at least one descriptor (1.8% of total works presented in the same period in all fields). Filtering only for researchers that supervised at least 10 PhDs, we selected 250 advisors. By automated analysis of thesis databases, we could find students that these scientists have supervised (descends). By automated analysis of their Lattes CV we could also follow advisors ascendance. These analyses provided a list of 1998 names that were manually inspected through Lattes CV examination. From these initial 1998 names, 10.9% could not be found either in Lattes Platform or CAPES database. The analyses allowed the classification of these researchers into 5 different categories:(a) scientists that are working or worked (in past) in Protozoology in Brazil (45.5%);(b) scientists working in Protozoology outside Brazil that were advisors of Brazilians (1.15%); (c) scientists who developed PhD in Protozoology but established a research in other field (9,3%);(d) persons who developed PhD in Protozoology but are now in other activities than science (8.4%); (e) scientists that did not worked in Protozoology during PhD neither established a research in this field but were advisors of scientists that migrated to Protozoology (24.7%). The group (a) could be sub-classified into: (1) the scientists that did the PhD in Protozoology (85%); (2) scientists that migrated to Protozoology (15%). From the last sub-group, we could identify names of the Pioneers of Protozoology. Based in these categories we intend to show the dynamic of Brazilian Protozoology presenting the contribution of Protozoology for itself (69%) as well as to other scientific fields (16,5%). In addition, a graph showing the genealogy of Brazilian Protozoology will be presented. **Supported by:**FAPESP
Keywords: Protozoology; pioneers; brazilian Science

PV022 - BIOCHEMICAL CHARACTERIZATION OF PHOSPHATE UPTAKE IN FREE-LIVING AMOEBA *ACANTHAMOEBA CASTELLANII*

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Acanthamoeba castellanii is a free-living amoeba and the etiological agent of granulomatous amoebic encephalitis and amoebic keratitis. In their life cycle *A. castellanii* has one host, the mammalians and can be colonize different tissues and the cells presents two forms, trphozoite and cysts. The trophozoite is a vegetative form of the cell and has a great infective capacity, the cysts are dormant forms that protect the cell form environmental changes and is a escape mechanism to the host immune response. The phosphate transports are a group of proteins able to internalize phosphate from the extracellular medium to intracellular medium. There are two big families of these proteins the phosphate proton simporter family (PHS) and the inorganic transporter family (PiT). The proteins are responsible for maintain the phosphate homeostasis and in some organism they have a function of regulating the cellular growth. Here our group characterizes the biochemical mechanisms of phosphate uptake on *A. castellanii* cells and the role of these proteins in the growth cellular processes of the amoeba. To measure inorganic phophate (Pi) uptake the cells were grown in liquid peptone-yeast-glucose (PYG) medium, at 28^{o}C for 2 days. The activity was measured by rapid filtration of intact cells incubated with 0,5 µCi 32Pi for 1 hour. Control activity was 256,99±1,72 pmol Pi/h-1 x 10^6 cells. *A. castellanii* presents a linear activity until 1 hour with a cell density ranging from 10^5 to 2x10^6 amoeba/ml. The uptake it's unaffected for pH variation ranging from 6.4 to 8.6. FCCP, a proton ionophore, inhibited the uptake, suggesting that the Pi uptake is coupled to a proton uptake. The phosphate uptake rate is high in the initial days of the culture when compared to the rate when the culture reaches the logarithm phase. It is a high affinity phosphate transport with values of a $K_{0,5}$ and $V_{máx}$ of 0,076 ± 0,006 mM Pi and 639 ± 18,96 pmol x min-1 x 10^6 cells respectively. **Supported by:**CNPq
Keywords: Acanthamoeba castellanii; phosphate transporters; cellular growth

PV023 - REPERTOIRE OF TRYPANOSOMA CRUZI DHHC PALMITOYL TRANSFERASES AND EFFECT OF THE SPECIFIC INHIBITOR 2-BROMOPALMITATE ON EPIMASTIGOTES AND IN VITRO-DERIVED METACYCLIC TRYPOMASTIGOTES

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Palmitoylation is a post-translational modification that consists on the insertion of palmitic acid through a thioester linkage with a cysteine of the modified protein. This modification regulates protein localization and function and is catalyzed by DHHC-palmitoil transferases (PATs), which are transmembrane proteins with an aspartate-hystidine-hystidine-cysteine motif in a cysteine rich domain. Several studies showed that palmitoylation is crucial for important biological functions of different eukaryotic cells, from yeast to humans. However, there are no global studies of protein palmitoylation focused on the parasitic protozoan Trypanosoma cruzi, the etiological agent of Chagas disease. So far only a putative PAT has been characterized as a Golgi marker of T. cruzi (TcHIP or TcPAT1) and only two proteins are known to be palmitoylated in this parasite: Flagellar Calcium Binding Protein (FCaBP) and Phosphoinositide-specific Phospholipase C (PI-PLC). Aim of this work was to determine the repertoire of T. cruzi PATs and the effect of the specific inhibitor 2-bromopalmitate (2-BP) on this parasite. Fifteen genes of putative PATs with CRD-DHHC and predicted transmembrane domains were found (TcPAT1-15). Fourteen of these genes could be isolated by PCR, except for TcPAT6. Plasmid vectors are under construction to fuse the PAT genes with FLAG tags to evaluate the protein subcellular localization. Preliminary data on the effect of the inhibitor 2-BP in epimastigotes growth allowed to calculate the 50% inhibitory concentration (IC50) as approximately 130 µM. Surprisingly, incubation of in vitro-derived metacyclic trypomastigotes with 2-BP resulted in 50% lytic dose (LD50) of about 216 nM. This result indicates that palmitoylation can be crucial for survival of metacyclic trypomastigotes. Further experiments are underway to determine the effect of 2-BP on the T. cruzi metacyclogenesis process and on the parasite-host cell interaction.**Supported by:**CAPES, FIOCRUZ **Keywords:**Palmitoylation; dhhc palmitoil transferases; trypanosoma cruzi

PV024 - NEW INSIGHTS OF *TRYPANOSOMA CRUZI* CYTOSKELETON BY HIGH-RESOLUTION MICROSCOPY

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Trypanosomatids possesses a network of stable microtubules organized just below the plasma membrane called subpellicular microtubules. The association between these microtubules and the plasma membrane impairs the budding or fusion of vesicles to/from the plasma membrane. In Trypanosoma brucei and Trypanosoma cruzi the endocytic process occurs through a region of the plasma membrane, the flagellar pocket. Near to this structure, T. brucei possesses a cytoskeletal feature with unclear function, called bilobe, which aligns closely with the Golgi and is thought to function in Golgi replication and as an adaptor during cytokinesis. In T. cruzi, an additional endocytosis entrance, the cytostome-cytopharynx, is present and is physically linked to the flagellar pocket by four microtubules. The use of detergent-extracted T. cruzi preparations was found to be an effective way of discerning features of the cytoskeletal ultrastructure that are otherwise obscure. By negative staining, we could clearly observe two never seen cytoskeletal structures: 1) A hook around the axoneme of epimastigotes, followed by microtubules that were probable constituents of the cytopharynx. It is possible that this structure is homolog to the T. brucei bilobe. 2) A strap associated to the microtubule quartet of cytopharynx; this structure probably maintains the microtubules stably assembled. By electron tomography and 3D reconstruction we observed this strap underneath the preoral ridge and it seemed to limite this membrane domain. In T. cruzi trypomastigotes we could clearly observe the microtubule quartet of the cytopharynx going from the flagellar pocket towards the nucleus. We conclude that the microtubules of cytostome-cytopharynx complex are never disassembled, even in trypomastigote forms that do not present this invagination. These findings may contribute to reveal the structures essential for T. cruzi endocytic biology and functionality.**Supported by:**CNPq **Keywords:**Trypanosoma cruzi ; high-resolution microscopy; cytoskeleton

PV025 - ISOLATION OF TRYPANOSOMA CRUZI INTRACELLULAR AMASTIGOTES BY NITROGEN DECOMPRESSION

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Previous protocols for isolation of intracellular amastigotes of Trypanosoma cruzi from host cells using density gradients or anion-exchange chromatography are very laborious, time consuming and involve several medium changes. In this work we describe the fast and efficient isolation of viable intracellular amastigotes by nitrogen decompression (cavitation) followed by a few centrifugation steps, without density gradients. Five culture flasks with 1x106 infected Vero cells each were washed three times with PBS under gentle agitation, 48 hours post-infection, in order to eliminate contamination with extracellular amastigotes. The cultures were then trypsinized for 10 min at 37ºC and the trypsinization was halted by addition of cold fetal bovine serum 1:1 (v/v). The infected cells in suspension were lysed by nitrogen decompression (cavitation) in a 4639 cell disruption vessel (Parr Instrument Company, Moline, IL, USA), using 180 psi pressure for 5 min. Intact Vero cells were removed by low speed centrifugation (10 min, 800g), and intracellular amastigotes were recovered from the supernatant. Cell debris were removed from the amastigote fraction by three centrifugation steps at 2,000g for 5 minutes, to produce a supernatant of isolated intracellular amastigotes. Flow cytometry using a GFP-fluorescent T. cruzi clone showed that after cavitation almost all infected Vero cells were disrupted, releasing GFP-positive viable intracellular amastigotes. Starting from 5x106 infected Vero cells this isolation methodology routinely yielded 5x107 amastigotes, with typical shape and positive for the amastigote marker Ssp4 and for cruzipain (a reservosomal marker). The nitrogen decompression method for intracellular amastigote isolation described here is easier and faster than previously described protocols, yields viable amastigotes efficiently, and is likely to represent an excellent tool for studies on different aspects of amastigote biology. **Supported by:**CAPES, FIOCRUZ
Keywords: Intracellular amastigotes ; nitrogen decompression; isolation

PV026 - CHARACTERIZATION OF *LEISHMANIA AMAZONENSIS* PINX1 ORTHOLOG, A REGULATOR OF TELOMERASE ACTIVITY

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PinX1 is a telomerase inhibitor and has been implicated in telomere maintenance since in humans and yeast it is directly involved in the cell cycle-dependent trafficking of hTERT (human Telomerase Reverse Transcriptase) to telomeres. Thus, PinX1 regulates telomerase function by mediating its localization inside cells and loss of PinX1 leads to increased telomere length along with defects in chromosome dynamics. Since telomerase recruitment to telomere is the prerequisite for telomere extension, but the proteins involved in this process are still largely unknown in *Leishmania spp*., we decide to characterize the parasite PinX1 othologue. Among different *Leishmania* species we can find the ethiologic agents of leishmaniases, neglected tropical diseases of distinct clinical forms that affect million people around the world and to which there is no effective control or treatment. *Leishmania amazonensis* PinX1 orthologue contains both the G-patch (Glycine-rich) and the TID (Telomerase Interaction domain) domains that characterize it as an ortholog of eukaryotes PinX1. In addition, the protein PinX1 is predominantly nuclear in *L. amazonensis*. We are actually doing chromatin immunoprecipitation and FISH-IF to verify if LaPinX1 binds and co-localizes with parasite telomeres in vivo and also performing co-immunoprecipitation using specific immune serum to check for PinX1 and telomerase interactions. In the future, we intend to use the potential effects of PinX1 as a telomerase regulator to raise new models and questions of how telomeres and telomerase are important for parasite homeostasis. **Supported by:**PIBIC/CNPQ
Keywords: Telomere; leishmania amazonensis; lapinx1

PV027 - PHAGE DISPLAY TECHNOLOGY APPLIED TO SELECT NEW ANTIGENS TO BE USED AS VACCINE AGAINST LEISHMANIA INFANTUM INFECTION

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The development of cost-effective prophylactic strategies to prevent leishmaniasis has become a high priority. The present study used the phage display technology to identify new immunogens, which were evaluated as vaccine in BALB/c mice. Epitope-based immunogens, represented by phage-fused peptides that mimic Leishmania infantum antigens, were selected according to their affinity to antibodies from asymptomatic and symptomatic visceral leishmaniasis (VL) dogs' sera, by bio-panning cycles of phage display, using negative and positive selection processes. Twenty phage clones were selected, and they were evaluated by means of in vitro assays of the immune stimulation of spleen cells derived from naive or chronically infected with L. infantum BALB/c mice. Clones that were able to induce specific Th1 response, represented by high levels of IFN-γ and low levels of IL-4 were selected, and based on their selectivity and specificity, two clones, B10 and C01, were further employed in the vaccination protocols. BALB/c mice vaccinated with clones plus saponin showed both a high production of IFN-γ, IL-12, and GM-CSF, after in vitro stimulation with clones or L. infantum extract. These animals, when compared to controls (saline, saponin, wild-type phage plus saponin, or non-relevant phage clone plus saponin), showed significant reductions in the parasite burden in the liver, spleen, bone marrow, and paws' draining lymph nodes. Protection was associated with an IL-12-dependent production of IFN-γ, mainly by CD8+ T cells. These animals also presented decreased parasite-mediated IL-4 and IL-10 responses, and increased levels of parasite-specific IgG2a antibodies. This study describes two phage clones that mimic L. infantum antigens, which were directly used as immunogens and significantly reduced the parasite burden in the infected animals. This is the first study that describes phage-displayed peptides as successful immunogens in vaccine formulations against VL. **Supported by:**cnpq
Keywords: Phage display; vaccine; visceral leishmaniasis

PV028 - CLINICAL, EPIDEMIOLOGICAL AND MOLECULAR ASPECTS OF CUTANEOUS LEISHMANIASIS IN THE CENTRAL WESTERN REGION, BRAZIL.

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Protozoa of the genus Leishmania, which affects the skin and/or mucous membranes, cause American Cutaneous Leishmaniasis (ACL). In the Goiás state, 2798 ACL cases were been reported between 2007 and 2013. We investigated the epidemiological and molecular characteristics of ACL patients attended at the Hospital Anuar Auad in the period 2000 at 2006 and identify the species of Leishmania spp. The study included 152 patients with ACL, 124 were from of the state of Goiás and 28 at Mato Grosso, aged between 6-79 years, and 113 were male. For diagnosis, clinical and epidemiological, the laboratory data were collected, such as direct examination (DE), histopathology (AH), Montenegro skin test (MST), indirect immunofluorescence (IIF). ELISA using crude extract of L. (Viannia) braziliensis was performing. Characterization of Leishmania species was carried out by polymerase chain reaction (PCR). The positivity of ED, AH, MST, IIF and ELISA was 70.6%, 80.9%, 68.9%, 44.2% and 73.0%, respectively. Specific IgG to L. (V.) braziliensis were detecting in 84.7% of patients with mucosal leishmaniasis (ML), significantly higher than those found in patients with cutaneous leishmaniasis (CL), which was 69.0% ($p < 0.05$). Detection of IgG before and after treatment was performed and was observed a significant difference only in samples obtained from patients with CL after 6 and 18 months of treatment. PCR was performed on 69 samples obtained from scraping the edge of lesions of patients with ATL, these, 62 (89.8%) were positive, 53 patients with LC and 9 patients with ML. The samples were characterized by PCR as L. (V.) braziliensis (93.5%) and L. (L.) amazonensis (6.5%). It was found that 25.8% of cases of ACL in the state of Goiás occurred in Campestre, Goiânia, Aparecida de Goiânia and Niquelândia. PCR represents important and powerful tool in the diagnosis and species identification in ACL in endemic areas. **Supported by:**FAPEG
Keywords: Acl; pcr; central western

PV029 - CROSS-PROTECTIVE EFFICACY OF A MIMOTOPE-BASED VACCINE SELECTED IN LEISHMANIA INFANTUM AGAINST MURINE TEGUMENTARY LEISHMANIASIS

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Leishmaniasis presents a high morbidity and mortality throughout the world, where about 350 million people in 98 countries are at risk of contracting the infection. Among them, the Leishmania amazonensis species presents particular importance, since it is able to cause human disease, as well as a broad spectrum of clinical manifestations, from cutaneous to visceral leishmaniasis. The present study has used two mimotopes of Leishmania infantum identified by phage display technology as immunogens in BALB/c mice to cross-protect against Leishmania amazonensis. The epitope-based immunogens, B10 and C01, presented as phage-fused peptides; were used without association of a Th1 adjuvant, and clones were administered isolated or in combination into animals, in three doses regimen and immunological and parasitological parameters were evaluated, before and/or after challenge infection. Both clones showed a specific production of IFN-□ IL-12, and GM-CSF after in vitro spleen cells stimuli, and were able to induce protection. Significant reductions of parasite load of infected footpads, liver, spleen, bone marrow, and paws' draining lymph nodes were observed in the immunized mice, in comparison to the control groups (saline, saponin, wild-type and non-relevant clones). Protection was associated with an IL-12-dependent production of IFN-□ mediated mainly by CD8+ T cells, against parasite proteins. Protected animals also presented low levels of IL-4 and IL-10, as well as increased levels of parasite-specific IgG2a antibodies. The association of both antigens resulted in an improved protection in relation to their individual use. Besides, the absence of adjuvant did not diminish the cross-protective efficacy against infection. The present study describes for the first time two epitope-based immunogens selected by phage display in L. infantum, which cross-protected BALB/c mice infected with L. amazonensis. **Supported by:**cnpq **Keywords:**Phage display,; vaccine, ; tegumentary leishmaniasis.

PV030 - 24Sβ RIBOSOMAL GENE AS A POTENTIAL MARKER FOR INTRA AND INTERSPECIFIC DISCRIMINATION OF *TRYPANOSOMA RANGELI*

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Trypanosoma rangeli is a flagellate protozoan with wide distribution in Americas. Unlike other trypanosomatids causing severe and debilitating illness such as *T. cruzi* and *Leishmania* spp., this parasite is not pathogenic to humans. However, *T. rangeli* has high similarity of surface antigens with *T. cruzi*, which can lead to false-positive results in serological diagnosis of Chagas' disease. Ribosomal genes and transcripts are valuable tools to show variability and generation of molecular markers useful in phylogenetic analysis and differential diagnosis. The aim of this work was sequencing regions of 24Sβ gene from different genotypes of *T. rangeli* and compare them with homologous regions of other trypanosomatids. Several strains from *T. rangeli* were grown in LIT medium, 28°C. Nucleic acids were purified, DNA was amplified with specific primers and total RNA were analyzed in denaturing agarose gel. Amplicons were purified and sequenced. Two regions of 24Sβ gene were analyzed (24Sβ-1 and 24Sβ-2). Regarding interspecific variability, it was observed differences in rRNA size between *T. rangeli* and *T. cruzi*. PCR showed significant differences in 24Sβ-1 size between *T. rangeli* and *T. cruzi*, and sequencing confirmed a 106 bp insertion/deletion. Comparison with other trypanosomatids shows that *T. rangeli* rDNA sequences estudied are more related to *L. major* sequences (85% and 69% identity for 24Sβ-1 and 24Sβ-2, respectively) than to *T. cruzi* sequences (64% and 70% identity for 24Sβ-1 and 24Sβ-2, respectively). When compared with different genotypes of *T. rangeli*, both regions presented Group Specific Polymorphisms (GSPs), distinguishing KP1+ and KP1- genotypes. 24Sβ-2 presented more GSPs (6%) than 24Sβ-1 region (0,3%), showing its potential for intraspecific differentiation using techniques such as SNP-RFLP. Applying adequate molecular techniques, both regions have potential for intra and interspecific differential diagnosis. **Supported by:**FAPEMIG
Keywords: Ribosomal locus; polymorphism; trypanosoma rangeli

PV031 - THE BIOGENESIS OF THE CYTOSTOME-CYTOPHARYNX COMPLEX OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES DURING CELL DIVISION

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The cytostome-cytopharynx complex is the main site for endocytosis in epimastigote forms of Trypanosoma cruzi. The cytostome consists of an opening at the plasma membrane surface, near the flagellar pocket, followed by a profound and helical shaped invagination called cytopharynx. We have shown that this structure varies in length from cell to cell and is supported by two microtubule sets: a triplet that started underneath the cytostome membrane, and a quartet whose microtubules originated from staggered positions underneath the flagellar pocket membrane and followed the preoral ridge before reaching the cytopharynx. These two microtubule sets accompanied the cytopharynx forming a `gutter` and leaving a microtubule-free side, where vesicles bud or fuse. In the present work, we investigate how the cytostome-cytopharynx duplicates during epimastigote cell division by using advanced electron microscopy. Cells were synchronized using hydroxyurea. After synchronization the material was processed to transmission electron microscopy and observed using FIB-SEM microscopy and electron-tomography. We observed that at the end of G2, when parasites already have two flagella but a dividing kinetoplast, the cytostome closes and the cytopharynx vesiculates. At this stage, the accompanying microtubules remained with the usual helical format and disposition. During nuclear mitosis, the microtubules depolymerized, remaining only a small part of the quartet, near the old flagellar pocket. The new flagellar pocket also possessed a short microtubule quartet. After nuclear mitosis, the microtubules started to grow again and were directed towards the Golgi complex. A new cytostome-cytopharynx was forming in each cell from the flagellar pocket membrane during cytokinesis. We could conclude that the cytostome-cytopharynx complex is disassembled during the cell division and formed de novo during the cytokinesis **Supported by:**CNPq
Keywords: Endocytosis; tridimensional reconstruction ; cell cycle

PV032 - LIPID DROPLETS AND PROSTAGLANDINS ARE KEY MEDIATORS IN THE ELICITATION OF THE HUMORAL IMMUNE RESPONSE IN AEDES AEGYPTI

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In mammals, lipid droplets (LDs) are organelles that play key roles in the modulation of immune response, through the production of lipid mediators such as prostaglandins. The role of LDs and prostaglandins mediating insect immune responses is still unknown. We show that *Aedes aegypti* Aag2 cells and midgut epithelia accumulate LDs when challenged with bacteria and viruses. LD enrichment correlates with prostaglandin production by Aag2 cells, an immune responsive cell line from *Aedes aegypti,* and by midgut cells. Acetylsalicylic acid (ASA), an inhibitor of prostaglandin production, decreases the expression of several components of the mosquito's major immune pathways, thereby rendering Aag2 cells more permissive to bacterial and viral infection. Similarly, in mosquitoes, the down-regulation of genes involved in immune response, by ASA, compromises the insect's survival after immune challenges. In this study we show for the first time that LDs and prostaglandins are key components for generation of an effective immune response against bacteria and Dengue virus, opening a new venue for development of novel vector control strategies.**Supported by:**CNPq, Faperj
Keywords: Aedes aegypti; prostaglandin; lipid droplets

PV033 - DYNAMICS OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* CO-CULTURE *IN VITRO*

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Trypanosoma cruzi (*Tc*) and *Trypanosoma rangeli* (*Tr*) are bug-transmitted kinetoplastid parasites occurring in sympatry. These species are morphologically similar, especially on the replicative epimastigote stage. Due the occurrence of mixed infections, we have assessed the dynamics of co-culture *in vitro* using parasites expressing distinct fluorescent proteins. Parasites from *Tc* CL Brener, Y and SC 28 strains and *Tr* Choachí strain were transfected with pTREX-eGFP/pTREX-eRFP or pROCK-eGFP plasmids. After selection with G-418, at least 91.80 % of *Tr* and 98.83% of *Tc* transfectants were fluorescent as revealed by flow cytometry. Also, transfectants maintained the same fluorescence intensity after cyclic passages in *Rhodnius prolixus* and Balb/C mice. Genomic integration of GFP/RFP was confirmed by PFGE for *Tc* CL Brener and *Tr* Choachí strains. The dynamics of co-culture was evaluated using different proportions of each species (10:1, 5:1, 1:1, 1:5, 1:10) in LIT medium (10% FCS at 27.5oC). The transfectants revealed no difference on their growth patterns when compared to the wild type cells but, despite the proportion of the *Tc* strain used, *Tr* revealed a reduced growth when in co-culture with *Tc*. Despite the parasites strains, co-infection of THP-1 cells using different proportions of transfectants revealed a lower number of amastigotes/cell when compared to the control *Tc* infection, but not differing on the number of infected cells. Preliminary assays of in vitro interaction of *R. prolixus*-derived hemocytes and *Tr* (GFP) showed no signs of multiplication of the parasite within these cells. Our results suggest that *in vitro* dynamics of axenic *Tc* and/or *Tr* culture and the interaction of these parasites with a phagocytic cell line (THP-1) are affected during co-cultures or co-infections, despite the proportion of each species. In vivo studies of *Tc*/*Tr* co-infection in mice and triatomines are in progress. **Supported by:**CAPES, CNPq, FINEP
Keywords: Trypanosoma cruzi; trypanosoma rangeli; co-culture/co-infection

PV034 - FUNCTIONAL ANALYSIS OF A NOVEL CYTOSOLIC PARTNER FROM A NON-CONVENTIONAL EIF4F-LIKE COMPLEX FROM TRYPANOSOMA BRUCEI

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TbEIF4E5 (TbE5) is a Trypanosoma brucei homologue of the initiation factor eIF4E, a subunit of the tripartite eIF4F complex, which acts in the recognition of the cap structure during translation initiation. Initial studies aiming to identify specific complexes which include TbE5 identified the protein named Tb117.5 (Tb927.11.6720) as constituent of a complex involving also homologues of eIF4G (TbEIF4G1 and TbEIF4G2), the second subunit of eIF4F and which functions as a scaffold for the complex, among other proteins. Tb117.5 is annotated as “hypothetical” and bioinformatic analysis by PHYRE2 revealed the presence of methyltransferase and guanylyltransferase domains which are associated with mRNA 5’ cap formation in higher eukaryotes. The reciprocal Mudpit analysis of these partners confirmed the interactions of these proteins and blue native gel migration was also consistent with the formation of a unique complex. Immunolocalization of the Tb117.5 fusion protein in the Tb117.5−/PTP line via the PTP tag revealed a diffuse cytosolic distribution with numerous foci of greater intensity, and apparent exclusion from the nucleus. RNA interference (RNAi) directed against the Tb117.5 transcript in procyclic cells showed no dramatic effect on cell growth in standard growth medium. Double allele knockout of the Tb117.5 gene was attempted two times, and all attempts failed in the elimination of the second targeted allele. We interpret this to signify that the Tb117.5 protein is essential, and the lowered protein levels achieved by our RNAi inductions allowed the cells to keep viable. We also found Tb117.5 respond to nutritional stress forming nuclear peripheral granules, thought to be involved in mRNA maturation. This data is intriguing as it shows a cytoplasmic localization for a protein that possess domains thought to be involved in the maturation of mRNA and also as part of a eIF4F like complex. **Supported by:**CAPES
Keywords: Trypanosoma brucei; eif4f; tb117.5

PV035 - DCS TRANSFERRED FROM MICE VACCINATED WITH THE C-TERMINAL MOIETY (F3) OF NUCLEOSIDE HYDROLASE NH36 REDUCE THE PARASITE LOAD OF L.(L.) CHAGASI INFECTED MICE

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Visceral leishmaniasis (VL) is a chronic and lethal parasite disease against which no human vaccine is available. The characteristic cellular immunosupression was described as being mediated in part, through the spatial segregation of dendritic cells (DCs) and T cell lymphocytes due to altered frequencies and migration capabilities of DCs. Recently, we demonstrated that infected animals show a 5.08 fold increase of spleen relative weight, a 19.6 fold increase of parasite load and a 4.55 increase of total DCs counts, when compared to naïve controls. The NH36 and F3 vaccines formulated in saponin prevented VL in mice. The F3 vaccine induced the highest efficacy showing 95% and 49% reduction the parasite load and splenomegaly, respectively. The NH36 vaccine reduced by 87% the parasite load and by 39% the spleen relative weight. Both vaccines also prevented the increase in total counts of DCs and their migration defect. In this investigation we transferred splenic DCs from normal, L.(L.) chagasi infected and NH36 or F3-saponin vaccinated and infected mice, into BALB/c mice previously infected with L.(L.) chagasi. Seven days after transfer, animals were euthanized and analyzed for their parasite load. Significant decreases of the spleen (64%) and liver parasite load (29 %) were detected only in mice that received DCs from animals vaccinated with the F3-saponin vaccine. Our results suggest that the potential immunotherapeutic efficacy of the NH36 vaccine on VL is partially mediated by the sustained DCs migratory capabilities and APC activities related to the C-terminal moiety epitopes. **Supported by:**FAPERJ; CAPES; CNPq
Keywords: Visceral leishmaniasis; murine; dendritic cells

PV036 - COMPARING THE USE OF ZINC FINGERS AND CAS9 NUCLEASES TO IMPROVE GENETIC MANIPULATION IN TRYPANOSOMATIDS

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Stable transfection of *T. brucei* and *T. cruzi* relies in the integration, by homologous recombination (HR), of linear plasmids into the parasite genome. However integration events occur at low rates and therefore, overall efficiency for generating stable transfectants by conventional electroporation is generally low. Zinc finger nucleases (ZFNs) and the CRISPR-associated protein Cas9 - mediated genome modification have been used to improve the efficiency of genetic manipulation in different cell types including protozoans such as *Plasmodium spp*. ZFNs are synthetic endonucleases that have large recognition sites, whereas Cas-9 are RNA-guided endonucleases (RGENs) that require the formation of a complex with a single guide RNA (sgRNA) that directs the enzyme to specific DNA sites. Two major concerns about these systems are their toxicity and specificity, since expression of both nucleases may result in off-target mutations. We have tested the use of ZFNs and CRISPR/Cas9 as new tools to facilitate the generation of gene knockouts and DNA integration into *T. cruzi* genome by transfecting *T. cruzi* epimastigotes expressing eGFP with the pROCK-hygro plasmid containing a pair of ZFNs designed to cleave the eGFP sequence and with the pROCK-hygro plasmid containing the Cas9 sequence. Although expression of Cas9 was readily detected in transfected parasites, no evidence for the ZFNs expression was obtained either by western blot or northern blot analyses. To verify if ZFNs may be toxic to trypanosomes, we expressed a pair of ZFNs in *T. brucei* under the control of tetracycline and demonstrated that, although ZFNs expression causes cell growth inhibition, it also resulted in a 3-5 fold increase in transfection efficiency of bloodstream forms. Currently we are comparing the efficiency of integration of drug resistance or fluorescent protein markers into the eGFP locus of *T. cruzi* using parasites transiently expressing ZFNs and a cell line stably expressing Cas9. **Supported by:**Cnpq
Keywords: genetic manipulation; zinc finger nucleases; crispr-associated protein cas9

PV037 - IMMUNOTHERAPY AGAINST MURINE CUTANEOUS LEISHMANIASIS BY L.(L.) AMAZONENSIS WITH THE RECOMBINANT CHIMERA CONTAINING THE N- AND C-DOMAINS OF NUCLEOSIDE HYDROLASE FROM LEISHMANIA (L.) DONOVANI

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The Nucleoside Hydrolase (NH36) is the main marker of the FML complex (Fucose and Mannose-binding) of Leishmania (L.) donovani antigen of Leishmune® (vaccine, licensed for prophylaxis of canine visceral leishmaniasis). A recombinant chimera F1F3 including the F1 and F3 domains in tandem and cloned in the pET28b expression system was obtained with optimized codons for E. coli. Its immunogenic potency and vaccine efficacy were compared with F1 and F3 peptides independently expressed and with the addition of both peptides for immunotherapy of cutaneous leishmaniasis by L. (L.) amazonensis. The chimera induced the highest IgG2a antibody titers, the strongest IDR and the secretion of IFN-γ, TNF-α and IL-10. The F1 or F1+F3 addition vaccine generated the earliest intracellular citokyne expression in T CD4+ cells while the chimera induced an stronger expression later. The chimera induced the highest proportions of CD8+-IL2+ T cells, and together with the F1+F3 vaccine, of T CD8+-IFN-γ+ secreting cells. The frequencies of CD8+-TNF-α+-IL2+ and CD8+-IFN-γ+-IL-2+ were decreased, indicating the curative potential on the infection. The CD8+ IFN-γ+-TNF-α+ secreting cells were increased after immunotherapy with all vaccines. Increases in the frequencies of CD8+ T cells TNF-α+-IL2+-IFN-γ were observed only in mice treated with F1, F3 or F1+F3 vaccines. The F3 vaccine was superior to the chimera, and this, stronger than the addition, in reducing the lesion sizes. The chimera was equivalent to the addition F1+F3 and the F1 vaccine in the reduction of parasite load; confirming the relevance of the F1 domain in the cure of advanced cutaneous leishmaniasis. **Supported by:**FAPERJ; CAPES; CNPq
Keywords: Cutaneous leishmaniasis; immunotherapy; chimera

PV038 - FUNCTIONAL ANALYSIS OF ATEXIA TELANGIECTASIA MUTATED (ATM) AND ATEXIA TELANGIECTASIA MUTATED-RELATED RAD3 (ATR) HOMOLOGOUS IN TRYPANOSOMA CRUZI

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The Trypanosoma cruzi cell cycle includes proliferative form and forms that are not able to proliferate. The signaling pathway that leads disruption of DNA replication in non-proliferating forms has not been elucidated. Since DNA damage can arrest the cell cycle, we are interested in evaluating whether the signaling pathways of DNA damage are present and may be activated in non-proliferative forms of T. cruzi. Here we searched for homologous of ATM and ATR, which are proteins involved in the DNA damage signaling pathway. We have identified sequences homologous to ATM (TcCLB.506533.34) and to ATR (TcCLB.506223.120). We found that ATM homologous contains the PI3Kc_like super family domain and the PIKKc_ATR domain was found in ATR homologue. Therefore, primers were designed in order to amplify fragments of these putative genes that are not present in other unrelated sequences. Then, these amplicons were cloned (using pCR 2.1 plasmid) and sub-cloned in expression vector (using pET 28a+ plasmid). Partial putative proteins are now being expressed in order to raise specific antibodies. The pattern of ATM or ATR distribution in epimastigote-proliferative cells under DNA damage will be evaluated in order to check the role of these proteins in the DNA damage signaling. We will also check if ATM/ATR proteins are activated in non-replicative trypomastigote form. FAPESP 2014/21557-6 **Supported by:**Fapesp
Keywords: Tripanossoma cruzi; atm; atr

PV039 - THE NATURAL COMPOUND, PHYSALIN F, ALTER *RHODNIUS PROLIXUS* MICROBIOTA AND THE INSECT IMMUNE RESPONSE

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The triatomine, *Rhodnius prolixus*, is vector of the parasite *Trypanosoma cruzi*, which is the causative agent of Chagas disease. Vector control is the most useful method to prevent this disease. In this context, the natural compound, physalin B, has shown potential activity through inhibition of the parasite development in the insect vector, modulation of the insect immune responses, and its microbiota density. Herein, we investigated the effect of the physalin F on immune responses and microbiota in the digestive tract of *R. prolixus*. The physalin F (1 or 10ug/mL of blood) was administered via the insect blood feeding. Control insects were orally treated with the solvent used to solubilize physalin F (0.5%DMSO). The flora of the digestive tract was analyzed by counting colony forming units (CFU) eight days after treatment with physalin F. The insect immune responses such as antibacterial activity, reactive nitrogen species and phenoloxidase activity was analyzed by using turbidometric assay, quantification of the nitrite production by *Griess* colorimetric kit and L-DOPA melanization in insect samples collected nine days after treatment. The results were statistically analyzed using one way ANOVA on the GraphPad Prism program. Treatment with the lowest concentration of physalin F (1µg/mL of blood) significantly increased the activity of phenoloxidase and enhanced the production of reactive nitrogen species when compared to control insects. However, the group of insects treated with physalin F (10mg/mL of blood) decreased the antibacterial activity when compared to control and increased the number of bacteria in the gut when compared to the insect control (DMSO). These results showed the effect of physalin F in the *R. prolixus* immune response and alteration of microbiota density in the insect digestive tract. These insect physiological modification caused by physalin F could interfere on parasite development in the invertebrate host, which will be further investigated. **Supported by:**FAPERJ
Keywords: Rhodnius prolixus; physalin; insect immunity

PV040 - THE RECOMBINATION PROCCESS COULD BE INVOLVED IN GENERATION OF HYBRID STRAINS OF TRYPANOSOMA CRUZI

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Trypanosoma cruzi is the etiological agent of Chagas Disease. The variety of symptoms of Chagas disease can be associated with the genetic variability of different strains of Trypanosoma cruzi. Studies subdivided T. cruzi into six discrete taxonomic units (T. cruzi I to T. cruzi VI). The occurrence of recombination events are rare in the literature, but it is known that they occur between populations, creating hybrids populations of the parasite. In others organisms, the recombination process is associated with the generation of hybrid cells. The homologous recombination repair (HRR) is also involved in the repair of the DNA double strand break. While other organisms have other pathways, such as non-homologous end joining to deal with double strand breaks, Trypanosoma cruzi relies only on HRR to deal with this kind of damage. We studied the response to ionizing radiation, an agent capable of causing double strand breaks, in different strains of Trypanosoma cruzi. Although the parasite does not face high doses of radiation on his life cycle, it can resist to doses as high as 500Gy. It was observed that non-hybrid lineages have a distinct phenotype when compared with a hybrid strain in response to this kind of damage, i.e, the hybrid cell respond faster to this lesion. The analysis of genes involved in the repair revealed differences in the sequence of certain proteins between strains, and the prediction of the impact of these changes showed that the interaction between the proteins of each strain could be different. In addition, the expression of some HRR essential genes are high in the hybrid strain. These data suggest that a more efficient recombination process is important to the generation of hybrid strains in Trypanosoma cruzi. **Supported by:**CNPq, CAPES, FAPEMIG
Keywords: Trypanosoma cruzi; recombinação homóloga; brca2

PV041 - STUDY OF THE IMMUNE RESPONSE OF *RHODNIUS PROLIXUS* INDUCED BY NITROGEN REACTIVE SPECIES TO INFECTON BY *TRYPANOSOMA CRUZI*

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The study of *Rhodnius prolixus* immune response allows us to understand the physiology of this triatomine specie, its interaction with microbiota and *Trypanosoma cruzi,* the etiologic agent of Chagas disease. The production of reactive nitrogen species (RNS) by triatomines seems to be crucial for a successful *T. cruzi* establishment in the intestinal tract of the vector. Further investigations are needed to understand the action of RNS on both the parasite and the microbiota in relation to other insect's immune responses. The objective of the present work was to analyze the importance and influence of RNS on the vector, the parasite and microbiota. Therefore, fifth instar nymphs of *R. prolixus* were treated with enhancers and inhibitors of nitric oxide synthesis. The amount of nitrite, phenoloxidase and antimicrobial activity were assessed at different days after feeding in different tissues. Numbers of microbiota and *T. cruzi* were determined using the UFC method, parasite counting by microscopy. Transcript abundance of nitric oxide synthase (NOS) was analyzed by RT-PCR. The highest amount of nitrite corresponding to RNS in the anterior midgut of control insects was found at the first days after feeding. In the posterior midgut, the highest amount of nitrite was found at the thirteenth day after feeding. The transcript abundance of NOS encoding cDNA was higher in fat body than in other tissues, highest at one day after feeding decreasing with time, simultaneously increasing in other tissues. Insects infected with *T. cruzi* showed a lower production of nitrite, lower activity of phenoloxidase enzyme and lower microbiota. After induction of the RNS synthesis using L-arginine, in *T. cruzi* infected insects the parasitemia decreased and numbers of intestinal bacteria increased, as well as phenoloxidase and antibacterial activity. Inhibition of RNS by treatment with L-NAME increased parasitemia and decreased phenoloxidase activity in uninfected insect **Supported by:**CAPES
Keywords: Rhodnius prolixus; insect immunity; reactive nitrogen species

PV042 - INTRACELLULAR LOCALIZATION OF A NEW FLUORESCENT CHALCONE PROBE FOR DETERMINATION OF CHALCONE TARGETS IN LEISHMANIA.

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Chalcones are a potential class of new antileishmanials whose target is still unknown. We have reported the selective activity of the chalcone (CH8) against Leishmania spp., suggesting a conserved drug target. In this work, we proposed to synthesize fluorescent probes based on CH8 and to image its intracellular localization into the parasite with the goal of identifying new drug targets. CH8 analogues were synthesized by aldol condensation. Of all the synthetic analogues, the nitrochalcone (NAT22) was chosen for its highest activity. Introduction of a linker unit afforded the intermediate PGS12.2. Subsequent coupling with a fluorescein containing moiety yielded the fluorescent probe PGS14.4. To antileishmanial activity, promastigotes of L.amazonensis, L.donovani, L.braziliensis and L.infantum were incubated at 26°C with different concentrations of NAT22, PGS12.2 and PGS14.4 for 72 hours when the cell viability was assayed by AlamarBlue. NAT22 showed excellent activity against all the tested species, with IC50 varying from 0.2µM to 2.2 µM. PGS12.2 activity was equal to original NAT22, indicating that addition of the spacer did not affect the pharmacophore group. Nonetheless, the PGS14.4 probe showed significantly decreased activity against all species, likely due to its large molecular size (MW>1000) that inhibits membrane permeation. To evaluate the intracellular localization, L. amazonensis promastigotes were permeated with digitonin prior to incubation with PGS14.4 and the organelle-specific dyes Mitotracker, ER-Tracker, Lysotracker and Hoescht and imaging with confocal microscope. In agreement with our early results of ultrastructural mitochondria changes in L. amazonensis promastigotes treated with a natural chalcone, the results showed that PGS14.4 molecule preferably concentrates in this organelle, suggesting the localization of the molecular target. In summary, PGS14.4 appears to be a useful tool for the identification of the chalcone target in Leishmania. **Supported by:**CAPES, CNPq
Keywords: Leishmania promastigotes; chalcone; pgs14.4

PV043 - INTERCLONAL VARIATIONS IN THE MOLECULAR KARYOTYPE OF TRYPANOSOMA CRUZI: CHROMOSOME REARRANGEMENTS AMONG CL-STRAIN DERIVED CLONES

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T. cruzi exhibits remarkable inter- and intra-strain karyotypic heterogeneity. Most karyotypic polymorphisms are currently uncharacterized with respect to content and origin. Lima et al. (Plos One 2013, 8|5|e63738) have reported karyotypic variations in clones of the same parental strain (G strain), suggesting the occurrence of aneuploidy and mosaicism in T. cruzi. The present studies were designed to extend this analysis to CL-strain derived clones CL Brener (CLB) and CL14 and subclones of CLB isolated after nutritional stress. The karyotypes of CL strain, CLB and CL14 and CLB subclones were analyzed by hybridization of chromosomal bands with chromosome specific-markers. This analysis revealed minor karyotypic differences between CL strain and its clones, most of chromosome length polymorphisms were of small amplitude. For instance, CL strain and CLB exhibited a large chromosomal band of 2.09 Mb which underwent a deletion resulting in a band of <2 Mb in CL14. The karyotypes of CLB and its subclones are generally similar, the syntenic groups are conserved among the isolates. However, large chromosome deletions were observed in subclones isolated after nutrient stress. We used comparative genomic hybridization (aCGH) to compare CL strain, CLB and CL14 . We found 145 chromosomal abnormalities with average size of 75.64 kb, 80 of them (55.17%) were related to gain/amplification of DNA and 65 (44.83%) with loss/deletions. The most of chromosomal abnormalities had between 10 and 50 kb (33.10%) and only 4 rearrangements were larger than 300 kb. We identified a 295 kb deletion in one of homologous chromosomes located on the band of <2 Mb in clone CL14. This deletion occured in an enriched region of calpain-like genes indicating a possible fragile site. Integration of aCGH data with those from hybridization of chromosomal bands provided significant information regarding the frequency and variety of chromosomal rearrangements observed in this protozoan parasite. **Supported by:**FAPESP, CNPq, CAPES **Keywords:**T. cruzi; polymorphisms; acgh

PV044 - EVALUATION OF THE FUNCTIONS OF XPC AND CSB PROTEINS IN TRYPANOSOMA CRUZI.

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Nucleotide Excision Repair (NER) is a versatile DNA repair pathway, responsible for detecting and removing distorting lesions in the DNA double helix. The pathway is divided into two subpathways, global genome repair (GGR) and the transcription coupled repair (TCR). In most eukaryotes, XPC protein functions to detect lesions and initiate GGR throughout the genome, while CSB protein detects and directs TCR in actively transcribed genes. Trypanosoma cruzi is the etiological agent of Chagas' disease, a tropical infirmity that affects 10 million people in tropical regions of the globe. Like all kinetoplastids, T. cruzi displays highly unusual gene expression, with virtually all its nuclear genes transcribed in multigenic gene clusters and little evidence for control of gene expression at the small number of poorly defined promoters. In this study, we show that NER in T. cruzi, similar to T. brucei, is organized in a different way from most characterised eukaryotes. Depletion and overexpression of T. cruzi XPC and CSB proteins is described, revealing no evidence that XPC is involved in UV- or ciplatin- induced damage, but depletion of XPC results in delayed cell cycle progression and multinucleated cells. Depletion of CSB causes increased sensitivity to UV, even though the lesions induced are slowly repaired. In addition, depletion of CSB increases sensitivity to cisplatin and MMS, which are rapidly repaired. Overexpression of CSB caused elevated mortality at high levels of UV in an ATM/ATR-dependent manner, since that death can be abolished in presence of caffeine. These results indicate the predominant use of TCR in T. cruzi, perhaps due to the transcriptional processes in the parasite. **Supported by:**CNPq, CAPES, FAPEMIG, Fundo Newton **Keywords:**Trypanosoma cruzi; xpc; csb

PV045 - CLONING AND EXPRESSION OF GLUCOSAMINE-6-PHOSPHATE *N*-ACETYL TRANSFERASE FROM *TRYPANOSOMA CRUZI*

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It's well established that amino acids are crucial during *Trypanosoma cruzi* life cycle, since they can be used as carbon and energy sources and still can participate in several biological processes that help the parasite to adapt to different changes of environment. A topic relatively unexplored is the relationship between amino acids and amino sugars biosynthesis. Amino sugars are important for glycosylation of glycoconjugates, including membrane proteins. In this proposal, we focus on one of the initial steps of the amino sugar biosynthesis. Firstly, L-glutamine and D-fructose-6-phosphate are substrate of glucosamine-6-phosphate aminotransferase (already being studied in our laboratory), yielding glutamate and glucosamine-6-phosphate. This product is acetylated by glucosamine-6-phosphate N-acetyltransferase (GNA, EC 2.3.1.4), producing *N*-acetylglucosamine-6-phosphate, one precursor of UDP-GlcNAc biosynthesis. Our goal is to clone and characterize the GNA from *T. cruzi*, and evaluate their role in parasite biology. GNA was cloned in pET24a$_{(+)}$ vector and expressed with activity in Escherichia coli BL21codon plus strain. We also measured the GNA specific activity using extract of epimastigotes forms. Presently, we are looking for optimizing the purification conditions to further to characterize its kinetic and thermodynamic parameters. **Supported by:**CNPq, Fapesp, CAPES
Keywords: amino sugar; gna; glucosamine

PV046 - GLUTAMATE AND GLUTAMINE ANALOGUE AZASERINE INTERFERE IN CELL PROLIFERATION AND DIFFERENTIATION OF TRYPANOSOMA CRUZI, AND INTERACT WITH STRESS CONDITIONS.

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Chagas' disease or American trypanosomiasis is an antropozoonosis that affects millions of people in Americas, mainly Central and South America and Mexico. Nowadays, two drugs are available for treatment of Chagas' disease: benznidazole and nifurtimox. Chagas' disease is caused by the flagellate intracellular parasite *Trypanosoma cruzi*, which have a complex life cycle alternating between invertebrate and mammalian hosts. During the life cycle, the parasite needs to survive in different conditions including the availability of different energy and carbon sources, such as carbohydrates or amino acids. The study of the role of amino acids metabolism in different biological processes, such as, energy production, stress resistance, differentiation and infectivity is important to better understand how the parasite survives in different environmental conditions. The amino acids glutamate and glutamine have been studied in some extension but little is known about their importance in the life cycle of *T. cruzi*. The aim of this work was to evaluate the interference of glutamine and glutamate analogue Azaserine in cell proliferation, differentiation and resistance to stress conditions in epimastigotes forms of *Trypanosoma cruzi*. Azaserine inhibited the proliferation of epimastigotes (IC_{50} 7.9 µM) and also diminished the parasite´s viability when the parasites was exposed to thermal and nutritional stress. Moreover, this analogue diminished metacyclogenesis, the differentiation process from epimastigotes to metacyclic tripomastigotes occurring in the insect vector. **Supported by:**CNPq
Keywords: Amino acids; analogues; azaserine

PV047 - INTERACTION BETWEEN PROTEINS AND THE EFFICIENCY OF CAP4-SL RNA PROCESSING IN TRYPANOSOMATIDS

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Before being added to the 5' end of every kinetoplastid mRNAs by trans-splicing, Spliced Leader (SL) RNA undergoes modifications such as methylations of the first 4 nt providing maturation of cap4. MTR1 is responsible for the 2'-O-ribose methylation of the first nt. MTR1 knockout/ knockdown resulted in impaired SL RNA processing, retarded growth under stress conditions and 50% less methionine incorporation. Mass spectrometry identified the complex associated to MTR1, which is composed by the pseudouridylation machinery and a methyltransferase associated protein called MTAP. Bioinformatic analyzes predict that MTAP may be physically associated with MTR1 being the link between these machineries. Our proposal was to confirm this interaction using the yeast two-hybrid assay (Y2H).
MTAP and MTR1 were cloned into Y2H vectors and their expression checked by western blotting. The yeast used in Y2H does not produce histidine and die in the presence of 3AT (competitive inhibitor of the HIS3 gene product). Only when bait and prey interact with each other HIS3 is expressed in enough amounts to suppress the inhibitory effect of 3AT enabling yeast to grow in selective medium. The interaction between MTAP and MTR1 can be visualized by growing colonies on plates containing increasing concentrations of 3AT. No growth was observed in the negative control.
MTR1 mutated in the catalytic core abolish methyltransferase activity. K95A, D207A, E285A, and K248A mutated genes were cloned into Y2H vectors. Growth of colonies on plates containing increasing concentrations of 3AT showed that mutations in the catalytic site did not abolish the interaction between MTR1 and MTAP suggesting that the protein conformation was not altered as well as the complex formation. In order to know which domains are responsible for the interaction with MTAP, MTR1 fragments cloning is underway. **Supported by:**CNPq, FAPESP and NIH
Keywords: Methyltransferase; spliced leader rna; yeast-two-hybrid

PV048 - USE OF DICRE-BASED CONDITIONAL KNOCKOUT TO STUDY THE ROLE OF THE C-TERMINAL DOMAIN OF LMRAD9 IN THE LEISHMANIA DNA DAMAGE RESPONSE

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The integrity of the eukaryotic genetic information relies on molecular mechanisms that sense, signal and repair DNA injuries. One major player in these processes is the heterotrimeric complex Rad9-Hus1-Rad1 (9-1-1 complex), which participates in the activation of the ATR signalling pathway and also interacts with DNA repair proteins. We have identified the 9-1-1 homologue in the protozoan *Leishmania (*please refer to the Damasceno et al., abstract in this meeting), though initial characterization of the subunits suggests marked structural and functional divergence in this parasite. For instance, the LmRad9 subunit is predicted to be composed of 718 amino acids, twice the size of its human homologue. Such dissimilarity is mainly due to a longer C-terminal domain, which corresponds to 44% of the protein and is predicted to be an unstructured region. We are interested in studying the function of LmRad9 C-terminal domain, as it is the main target for regulatory phosphorylation in other eukaryotic counterparts. In order to improve our understanding of LmRad9 function, we have generated a cell line that allows DiCre-based conditional knockout. After induction of LmRad9 knockout, cells were unable to sustain proliferation, indicating that this protein is essential for parasite survival. Ongoing work is focused on the generation of conditional LmRad9 knockout cell lines that also express C-terminally truncated versions of LmRad9. The effect of these deletions will be investigated by evaluating interaction with LmHus1 and LmRad1, cell cycle progression profile and response to genotoxic stress. We are also working on the identification of new LmRad9-interacting factors. The role of the C-terminal domain of LmRad9 on the establishment of these interactions is also going to be evaluated. **Supported by:**CAPES, FAPESP
Keywords: Dna damage; 9-1-1 complex; leishmania

PV049 - LOW PREVALENCE OF LRV1 IN LEISHMANIA BRAZILIENSIS STRAINS ISOLATED IN THE STATE OF MINAS GERAIS

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Leishmania RNA virus (LRVs) are commonly found infecting Leishmania species, especially from the subgenus Viannia. LRV1 is a double-stranded RNA virus (Totiviridae) first described in L. guyanensis and L .braziliensis strains from the Amazon region. Previous reports have shown that L. guyanensis infected with LRV1 causes a higher pro inflammatory profile partially caused by the activation of Toll like receptors 3 (TLR3) with the viral dsRNA. The presence of LRV1 infected strains was already detected in biopsy from patients with cutaneous leishmaniasis (CL) from Caratinga, Minas Gerais state. However, no information for LRV1 presence are available for other endemic regions of the State, such as São João das Missões, where many cases of CL are reported in the Indigenous Reserve of Xacriabá. The main objective of this study is to prospect the presence of LRV1 in Leishmania strains isolated from two endemic regions in the state of Minas Gerais (Caratinga and Xacriabá) and others regions of Brazil. The RNA from the strains was extracted by Trizol and cDNA synthesized using the Super script III kit from Invitrogen. PCR reactions used primers for the viral capsid and cDNA from L. guyanensis (MHOM/BR/75/M4147) was used as positive control. The band profiles were vizualized in agarose gels (1.0%) using gel red. Beta-tubulin gene was used as control. To this date, we prospected 9 strains from Xacriabá, 12 strains from Caratinga and one isolated from a sand fly from Pará. Different from the previous studies observed in Caratinga, the presence of LVR1 in parasites isolated from patients was not detected. Similar results were observed for the patients from Xacriabá and other Brazil regions. Those results suggest that the prevalence of LRV1 in L. braziliensis strains seem to be very low at least in the Southeast of Brazil. **Supported by:**CNPq and FAPEMIG **Keywords:**Leishmania braziliensis; lrv1; prevalence

PV050 - FUNCTIONAL CHARACTERIZATION OF TCRLP, A RAS RELATED GENE FROM *TRYPANOSOMA CRUZI*: PRODUCTION OF PARASITE LINEAGES EXPRESSING DOMINANT POSITIVE AND NEGATIVE MUTANTS.

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Ras family GTPases are key regulators in signaling pathways leading to cellular proliferation and/or differentiation in many eukaryotic organisms. These GTPases undergo a classical activation cycle in which they are active when bound to GTP and inactive after GTP hydrolysis (GDP bound). Much of our current knowledge about the cellular functions of Ras family GTPases derives from analysis of cells expressing positive dominant (GTP locked) and negative dominant (GDP locked) versions of *Ras* genes. The genome of the parasite *Trypanosoma cruzi* possess only one Ras family gene, designated *TcRlp* (T. cruzi Ras-like protein). As the role of *TcRlp* in parasite physiology is still unknown, we aim to initiate the characterization of its function by producing genetically modified parasite lineages expressing dominant positive and negative forms of this gene. *TcRlp* ORF was PCR amplified from Dm28c genomic DNA, cloned into *pGEM-T-Easy* vector and sequenced to confirm identity and integrity. We performed PCR-based site-directed mutagenesis to produce *TcRlp-S17N* (negative dominant), *TcRlp-G12V* and *TcRlp-Q61K* (two positive dominants) versions of the gene. These mutants were first cloned into *pGEM-T-Easy* vector and sequenced to confirm mutagenesis procedure. The three mutant genes were further subcloned into pTEX-GFPn expression vector to produce fusion constructs with the Green Fluorescent Protein gene. Resulting constructs were transfected into Dm28c epimastigotes by electroporation. Epimastigotes expressing these constructs were selected with G418 and expression of recombinant mutant genes was confirmed with RT-PCR and fluorescence microscopy. These mutant lineages will be employed to investigate the cellular role of *TcRlp.* If we detect an involvement of this gene in critical events of *T. cruzi* physiology, it will be considered as a potential new molecular target in the drug search of drugs for Chagas disease chemotherapy, as occurs with the Ras proto-oncogene in human tumors. **Supported by:**FAPERJ - FUNEMAC
Keywords: Trypanosoma cruzi; ras; gtpases

PV051 - COMPARATIVE GENOMIC HYBRIDIZATION (ACGH) AND CHROMOBLOT HYBRIDIZATION (CH) ANALYSIS OF *TRYPANOSOMA CRUZI*, *TRYPANOSOMA RANGELI* AND *TRYPANOSOMA CONORHINI* GENOMES.

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T. rangeli and *T. cruzi* share vectors and hosts over a wide geographical area in Latin America. Comparative sequence analysis revealed that 93% of *T. rangeli* CDS are shared with other human pathogenic trypanosomes (Stoco et al. 2014 PLoS NTD 8|9|e3176), being more closely related to *T. cruzi* than to *T. brucei*. Recently it has been suggested that *T. rangeli* is more closely related to *T. conorhini* than to *T. cruzi* (Caballero et al. 2015 Parasit Vectors 8:222). In the present study we compare the karyotype and the genome of *T. rangeli, T. cruzi and T. conorhini* by hybridization of chromosomal bands with chromosome specific-markers and comparative genomic hybridization (aCGH). *T. rangeli and T. conorhini* karyotypes differ from that of *T. cruzi* in the number and size of chromosomes. *T. rangeli* and *T. conorhin*i karyotypes have 16-18 chromosomal bands, whose sizes varied from 0.31 to 3.44 Mb. The synteny among these species was analyzed by hybridization of chromosomal bands with genetic markers from two large syntenic blocks conserved among *T. cruzi* isolates (Souza et al. 2011 PLoS One |6|8|e23042). Although partially conserved, synteny seems to be broken by fissions or translocations in *T. rangeli* and *T. conorhini*, suggesting that orthologous counterparts were joined in different combinations to form chromosomes of these parasites. The genomes of these species were also compared by aCGH. We found 129 chromosomal abnormalities, 93.8% of them related to loss/deletions. The average size of loss/deletions was 316 kb and 42.7 kb for alterations related to gain/amplification. Our results suggest the occurrence of fusion and split events in these species. We could suggest that the common ancestor of trypanosomes had small chromosomes; during speciation these fragments joined in different combinations, forming different genomes at the same time. **Supported by:**CAPES/ FAPESP/ CNPq
Keywords: Trypanosoma cruzi; trypanosoma rangeli; trypanosoma conorhini

PV052 - LMRAD1 IS PART OF THE 9-1-1 CHECKPOINT CLAMP OF *LEISHMANIA* AND PARTICIPATES IN THE PARASITE DNA DAMAGE RESPONSE.

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The 9-1-1 complex (formed by Rad9, Rad1 and Hus1 proteins) is a PCNA-like clamp that, in other eukaryotes, binds to sites of DNA damage to activate DNA damage checkpoint signalling and DNA repair. The predicted Rad1 homologue of *Leishmania* has a ~21% identity with the human Rad1. Similarly to what was found for LmRad9, most of the conservation in LmRad1 was confined to the amino-terminal region, whereas the carboxi-terminal presented a marked divergent structure. Immunofluorescence experiments with cell lines expressing tagged versions of LmRad1 indicated that this protein is diffusely distributed through the cell. We also observed that LmRad1 overexpression interferes with the parasite recovery from HU (deoxynucleotide synthesis inhibitor) or CPT (topoisomerase I inhibitor) treatment. To further characterize LmRad1 protein, we raised anti-LmRad1 antibodies. Western blot analysis using these antibodies revealed that LmRad1 associates to chromatin in response to either HU or CPT exposure. These data indicate that LmRad1 is involved in genotoxic stress response in *L. major*. We also performed size-exclusion chromatography analysis and observed that endogenous LmRad1 have a coincident elution peak with LmRad9 andLmHus1 (see Damasceno JD abstract for details).Consistent with this, in vivo and in vitro co-immunoprecipitation assays demonstrated that LmRad1 is able to interact with LmRad9 and LmHus1. Altogether, our data indicated that LmRad1 is involved in genome maintenance-related pathways and, together with LmRad9 and LmHus1, participates in the formation of the 9-1-1 complex in this parasite. Our current work is focused on the generation of LmRad1-deficient cell lines to better understand the mechanisms by which this protein contributes to genome maintenance in *L. major*. **Supported by:**FAPESP, CAPES
Keywords: Leishmania; lmrad1; 9-1-1 complex

PV053 - SUBCELLULAR LOCALIZATION OF ACTIN 2 IN *TRYPANOSOMA CRUZI*: TRANSFECTED PARASITES AND POLYCLONAL SERUM

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Trypanosoma cruzi possess a differentiated cytoskeleton, based on a sub-pellicular corset of microtubules, therefore, the role of others cytoskeleton components, such as actin, still remains poorly understood. At least four genes that codify to different isoforms of this protein were found in the genome of *T. cruzi*. However, a full characterization of these isoforms has not yet been concluded. In this context, the present study aims to produce tools to determine a more specific immunolocalization of one of the actin isoforms. By in silico analysis, it was found that actin 2 is an exclusive isoform of *T. cruzi* with 51% of identity when compared with the most conserved isoform (actin 1). Thus, a sequence of 14 amino acids corresponding to a non-conserved region of actin 2 was utilized to immunize mice and obtain the polyclonal serum. Immunofluorescence showed that actin 2 was distributed along the parasite cell body, predominantly in the perinuclear region of the epimastigote forms. In addition, the entire sequence of the actin 2 was inserted into a cassette, based on Gateway technology, containing the commercial tag FLAG. The cassette was transfected into epimastigote forms and after selection, the presence of the cassette was confirmed by Western Blot and PCR assays. Subcellular localization of FLAG by indirect immunofluorescence showed that the insertion of the tag did not affect the protein addressing when compared with the results obtained with the polyclonal serum. These results indicate that the use of genetically modified parasites and specific polyclonal antibodies allow a more accurate evaluation of location of different isoforms of the same protein in *T. cruzi*. Still, the immunolocalization of actin 2 differs from actin 1 localization, suggesting that these isoforms may have distinct functions. This work opens up a range of possibilities to study the sub-cellular localization and function of each one of the actin isoforms in *T. cruzi*.
Keywords: Actin; trypanosoma cruzi; cytoskelleton

PV054 - QUANTITATIVE PROTEOMIC ANALYSIS OF *T. CRUZI* IN EXPONENTIAL AND STATIONARY PHASE REVEALS COMPLETE REMODELING OF THE CELL METABOLISM

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The processes of growth and proliferation of different microorganisms such as *Saccharomyces cerevisiae* are controlled by the availability of resources, nutrients among them. Strains selected by surviving the shortage of nutrients showed at least seven different cellular processes affected. In nature bacteria are rarely found in the exponential growth phase and many microorganisms tend to spend most of their life cycle in stationary phase, but this phase is still poorly characterized from the point of view of the physiology of these cells. In *T. cruzi* epimastigotes, the deprivation of nutrients simulates in vitro the conditions in the midgut of its invertebrate host, leading to an arrest of cell proliferation and to differentiation to infectious metacyclics forms. Thus, it is clear that the epimastigotes transit from exponential to stationary phase have been critical for its biology over centuries. We investigate by quantitative proteomics analysis the proteins of exponential and stationary phase in these forms of *T. cruzi*. More than five thousand proteins were identified in both populations. We defined a group of fourteen up and down regulated proteins resulting from a comparative analysis of both groups. An analysis of the relation between exponential versus stationary using the Gene Onthology categories, the major variations were found in ribosomal proteins, protein from nucleus, membrane and cytoplasm and the principal biological process envolved was metabolic process. The major molecular function involved was belonged to the category catalytic activity. Based on our results, we conclude that both groups are constituted by cells that diverge not only in their metabolic status, but in their whole constitution. The variations of some of the identified proteins such as proline oxidase and cysteine protease are currently being confirmed by measuring their specific activities. **Supported by:**CAPES/CNPQ/ FAPESP
Keywords: Quantitative proteomic; t. cruzi; cell metabolism

PV055 - MOLECULAR IDENTIFICATION OF BRAZILIAN CLINICAL ISOLATES BASED ON HSP70 PCR-RFLP: SOME UNEXPECTED OBSERVATIONS

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The 70 kDa heat-shock protein 70 (*hsp70*) is a multicopy tandemly repeated gene in *Leishmania* spp., present in 5 to 10 copies in the genome of the parasite. The gene is widely used for Leishmania species typing using different PCR protocols followed by RFLP and/or sequencing. In this study, we applied one of most used PCR-RFLP methods described (Montalvo et al., 2012; Eur J Clin Microbiol Infect Dis 31:1453-1461) for typing Brazilian clinical isolates of *Leishmania (Leishmania) amazonensis* and *L. (Viannia)* spp. We found that some isolates of *L. amazonensis* studied, presented two *hsp70* alleles due to a polymorphism at a *Hae*III site. We also identified a *L. (V.) guyanensis* isolate presenting an additional *Hae*III site not detected in other isolates of this species. Nucleotide sequencing of the *hsp70* gene confirmed this isolate as *L. (V.) guyanensis*, differently from what was expected using the PCR-RFLP approach. An alternative *Hae*III digestion pattern was also found for a clinical isolate of *L. (V.) braziliensis*. In all cases described above, the RFLP pattern could lead to misinterpretations and identification mistakes.**Supported by:**FAPESP,CNPq and CAPES
Keywords: Molecular identification; hsp70; leishmania

PV056 - THE P-TYPE H+-ATPASE IN *TRYPANOSOMA CRUZI* DM28C

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Trypanosoma cruzi epimastigotes have a lysosome-like organelle, known as reservosome, that accumulates internalized nutrients and proteases. However, they lose the endocytic capacity during the metacyclogenesis. The lack of molecular markers for this endocytic compartment makes it difficult to understand the organelle biogenesis and functions. Previous studies with the *T. cruzi* Y strain identified two genes that encode P-type H^+-ATPases, called *Tc*HA1 and *Tc*HA2. ATP-dependent proton transport was detected in isolated reservosomes and immunofluorescence assays revealed that the gene product of epitope-tagged *Tc*HA1-Ty1 was localized in reservosomes and in the plasma membrane, whereas *Tc*HA2-Ty1 was exclusively seen in reservosomes, being suggested that the endocytic pathway in *T. cruzi* is acidified by these enzymes. In order to study the protein sorting and validate a candidate molecular marker for the reservosomes, we amplified by PCR the *Tc*HA2 from the Dm28c genome and constructed a transfection cassette of the gene in fusion with eGFP. Surprisingly, the fluorescence was not detected in the reservosomes, but distributed along the plasma membrane and in a prominent dot that did not co-localize with endocytic tracers. Transgenic epimastigotes overexpressing *Tc*HA2 without any tag likewise displayed *Tc*HA2 at the membrane as detected by immunofluorescence microscopy using antibody raised in mice immunized with a recombinant peptide. We also performed subcellular fractionation of wild type and overexpressor lines and the fractions were analyzed by transmission electron microscopy and Western blotting. The anti-*Tc*HA antibodies did not react with proteins in reservosome enriched samples but in a microsomal fraction constituted mainly of membrane vesicles. Immunoelectron microscopy is being performed to determine the cellular locations of *Tc*HA2. Taken together, our data indicates that in Dm28c, *Tc*HA2 is located primarily at the parasite surface and is absent from reservosomes.
Supported by:CNPq e FAPERJ
Keywords: Trypanosoma cruzi; reservosome; p-type h+-atpase

PV057 - IMMUNOGENIC PROPERTIES OF A RECOMBINANT FUSION PROTEIN CONTAINING THE C-TERMINAL (F3) OF NUCLEOSIDE HYDROLASE AND THE INNATE IMMUNITY AGONIST FLIC FLAGELLIN OF SALMONELLA TYPHIMURIUM

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The nucleoside hydrolase (NH) of Leishmania (L.) donovani (NH36) is a phylogenetic marker of high homology among Leishmania parasites. In mice vaccination NH36 induced a main CD4(+) T cell driven protective response against L. (L.) chagasi infection which is directed against its C-terminal domain. In this investigation, we tested a strategy based on a recombinant fusion protein containing the F3 domain from NH36 and an innate immunity agonist, the Salmonella enterica serovar Typhimurium flagellin (FliC). Bacterial flagellins have shown to bind extracellular TLR5 leading to strong inflammatory responses. This recombinant protein was successfully expressed in Escherichia coli DE3 as soluble protein and was purified by affinity to Ni-NTA by chromatography. BALB/c mice were subcutaneously immunized three times, one week apart, with the recombinant fusion protein (F3FLIC), or recombinant F3 in formulation with saponin, or only saline. After complete immunization all animals were challenged with 3x107 L. (L.) chagasi amastigotes. The F3sap and F3FLIC vaccines showed similar levels for IgG, IgG1, IgG2a and IgG2b anti-NH36 antibodies and equal intradermal reponse to Leishmania lysate. The parasite load in liver was also similar to both vaccines, however the levels of cytokines were measured in supernatants of splenocyte cultures. After immunization, and compared to the saline controls, higher concentrations of IFN-γ were detected only in the F3FliC vaccinated mice (mean= 18837.18 pg/ml). After infection, the F3Flic also showed higher concentrations of IFN-γ (mean=15223,41 pg/ml), this being the differential promoted by the presence of flagellin in this vaccine. At long last, after challenge, the F3FliC vaccine showed higher frequencies of CD4+ T cells IFN-γ+; IFN-γ-TNF-α+; IFN-γ-IL-2+ and IL-2-IFN-γ-TNF-α+, resulting in a higher adaptive immune response. **Supported by:**FAPERJ; CAPES; CNPq
Keywords: Visceral leishmaniasis; flagellin; vaccine

PV058 - COMPARATIVE MOLECULAR MODELING OF A PHOSPHOLIPASE A2 OF *TRYPANOSOMA CRUZI*

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Phospholipase A_2 (PLA_2) is a class of enzymes that hydrolyze phospholipids, generating free fatty acids and lysophospholipids, which have important physiological roles. *T. cruzi* PLA_2 activity has been related to the association of these parasites with theirs host. In the present study, we built a three-dimensional structure model for a putative PLA_2 of *T. cruzi* (Dm28c), using a comparative molecular modeling method as strategy. A search for PLA_2 gene sequences was performed on TritrypDB, a PLA_2-like gene sequence was found in the CL Brenner genome. To confirm the existence of this gene in *T. cruzi* Dm28c, we performed a PCR and the product was sequenced. The ClustalW program was used to generate alignment sequences between the putative Dm28c PLA_2 and the CL Brenner PLA_2-like protein sequences. This alignment did not show any conserved regions in the beginning or in the end of the enzymes. So, we decided to use only the region between the amino acids 31 and 85 to increase the efficiency of the comparative modeling technique. The construction of the three-dimensional model was carried out using the MHOLLINE program, for modeling from the alignment of primary amino acid sequences that 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 both 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, identification of 98.1% of residues falling within favorable and allowed regions of Ramachandran plots indicates 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: Molecular; modeling; phospholipase a2

PV059 - ANALYSIS OF THE IMMUNOGENICITY OF INCREASING DOSES OF THE CHIMERA F1F3 IN THE MODEL OF VISCERAL LEISHMANIASIS BY L. (L.) CHAGASI INFANTUM CHAGASI

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The Nucleoside Hydrolase (NH36) is the main marker of the FML complex (Fucose and Mannose-binding) of Leishmania (L.) donovani antigen of the Leishmune® vaccine licensed for the prophylaxis of canine visceral leishmaniasis. A recombinant chimera F1F3 including the F1 and F3 domains of the NH36 sequence was cloned in the pET28b expression system and was obtained with optimized codons of Escherichia coli. In this investigation, BALB/c mice were subcutaneously immunized three times with the recombinant chimera F1F3 (100µg, or 50µg, or 25 µg) in formulation with saponin or only saline. After completing immunization, all animals were challenged with 3 x 107 L. (L.) infantum chagasi amastigotes. After immunization, intradermal response showed that all doses were more potent than the saline control; however, the dose of 100ug of the F1F3 protein was the most potent after 24h of the antigen injection. Although the conclusion showed that the dose of 25µg of the chimera was already enough to generate a strong IDR, only the doses of 50µg and 100µg of the chimera decreased parasite load compared to saline control. IDR has strong correlate with protection and our results there was a strong IDR already with 25µg of the recombinant chimera. Our results showed an excellent protection against infection by L. (L.) infantum chagasi. Thereby the chimera F1F3 is an excellent candidate for a vaccine against leishmaniasis. **Supported by:**FAPERJ; CAPES; CNPq
Keywords: Chimera; doses; visceral leishmaniasis

PV060 - RNA-BINDING PROTEINS DUPLICATION IN TRYPANOSOMA CRUZI REVEAL REGULATORY NETWORKS EVOLUTION

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A key-step of cell adaptation is reprogramming gene expression. It occurs in every instances of life: organisms facing adverse conditions, with varied cell types, or adapting to different host responses. One main issue in biology is how regulatory proteins evolve so that they regulate new gene networks. Regulatory networks coordinate post-transcriptional gene expression by means of RNA regulons, where functionally related transcripts are co-regulated on the same ribonucleoprotein (RNP) particle, orchestrated by RNA binding proteins (RBPs), which recognize specific elements on transcripts and determine their fates. Gene duplication may lead to different outcomes, due to a relaxation of selective pressures acting on one isoform, allowing it to change. So what happens when RBPs duplicate? We have found such occurrence on Trypanosomatids, organisms which relies their gene expression regulation mainly on post-transcriptional mechanisms. TcRBP40, a previously characterized RBP from *Trypanosoma cruzi*, revealed to be a paralogous of TcRBP7, a duplication that is also observed in other related species. Sharing 73% identity, they have conserved tridimensional structure on its RNA Recognition Motif, show similar expression patterns and co-localize in *T. cruzi* epimastigotes' reservosomes, apparently showing functional redundance. However, when performing co-IP and RNA-Seq of their mRNP complexes, we found out that they associate to distinct transcript sets, with TcRBP40 binding mainly to mRNAs coding for MASPs, and TcRBP7 not presenting a significant particular functional group. Their recognition elements on transcripts apparently are quite similar, but distinct on composition. Further analysis will confirm those elements and the kinetics of cross-recognition. These data provide a model for showing the power of regulatory genes duplication, where mild changes in sequence or structure of its encoded proteins might be sufficient for reprogramming a cell expression network. **Supported by:**CNPq, Fiocruz
Keywords: Rna-binding proteins; gene duplication; trypanosoma cruzi

PV061 - ZINGIBER OFFICINALIS ROSCOE PRESENTS AN EFFECTIVE ANTILEISHMANIAL ACTIVITY AGAINST LEISHMANIA AMAZONENSIS

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Leishmaniasis is a major public health problem, and the alarming spread of parasite resistance underlines the importance of discovering new therapeutic products. The present study aims to investigate the in vitro antileishmanial activity of Zingiber officinalis Roscoe water extract and its purified fraction (namely F10) against Leishmania amazonensis species. The water extract and F10 were prepared and evaluated against stationary-phase promastigotes of L. amazonensis. Also, the cytotoxicity and hemolytic activity in type O+ human red blood cells were analyzed, as well as the treatment of the infected macrophages with L. amazonensis. The products showed to be effective against parasites, presenting IC50 values of 80.0 and 48.0 µg/mL, respectively; to water extract and F10 fraction. The percentage of infection with L. amazonensis in murine macrophages after treatment with the extract or F10 was of 50.0% and 12.7%, respectively; while the control cells had 75% of degree of infection. The chemical characterization of F10 fraction showed the presence of tannis, anthraquinones, triterpenoids, coumarins, alkaloids, saponins, and cardiotonic glycosides. The applied extract and fraction presented a low toxicity in macrophages and a null hemolytic activity. Our studies also showed that fraction F10 induced the production of nitric oxide (NO) after stimulation of murine macrophages, suggesting that this could be the reason for the in vitro elimination of parasites. The results demonstrated that the F10 purified from Zingiber officinalis Roscoe could potentially be used as a new therapeutic alternative on its own, or in association with other drugs, to treat leishmaniasis caused by L. amazonensis.**Supported by:**FAPEMIG, INCT-NanoBiofar, CNPq and PRPq/UFMG

Keywords: Zingiber officinalis roscoe; antileishmanial activity; natural product

PV062 - FUNCTIONAL CHARACTERIZATION OF LEISHMANIA AMAZONENSIS PROMASTIGOTES OVEREXPRESSING THE TELOMERIC PROTEIN LARPA-1

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Leishmania spp. are protozoa parasites that cause leishmaniasis, that is endemic in many countries. Disease control and treatment are still inefcient and parasite drug resistance is a challenge. Therefore, efforts for the establishment of intensive research to better understand the molecular biology of these parasites are encouraged. One possible strategy is to study the role played by Replication Protein A subunit 1 (RPA-1), a single-stranded DNA-binding protein that plays multiple roles in eukaryotic DNA metabolism, including telomere maintenance and DNA damage signaling. RPA-1 is part of a conserved heterotrimeric complex which is present in most eukaryotes including Leishmania spp. Recently, we showed by molecular dynamics simulations that the tertiary structure of LaRPA-1 differs from human and yeast RPA-1 and also shows parasite-specific protein:telomeric DNA interaction, which in the absence of real homologues to telomere-end binding proteins elect LaRPA-1 as a potential candidate. Thus, our goal is to study the roles played by LaRPA-1 using Leishmania amazonensis promastigotes overexpressing the protein. LaRPA-1 was cloned in a parasite expression vector (pX63) that confers resistance to G418 and log phase promastigotes were transformed with pX63:LaRPA-1 and with the empty vector. Preliminary results showed that parasites from a non-cloned population had an increase in LaRPA-1 expression in addition to greater resistance to genotoxic agents when compared with the controls. In order to obtain a cloned overexpression population, we first determined the IC50 for G418 and then isolated clones using serial dilution.After this, we confirm that the plasmid are in our populations and made a Growth Curve that shows differences between the populations . We are now examining the protein and RNA expression profile and protein colocalization of independent isolated clones to further verify if the overexpression of LaRPA-1 causes alterations in parasite telomere length. **Supported by:**FAPESP

Keywords: Telomeres; leishimania amazonensis; rpa-1 overexpressing

PV063 - AGARICUS BLAZEI MURILL IS EFFECTIVE IN THE IN VITRO TREATMENT AGAINST DIFFERENT LEISHMANIA SPP. SPECIES CAUSING TEGUMENTARY AND VISCERAL LEISHMANIASIS

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Leishmaniasis is a neglected disease that presents a high incidence in Brazil. The drugs of choice for its treatment are the pentavalent antimonials that may induce renal and cardiac toxicity. The purpose of this study was to identify new and safe alternative treatments against leishmaniasis based on natural products of the Brazilian flora. The aqueous extract of Agaricus blazei Murill was fractionated in AMICON® filters and five purified fractions were obtained. Biochemical characterization of the fractions was performed by SDS-PAGE and mass spectrometry. The Leishmania mortality rate induced by the fractions was evaluated by MTT assays in promastigotes and like-amastigotes forms of L. amazonensis, L. chagasi and L. major . The fractions cytotoxicity was tested in murine macrophages and the treatment of macrophages infected with Leishmania was also assayed. The aqueous extract of A. blazei showed a significant death rate (about 50%) against promastigotes and like-amastigotes forms of the three parasite species. The fraction namely F5 showed the best antileishmanial activity when used in a low concentration (5 µg), inducing a viability loss of 60% in promastigotes and 90% in like-amastigotes forms. The fractions showed no cytotoxicity in murine macrophages and did not induce nitric oxide production, indicating the existence of a direct mechanism of parasites death. The results demonstrated that the aqueous extract and the purified fractions of A. blazei produced a significant death rate in different Leishmania species, presenting no toxicity in murine macrophages and being effective in inducing the killing of internalized parasites. The great death rate of Leishmania species in the presence of Agaricus blazei fungus provides good perspectives for the development of drugs obtained from natural and non-cytotoxic products. **Supported by:** FAPEMIG, CNPq, INCT-NanoBiofar, and Minasfungi do Brasil Ltda **Keywords:**Agaricus blazei murill; antileishmanial activity; natural product

PV064 - METABOLISM OF SERINE IN TRYPANOSOMA CRUZI: BIOCHEMICAL AND 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 Latin America. T. cruzi is able to use efficiently use carbohydrates and amino acids as energy source. In addition, amino acids can be involved in several biological processes beyond their participation as building blocks for protein and oxidizable metabolites. Among these non-canonical roles, it is worth mentioning their participation in differentiation, resistance to several stress conditions and osmoregulation. In the particular case of L-serine, it is well known up to now that it is essential for the syntheses of proteins, lipids, nucleic acids and other amino acids. One of the functions of serine in 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 and act as the first enzyme in the assimilation of C1 compounds. SHMT activity was detected in different strain of Leishmania spp e T. cruzi, but the role of SHMT and L-serine in the biology of parasite remains unclear. In this work we identified a putative gene encoding SHMT. We determined that TcSHMT is mainly cytoplasmic with partial co-localization with MitoTracker. Interestingly, quantitative PCR revealed that TcSHMT is less expressed in the intracellular stages than in trypomastigotes or epimastigotes. Currently we are functionally expressing the enzyme to determine the kinetic parameters as well as other biochemical characteristics of the enzyme to unveil its role in the biology of T. cruzi. **Supported by:**CNPQ

Keywords: Trypanosoma cruzi; metabolism; molecular biology

PV065 - GENOME EDITING BY CRISPR/CAS9 IN *TRYPANOSOMA CRUZI*: A STEP FORWARD IN THE REVERSE GENETICS OF A REFRACTORY PARASITE

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Trypanosoma cruzi -the etiologic agent of Chagas disease- exhibits unique characteristics that cannot be studied in other models. However, current methods for its genetic manipulation have been highly inefficient. The advent of the prokaryotic CRISPR/Cas9 system has been transformative in biology, being used for genome editing in diverse organisms, including pathogenic protozoa. We report here the use of the CRISPR/Cas9 system for disrupting genes in *T. cruzi*. The system consists of the prokaryotic endonuclease Cas9 and an engineered RNA chimera or single guide RNA (sgRNA) conforming a ribonucleoprotein complex able to recognize a target sequence and produce double strand breaks, that in trypanosomes can be repaired by homologous recombination using donor DNA or by microhomology-mediated end joining (MMEJ). We used either vectors containing sgRNA and Cas9 (separately or together) or one vector containing sgRNA and Cas9 plus a DNA donor for homologous recombination, to rapidly generate mutant cell lines in which endogenous genes have been disrupted. Up to date we have successfully disrupted genes encoding paraflagellar rod proteins 1 (TcPFR1) and 2 (TcPFR2), GP72 protein (TcGP72) and mitochondrial calcium uniporter (TcMCU), without detectable toxicity of Cas9. Our results indicate that TcPFR1, TcPFR2 and TcGP72 contribute to flagellar attachment to the cell body and motility of the parasites, while TcMCU is entirely responsible for mitochondrial calcium uptake without affecting the mitochondrial membrane potential. In conclusion, CRISPR/Cas9 allows efficient gene disruption in *T. cruzi*, a parasite that has been highly refractory to genetic manipulation. We anticipate that this method will significantly improve the functional analyses of its genome. We are currently using this system to investigate the role of different proteins involved in calcium signaling in *T. cruzi.* **Supported by:**FAPESP (2013/50624-0 and 2014/08995-4) and NIH (AI107663).
Keywords: Trypanosoma cruzi; crispr/cas9; genome editing

PV066 - CLONING, EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF ENZYMES WITH 3-METHYLGLUTACONYL-COA HYDRATASE ACTIVITY IN TRYPANOSOMA BRUCEI

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Trypanosoma brucei is mainly found as a procyclic form, which grow in the gut of tse-tse flies, and as bloodstream trypomastigotes, which proliferate in the blood of mammalian hosts. When changing hosts, the parasite senses nutritional variations and performs metabolic adaptations. The bloodstream form generates energy through glycolysis, while procyclics perform oxidative phosphorylation, using mainly amino acids as energy source. Therefore, changes in amino acid catabolism must be one of the required adaptations. Here we characterized one of the enzymes involved in the leucine catabolism. 3-methylglutaconyl-CoA hydratase (3-MGCoA, Tb427.10.4000) catalyzes the hydration of 3-methylglutaconyl-CoA to 3-hydroxy-3-methylglutaryl-CoA (3-HMGCoA). This later is an intermediate in the generation of acetyl-CoA and substrates for the mevalonate biosynthesis, which are used for sterol and isoprenoid formation. In contrast to what is predicted in the genome, we found at least two different genes coding the enzyme by Southern blotting analysis. When we expressed double strand RNA to inhibit enzyme expression, the activity was significantly reduced. However, knockouts of the predicted gene, still presented enzymatic activity, suggesting that another gene could encode for the enzyme. To search for the second gene, we reanalyzed the T. brucei genome looking for crotonases and found 8 other genes predicting proteins that have conserved regions that recognize acyl-CoA. We observed that an enoil-CoA hydratase (Tb927.3.4850) is more similar to 3-MGCoA from T. brucei and other species. The predicted 3-MGCoA and enoil-CoA hydratases genes were amplified, cloned in pET28a and used to express the respective recombinant proteins. Both proteins were purified by Ni-affinity chromatography and tested for enzymatic activity. We will present results of their activities using 3-HMGCoA and other substrates, which will help to define the enzyme involved in the leucine catabolism. **Supported by:**FAPESP **Keywords:**Trypanosoma brucei; amino acid; enzyme

PV067 - ESTABLISHMENT OF MOLECULAR KARYOTYPE AND SYNTENIC ASSOCIATIONS AMONG TRYPANOSOMA CRUZI AND TRYPANOSOMES ISOLATED FROM BATS.

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Chromosomal polymorphism is a common feature among Trypanosoma cruzi isolates and related trypanosomes. Chromosomal rearrangements play key role in genome evolution; however, the molecular mechanisms underlying this structural genomic variation are not fully understood. Comparative karyotypic analysis is a useful tool that can facilitate the reconstruction of the evolutionary history of the genomes of trypanosomes. In this work, we determined the molecular karyotype of trypanosomes isolated from bats such as T. cruzi bat (Tc bat), Trypanosoma cruzi marinkellei (Tcm) and Trypanosoma dionisii (Td). Tc bat and Tcm karyotypes are very similar consisting of 17 bands whose sizes ranging from 0.41 Mb to 1.95 Mb in Tc bat, and from 0.63 Mb to 2.47 Mb in Tcm. Taking into account the number and size of chromosomal bands, the karyotypes of Tc bat and Tcm are closest to those of T. cruzi lineage TcI, for instance G strain and clone Sylvio X10. The karyotype of the Td has 14 chromosomal bands ranging from 0.39 Mb to 1.25 Mb. The number and size of bands were lower than those set for T. cruzi. The synteny among these species was analyzed by hybridization of chromosomal bands with genetic markers from three large syntenic blocks conserved among T. cruzi isolates (Souza et al. 2011 PLoS One |6|8|e23042). The syntenic groups formed by TcChr4+TcChr37 and TcChr39 were conserved among T. cruzi, Tcm, Tc bat and T. dionisii. Although partially conserved, the syntenic group TcChr40 seems to be broken by duplications or deletions in Tcm and Td. Despite the chromosomal polymorphism, the syntenic groups are relatively well conserved among T. cruzi and trypanosomes isolated from bats. Further studies will be designed to compare the genomes of these species by comparative genomic hybridization (aCGH). **Supported by:**FAPESP
Keywords: Trypanosoma cruzi; synteny; chromosomal polymorphism

PV068 - EFFECT OF THE IRON CHELATOR 2,2-DIPYRIDYL ON *LEISHMANIA (VIANNIA) BRAZILIENSIS* GROWTH, ULTRASTRUCTURE AND IRON UPTAKE

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Background: *Leishmania* requires iron for the generation of infective forms, the colonization of macrophages and the development of lesions in mice. Iron also composes the mitochondrial enzymatic complexes and the superoxide dismutase isoforms. Previous works have showed that iron transporter LIT1 and heme transporter LHR1 are expressed by promastigotes and amastigotes cultured in poor iron environments. In the present study, we investigate how iron concentration is affected by the treatment with the chelator 2,2-dipyridyl and how it influences the ultrastructure and the expression of genes involved with the metabolism of iron.
Methods: Promastigotes were treated with distinct concentrations of the iron chelator and cellular density was determined. Intracellular concentrations of iron and heme were quantified. Changes in the ultrastructure of the parasite were determined by transmission electron microscopy and flow cytometry. The gene expression of LIT1, LHR1 and iron superoxide dismutase (FeSOD) was analyzed by qPCR after 24 and 48 hours.
Results: The iron chelator affected proliferation in a dose and time-dependent manner, but growth was restored after inoculation of the parasites in fresh culture medium. The intracellular concentrations of iron and heme in treated parasites were equal or greater than those observed in control parasites. Promastigotes treated for 24 hours revealed severe damage to the mitochondrion. The iron chelator also collapsed the mitochondrial membrane potential (Δψm) after 24 hours. However, after 48 hours, the parasites partially recovered from the dissipation of the Δψm. Finally, gene expression of LIT1, LHR1 and FeSOD increased in parasites treated with the chelator. Conclusion: Iron depletion by 2,2-dipyridyl inhibits the growth and dissipates ΔΨm of L.(V.) braziliensis promastigotes. However, the parasites may increase the uptake of iron and the antioxidant defenses to resist the nutritional stress and prolong their survival. **Supported by:**FIOCRUZ CNPq/ Papes VI and CAPES
Keywords: Leishmania ; iron uptake; mitochondrion

PV069 - EVALUATION OF PARASITE LOAD IN BLOOD SAMPLES FROM CHRONIC CHAGAS DISEASE PATIENTS BY QUANTITATIVE REAL TIME PCR AND HEMOCULTURE.

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Difficulties in the diagnosis of chronic phase of Chagas disease justify the interest and need to implement a direct and more sensitive method that enables to monitor the parasite presence in blood of patients. In this work we use methodologies based on parasitological diagnosis (hemoculture) and molecular diagnosis of Trypanosoma cruzi (quantitative PCR-qPCR) for detecting the parasitemia and to quantify the parasite load of 93 chronic chagasic patients, untreated and with clinical forms defined, indeterminate (25) and cardiac (68) from the State of Minas Gerais, Brazil. Two blood samples were collected in 44 of these patients at different time periods in trying to demonstrate the evolution of the parasitemia in the chronic phase of Chagas disease, totaling 137 samples. DNA extraction was performed with blood samples preserved in Guanidine/EDTA buffer, for quantifying the parasite load by qPCR in a multiplex reaction with two targets, satellite DNA for T. cruzi and IAC (Internal Amplification Control). Positivity of qPCR was 59% (81/137) and 41% (56/137) negative. In contrast, the positivity of hemoculture was 49% (67/137) with 51% (70/137) negative. The median parasite load of all positive patients was 1.18 par. eq./mL ranging from 0.007 to 116.102 par. eq./mL. Hemoculture and qPCR results were concordant, since 38% (52/137) and 30% (41/137) of samples were positive and negative respectively, for both techniques. Hemoculture and qPCR were discordant in 32% (44/137) of the samples. Hemoculture were positive and negative in qPCR in 11% (15/137) of the samples, and in 21% (29/137) of them the hemoculture was negative and positive qPCR. We conclude that qPCR is more sensitive to evaluate and quantify the parasite load in chronic patients, and may be a useful tool for medical decision regarding the introduction or not of anti-T. cruzi specific therapeutic. Besides, it was not possible to establish a correlation between parasite load and clinical forms of patients. **Supported by:**CNPq, FAPEMIG, MCTI/CNPq/MS-SCTIE-Decit **Keywords:**Trypanosoma cruzi; quantitative pcr-qpcr; hemoculture

PV070 - LEISHMANIA MAJOR PROTEINS ENCODED BY HEMOLYSIN TYPE III-RELATED GENES ARE HEMOLYTIC AND POSSIBLY ACCOUNTS FOR LEISHPORIN ACTIVITY

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Our previous work has shown that *Leishmania amazonensis* promastigotes express a cytolytic molecule that lyses erythrocytes/damages macrophages, named leishporin. Leishporin has already been characterized in a number of biochemical aspects. However, its molecular identity remained unknown. In attempt to identify gene(s) that would possibly code for leishporin, we have performed a data mining to search for putative cytolysin genes in *L. major*. We have found 3 sequences, LmjF36.5500, LmjF36.5510 and LmjF36.5520, containing the Pfam domain PF03006, named hemolysins type III-related. It is worth mentioning that these sequences show a significant homology to other characterized pore-forming-proteins. Real time PCR and Northern blots were performed to investigate the relative amounts of these genes in L.major promastigotes and amastigotes. Our results demonstrated a higher expression of 5500 and 5520 mRNA in amastigotes, whereas 5510 mRNA was not detected. In order to verify the hemolytic activity of the encoded proteins, transfected parasites, overexpressing the hemolysins type III genes were generated. Our results show that parasites overexpressing the proteins 5500 and 5520 express a hemolytic activity higher than WT parasites. This indicates that 5500 and 5520 may be the genes coding for leishporin. Polyclonal antibodies to N-terminal peptides from the three proteins were produced and used in confocal immunofluorescence microscopy, aiming to determine the subcellular localization of the hemolytic proteins. Confirming the qRT PCR and Northern blot results, expression of 5510 protein was not detected. 5520 and 5500 proteins are localized to a structure morphologically similar to a lysosome compartment, termed multivesicular tubule (MVT) that extend from near the flagelar pocket to the posterior end of the cell. We are currently investigating whether the mechanism that accounts for the hemolytic activity of these proteins is by pore formation. **Supported by:**CNPq, CAPES, FAPEMIG
Keywords: Leishmania; hemolisina; proteína formadora de poros

PV071 - EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS IN THE MUNICIPALITY OF BRASILéIA, STATE OF ACRE: STUDY ON THE SANDY FLY FAUNA

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In Acre there are few studies concerning the situation of cutaneous leishmaniasis (CL), despite the increase of cases in recent years with 1,009 notifications in 2013. Herein, we proposed to investigate the heterogeneity of sandflies from the municipality of Brasiléia to bring more insights concerning the epidemiology of CL. We will evaluate sandfly taxonomy, rates of natural infection by Leishmania followed by species identification, blood sources of vectors and finally, correlate the parasite species found in insects with the one detected in patients lesions from the same region. Sandfly collections are being held since September 2013, using light traps and Castro manual capture and taxonomically identified following Galati(2003). In parallel, biopsy imprints are being collected from patients with CL lesions. Molecular diagnosis in insects was performed individually in non-blood-fed females using a multiplex PCR with primers for Leishmania genus (kDNA) and sandfly gene (cacophony). For the identification of Leishmania species, a PCR targeting the parasite hsp70 gene followed by sequencing was assayed. An amount of 6,058 sand flies was collected in 4 capture points, being 2,170 males; 3, 610 non-blood-fed females (for molecular diagnosis) and 278 blood-fed females (for the search of food source by HRM method). So far, we identified 14 sandflies genera and 63 species by morphological identification. For the evaluation of natural infection indexes, to date, 297 females were processed individually for DNA extraction and PCR. Positive results for the presence of Leishmania DNA were identified in 13 specimens (4,4%). At present, 23 clinical samples were obtained from CL patients. From these, 8 represented infection by L. (V.) braziliensis, one corresponded to L. (V.) guyanensis, 10 Leishmania spp., and four non-infected samples. With the conclusion of this study, we expected to provide new elements for better understanding the CL transmission cycle in the study area. **Supported by:**CNPQ; CAPES; FAPERJ; IOC-FIOCRUZ
Keywords: Sand flies; cutaneous leishmaniasis; brasiléia-ac

PV072 - THE METABOLISM OF EXPONENTIAL AND STATIONARY EPIMASTIGOTES OF *T. CRUZI:* A TARGETED METABOLOMICS APPROACH

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Axenic cultures of epimastigotes from *Trypanosoma cruzi* present two main phases during growth: the exponential phase (EP, 4.5-6.5 x 10^7 cells/mL) and the stationary phase (SP, 8.0-10.5 x 10^7 cells/mL). During the EP, parasites primarily metabolize glucose as energy source producing reduced metabolites such as alanine and succinate. As proliferation increases, nutrients in the medium become limiting and the parasites enter into SP growth. This phase is characterized by amino acid degradation, activation of mitochondrial enzymes, and respiration that is dependent on cytochromes. In the present work, we used a targeted metabolomics approach to study the metabolic changes in parasites between the EP and the SP. We detected high levels of glycolysis and Krebs's cycle intermediates (as expected) from the glucose-dependent metabolism during the EP. During the SP, some amino acids and related metabolites were at higher levels compared to the EP, supporting their relevant role during this phase of growth. Sulfur containing metabolites such as cysteine, gluthathione, and trypanothione, which are all related to resistance to oxidative unbalance, were also analyzed. We observed that these molecules increased in the SP, probably in response to the oxidative environment resulting from metabolism in the EP. In addition, some amino acids, such as proline, were considerably increased in the SP compared to the EP. As described in the literature, Pro participates in both energy and redox metabolism. Therefore, we analyzed proline's metabolism in more detail. We observed higher Pro biosynthesis and lower degradation during the EP. We observed the opposite during the SP as well as a higher uptake of this amino acid, confirming that epimastigotes use Pro during the SP and maintain basal levels through incorporation from the extracellular media. Our data suggest that Pro levels are regulated during the SP as a pre-adaption for metacyclogenesis, the next step in *T. cruzi*'s life cycle. **Keywords:**Trypanosoma cruzi; metabolomics; energy metabolism

PV073 - THE SAP MULTIGENE FAMILY IS FOUND ONLY IN *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA CRUZI MARINKELLEI* BUT NOT IN OTHER SPECIES OF THE GENUS *TRYPANOSOMA*

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The members of *T. cruzi* SAP (Serine-, Proline- and Alanine-rich Protein) gene family share a central domain of 513 bp (SAP-CD). SAP-CD is capable of binding to the host cell and inducing activation of signal transduction pathways which lead to an increase of intracellular calcium concentration. These events will be induced exocytosis of lysosomes participating directly in the internalization of the parasite. Previous work of our laboratory suggested that SAP sequences are species-specific. To investigate whether SAP is present in *T. cruzi* lineages and other species of the genus *Trypanosoma*, we carried out BLASTN searches using the whole SAP and SAP-CD as query sequences. It revealed the presence of SAP in the strain G and clone Sylvio X10 (Tc I), Esmeraldo (Tc II), TCC 2177 (Tc III), Can III (Tc IV), clone SO3 (Tc V) and CL Brener (Tc VI), and in the subspecies *T. cruzi marinkellei*. The sequence similarity revealed no orthologous in the genomes of *T. rangeli, T. conorhini, T. dionisii, T. grayi* and mammal-dwelling African trypanosomes. The species-specificity of SAP was confirmed by PCR genomic amplification using specific primers.
At the carboxy-terminal domain of SAP there is a 70-200 amino acids sequence that shares 50-80% identity with a few members of MASP (Mucin Associated Surface Protein) and mucins (TcMUC) families. A phylogenetic tree was generated from the alignment of the sequences of SAP variants and members of multigene families MASP, TcMUC, mucin-like glycoprotein and TcTASV (Trypomastigote Alanine, Serine, Valine rich protein). Both SAP proteins from *T. cruzi* and *T. c. marinkellei* and 3 MASP that share identity with SAP appear to cluster as an independent clade. The closest branch to the SAP contains the remaining TcMUC and MASP that have similarity with SAP. Taking into account the results we suggest that SAP could have originated from an ancestor gene of MASP, followed by duplication and diversification of the current SAP repertoire. **Keywords:**Trypanosoma cruzi; multigene family; t. cruzi lineages

PV074 - GENOME WIDE IDENTIFICATION OF PEPTIDASES IN RHODNIUS PROLIXUS AND THEIR ROLE IN HEMATOPHAGY.

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Triatominae is a subfamily of Hemiptera order whose species are able to feed in vertebrate blood (i.e. hematophagy). This feeding behavior presents a great physiological challenge to insects, especially in Hemipteran species with a digestion performed by lysosomal-like cathepsins instead of the more common trypsin-like enzymes. Peptidase families of Rhodnius prolixus were screened and characterized by common blast (NCBI) and conserved domain analyses using a HMMER/blast manager software (FAT) and PFAM database. The results were compared with 18 arthropod genomes which are available in the MEROPS database.
Rhodnius prolixus contains at least 433 protease coding genes, belonging to 71 protease families. A higher number of peptidases in Rh. prolixus were found in 13 peptidase families when compared with the other arthropod species. Further analysis indicated that a gene expansion of the protease family A1 (Eukaryotic aspartyl protease, PF00026) may play an important role in the adaptation to hematophagy since most of these peptidases were expressed in the Rh. prolixus gut and presented secretory pathway signal in their sequences. The other two families expanded in Rh. prolixus (i.e. C2 and M17) also presented significant differences between hematophagous (higher number of peptidases) and non-hematophagous species. This study also provides evidence for gene acquisition from microorganisms in some peptidase families in Rh. prolixus: 1) family M74 (murein endopeptidase), 2) family S29 (Hepatitis C virus NS3 protease), and 3) family S24 (repressor LexA). This study revealed new targets for study the adaptation of these insects for digestion of blood meals and their competence as vectors of Chagas disease. **Keywords:**Rhodnius prolixus; peptidase; gene transfer

PV075 - TRYPANOSOMA BRUCEI ORC1/CDC6 (TBORC1/CDC6) REQUIRES BINDING TO AND HYDROLYSIS OF ATP TO LICENSE DNA REPLICATION

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DNA replication in Trypanosoma (T.) brucei, as in other eukaryotes, starts with the licensing of a pre-replication machinery (pre-RC) in specific regions of DNA called replication origins. The dynamics and the proteins involved in pre-RC assembly are not well established in these parasites. Our group has demonstrated that the protein Orc1/Cdc6 from T. brucei (TbOrc1/Cdc6), which is responsible for the recognition and activation of replication origins, binding to and hydrolysis of ATP in vitro. Our purpose is to evaluate the importance of these in the assembly and stability of pre-RC. To check this, we analyzed the primary sequence of TbOrc1/Cdc6 and replaced, by PCR overlap, one aminoacid at domain important for ATP binding (TbOrc1/Cdc66K79T) and two aminoacids at region essential for ATPase activity (TbOrc1/Cdc6R251,252E). In vitro assays showed, as expected, that recombinant TbOrc1/Cdc66K79T is not able to bind ATP while recombinant TbOrc1/Cdc6R251,252E lost their ability to hydrolyze ATP. Plasmids containing these mutated genes were transfected into T. brucei procyclic cells. After expression of these mutated genes, cells showed problems in proliferation resulting in an inefficient loading of MCM complex onto DNA and alteration in DNA synthesis, analyzed by FACS. These data suggest that TbOrc1/Cdc6 need binding to and hydrolysis of ATP to bind into DNA, recruit MCM complex and then, license DNA replication. Processo FAPESP 2014/24170-5. **Supported by:**FAPESP
Keywords: Dna replication; trypanosoma brucei; origin recognition complex (orc)

PV076 - DNA IDENTIFICATION OF TRYPANOSOMA CRUZI AND HUMAN IN PANSTRONGYLUS MEGISTUS FECES CAPTURED IN SOUTH OF MINAS GERAIS

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American Trypanosomiasis is a complex zoonosis and one of the most important parasitic infectious diseases in Latin America. It is present in all Brazil regions and the occurrence depends also on the distribution and positivity of the domiciled vectors. From January 2013 to July 2014 we received 324 insects captured in the municipalities of origin, packed and sent to the Laboratory of Clinical Parasitology of Universidade Federal de Alfenas (UNIFAL-MG). From 324 insects received, eleven were classified as predators, two as phytophagous and 311 hematophagous, which were identified as *Panstrongylus megistus*, the most important vector of Chagas disease in Brazil since *Triatoma infestans* eradication. Thirty five insects were dry and unable to perform the tests and other 276 feces samples were collected. Part of such intestinal contents were collected in 300 µl of saline solution (0.85%) and stored by freezing (-20oC) for use in molecular tests. All the samples were processed according to the protocol for genomic DNA extraction by phenol/chloroform/isoamyl. The detection of T. cruzi DNA from feces was performed by conventional PCR using TCZ1/TCZ2 primers, which amplified a 188 base pairs fragment. The TCZ real-time PCR assay was performed with a probe FAM labeled. From the 276 feces samples, in molecular analysis, 45 (16.3%) were positive for conventional TCZ1/TCZ2 primers and 120 (43,4%) positive for qPCR. For source supply control were used primers that amplify a sequence of 140 pairs of bases of human β –globulin gene from positive samples in qPCR and 31 (25,8%) showed amplification compatible with human genetic material. These data shows a superior performance of the qPCR and a high rate of T. cruzi in intestinal contents from the collected insects and also suggested that vectorial transmission may be occurring because there is evidence of human blood intake by these insects. **Supported by:**PNPD CAPES, UNIFAL-MG, FAPEMIG
Keywords: Trypanosoma cruzi; qpcr; chagas disease

PV077 - IMMUNOPROTEOMIC ANALYSIS OF ANTIGENS IDENTIFIED IN THE PROMASTIGOTE AND AMASTIGOTE STAGES OF LEISHMANIA INFANTUM

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Visceral leishmaniasis (VL) is an important parasitic disease, with a worldwide distribution in 98 countries, where a total of 350 million people may be at risk. In Brazil, the disease is caused by the parasitic protozoa Leishmania infantum. Dogs are the main parasite domestic reservoirs and culling of seropositive animals, as detected by means of serological tests using promastigote antigens, is a VL control measure adopted. Therefore, to reduce the transmission of parasites between dogs and humans, it is necessary to diagnose canine visceral leishmaniasis (CVL) as early as possible, by means of sensitive and specific diagnostic tools. This study aims to identify antigens in protein extracts of promastigote and amastigote-like L. infantum recognized by antibodies present in the sera of asymptomatic and symptomatic VL dogs. Immunoblotting experiments using the sera samples of CVL were performed and the target proteins were identified by mass spectrometry. A total of five hundred and fifty spots were observed in the 2DE gels, and approximately one hundred and four proteins were identified. Several stage-specific proteins could be identified by either or both classes of sera, including, as expected, previously known proteins identified as diagnosis, virulence factors, drug targets, or vaccine candidates. Three, seven, and five hypothetical proteins could be identified in promastigote antigenic extracts; while two, eleven, and three hypothetical proteins could be identified in amastigote-like antigenic extracts by asymptomatic and symptomatic sera, as well as a combination of both, respectively. Therefore, the present study represents a significant contribution in identifying stage-specific L. infantum molecules, as well as in revealing the expression of a large number of hypothetical proteins. **Supported by:**FAPEMIG, INCT NanoBiofar, CNPq and PRPq/UFMG.
Keywords: Immunoproteomic approach; leishmania infantum; vaccine

PV078 - PROLINE-P5C CYCLE IS AN ELECTRON SHUTTLE MECHANISM IN TRYPANOSOMA CRUZI

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In most of the organisms, redox homeostasis is maintained by different redox transfer mechanisms. It is well known that in *Trypanosoma cruzi*, L-proline (Pro) is converted in Δ^1-pyrroline-5-carboxylate (P5C), reducing FAD to $FADH_2$ by a mitochondrial P5C-dehydrogenase. Therefore, these electrons are further transferred from $FADH_2$ to the cytochrome c through the coenzyme Q and the mitochondrial complex III. In this work, we show that P5C also can be reduced to Pro, oxidizing NADPH to NADP only through a reaction catalyzed by a Δ^1-pyrroline-5-carboxylate reductase (*Tc*P5CR, E.C. 1.5.1.2). Digitonin permeabilization experiment showed that the enzyme was located in the cytoplasm. In addition, *Tc*P5CR was expressed in *E. coli* and purified in an active form. The parameters K_M, k_{cat}, Ea and optimum pH were estimated using the recombinant *Tc*P5CR. Some of these parameters were also determined in cell-free extracts. The regulatory influence of NADPH on the reaction was likewise analyzed showing that *Tc*P5CR is a NADPH-dependent enzyme. Interestingly, this cofactor has a substrate-inhibitory effect on *Tc*P5CR (Ki^{app} = 39,5 µM), which suggests that this enzyme is an important point of regulation in the Pro-P5C cycle. In synthesis, the characterization of this enzyme allows us to propose that Pro and P5C constitute a redox couple, being the former oxidized in the mitochondria and the latter reduced in the cytosol. The result of the entire cycle is a transfer of electrons from the cytosolic NADPH pool to the mitochondrial $FADH_2$ pool, which can feed the respiratory chain at the level of the complex II. The cycle results also in an electron transport from the cytosol to mitochondria via Pro, which could affect the whole bioenergetics of the parasites. **Supported by:**CNPq, FAPESP, INBEQMEDI
Keywords: Metabolism; l-proline; trypanosoma cruzi

PV079 - AN EFFECTIVE IN VITRO ANTILEISHMANIAL ACTIVITY FROM 8-HYDROXYQUINOLINE AGAINST LEISHMANIA AMAZONENSIS

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Leishmaniasis is a major public health problem, and the alarming spread of parasite resistance underlines the importance of discovering new therapeutic products. The present study investigated the in vitro antileishmanial activity of 8-hydroxyquinoline (8-HQN) against Leishmania amazonensis. Experiments were performed in order to evaluate the minimum inhibitory concentration (IC50) of 8-HQN in L. amazonensis, as well as their leishmanicidal effects on the intra-macrophage Leishmania stage, the cytotoxic effects on murine macrophages (CC50), and in O+ human red blood cells. The 8-HQN proved to be effective against stationary promastigotes of L. amazonensis, with IC50 values of 0,34 nmol/mL. The infectivity of the parasites before and after treatment was analyzed. It could be observed that 94% of the macrophages were infected with L. amazonensis; however, when parasites were pre-incubated with 8-HQN and later used to infect macrophages, they were able to infect only 15.4% of the phagocytic cells. When macrophages were previously infected with L. amazonensis, and later treated with 8-HQN, only 15.0% of macrophage were infected. In addition, a low cytotoxicity in murine macrophages, and a null hemolytic activity in type O+ human red blood cells; were also observed. In conclusion, the results demonstrate that 8-HQN could potentially be used as a therapeutic alternative on its own, or in association with other drugs, to treat disease evoked by L. amazonensis. **Supported by:**FAPEMIG, INCT NanoBiofar, CNPq and PRPq/UFMG.
Keywords: Tegumentary leishmaniasis; treatment; 8-hydroxyquinolin

PV080 - STRUCTURAL ORGANIZATION OF THE ENDOCYTIC SYSTEM IN *PLASMODIUM CHABAUDI*

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In the course of its intraerythrocytic development, parasites from de genus *Plasmodium* incorporate massive amounts of the host cell cytoplasm. Internalized hemoglobin is digested in a compartment with acidic pH termed the food vacuole, producing aminoacids and other by products, namely heme. Due to its toxic effects, free heme is immobilized and stored in a crystal form known as hemozoin. This mechanism is essential to parasite development and represents a physiological step used as target for many antimalarial drugs. The fine mechanisms underlying this process in *Plasmodium* species are still under discussion. In addition, most of the structural data presented so far results from observation of chemically fixed samples, a technique that has a large potential of generating artifacts. Here, we studied the events of hemoglobin uptake and hemozoin nucleation in the different stages of the intraerythrocytic cycle of the murine malaria *Plasmodium chabaudi* using transmission electron tomography of cryofixed and freeze substituted cells. Cryofixation of samples provided a better preservation of the cells, allowing a better analysis on the mechanism of hemozoin nucleation, whereas electron tomography showed the three-dimensional organization of hemoglobin uptake mechanisms. Results showed that hemoglobin uptake is a dynamic process, being observed since the early ring stage. Hemozoin crystals are seen as early as in the ring stage and are present in all developmental stages, including the replicative schizont stage. The hemozoin nucleation process occurs near the membrane of small food vacuoles, that latter fuse, originating a single food vacuole completely filled with hemozoin crystals. Taking together, these results provide new insights on the mechanisms of hemoglobin uptake and degradation in rodent malaria parasites. **Supported by:**Capes, CNPq, FAPERJ
Keywords: Plasmodium; malaria; eletron tomography

PV081 - STUDY OF MECHANISMS OF THE GENE EXPRESSION AND EPIGENETIC REGULATION OF FLI1 GENE IN TEGUMENTARY LEISHMANIASIS

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Previous studies have demonstrated a role for wound healing genes in resolution of cutaneous lesions caused by Leishmania spp., including FLI1 gene. Reduction of Fli1 expression in mice has been shown to result in up-regulation of Col1a1 and Col1a2 genes and, conversely, in down-regulation of Mmp1 gene, suggesting that Fli1 suppression is involved in activation of the profibrotic gene program. Moreover, it was also shown that the CpG islands in the FLI1 promoter region are susceptible to epigenetic regulation in systemic sclerosis fibroblasts and skin biopsy specimens. The objectives of this work were to evaluate the gene expression of FLI1 and genes under its regulation (COL1A1, COL1A2 and MMP1) and to evaluate the epigenetic regulation of FLI1 gene in biopsies of normal skin and lesions of cutaneous leishmaniasis (CL). For the gene expression study (N = 17) and for the epigenetic regulation study (N = 8) normal skin biopsies and CL biopsies were obtained by punch from CL patients from Corte de Pedra, Bahia, where L. braziliensis is endemic. Methylation profile, as well as gene expression, was evaluated by real time PCR. We observed that gene expression of COL1A2 was increased in normal skin biopsies (P = 0.0013), whereas gene expression of MMP1 was increased in CL biopsies (P = 0.0004). The epigenetic regulation data showed that the percentage of methylated DNA was significantly higher in normal skin biopsies compared to CL biopsies (P = 0.0011). The methylation profile of the FLI1 gene demonstrated here can be responsible for the disequilibrium of COL1A2 and MMP1 expressions observed in these biopsies, which would promote the extensive tissue damage presented in the disease. These observations emphasize the role of FLI1 pathway in the pathogenesis of L. braziliensis infection and contribute to our further understanding of the role of wound healing in the resolution of CL disease, providing potential for therapies acting on FLI1. **Supported by:**NIH Grant AI 30639 and CNPq/INCT-DT. **Keywords:**Cutaneous leishmaniasis; wound healing; epigenetic regulation

PV082 - *TRYPANOSOMA CRUZI* TCI, TCII, TCIII AND TCV ISOLATED FROM *TRIATOMA LENTI*, *TRIATOMA MELANOCEPHALA*, *TRIATOMA RUBROVARIA* AND *TRIATOMA SORDIDA* COLLECTED IN BAHIA AND RIO GRANDE DO SUL, BRAZIL.

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Trypanosoma cruzi, etiologic agent of Chagas disease has vast intraspecific variability, including differences related to morphology, virulence, pathogenicity, antigenic composition and biochemical properties. It is possible that such diversity is associated with their adaptation and survival in different hosts. This study was conducted to provide the molecular characterization of 18 strains of *T. cruzi* isolated from *Triatoma lenti*, *T. melanocephala*, *T. rubrovaria* and *T. sordida* collected in Bahia and Rio Grande do Sul, Brazil. DNA from culture trypanosomes (1×10^6 parasites) was extracted using the phenol/chloroform method. Genotyping the *T. cruzi* was done using PCRs based on LSU 24 Sα-rDNA, HSP60 and GPI gene sequences with 10-100 ng of genomic DNA; 0,2 mmol/L of dNTP; 1 pmol/µL of each primer; 1,5 mmol/L of MgCl2; 1U Taq DNA polimerase and buffer 10X. The genes were analysed by electrophoresis in 1-3% agarose gels stained with ethidium bromide. Amplified HSP60 and GPI genes were digested with several restriction enzymes. The enzyme EcoRV was selected using the PCR-RFLP for the HSP60 gene and the enzyme HhaI was selected for the GPI gene. The amplified products presented 110-125 bp for the 24 Sα rRNA gene, 432–462 pb for the HSP60 gene, and 1264 bp for the GPI gene. According to the DNA analysis by PCR amplification reaction, the 18 strains isolated can be classified as belonging to *T. cruzi* group I (TIenti; Tm), TcII (SIGR3; SIGR5; SI1; SI8; SI9), TcIII (SI5; QMM2; QMM6; QMM9; QMM10; QMM13) and TcV (QMM1; QMM3; QMM4; QMM7; QMM11). The results expand the knowledge about the infection by *T. cruzi* in Macaúbas, Poções and Santo Inácio in Bahia, Quaraí and Santana do Livramento in Rio Grande do Sul. The 18 strains were classified as belonging to *T. cruzi* groups: TcI (*T. lenti*; *T. melanocephala* - Northeast), TcII (*T. sordida* - Northeast), TcIII (*T. rubrovaria*; *T. sordida* – Southern and Northeast) e TcV (*T. rubrovaria* - Southern). **Supported by:**CAPES**Keywords:**Trypanosoma cruzi; characterization; triatomine

PV083 - RPA-1-TELOMERE ASSOCIATION: POSSIBLE NEW FUNCTIONS FOR AN OLD PLAYER

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Replication protein A (RPA), the major eukaryotic single-stranded binding protein, is a heterotrimeric complex formed by RPA-1, RPA-2 and RPA-3. RPA is a fundamental player in replication, repair, recombination and checkpoint signaling. In this sense, RPA interacts with DNA at S-phase, but its presence outside replication triggers the DNA damage response, resulting in cell cycle arrest. Genome databases of T. cruzi revealed that this parasite contains RPA-1 and 2 homologous, but no sequence codifying to RPA-3 was found. T. cruzi RPA-1 present significant structural peculiarities compared to other eukaryotes: (i) lacking of DBDF-domain that interacts with the checkpoint and repair proteins, (ii) more flexible linkers between DNA binding domains, and (iii) some aminoacids substitutions in conserved regions. Here we show that in spite of all structural peculiarities as well as absence of RPA-3 homologous, RPA-1 and 2 are found at replication foci and change its pattern in Immunofluorescence assays after UV-induced damage and hydroxyurea treatment, suggesting their participation in DNA replication and damage response. Although involved with DNA replication and repair, RPA-1 was also found at telomeres in vivo during the entire cell cycle, while RPA-2-telomere association is only enriched at G1/S phase. Since T. cruzi does not contain homologous to telomere overhang binding proteins, and these proteins are considered RPA-like due to structural similarities with components of the RPA complex, we deeply investigated RPA-1-telomere association. One telomeric repeat is sufficient to bind RPA-1. Telomeric DNA induces different secondary structural modifications on RPA-1 in comparison with other types of DNA. In addition, RPA-1 presents a higher affinity for telomeric sequence compared to other genomic randomic/repetitive sequences. Based on these data we propose that RPA-1 may play additional roles, than replication and repair, in T. cruzi telomeric region. FAPESP2014/02978-0
Supported by:FAPESP **Keywords:**Replication protein a; telomeres; t. cruzi

PV084 - CHARACTERIZATION OF GENES EXPRESSED BY *LEISHMANIA INFANTUM CHAGASI* DURING INFECTION OF *LUTZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF VISCERAL LEISHMANIASIS IN BRAZIL

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Lutzomyia longipalpis is the main vector of *Leishmania infantum chagasi* in the Americas. The parasite amastigote forms are ingested by the insect vector, survive through the insect's digestive process, differentiate into promastigote forms, and at the end of digestion differentiate into metacyclic forms. Some published work has described *Leishmania* molecules that participate in the interaction with the insect vector. FLAG1 gene encodes a flagellar protein that participates in the interaction between *Leishmania major* and the gut of *Phlebotomus papatasi*. GP63 is a zinc-dependent metalloprotease described as a major antigen expressed by *Leishmania* promastigotes and is involved in the interaction between the parasite and the vertebrate and invertebrate hosts. Elongation factor 1-α (EF1-α) which is known to bind to SHP1 reducing the host immune response. Our objective is to investigate *L. i. chagasi* genes that may play a role in the parasite-vector interaction. We performed qPCR to study the expression of FLAG1, GP63, and EF1-α expressed by *Leishmania* during promastigote initiated infection in *L. longipalpis*. We also used *Leishmania* procyclic forms or metacyclic-like forms from culture. Our results show that there was no difference in expression levels of FLAG1 during the course of infection in the insect when comparing procyclic and metacyclic forms. and did not vary in parasite samples obtained at the initial and late phase culture. Intriguingly, EF1-α expression decreased post infection in insects while increased in metacyclic-like forms of parasites grown in culture. The GP63 had higher expression in metacyclic-like forms obtained from culture and also inside the insect at late infection. Our findings indicate that there is no significant modulation of FLAG1 from procyclic to metacyclic-like forms in culture or in the insect. Nevertheless *L. i. chagasi* expresses two genes that encode molecules potentially capable of suppressing the insect immune response. **Supported by:**Programa Ciência sem Fronteiras-CNPq, IOC-Fiocruz,FAPERJ.
Keywords: Leishmania infantum chagasi; lutzomyia longipalpis; interaction

PV085 - CHARACTERIZATION OF INORGANIC PHOSPHATE TRANSPORT IN G. DUODENALIS

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INTRODUCTION: *Giardia duodenalis* is a flagellate protozoan that infects the small intestine of vertebrates, the most common cause of epidemics diarrhea in the world. The infection begins by cysts ingestion, followed by excystation and colonization of the small intestine by trophozoites, which are responsible for the clinical manifestation of the disease. As other parasites, G. duodenalis needs to adapt to environmental changes, in order to survive. One important environmental change is availability of nutrients, as inorganic phosphate (Pi). Pi is an extremely important nutrient in the cellular metabolism and it is required involving for the synthesis of DNA, RNA, lipids, sugars and proteins; in addition, Pi is involved in biochemical reactions transfer of phosphoryl grouping. OBJECTIVE: The aim of this work is characterize biochemically the Pi transport in *G. duodenalis,* by investigating the kinetics characteristics of Pi transport, as time course, cell density, pH and the substrate affinity of the Pi transporter ($K_{0,5}$ and V_{max}). MATERIALS AND METHODS: It was used the trophozoite form to accomplish the quantification of Pi transport in *G. duodenalis*. The Pi transport was uptake by the of 32Pi: the cells were incubated at room temperature for 15 minutes in a reaction mixture containing 140 mM choline chloride, 5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES (pH 7.2), 0.1 mM KH_2PO_4, 2.5 mCi/nmol^{32}Pi. RESULTS: The Pi transport in *G. duodenalis* is linear up to 15 minutes, reaching a value of 750 pmol Pi / 10^7 cells. This Pi transport also varies linearly with the increase in cell density, but it is not modulated by different pH values, suggesting that Pi transporter has no preference by Pi in the monovalent ($H_2PO_4^-$) or divalent (HPO_4^{2-}) form. PERSPECTIVES: The perspectives of this study are to evaluate the $K_{0,5}$ and V_{max}, besides to investigate the mechanism of Pi transport in different forms present in the life cycle of *G. duodenalis*. **Supported by:**CAPES, CNPQ, FAPERJ
Keywords: Pi transporter; giardia duodenalis; cellular metabolism

PV086 - TRANSCRIPTIONAL PROFILING OF *TRYPANOSOMA CRUZI* CLONES WITH DISTINCT VIRULENCE PHENOTYPES

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Trypanosoma cruzi has a life cycle involving several biochemically distinct stages that establish complex interactions with its hosts. We present a *T. cruzi* transcriptome generated from two cloned strains showing specific virulence phenotypes: CL Brener, a virulent clone used as reference for the genome project and CL-14, which is neither infective nor pathogenic in vivo. RNAs extracted from epimastigotes, trypomastigotes and intracellular amastigotes harvested at 60h and 96 hours post-infection (hpi) of fibroblasts were sequenced. The transcriptomes from the epimastigote stage showed similar profiles, with most highly transcribed genes expressed at same level in both. Amastigotes 60hpi also present no significant differences when the highly expressed genes are compared in CL Brener and CL-14. However, in amastigotes 96hpi, mRNA levels of trans-sialidase, cytoeskeleton proteins, MASPs and mucins are more expressed in CL Brener than in CL-14. A few genes involved with protein synthesis, DGF-1 and HSP are more expressed in amastigotes 96hpi of CL-14. In trypomastigotes, trans-sialidases, genes involved with protein synthesis, TASV and DGF-1 are more expressed in CL Brener, whereas HSP and MASPs are more expressed in CL-14. During differentiation within the mammalian stages, we observed that, in CL Brener, mucins, MASPs and trans-sialidases have increased their expression when amastigotes 60hpi differentiate into amastigotes 96 hpi. None of these sequences were detected when the same comparison was made with CL-14. Comparison of the transcriptional profiles during differentiation of amastigotes 96hpi into trypomastigotes, revealed that in both clones expression of trans-sialidases, MASPs, TASV and GP63, were induced, however, with higher differences observed in CL-14. Together, these analyses suggest that the low virulence phenotype of CL-14 may be due to, at least in part, to a delay in the expression of genes required for the transition amastigote to trypomastigote. **Supported by:**CAPES **Keywords:**Virulence; rna-seq; trypanosoma cruzi

PV087 - IN THE SEARCH FOR RHODNIUS PROLIXUS GENES RELATED TO HEMATOPHAGY AND TO THE RESPONSE TO TRYPANOSOMA CRUZI INFECTION

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The triatomine Rhodnius prolixus (Rp) is a major vector of Chagas disease (CD) whose the etiological agent is the parasite Trypanosoma cruzi (Tc). Actually, CD affects about 7 million people worldwide, principally in the Latin America, and submits 100 million people to the risk of infection. Annually, 10 thousand patients die due the disorders caused by Tc infection. Recently, it was sequenced the Rp genome, which revealed 15,456 putative protein-coding genes. The genomic data obtained offered a large volume of information that we used here to study at the molecular level the physiology of blood digestion and the interaction between the insect and the parasite, through a RNAseq transcriptome of the gut of Rhodnius first instar nymphs. We analyzed samples from both unfed and insects at several time points after a blood meal. We also studied the effect of Tc infection with nymphs that were artificially infected with epimastigotes or trypomastigotes at concentrations of 103 or 107 Tc cells/ml of blood. Gene expression changes were followed in the midgut at several different time points after the ingestion of the infectious blood meals. Data obtained revealed that the presence of the parasite induced significant global changes in the insect gene expression, with distinct responses being obtained according to time post-infection, parasite developmental form or parasites levels in the blood meal. Altogether, these data presents the first comprehensive molecular analysis of the impact of Tc infection on the physiology of its insect vector. The present study may unveil important goals to prevent vector-based CD transmission. **Supported by:**CNPq;FAPERJ;CAPES;NIH;INCTEM
Keywords: Rhodnius prolixus; trypanosoma cruzi; parasite-host relationship

PV088 - *LEISHMANIA AMAZONENSIS* TELOMERASE ACTIVITY AND TELOMERE LENGTH ARE REGULATED DURING THE PARASITE DEVELOPMENTAL CYCLE

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The *Leishmania* genus comprises species that cause leishmaniasis, a spectrum of diseases that affects million people worldwide and to which there is no effective treatment and control. *Leishmania* spp. developmental cycle generally involves an insect and a mammalian as hosts. Inside the insect vector, *Leishmania* promastigotes multiply and transform into infective and non-proliferative metacyclics. During blood feeding, metacyclics are phagocytized by macrophages of the mammalian host and differentiate into amastigote forms. Amastigotes multiply and are able to infect new cells and other sandflies in another round of infection. Interrupting this cycle has been challenging for parasitologists and key for the discovery of new anti-parasite therapy. Telomeres, the nucleoprotein structures at the end of eukaryote chromosomes, have been considered potential targets for drug design as they maintain genome stability and cell proliferation. The effect of drugs directed against telomeres can be estimated by measuring telomere length. Here we estimated telomere length in axenic promastigotes and in lesion-derived amastigotes using three different approaches: Southern blot revealed by chemiluminescence, quantitative PCR and flow-FISH. All of these methods have been widely used to study human telomeres with the advantages of requiring low amount of DNA, are highly reproducible and accurate. Our results showed that, no matter the assay performed, amastigotes telomeres are shorter than promastigotes telomeres, with a difference of about >0,3 kb. As we could show, this intriguing phenomenon was not attributed by a defective telomerase, since we did not detect differences in enzyme activity when purified extracts of both forms were tested in TRAP assays. We speculate that the detected difference between promastigotes and amastigotes telomere length is probably a natural event that occur due to variations in telomerase holoenzyme biogenesis and composition during parasite development.
Keywords: Leishmania; diferent telomere length; telomerase

PV089 - IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF GDP- MANNOSE TRANSPORTERS OF TRYPANOSOMA CRUZI

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Glycoconjugates play important roles for survival and infectivity of parasites. Their synthesis occurs in the endoplasmic reticulum (ER) and Golgi apparatus using nucleotide sugars as substrates. These activated sugars, however, are mostly synthesized in the cytosol and must be transported across the ER and Golgi membranes. This intracellular transport is essential for proper glycosylation and it is carried out by nucleotide-sugar transporters (NSTs). Our group has been working on the identification and functional characterization of NSTs in Trypanosoma cruzi, the etiological agent of Chagas' disease. We have previously identified a UDP-GlcNAc (TcNST1) and a UDP-Gal (TcNST2) transporters . In this work, we aim to study GDP-Man carriers, since mannose residues are present in N-glycans, GPI anchors and in NETNES, which are specific glycoconjugates from T. cruzi. Furthermore, GDP-mannose transport is not present in mammals , making these transporters potential therapeutic targets. Based on a phylogenetic analysis, three genes were selected as candidates for GDP-Man transport: TcCLB.510355.220, TcCLB.510611.20, and TcCLB.504057.120. Only the TcCLB.504057.120 gene was able to transport GDP-Man, according to complementation experiments in Saccharomyces cerevisiae . RT-PCR data suggest that the transporter, named TcNST3, is constitutively expressed during the cycle life of the parasite. TcNST3 was labed with a C-terminal tag and its subcellular localization was analyzed. By fusion proteins with. Our results showed a specific localization at the Golgi apparatus Functional characterization of TcNST3 is currently under way by gene knockout experiments. **Supported by:**FIOCRUZ/ Cnpq/ Capes
Keywords: Glycoconjugates; nucleotide-sugar transporters; gdp-man

PV090 - STACHYTARPHETA CAYENNENSIS, A TRADITIONAL MEDICINE PLANT USED AS ANTILEISHMANIAL, TARGET ARGINASE ENZYME FROM LEISHMANIA (LEISHMANIA) AMAZONENSIS

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Introduction: Stachytarpheta cayennensis is a plant traditionally used to treat tegumentary leishmaniasis and as an anti-inflammatory. The aim of this study was to evaluate the mechanisms of action of extracts from S. cayennensis on the arginase enzyme of the trypanosome parasite Leishmania (Leishmania) amazonensis. Material and method: S. cayennensis was collected in Formosa da Serra do Maranhão, Brazil. Crude water extract was fractionated with n-butanol and fractions were tested against arginase enzymes from L. (L.) amazonensis. The most potent arginase inhibitor fraction was then tested against L. (L.) amazonensis promastigotes in axenic culture. To verify arginase inhibition in L. (L.) amazonensis, promastigote cultures were supplemented with L-arginine (substrate) and L-ornithine, a product of hydrolysis of L-arginine by the arginase enzyme. Results: butanolic fraction (BUF) is a potent L. (L.) amazonensis arginase inhibitor (IC50 = 5 ± 1 µg/mL). Verbascoside is the major constituent of BUF and exhibits competitive arginase inhibition (Ki= 4.2 ± 0.2 µg/mL). BUF showed an IC50 of 57 µg/mL against L. (L.) amazonensis promastigotes. The inhibition of arginase by BUF in the promastigote cultures was demonstrated by adding L-ornithine, which enhances parasite growth, whereas cultures supplemented with substrate L-arginine did not restore parasite growth interrupted by BUF.
Conclusions: Verbascoside present in S. cayennensis extracts (BUF) target the arginase enzyme of L. (L.) amazonensis, resulting in the death of the parasites.
Supported by:FAPESP e CNPQ
Keywords: Leishmania; arginase; verbascoside

PV091 - FUNCTIONAL CHARACTERIZATION OF ZFP29 AND ZFPTTP ZINC FINGER PROTEIN IN TRYPANOSOMA CRUZI

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Trypanosoma cruzi has a complex life cycle, involving at least four distinct development stages and two hosts. The alternations between the distinct forms indicate that changes occur in gene expression, since the forms throughout the life cycle differ functionally and morphologically. The gene expression regulation relies on post-transcriptional mechanisms. RNA binding proteins are key players involved in these regulatory mechanisms, including the group composed by zinc finger proteins with CCCH domain, which are associated to post-transcriptional regulation. The aim of this work is to investigate and characterize the zinc finger proteins ZFPTTP and ZFP29, which present a CCCH domain in T. cruzi, and their role in the complex regulatory mechanism that occurs in the differentiation process from the replicative (epimastigotes) to the infective form (metacyclic trypomastigotes). Expression analysis showed that both proteins are regulated throughout the life cycle, being expressed in epimastigotes, but not in metacyclic trypomastigotes. Ribosome profiling data from epimastigotes and metacyclic trypomastigotes showed that the ZFP29 and ZFPTTP mRNAs are in fact, associated with ribosomes in both forms, however the proteins are absent in metacyclic trypomastigotes. This result indicates that the mRNAs are regulated at the translation level in metacyclic trypomastigotes, stalled on polysomes but not engaged on translation. Immunolocalization of ZFP29 and ZFPTTP have shown cytoplasmic localization with a granular pattern for both proteins in epimastigotes. Interestingly, when the epimastigotes are subjected to nutritional stress ZFPTTP changes its localization and migrates to the nucleus. Altogether the results suggest a role for ZFP29 and ZFPTTP in the differentiation process of T. cruzi. **Supported by:**CAPES
Keywords: Trypanosoma cruzi; rna binding protein; zinc finger protein

PV092 - EFFECTS OF TRICHOSTATIN A, A HISTONE DEACETYLASE INHIBITOR, ON TRYPANOSOMATIDS

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DNA phosphorylation, methytlation, and deacethylation/acethylation are important epigenetic events which regulate 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, such as tubulin and actin. For these reasons, HDAC inhibitors (HDACi), such as Trichostatin A (TSA), have been used as chemotherapeutic agents in cancer treatment by blocking cell proliferation, cell cycle and also promoting intense ultrastructure alterations to the cells. Recently, it was shown that HDACi can have an antileishmanicidal activity, mainly by targeting the parasite HDAC6-like. In this study, we evaluated the effects of TSA on proliferation, viability, cell cycle and ultrastructure of two trypanosomatid species; *Trypanosoma cruzi* and *Angomonas deanei*. Our data with *T. cruzi* suggest that TSA can stabilize the microtubule cytoskeleton and also promote an accumulation of cells on G2/M phase of the cell cycle. Furthermore, the protozoa proliferation and viability were reduced after 72 h of treatment with 50 and 100 µM of the inhibitor. TSA was also tested in *A. deanei* considering two essential characteristics of this trypanosomatid: the atypical cytoskeleton arrangement and the presence of an endosymbiotic bacterium, which divides in synchrony with the host cell cycle in a microtubule-dependent manner. Preliminary results suggest that TSA can also inhibit *A. deanei* proliferation after treatment for 72 h with 100 µM. Transmission and scanning electron microscopy analyses will be performed to check eventual effects of this inhibitor on the symbiont and its host protozoan ultrastructure. In conclusion, our results reinforce the idea that histone deacetylases can not only be explored as chemotherapeutic targets but also to better understand the cell biology of trypanosomatids. **Supported by:**CNPq and FAPERJ
Keywords: Trichostatin a; trypanosoma cruzi; angomonas deanei

PV093 - MOLECULAR MODELLING OF LEISHMANIA MAJOR TELOMERASE RNA BINDING DOMAIN (TRBD) SHOWS TERTIARY STRUCTURE CONSERVATION BUT AMINOACID SUBSTITUTIONS SPECIFIC TO THE GENUS

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The parasites of the Leishmania genus are the causative agents of leishmaniasis, a disease that is of importance in public health and to which there is no effective treatment or vaccine available. Therefore to explore new approaches to treat these diseases is needed. Because of their importance in cell division and genomic stability, telomeres are considered potential targets for the development of new therapies against leishmaniasis. Telomeres are maintained by the telomerase complex which is minimally composed, by the protein component TERT (telomerase reverse transcriptase), and TER, the telomerase RNA component. The interaction between these components is vital for telomerase action. We have previously cloned and characterized both Leshmania major LmTERT and LeishTER components and we were able to show that they interact and co-localize in vitro. To decipher the dynamic of interactions between TERT and TER, can help us to understand how telomerase acts in a specific organism. There is no information available about the LmTERT tridimensional structure. In order to provide information about its interaction with TER we generated a in silico model of the RNA binding domain (TRBD) of LmTERT by molecular modelling and molecular dynamics simulations. Structural comparisons of LmTERT model and crystallographic structures of TRBDs from different eukaryotes show conservation on LmTERT tertiary structure. However, LmTERT has aminoacid substitutions specific to the Leishmania genus that are located on motifs involved on TER binding. Currently, different domains of LmTERT are being expressed in an heterologous system in order to perform in vitro protein:RNA interaction assays. The characterization of the interactions between the Leishmania telomerase complex components might reveal the nature of these interactions and the physiological importance of the complex. Finally, these results can provide insights about the evolution of the eukaryotic telomerase enzymes.
Supported by:FAPESP
Keywords: Leishmania; telomeres; tert

PV094 - AN ULTRASTRUCTURAL STUDY OF TRYPANOSOMA CRUZI DEVELOPMENTAL STAGES DURING METACYCLOGENESIS

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Trypanosoma cruzi is an excelent model to study cell differentiation, since it presents distinct developmental stages during its life cycle. At the insect midgut, epimastigotes undergo differentiation to metacyclic trypomastigotes, a process known as metacyclogenesis that involves differential gene expression and ultrastructural alterations. The kinetoplast corresponds to an enlarged portion of the protozoa mitochondrion that contains DNA (kDNA) organized in a network of interlocked circular molecules. In epimastigotes the kinetoplast is in the anterior end of the cell, presents a disk-shaped morphology and contains condensed kDNA fibers, whereas trypomastigotes present a more relaxed kDNA arrangement, situated into a globular structure in the posterior region of the cell. In this work different ultrastructural techniques were used to study T. cruzi during metacyclogenesis, focusing on kinetoplast. Since the beginning of the differentiation process, we observed a decrease of cell body length by scanning electron microscopy. In addition, transmission electron microscopy and DAPI staining revealed that changes in KDNA topology only occurred after kinetoplast migration to the posterior cell end. The deep-etching technique confirmed that kDNA fibrils in trypomastigotes are more loosely packed than in epimastigotes and showed association with globular structures that probably correspond to proteins. Regarding distribution of basic proteins, which can be involved in the kDNA condensation, cytochemistry assays showed different patterns of labeling in kinetoplasts of epimastigotes and intermediate forms. Analysis by atomic force microscopy showed that trypomastigote networks are more compacted than epimastigotes. Our next steps include the investigation of molecular mechanisms involved in the kDNA rearrangement. **Supported by:**FAPERJ e CNPq
Keywords:T. cruzi; differentiation; kdna

PV095 - HEME STIMULATES NA+/K+ ATPASE ACTIVITY TROUGH HYDROGEN PEROXIDE GENERATION IN *LEISHMANIA AMAZONENSIS*

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Leishmania amazonensis is a protozoan parasite that occurs in many areas of Brazil and causes skin lesions. Using this parasite, our group showed the activation of Na^+/K^+ ATPase through a signaling cascade that involves the presence of heme and protein kinase C (PKC) activity. Heme is an important biomolecule that has pro-oxidant activity and signaling capacity. Reactive oxygen species (ROS) can act as second messengers, which are required in various signaling cascades. Our goal in this work is to investigate the role of hydrogen peroxide (H_2O_2) generated in the presence of heme in the Na^+/K^+ ATPase activity of *L. amazonensis*. Our results show that increasing concentrations of heme stimulates the production of H_2O_2 in a dose-dependent manner until a concentration of 2.5 µM heme. To confirm that the effect of heme on the Na^+/K^+ ATPase is through the generation of H_2O_2, we measured enzyme activity using increasing concentrations of H_2O_2 and, as expected, the activity increased in a dose-dependent manner until a concentration of 0.1 µM H_2O_2. To investigate the role of PKC in this signaling pathway, we observed the production of H_2O_2 in the presence of its activator phorbol 12-myristate 13-acetate (PMA) and its inhibitor calphostin C. Both showed no effect on the generation of H_2O_2. Furthermore, we found that PKC activity is increased in the presence of H_2O_2, and that in the presence of calphostin C, H_2O_2 is unable to activate the Na^+/K^+ ATPase. 100 µM of Mito-TEMPO was capable of abolishing the stimulatory effect of heme on Na^+/K^+ ATPase activity, indicating that mitochondria might be the source of the hydrogen peroxide production induced by heme. The modulation of *L. amazonensis* Na^+/K^+ ATPase by H_2O_2 opens new possibilities for understanding the signaling pathways of this parasite. **Supported by:**CAPES, FAPERJ, CNPq
Keywords: Hydrogen peroxide; heme; na-k atpase

PV096 - *IN VITRO* RECENTLY DIFFERENTIATED *TRYPANOSOMA CRUZI* EPIMASTIGOTES ARE INFECTIVE TO THE MAMMALIAN HOST

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Trypanosoma cruzi has a complex biphasic life cycle in which four distinct developmental forms alternate between the insect vector and the mammalian host. It is assumed that replicating epimastigotes present in insect gut are not infective to the mammalian host, a paradigm corroborated by the widely acknowledged fact that only this stage is susceptible to the complement system. In the present work, we establish a *T. cruzi in vitro* epimastigogenesis model aimed mainly at characterizing the molecular (mRNA and protein) changes in a large scale perspective associated with this differentiation, and also to analyze the biological aspects of recently differentiated epimastigotes (rdEpi). We show that both trypomastigote stages of *T. cruzi* (cell derived and metacyclic) are able to transform to epimastigotes (named primary and secondary epimastigogenesis, respectively), but with distinct efficiency and kinectis, and that rdEpi have unusual properties when compared to long term cultured epimastigotes, as resistance to complement-mediated lysis and both *in vitro* (macrophage) and *in vivo* (mouse) infectivity. Shotgun proteomics analysis of all *T. cruzi* stages reveled a remarkable cluster of thirty proteins up-regulated only in rdEpi (including ABC transporters and ERO1), suggesting a role for them in rdEpi virulence. This work introduces a new experimental model for the study of host-parasite interactions, showing that rdEpi could be infective to mammalian host, at least *in vitro*. **Supported by:**CNPq, Fundação Araucária, Fiocruz
Keywords: Trypanosoma cruzi; epimastigogenesis; omics analyses

PV097 - FUNCTIONAL CHARACTERIZATION OF A UDP-GLCNAC TRANSPORTER IN TRYPANOSOMA CRUZI

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Nucleotide sugar transporters (NSTs) play an essential role in translocating nucleotide sugars into the lumen of the endoplasmic reticulum and Golgi apparatus to be used as substrates in glycosylation reactions. This intracellular transport of nucleotide sugars is essential for proper protein and lipid glycosylation. In this study, we have identified and characterized a UDP-N-acetylglucosamine transporter (named TcNST1) from Trypanosoma cruzi, the etiological agent of Chagas' disease. TcNST1 was identified by heterologous expression of putative T. cruzi NSTs in a Kluyveromyces lactis mutant strain. A GFP-TcNST1 fusion protein was shown to be localized specifically at the Golgi apparatus. Knockout experiments suggest that the TcNST1 gene is essential. Single knockout (sKO) mutants display normal growth rates but are partially impaired in cellular differentiation and infection of mammalian VERO cells. Also in opposition to wild-type cells, metacyclic forms of Dtcnst1 are susceptible to complement mediated lysis. Furthermore analysis of specific glycoproteins by western blot indicate abnormal patterns of glycosylation in the mutant. The fact that the TcNST1 gene is essential and that sKO mutants are compromised in differentiation and infection reveal the importance of TcNST1, and NSTs in general, in these parasites.**Supported by:**Fiocruz, CNPq and Fundação Araucária
Keywords: Nucleotide sugar; transporter; trypanosomatids

PV098 - FUNCTIONAL CHARACTERIZATION OF THE TCRAB14 GENE FROM TRYPANOSOMA CRUZI: PRODUCTION OF PARASITE LINEAGES EXPRESSING DOMINANT POSITIVE, DOMINANT NEGATIVE AND PRENYLATION DEFECTIVE MUTANTS.

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Rab family GTPases are fundamental to intracellular transport, regulating vesicle targeting and fusion. These proteins undergo an activation cycle in which they are active when bound to GTP and inactive after GTP hydrolysis (GDP bound). Rab proteins are also post-translationally modified with two geranylgeranyl tails on C-terminal cysteine residues, which are crucial for cell localization and function. Although Rab genes are widely distributed, only a small group of Rab subfamilies are conserved through the eukaryotic domain. One of these groups comprises Rab14 proteins, a Rab subfamily whose cellular function is not well understood. Much of our current knowledge about the cellular functions of Rab proteins was obtained by analysis of cells expressing positive and negative dominant mutants of Rab genes. We initiate the characterization of Trypanosoma cruzi TcRab14 gene by producing parasite lineages expressing a dominant positive mutant (locked on GTP bond stage), a dominant negative mutant (locked on GDP bond stage) and a prenylation defective mutant (unable to associate with membranes). Initially, T. cruzi (Dm28c) TcRab14 gene was cloned in commercial vector pGEM-T-Easy and submitted to PCR based site-directed mutagenesis to produce TcRab14-Q65K (dominant positive), TcRab14-S20N (dominant negative) and TcRab14-ΔCXC (prenylation defective) mutants, with were also cloned into pGEM-T-Easy vector. These mutant genes were further subcloned in the pTEX-GFPn T. cruzi expression vector to produce chimeric constructs with the Green Fluorescent Protein gene. The resulting constructs were transfected in epimastigotes of Dm28c T. cruzi by electroporation. Genetically modified epimastigote lineages expressing these constructs were selected with the drug G418. Confirmation of the expression of recombinant mutant genes was performed with RT-PCR and fluorescence microscopy. We hope these mutant lineages will be valuable to investigate the role of TcRab14 in T. cruzi physiology. **Supported by:**FUNEMAC
Keywords: Trypanosoma cruzi; gtpase; rab

PV099 - UNRAVELLING THE TBTRL1 ENZYME FUNCTION IN *TRYPANOSOMA BRUCEI*

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Transfer RNAs (tRNAs) play a central role in protein synthesis as translators of the genetic code connecting the information found in genes to that ultimately deposited into proteins. The biosynthesis of mature and functional tRNAs involves many processing steps, including 5' and 3' end trimming, incorporation of numerous chemical post-transcriptional modifications and addition of a CCA sequence at the 3' end. A subset of tRNAs, that varies with different organisms, also contain introns that are cleaved by a tRNA splicing endonuclease, generating exon halves that are subsequently sealed by a splicing-specific tRNA ligase. We have identified a homolog of yeast tRNA ligase (Trl1) in trypanosomatids, that is presumably responsible for the joining of the two tRNA exon halves generated by endonuclease cleavage of $tRNA^{Tyr}$; the only intron-containing tRNA in these organisms. In the present work, we have preliminarily characterized the Trl-1 homolog of *T. brucei* (TbTrl1). We constructed a stable *T. brucei* RNAi knockdown strain of Tbtrl1 and show that RNAi induction leads to a severe growth defect. Unexpectedly, RNAi of TbTrl1 caused accumulation of intron-containing tRNA, as opposed to unligated exon halves, and almost a complete disappearance of mature tRNA. Furthermore, down-regulation of TbTrl1 also led to pronounced cell-cycle arrest. Taken together these results demonstrate the essentiality of the TbTrl1 homolog for cell viability. The observed accumulation of intron-containing tRNA is discussed in the context of the intra-cellular localization of TbTrl1and its potential role in tRNA export from the nucleus. The restricted phylogenetic distribution to fungi and trypanosomatids for Trl1-splicing ligases shown in this work makes this family of enzymes promising therapeutic targets for such medically important organisms. **Supported by:**CNPq, FAPERJ, NIH
Keywords: Trna; intron; brucei

PV100 - STUDY OF THE ROLE OF CHECKPOINT PROTEINS LMHUS1 AND LMRAD9 IN *LEISHMANIA* GENOME PLASTICITY

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Gene copy number variation (CNV) is commonly observed in Leishmania and is an important mechanism of adaptation to different environments. CNV is also a hallmark of tumor cells and mainly results from a deficient checkpoint response. In other eukaryotes, the 9-1-1 complex (Rad9, Rad1 and Hus1) plays a pivotal role in the ATR-mediated checkpoint signaling events. We showed the conservation of the 9-1-1 clamp in Leishmania and are currently investigating its role in the parasite DNA metabolism. We speculate on its participation in the mechanisms underlying genome plasticity in Leishmania. We have investigated CNV of the DHFR-TS locus upon methothrexate (MTX) selection in LmRad9 or LmHus1 deficient cell lines. Since both proteins are possibly involved in replication checkpoint, we also did the MTX selection in hidroxyurea-treated cells. Quantitative PCR and PFG-separated chromosomes were used to analyze the DHFR-TS CNV. We found that genotoxic stress led to DHFR-TS amplification in LmHus1-deficient cells but not in the LmRad9-deficient cell line. Considering that CNV may be a consequence of replication disturbance, we analyzed EdU incorporation in exponentially growing LmRad9 or LmHus1 defficient cells. EdU incorporation was increased in LmHus1-deficient cells, which agrees with the increased S phase progression rate observed in this cell line. Conversely, LmRad9-deficient cells incorporated less EdU, which agrees with the decrease in S-phase progression in these cells. Altogether, these findings suggest that these proteins may have separate roles in the maintenance of genome integrity. We have also used whole-genome sequencing to investigate global CNV in LmHus1 or LmRad9 deficient cells submitted to replication stress. Also, we are exploring the chromosome CNV in individual LmHus1-deficient cells using FISH to investigate the involvement of LmHus1 of LmRad9 in the pattern of aneuploidy or mosaicism in these protozoa. **Supported by:**FAPES
Keywords: Leishmania; 9-1-1 complex; gene amplification

PV101 - SUBCELLULAR LOCALIZATION OF ACTIN 4 IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi has a peculiar cytoskeleton based on a subpellicular stable microtubule network. Therefore, the role of other components of the cytoskeleton such as actin is still poorly understood in this protozoan. Although at least four genes coding for different actin isoforms have been found in *T. cruzi* genome, none of these isoforms have been deferentially characterized in terms of their expression levels, subcellular localization or functions. In this context, the present work aims to understand the issues discussed above in relation to the gene TcCLB.503841.40 annotated as “actin, putative” in the genome of *T. cruzi*, and informally named as actin 4 in this work. For this purpose, parasites genetically modified and specific polyclonal serum were produced. Epimastigotes forms of *T. cruzi* were genetically modified to express actin 4 fused to the commercial tag FLAG. After selection, positive parasites were confirmed by both Western Blot and PCR. For the production of a specific polyclonal serum, peptides containing a non-conserved region of actin 4 were used to immunize mice. Both tools allowed subcellular localization of actin 4 in wild type and genetically modified parasites. Indirect immunofluorescence with polyclonal serum showed that native actin 4 is preferentially observed at the perinuclear region and in a point at the anterior region, near to the flagellar pocket. On the other hand, there was no co-location of the recombinant protein using commercial anti-FLAG antibody and the policlonal serum. These results corroborate the importance of the development of different (but complementary) tools to study the biological diversity of isoforms of a same protein in *T. cruzi*. In the future, the polyclonal serum will be an important instrument for the determination of protein expression levels, and immunoprecipitation of the recombinant actin 4 will allow the analysis of proteins associated to it by mass spectrometry.
Supported by: Fiocruz
Keywords: Trypanosoma cruzi; actin; cytoskeleton

PV102 - AN IN VITRO HIGH CONTENT SCREENING ASSAY FOR THE SIRTUIN RP3 OF TRYPANOSOMA CRUZI

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Trypanosoma cruzi exhibits a complex life cycle alternating between invertebrate and vertebrate hosts. As T. cruzi faces distinct environment changing gene expression and metabolism, we hypothesized that sirtuins could have a regulatory role in these adaptations, as sirtuins are enzymes that exhibit NAD+ dependent deacetylase activity and are key regulators of diverse process from bacteria to higher eukaryotes. T. cruzi has two sirtuins, TcSir2rp1 found in the cytosol and TcSir2rp3 in the mitochondria. The TcSir2rp3 overexpressing parasites showed increased rates of cell invasion and multiplication of the intracellular amastigotes, which are reduced after treatment with salermide, a specific inhibitor of sirtuin. Therefore, TcSir2rp3 must play a key role in the T. cruzi development. Here, we established a plate assay using a recombinant form of TcSir2rp3 and an internally quenched fluorescent and acetylated peptide, as substrate. After incubation of the enzyme with the substrate, the fluorescent product was detected by addition of trypsin that only cleaved the deacetylated substrate. This assay was found highly sensitive with the enzymatic activity blocked by low (< 0.1 uM) concentrations of salermide. WE concluded that this assay is suitable to screen new compounds against Chagas disease. **Supported by:**CNPQ
Keywords: Trypanosoma cruzi; sirtuin; salermide

PV103 - DICRE-BASED CONDITIONAL KNOCKOUT OF LMHUS1 REVEALS FUNCTIONAL COMPARTMENTALIZATION OF THE 9-1-1 COMPLEX IN THE DNA DAMAGE RESPONSE OF *LEISHMANIA MAJOR*.

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Maintenance of the eukaryotic genome is essential for the faithful transmission of the genetic information. The 9-1-1 complex (formed by Rad9, Rad1 and Hus1 proteins) is pivotal for genome stability and participates in many roles in the DNA damage response, including cell cycle checkpoint and telomere homeostasis. We are interested in the mechanisms that give rise to and maintain the remarkable genome plasticity of Leishmania. We show here that LmRad9, LmRad1 and LmHus1 interact in a 9-1-1 complex in vivo in *L. major*. Surprisingly, size exclusion chromatography revealed that both LmRad9 and LmHus1 can also exist in alternative complexes in vivo. Consistent with functional compartmentalization of these subunits, the phenotypes observed upon LmRad9 deficiency are different from those resulting from LmHus1 deficiency. To further understand this, we generated a cell line in which LmHus1 expression can be conditionally abrogated by excision of the LmHus1 locus upon induction of DiCre activity. LmHus1 null cells were unable to sustain continuous proliferation, indicating that it is essential for *L. major* survival. Interestingly, when these cells were released from G1/S arrest, they resumed the cell cycle more quickly than control cells and presented a decrease in damage-related H2A phosphorylation levels. However, when LmHus1 null cells were released from mid-S phase arrest, they were unable to resume the cell cycle and showed an increase in H2A phosphorylation levels, indicating that LmHus1 has different roles through the cell cycle. Thus, our data demonstrate the existence of an active 9-1-1 clamp in Leishmania, and indicate that at least two of the subunits have evolved to participate in compartmentalized functions within this parasite. For at least LmHus1, such functional diversification relates to essential functions in cell cycle progression. Currently, we are characterizing the effects of DiCre-based conditional knockout of LmRad9. Supported by FAPESP and CNPq. **Supported by:**FAPESP **Keywords:**9-1-1 complex (rad9, rad1, hus1); dna damage response and genome maintenance; cell cycle and checkpoint control

PV104 - MOLECULAR CHARACTERIZATION OF ISOLATES FROM THE GENUS *TRYPANOSOMA* AT FIOCRUZ PROTOZOA CULTURE COLLECTION (FIOCRUZ – COLPROT).

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Trypanosomatids are flagellated protozoa that comprise two distinct groups: (1) the monoxenic, which presents typically only one host, mainly an insect and (2) the heteroxenic, whose life cycle alternates between an invertebrate host and a second host (which may be a vertebrate or a plant). The heteroxenic genus *Trypanosoma* has a large genetic diversity, which reflects its worldwide distribution and host range. The classical and nowadays outdated taxonomic identification of *Trypanosoma* spp. had been based on the combination of specific morphotypes, life cycle and hosts. However, these parameters do not always reflect the phylogeny. Here, we employed the multilocus sequencing to confirm the taxonomic status of several species of the genus *Trypanosoma* deposited at Fiocruz Protozoa Culture Collection (Fiocruz-COLPROT) and to identify new isolates. Up to now, we used three nuclear targets - Alpha-enolase, glycosomal glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) and the small subunit (SSU) of the ribosomal RNA (rRNA). We obtained sequences for five different species of the genus *Trypanosoma* (T. cruzi, T. rangeli, T.mega, T.cervi and T.conorhini) and from four others isolates without previous identification: one isolated from *Leptodactyllus ocelattus* (frog, anuran), one isolated from *Micronycteris* sp. (bat) and two others isolated from *Philander opossum* (opossum). Our data were compared with the GenBank database for SSU rRNA and gGAPDH markers, and the analysis confirmed the taxonomic status for previously identified COLPROT samples. The isolate from opossum presents high similarity with *Trypanosoma rangeli*, and the isolate from frog presents similarity to *Trypanosoma mega* (another trypanosomatid isolated from anuran). **Supported by:**FAPERJ, CAPES, CNPq and IOC/FIOCRUZ **Keywords:**Trypanosoma; molecular markers; phylogeny

PV105 - BIOCHEMICAL CHARACTERIZATION OF TWO PHOSPHATE TRANSPORT SYSTEMS IN *PHYTOMONAS SERPENS*

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Inorganic phosphate (Pi) is an essential nutrient for all organisms since it is required for a variety of biochemical processes, such as signal transduction and phosphate-containing biomolecules synthesis. Assays of 32Pi uptake performed in reaction buffer with or without Na+ indicated the existence of a Na+-dependent and a Na+-independent Pi transporter in Phytomonas serpens. Phylogenetic analysis of two hypothetical protein sequences of Phytomonas (EM1) showed high similarity with high-affinity Pi transporters, such as Pho84 (Na+-independent Pi transporter) and Pho89 (Na+-dependent Pi transporter). Plasma membrane depolarization by carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP, an H+ ionophore) strongly decreased Pi uptake via both Na+-independent and Na+-dependent carriers, indicating that a membrane potential is essential for Pi influx. In addition, furosemide-sensitive Na+-pump activity in membranes of cells grown at low Pi conditions was found 6-fold higher than that detected in membranes of cells cultivated at high Pi concentration, suggesting that up-regulation of Na+-ATPase pump enhances Pi uptake by Pho89p Na+:Pi symporter in alkaline conditions. Indeed, alkalization of the Pi uptake assay buffer enhanced Pi influx through Na+-dependent Pi transporter. Thus, the present work characterizes for the first time two inorganic phosphate transporters energized by Na+ and H+ gradients and activated by low Pi availability in the phytopathogen Phytomonas serpens. **Supported by:**CNPQ/CAPES/FAPERJ
Keywords: phytomonas serpens; inorganic phosphate (pi); na+ and h+ gradients

PV106 - ANALISIS OF THE INTERACTION BETWEEN THE TRANSLATION INITIATION FACTOR EIF4E4 AND A POLY-A BINDING PROTEIN HOMOLOGUE (PABP1) IN TRYPANOSOMATIDS

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A checkpoint in the gene expression in eukaryotes is the initiation stage of protein synthesis, whereas the complex eIF4F (formed by the subunits eIF4A, eIF4E and eIF4G) performs a crucial role by facilitating the recognition of the mRNA by the ribosomes. In trypanosomatids two eIF4F-like complexes were identified with distinct roles in translation initiation: one formed by EIF4E4, EIF4G3 and EIF4AI and another based on EIF4E3, EIF4G4 and EIF4AI, both complexes found to be associated with PABP (poly-A binding protein) homologues. The Leishmania PABPs have the typical N-terminal RNA binding region, linker segment and the C-terminal PABC domain. Recently, a direct interaction between EIF4E4 and a Leishmania PABP homologue (PABP1) was identified, which indicates novel aspects of translation initiation in these early diverging eukaryotes. This interaction was investigated in vitro and the interacting motifs between PABP1 and EIF4E4 were identified through pull-down assays. To identify which region of the PABPs mediates these interactions, PABP1 mutants were generated and mutations targeting conserved residues within its PABC domain abolished the binding to EIF4E4. To identify if this interaction also occurs in vivo in trypanosomatids a Fluorescence Resonance Energy Transfer (FRET) assay was set up in Trypanosoma brucei using cerulean and YFP fluorescent proteins to tag EIF4E4 and PABP1, respectively. The interacting partners were then analyzed using the Delta Vision Elite Imaging System. However the negative control, T. brucei wild type cells, was fluorescent even in the absence of any tagging. Therefore new FRET methods should be tried in order to figure out how these proteins interact in vivo.
Keywords: Trypanosomatids; eif4f complex and pabp; protein interaction

PV107 - *LEISHMANIA (VIANNIA) GUYANENSIS* ISOLATED FROM A PATIENT WITH TEGUMENTARY LEISHMANIASIS IN GOIáS: POSSIBILITIES OF SPREADING OF THE SPECIES IN BRAZIL

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The presence of *Leishmania* species in different areas depends on the ecosystem. The migratory behavior of vectors, animals and human beings infected with *Leishmania spp.* can facilitate the spreading of the species to distinct geographic regions. Here, we characterized clinical field isolates of *Leishmania spp.* obtained from patients with cutaneous Leishmaniasis who live in Goiás State, Brazil, but the presumed areas of infection were in Goiás, Tocantins and Pará states. Three strategies were used: sequencing of small subunit ribosomal RNA (SSU rDNA); PCR assays to discriminate *L. (V.) braziliensis* from other organisms of the *Viannia* subgenus, using sets of primers for G6PD; and ITS was amplified, cloned and the nucleotide sequence of three independent positive clones were phylogenetically analyzed. Three isolates of parasites were identified as L. (Viannia) braziliensis and one as *L. (V.) guyanensis*. In vitro, growth was similar among all parasites. However, in C57BL/6 mice *L. (V.) guyanensis* was better controlled than *L. (V.) braziliensis*. *L. (V.) guyanensis* caused faster lesion development in C57BL/6 mice deficient in interferon gamma than *L. (V.) braziliensis* isolates. In BALB/c mice, the development of the lesions was similar between isolates from both species, nevertheless amastigotes could not be observed in macrophages of *L. (V.) guyanensis*-infected mice on the 11th week of infection. Data suggest that *L. (V.) guyanensis* can be circulating in Goiás, a state where autochthonous cases of this species have not been reported. Considering the difficulties to differentiate *L. (V.) guyanensis* from *L. (V.) braziliensis* at molecular, morphological, and clinical (human and murine models) levels, the presence of *L. (V.) guyanensis* can be underestimated in Brazilian regions. Besides, the results also suggest that, at least in murine model, *L. (V.) guyanensis* can be more susceptible to microbicidal mechanisms induced by IFNγ than *L. (V.) braziliensis.* **Supported by:**CAPES, CNPQ, FAPEG
Keywords: Leishmania viannia guyanensis; tegumentary leishmaniasis; vectors

PV108 - RELATIVE EXPRESSION ANALYSIS OF MEIOTIC DIVISION RELATED GENES OF TRITRICHOMONAS FOETUS DURING DIFFERENTIATION OF TROPHOZOITES INTO PSEUDOCYSTS

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Tritrichomonas foetus is the causative agent of bovine genital trichomoniasis, a sexually transmitted disease that is one of the leading causes of abortion and infertility in bovine livestock. Although trophozoites are the main stage on T. foetus life cycle, the parasite can differentiate to a distinct morphology, known as pseudocyst, when submitted to stressful conditions. Albeit traditionally considered to proliferate only by means of mitotic division, several evidences indicate that sex and meiotic cell division occur in species closely related to T. foetus. In this work we have investigated the expression of a set of meiotic related genes during differentiation of T. foetus trophozoites in pseudocysts, as well as in regression of trophozoites from pseudocysts. Real time RT-PCR methodology was applied to parasite samples submitted to differentiation by incubation at 4 C in nine distinct moments. Genes encoding T. foetus actin, b-tubulin, GAPDH and eEF-1a were tested with DeltaCt, BestKeeper, NormFinder and Genorm programs to select for a suitable control for relative expression analysis. T. foetus actin mRNA presented the best stability values and was therefore adopted as the reference gene for subsequent relative expression analysis. Genes encoding T. foetus meiotic recombination proteins Dmc1, Hop1, Hop2, Mnd1, Mlh1, Mlh2, Mlh3, Mre11 and Pms1 were investigated by relative expression analysis. Cq values were normalized using Pfaffl method and relative expression values were analyzed with the Kruskal-Wallis test. Significant differences were not detected for any of the tested genes in these conditions. Curiously, cluster analysis suggests co-expression patterns similar to what is observed in other organisms for expression of mismatch repair genes and for genes involved with loop resolution in meiotic recombination. During this work, we have also applied PCR with degenerated primers to clone Actin and Dmc1 genes, previously not available for T. foetus. **Supported by:**CAPPES
Keywords: Tritrichomonas foetus; gene expression; meiosis

PV109 - ESTABLISHMENT OF A REFERENCE GENE FOR RT-QPCR ANALYSIS OF *TRICHOMONAS VAGINALIS* DURING DIFFERENTIATION OF TROPHOZOITES INTO PSEUDOCYSTS

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The real-time reverse transcription polymerase chain reaction (RT-qPCR) is a sensitive and accurate method for quantification of relative gene expression. The reliability of this analysis depends on the validation of suitable reference genes for relative expression studies. *Trichomonas vaginalis* is a parasite of the human urogenital tract and causative agent of trichomoniasis. This parasite is usually found as a motile flagellated trophozoite or as an adherent amoeboid stage. However, when subjected to stressful microenvironmental conditions, *T. vaginalis* trophozoites can differentiate into pseudocysts. Pseudocysts present internalized flagella, spherical morphology and some physiological resistance to unfavorable conditions. Our main objective is to investigate *T. vaginalis* gene expression during trophozoite to pseudocyst differentiation using the RT-qPCR approach. Here we establish asuitable reference gene to be used as a control of invariant expression during this differentiation process. We have selected nine genes to test for invariant expression: 18S rRNA, actin, b-tubulin, GAPDH, G6PDH, eIF2-a, eEF1-a, RNA pol-1 and ferredoxin. Differentiation was induced by incubating 10^7 parasites (JT strain) at 4°C during periods of 0h, 2h, 12h and after recovery of trophozoites at 37°C. Total RNA was extracted and cDNA was synthesized for each experimental point. Primer pairs were tested and showed adequate amplification profiles under our experimental conditions. The most appropriate dilution of cDNA for use in RT-qPCR assays was determined as 1:100. Analysis of expression stability was performed with *BestKeeper* software. Expression of GAPDH gene presented the best combination of standard deviation values (CqSD: 0.8; x-fold SD: 1.74) and coefficient of correlation (r = 0,87), and thus will be employed as reference in studies of relative analysis of gene expression during *T. vaginalis* differentiation. **Supported by:**CNPq/CAPES
Keywords: Rt-qpcr; trichomonas vaginalis; reference gene

PV110 - FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF TCSUB2: AN ESSENTIAL PROTEIN FOR MRNA EXPORT IN *T. CRUZI*

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Comparative genomic analyzes have showed that mRNA export pathway is less conserved than other RNA nuclear export pathways in several species of parasites as compared to other eukaryotes. However, the orthologue in *T.brucei* of the RNA Helicase DEAD box Sub2 in S. cerevisiae (UAP56 - mammals) is the most conserved throughout eukaryote phylogeny and essential for mRNA export in both species. RNAi assays in T. brucei have shown that the knockdown of TbSub2 is lethal and causes accumulation of mRNA in the nucleus. Even though TbSub2 is highly conserved, we cannot assume a priori that this protein has the same function in other species of trypanosomes. In this study, we have demonstrated by complementation assays that the expression of TcSub2 protein restores the wild type phenotype after TbSub2 RNAi induction in *T.brucei*, showing that these proteins have the same function in both T.cruzi and *T.brucei*. TcSub2 is a member of DEAD box helicases family that rearranges RNA secondary structure and RNA-protein interactions using ATP hydrolysis as an energy source (OR in an ATP-dependent reaction) Biochemical assays have confirmed that TcSub2 ATPase domain is functional with a Vmax = 0.5 µM/min that increases 0.3 times in the presence of RNAs. This value differs widely from UAP56 that has lower affinity for ATP (Vmax = 0.126 µM/min) compared to TcSub2, notwithstanding this affinity is increased up to 20 times in the presence of RNA. Structural and functional characterization of a mutant protein (TcSub2K87N) have demonstrated that the conserved residue K87 is essential for ATP hydrolysis, similar to yeast and mammalian homologues. The mutation K87N does not alter TcSub2 secondary and quaternary structures and, molecular modeling suggests that the lack of activity might be due to small changes in tridimensional structure precluding the correct binding of ATP in the active site of TcSub2. **Supported by:**CAPES, CNPq e Fundação Araucária
Keywords: Helicases; tripanosoma cruzi; exportação nucleocitoplasmática