PV1 - USE OF SIRTUINS AS POSSIBLE DRUG TARGET FOR CHAGAS DISEASE TREATMENT

MORETTI, N.S.[1]; AUGUSTO, L.S.[1]; CLEMENTE, T.M.[1]; ANTUNES, R.P.P.[1]; YOSHIDA, N.[1]; TORRECILHAS, A.C.T.[1]; CANO, M.I.N.[2]; SCHENKMAN, S.[1] *1.UNIFESP, SAO PAULO, SP, BRASIL; 2.UNESP, BOTUCATU, SP, BRASIL.* e-mail:nilbio85@yahoo.com.br

Lysine acetylation has emerged as a major post-translational modification involved in diverse cellular functions. While histone proteins are the founding members of lysine acetylation substrates, it is now clear that several other proteins can be acetylated. The protein acetylation is regulated through the activities of two families of proteins, lysine acetyltransferases (KATs) and lysine deacetylases (KDACs). Amongst the KDACs we have the Sirtuins, NAD+-dependent histone deacetylases involved in different cellular mechanisms. We investigated the protein acetylation during T. cruzi proliferation and differentiation and the involvement of sirtuins in these phenomena. Several changes in the acetylation were observed during parasite growth and metacyclogenesis, suggesting a role of this modification in these processes. Using cell lines overexpressing TcSir2rp1 and TcSir2rp3, the two putative T. cruzi sirtuins, we found that TcSir2rp1 is localized in the cytosol and TcSir2rp3 in the mitochondrion. Both proteins were able to modify the acetylation levels of specific proteins, but generated antagonistic effects on parasite development. TcSir2rp1 overexpression acts impairing parasite growth and differentiation, whereas TcSir2rp3 improve both. Moreover, overexpression of TcSir2rp3 expands the ability of metacyclic-trypomastigotes to invade and multiply inside the host cell in vitro. The same effects were not observed when we overexpressed a TcSir2rp3 mutant. Altogether, these data suggested a key role of sirtuins in T. cruzi biology, and to explore in the context of development of new treatments, we investigate the effect of salermide, a specific sirtuin inhibitor. Treatment of parasites with salermide during in vitro infection could negatively affects invasion and multiplication of intracellular parasites. Also, salermide reduced significantly the parasite infection during in vivo assays. Our findings prompted the use of sirtuins as drug targets for Chaga's disease.**Supported by:**FAPESP and CNPq

PV2 - INVOLVEMENT OF *T. CRUZI* AND *T. BRUCEI* DNA MISMATCH REPAIR PROTEINS IN OXIDATIVE STRESS RESPONSE

SILVA, V.G.[1]; ZEB, T.F.[2]; CAMPOS, P.C.[1]; MIRANDA, J.B.[1]; ALVES, C.L.[1]; MCCULLOCH, R.[2]; MACHADO, C.R.[1]; TEIXEIRA, S.M.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UNIVERSITY OF GLASGOW, GLASCOW, ESCOCIA. e-mail:vivianegrazielle@hotmail.com

DNA Repair mechanisms are crucial for genetic maintenance and to generate genetic variability. Its importance is highlighted in a context of parasitic infection since both genomic stability and sequence variation are crucial to maintain a successful infection. MSH2 and MSH6 are proteins of the Mismatch Repair Pathway (MMR) that form heterodimers responsible for the recognition of base mismatches that occur during DNA replication. Recent evidence suggests that in addition to mismatch repair, MMR pathway also plays an important role in mediating oxidative DNA damage repair. With the aim of investigating their involvement in the oxidative stress response *T. cruzi* and *T. brucei* must develop during infection, knockout mutants for two genes encoding MMR components were generated. Cloned $_{T.\ cruzi}$ cell lines in which one msh2 or msh6 allele was deleted (Tcmsh2+/- and Tcmsh6+/-) showed increased susceptibility to H_2O_2 when compared with wild type culture. Surprisingly, knocking out both Tcmsh2 alleles resulted in increased resistance to H_2O_2. Measurement of 8-oxo-guanine in gDNA and kDNA before and after exposure to H_2O_2 showed that Tcmsh2+/- parasites accumulate more 8-oxo-G in kDNA after exposure to H_2O_2 than WT cells. Tcmsh2-/- mutants are also more resistant to the highly oxidative environment inside infected macrophages when compared to WT parasites. To investigate if those proteins can be involved in repairing mitochondrial DNA we have determined their cellular localization and showed that, in the absence of oxidative stress, TcMSH2 and TcMSH6 have a nuclear localization. Similar to *T. cruzi* epimastigotes, an adaptation to the loss of MSH2 was also observed in the insect form of *T. brucei* msh2-/- mutants: whereas bloodstream forms of Tbmsh2 knockout are more susceptible to H_2O_2, procyclic forms are more resistant compared to WT. **Supported by:**CNPq, Capes, Fapemig

PV3 - COMPARATIVE GENOMIC ANALYSIS OF TRYPANOSOMA CRUZI SINGLE CELL CLONES DERIVED FROM CL BRENER CLONE SUBMITTED TO GAMMA IRRADIATION

MARINI, M.M.[1]; BARROS, R.R.M.[2]; ANTONIO, C.R.[1]; VAN SCHAIK, M.W.[1]; ROGERO, S.O.[3]; ROGERO, J.R.[3]; VILLACIS, R.A.R.[4]; ROGATTO, S.R.[5]; DASILVEIRA, J.F.[1]
1.UNIFESP, SAO PAULO, SP, BRASIL; 2.NIAID, NIH, ROCKVILLE, ESTADOS UNIDOS; 3.IPEN, SÃO PAULO, SP, BRASIL; 4.CIPE, SÃO PAULO, SP, BRASIL; 5.UNESP, BOTUCATU, SP, BRASIL. e-mail:marjoriemm@globo.com

Trypanosoma cruzi is highly resistant to the effects of ionizing radiation. After irradiation there is an extensive chromosomal fragmentation associated with the double strand breaks (DSBs) and arrest of the cell cycle (Regis-Da-Silva et al., Mol Bioch Parasitol 149, 91,2006). In this work, epimastigotes of clone CL Brener were exposed to radiation doses from 100, 250 and 500 Gy and after 10 days the karyotype was reconstructed as judged by the presence of chromosomal bands with the expected sizes. The parasites were then cloned by serial dilution and 18 single cell-derived clones were selected for further analysis. Hybridization of the chromosomal bands separated by PFGE with markers from three large linkage groups showed that synteny is conserved in the chromosomal bands XX, XVI and XIII. However differences with spliced leader and DNA satellite sequences were observed. Of the 18 clones, 6 showed alterations in the number and/or size of chromosomal bands with these two markers. Three clones (B2-100 Gy, C1-250 Gy and E5-500 Gy) were selected for comparative genomic hybridization based in microarray (aCGH) experiments that revealed many chromosomal alterations, especially associated with DNA gain. Chromosomal aberrations consistent with segmental aneuploidy associated with DNA gain were observed in 15 in silico chromosomes. TcChr6 was completely duplicated in clones B2 and E5. The pattern of small changes dispersed throughout the chromosome was found in 15 chromosomes and changes involving extensive regions were detected in 11 chromosomes. A small number of deletions were found: only 26 of the 555 recombination events and they also have small amplitude, with an average size of 21 kb. However on chromosome in silico TcChr39-P a deletion of approximately 266 Kb was observed in the chromosomal end of the three irradiated clones indicating a possible fragile site in the subtelomeric region.**Supported by:**fapesp

PV4 - PUTATIVE ROLE OF ARGININE METHYLATION BY *LMJ*PRMT7 IN RBP FUNCTION AND VIRULENCE DURING *LEISHMANIA MAJOR* DEVELOPMENT

FERREIRA, T.R.[1]; FERREIRA, E.V.C.A.[1]; DEFINA, T.P.A.[1]; SMITH, D.F.[2]; WALRAD, P.[2]; PAPADOPOULOU, B.[3]; CRUZ, A.K.[1]
1.BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGÊNICOS, FMRP-USP, RIBEIRAO PRETO, SP, BRASIL; 2.CENTRE FOR IMMUNOLOGY AND INFECTION, DEPARTMENT OF BIOLOGY, UNIVERSITY OF YORK, YORK, REINO UNIDO; 3.RESEARCH CENTER IN INFECTIOUS DISEASES, CHUL RESEARCH CENTER (CHUQ), LAVAL UNIVERSITY, QUÉBEC, CANADÁ. e-mail:tiago.rf@usp.br

The *Leishmania major* genome encodes five protein arginine methyltransferases (PRMT) homologs, including PRMT7, which is only described in a restricted group of eukaryotes. We have found that both *Lmj*PRMT7 expression and arginine monomethylation of cellular proteins are tightly regulated during promastigote development, reaching minimal levels at the stationary growth phase. On the other hand, procyclic promastigotes and amastigotes displayed elevated *Lmj*PRMT7 expression. We have found that *Lmj*PRMT7 co-immunoprecipitated with several RNA-binding proteins (RBPs), which might be putative substrates. We expressed five myc-tagged putative targets in *L. major* wild type and Δ*lmjprmt7* and assessed their methylation levels among samples by immunoblotting. Our data suggest that *Lmj*PRMT7 methylates at least one of the RBP binding partners *in vivo*. This indicates a role for arginine methylation in the post-transcriptional regulation of *Leishmania* gene expression. Considering RBPs are well-known mammalian PRMT substrates, our data suggest that arginine methylation may also modulate the interaction between RBPs and their target RNAs in *Leishmania*. Additionally, we aimed to evaluate the virulence of engineered transfectants. As a remarkably opposite phenotype, Δ*lmjprmt7* led to an increased infectivity in mice both *in vitro* and *in vivo*, while *Lmj*PRMT7-overexpressing parasites displayed attenuated virulence. To try to better understand this unusual virulence phenotype, we have performed RNA-seq experiments to compare the transcriptome of Δ*lmjprmt7* and WT parasites. This work is the first study to describe a possible role of *Leishmania* protein arginine methylation in the regulation of gene expression and to show a correlation between *Lmj*PRMT7 levels of expression and the parasite virulence. **Supported by:**FAPESP (2010/01129-9), CNPq, MRC

PV5 - IMMUCILLINS ARE POTENT COMPOUNDS THAT IMPAIR *LEISHMANIA INFANTUM* AND *LEISHMANIA AMAZONENSIS* MULTIPLICATION *IN VITRO*.

FREITAS, E.O.[1]; NICO, D.[1]; GUAN, R.[2]; CLINCH, K.[3]; EVANS, G.B.[3]; TAYLOR, P.C.[3]; SCHRAMM, V.L.[2]; *PALATNIK-DE-SOUSA, C.B.*[1] *1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.ALBERT EINSTEIN COLLEGE OF MEDICINE, YESHIVA UNIVERSITY, BRONX, ESTADOS UNIDOS; 3.THE FERRIER RESEARCH INSTITUTE, VICTORIA UNIVERSITY OF WELLINGTON, NOVA ZELANDIA, NOVA ZELÂNDIA.* e-mail:elisangelaoffreitas@micro.ufrj.br

Chemotherapy against visceral leishmaniasis is associated to high toxicity and resistance issues. Leishmania parasites are purine auxotrophs that must obtain their purines from exogenous sources. Nucleoside hydrolases release purines from nucleosides becoming a drug target for anti-leishmanial drugs, since they are absent in mammal cells. The inhibitory effect of the immucillins IA (ImmA), DIA (DADMe-ImmA), DIH (DADMe-ImmH), SMIH (SerMe-ImmH), IH (ImmH), DIG (DADMe-ImmG), SMIG (SerMe-ImmG) and SMIA (SerME-ImmA) on the enzymatic activity of recombinant NH36 and the in vitro multiplication of L. infantum and L. amazonensis promastigotes and L. infantum intracellular amastigotes were determined.
The NH36 shows specificity for a wide range of substrates: inosine, guanosine, adenosine, uridine and cytidine with a slight preference for adenosine and inosine. The first generation IA and IH, second generation DIH and DIG, and the third generation SMIH, SMIG immucillins inhibited the L.infantum and L. amazonensis promastigote growth in vitro at nanomolar to micromolar concentrations. IA and IH prevention of parasite replication is mediated by the inhibition of NH36 activity (Ki = 0.080 µM for IA and 0.019 µM for IH) while DIH, DIG, SMIG and SMIH act through unrelated mechanisms. IA, IH and SMIH also reduced the in vitro amastigote replication inside mice macrophages by 95% causing no damage to macrophage viability. Transmission electron microscopy revealed global alterations and swelling of L. infantum promastigotes after treatment with IA and IH, respectively. Treatment with SMIH determined intense cytoplasm vacuolization, enlarged vesicles and altered kinetoplasts. Our results disclose the potential use of IA, IH and SMIH immucillins in safe chemotherapy against visceral leishmaniasis. IH (Forodesine) is already on clinical trials against T- and B cell cancers and autoimmune diseases. **Supported by:**CNPQ, FAPERJ , CAPES, ASBMB E PABMB

PV6 - ENZYMES FROM PENTOSE-PHOSPHATE PATHWAY OF TRYPANOSOMATIDS AS POTENTIAL DRUG TARGETS

FERREIRA, M.A.[1]; SINATTI, V.V.[2]; DANTAS, C.L.[2]; GOMES, L.H.[2]; DEGRAVE, W.M.[2]; GUIMARÃES, A.C.R.[2] *1.CDTS/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.* e-mail:malves@cdts.fiocruz.br

Chagas disease and leishmaniasis are globally widely prevalent diseases while human African trypanosomiasis is endemic only on the African continent. The drugs in current use for treatment of these diseases have problems of toxicity, efficacy, administration or length of treatment; hence, some new, effective, safe and affordable drugs are needed. Therefore, we use an approach that was looking for metabolic enzymes from trypanosomatids as a new potential drug targets. We found specific and analogous proteins in genomes of those parasites in relation to their hosts. Here we are showing recent data concerning two enzymes from Pentose-Phosphate Pathway (PPP), ribose 5-phosphate isomerase (R5PI) and gluconate kinase (GK). R5PI catalyzes the reversible isomerization between ribulose 5-P and ribose 5-P. Two types of R5PI exist: RpiA that is broadly distributed among eukaryotic organisms and in some bacteria; and RpiB that is found in most prokaryotes. R5PI from trypanosomatids (RpiB) are analogous to the human isoform (RpiA). The enzymes from *Leishmania major* (LmRpiB) and *Trypanosoma brucei* (TbRpiB) were cloned and expressed on *E. coli*. Native LmRpiB was identified in L. major promastigotes using 2D-PAGE, western-blot and microscopy. The recombinant TbRpiB showed a Km=1.26±0.49mM, similar to Km described for *Trypanosoma cruzi* and bacteria enzymes. By site-direct mutagenesis we constructed a mutant substituting the catalytic residue Cys69 by Ala (TbRpiB-C69A). The recombinant TbRpiB-C69A showed no activity. The RNAi approach showed a short reduction in growth rate of bloodstrean and procyclic forms. GK is a specific enzyme that catalyzes the phosphorylation of D-gluconate to 6-phospho-D-gluconate. *T. cruzi* shows two genes coding for TcGK. Both were cloned and expressed on *E. coli*. Immunofluorescence assay revealed that native TcGK were located in the cytoplasm. Moreover, the structure of TcGK was deduced by homology modeling. **Supported by:**CNPq, PAPES V-FIOCRUZ, FAPERJ

PV7 - 17-AAG INDUCES INCIDENTAL CELL DEATH OF *LEISHMANIA* WITH AUTOPHAGIC FEATURES: EVIDENCE FOR A CROSSTALK BETWEEN AUTOPHAGIC AND PROTEOSOME PATHWAYS

PETERSEN, A.L.O.A.[1]; CULL, B.[2]; MOTTRAM, J.C.[2]; VERAS, P.S.T.[3]
1.CPQGM - FIOCRUZ, SALVADOR, BA, BRASIL; 2.UNIVERSITY OF GLASGOW/INSTITUTE OF INFECTION, IMMUNITY & INFLAMMATION, GLASCOW, REINO UNIDO; 3.GONÇALO MONIZ RESEARCH CENTER/FIOCRUZ, SALVADOR, BA, BRASIL.
e-mail:petersen.swe@gmail.com

Leishmaniasis has been treated by antimonial pentavalents for the last 70 years. However, the rise in resistant cases has come the efficacy of these drugs under scrutiny. We have previously observed an important leishmanicidal effect of 17-AAG, which is an HSP90 inhibitor. We showed that L. amazonensis(La)-infected macrophages, when treated with 17-AAG reduced infection up to 98% in a dose- and time-dependent manner. Electron microscopy revealed 17-AAG-induced alterations are suggestive of La cell death by a mechanism dependent in autophagy. To further investigate 17-AAG-dependent parasite death, we used mutants of the components of the autophagic pathway, such as GFP-ATG8 and ATG5-KO. We observed that 17-AAG induced an increase in the number of Lm bearing autophagic vacuoles. Also, ATG5-KO mutants unable to form autophagic vacuoles shown to be more resistant to 17-AAG than wild-type parasites, indicating that parasite death induced by 17-AAG appears to be linked to the autophagic process. We also showed that 17-AAG reduced the fusion between autophagosomes and lysosome/glycosome, contributing to the increased number of autophagosomes in parasites. Since autophagic can crosstalk with proteasome pathway we assessed the influence of this pathway in 17-AAG-induced Lm death. We evaluated the effect of MG132, a proteasome inhibitor, in the autophagosome formation and our findings demonstrated that MG132 induced autophagosome formation and, as expected, western-blot analysis demonstrated that MG132, as well as 17-AAG, induced ubiquitilated protein accumulation, and that the presence of ubiquitilated proteins was more evident in ATG5-KO Lm. These suggest that autophagy plays a role in ubiquitilated protein degradation, support the idea that 17-AAG-induced parasite death is dependent on the activation of the parasite autophagic pathway and, for the first time, evidence presented herein link the autophagic and the proteasome pathways in Leishmania. **Supported by:**CNPq - 306672/2008-1

PV8 - NEWER INSIGHTS INTO THE GENOME STRUCTURE AND DIVERSITY OF *TRYPANOSOMA CRUZI*

NEVES, J.W.L.; PRETI, H.; RAMPAZZO, R.C.P.; PAVONI, D.P.; KRIEGER, M.A.; PROBST, C.M.
INSTITUTO CARLOS CHAGAS, CURITIBA, PR, BRASIL.
e-mail:johnny.neves@hotmail.com

Trypanosoma cruzi is the etiological agent of Chagas disease, an important illness that affects more than 10 million people in Latin America. In relation to its phylogenetics, it is divided in 6 distinct discrete typing units (DTU), namely TcI to TcVI. The prototype genome sequence was obtained in 2005 for the CL Brener strain (TcVI) and after that we had the publication of other two genomes (Sylvio, TcI and a subspecies, T. cruzi marinkellei). Other genomes were sequenced and are available at specialized web databases. As a general trend, these assemblies are very fragmented (large number of contigs), with a small N50 value and with many truncated genes. Last year, we have presented a genome draft (v0.2) for the strain Dm28c (TcI), sequenced by PacBio SMRT technology. This technique represents a significant advancement for obtaining better genomes, specially when dealing with complex entities, as T. cruzi. We obtained a significant increase in the quality of the Dm28c genome. Here, we present its final draft version, with significant improvements, due to extra sequencing with a newer chemistry. Also, we present data about the sequencing of other 10 T. cruzi genomes, from DTUs TcI to TcV, using MiSeq technology (2x300 reads) at ~100x coverage. Comparisons between the DTUs, regarding genomic structure, gene repertoire and base variation (SNPs) will be presented. Next steps comprise sequencing these strains with PacBio for a hybrid assembly approach. **Supported by:**FIOCRUZ

PV9 - INFLUENCE OF A MAP KINASE DEPLETION ON *TRYPANOSOMA BRUCEI*

BATISTA, M.; KUGERATSKI, F.G.; DE PAULA LIMA, C.V.; PROBST, C.M.; KRIEGER, M.A.; MARCHINI, F.K.
ICC-FIOCRUZ, CURITIBA, PR, BRASIL. e-mail:batista.mic@gmail.com

Trypanosomatids find distinct environments in their life cycle, where quick adaptation is needed. Protein phosphorylation, driven by protein kinases and protein phosphatases, is an important process with potential to supply this necessity. The flagellate parasite *Trypanosoma brucei* possesses a diversity of kinase groups, among them the over-represented CMGC group, comprising different kinase families including the mitogen activated protein kinases (MAPKs). In eukaryotes, this family has been reported to be involved in cellular division and differentiation, in response to stress conditions, and other. Nevertheless, little is known about its function in trypanosomatids. In this sense, this work aimed to understand the role of MAPKs in *T. brucei* evaluating the effects of MAPK depletion on the cell. All the fifteen MAPKs of *T. brucei* were independently depleted by RNAi. Analysis on cell proliferation detected a decrease in seven of the fifteen analyzed knock downs. Initially, one of the affected knock downs (MAPK-A) had its transcriptome, proteome and phosphoproteome quantitatively analyzed by RNA-Seq and MS based proteomics combined to metabolic labeling (SILAC). In total, nearly of 8 million reads were generated, 1,511 proteins and 1,510 phosphorylation sites were identified. Despite the cell proliferation rate was strongly affected in this knock down, few modulations in the proteome, phosphoproteome or transcriptome were observed. In consonance with similar published works, this could represents the robustness of MAPK signaling pathway in *T. brucei*. Phosphoproteome analysis revealed greater modulation fold changes than proteome and transcriptome. From fourteen modulated phosphorylation sites (fold change>1.5), four of them are located in proteins related to mRNA either processing or stability control. Altogether, the finds of this work have the potential to drive future projects in order to well understand the MAPK signaling pathway in trypanosomatids. **Supported by:**PDTIS and PAPES Program from FIOCRUZ

PV10 - ARCHITECTURE OF A PARASITE CELL SURFACE: COMPLEX-TARGETING MECHANISMS REVEALED THROUGH PROTEOMICS

GADELHA, C.[1]; ZHANG, W.[2]; WICKSTEAD, B.[1]; CHAIT, B.T.[2]; FIELD, M.C.[3]
1.UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, REINO UNIDO; 2.THE ROCKEFELLER UNIVERSITY, NEW YORK, ESTADOS UNIDOS; 3.UNIVERSITY OF DUNDEE, DUNDEE, REINO UNIDO. e-mail:catarina.gadelha@nottingham.ac.uk

Surface membrane composition is key to parasite growth, immune evasion and transmission; and membrane proteins are promising therapeutic targets due to their exposure and roles in modulating essential parasitic processes. For extracellular African trypanosomes, the surface is, however, a double-edged sword; this is the primary interface between the parasite and its host, and those crucial cellular functions must be carried out while avoiding elimination by the host immune defenses. Despite its critical role in parasitism, very little is known about most of the proteins that reside at this interface. This severely limits functional and evolutionary studies, and hampers the development of treatments.

We used a combinatorial biochemical, proteomic and bioinformatic strategy to achieve a high-confidence surface proteome for the human parasite *Trypanosoma brucei*. This 'surfeome' contains previously known surface proteins and multiple novel components with membrane characteristics. Extensive validation indicates that the majority of surfeome constituents are bona fide surface-associated proteins and includes several ESAGs of unknown function. Interestingly, receptor-like molecules are almost exclusively species-specific, whereas transporter-like ones are conserved to model organisms. We also present a first examination of sorting signals and show that proteins with different biochemical characteristics access combinations of specialized membrane regions, suggesting the existence of distinct surface domains defined by boundaries which restrict diffusion. This paradigm has important implications for the function of the parasite cell surface.

PV11 - MECHANISM OF INHERITANCE OF THE APICOPLAST GENOME

DOS SANTOS MARTINS-DUARTE, E.[1]; SHEINER, L.[2]; REIFF, S.[3]; STRIEPEN, B.[3]
1.UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSITY OF GLASGOW, GLASGOW, REINO UNIDO; 3.UNIVERSITY OF GEORGIA, ATHENS, ESTADOS UNIDOS. e-mail:emartinsduarte@yahoo.com.br

Apicomplexan parasites harbor a non-photosynthetic secondary plastid (apicoplast), which originated from an ancient endosymbiosis between two eukaryotes: a heterotrophic protist engulfed a single-celled red algae containing a chloroplast. The maintenance of the apicoplast 35kb circular DNA transcription and replication is essential for parasite survival and a validated drug target for the treatment of toxoplasmosis and malaria. Using *Toxoplasma gondii* as our model, we characterized homologous of the prokaryote DNA polymerase I (TgPrex), single-stranded DNA binding protein and DNA gyrase domains A and B exploiting genetic and fluorescence imaging approaches. In prokaryotes these proteins are essential for DNA replication. Parasites expressing endogenous proteins tagged with a triple hemagglutinin (3xHA) at the c-terminal end were generated and the apicoplast localization of these proteins was confirmed by immunofluorescence. Conditional knockouts parasites for the subunits A and B of DNA gyrase and TgPREX showed remarkable growth impairment. Besides, the knockouts also showed decrease in the apicoplast genome copy number and apicoplast segregation defect. Our findings show that DNA gyrase and TgPREX are essential for apicoplast genome maintenance and parasite survival. **Supported by:**CNPq, CAPES, FAPERJ

PV12 - IDENTIFICATION AND CHARACTERISATION OF A PUTATIVE CELL SURFACE RECEPTOR FOR PAF AND LPC IN *TRYPANOSOMA CRUZI*

OLIVEIRA, M.M.[1]; KEMP, L.E.[1]; FRAGOSO, S.P.[2]; LOPES, A.H.C.S.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ PARANA, CURITIBA, PR, BRASIL.
e-mail:kemp.louise@micro.ufrj.br

The structural role of phospholipids is well understood, however the function of bioactive phospholipids during mammalian infection by *T. cruzi* is unknown. Platelet-activating factor (PAF), which acts through the PAF receptor (PAFR) in mammalian cells, is an important phospholipid that is involved in diverse cellular processes. Previous work from our group highlighted that PAF can stimulate cell differentiation in *T. cruzi*. We also showed that *T. cruzi* synthesizes several lysophosphatidylcholine (LPC) species and C18:1-LPC can aggregate rabbit platelets in a PAF-like manner. Recently we constructed a refined 3-D model of the mammalian PAFR that allowed us to use a molecular docking strategy to test several PAF and LPC ligands. We demonstrated that C18:1-LPC is capable of interacting with the mammalian PAFR in a fashion very similar to PAF, unlike other LPC species. In the current study we performed an *in silico* scan for potential PAFR candidates in the *T. cruzi* genome. Though no direct homologues were identified, a gene with similarity to IZH-2, a yeast steroid receptor that is predicted to have 7 transmembrane domains, was selected as the most likely candidate to interact with both PAF and C18:1-LPC. We named this candidate putative *T. cruzi* Surface Receptor 1 (TcSUR1). To study the function of this gene we created single allele knockout *T. cruzi* parasites (TcSUR1-sKO). These parasites showed an increased rate of natural cell differentiation as compared to the wild type (WT) strain. Double allele knockout parasites were not viable. Further characterisation of the TcSUR1-sKO strain, including protein localisation, determination of expression levels and identifying growth rates, differentiation and virulence in the presence and absence of LPC and PAF will allude to the function of this protein. The exact role of PAF and LPC with regard to the *T. cruzi* lifecycle has still to be defined. However, our results show that their influences may be regulated via TcSUR1.

PV13 - EX VIVO ACTIVITY OF QUINOLINE DERIVATIVES AND DRUG-RESISTANCE GENE CHARACTERIZATION OF PLASMODIUM VIVAX AND P. FALCIPARUM ISOLATES FROM BRAZILIAN PATIENTS.

AGUIAR, A.C.C.[1]; PEREIRA, D.B.[2]; MENEGHETTI, M.R.[3]; KRETTLI, A.U.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.CENTRO DE PESQUISAS EM MEDICINA TROPICAL DE RONDÔNIA, PORTO VELHO, RO, BRASIL; 3.UNIVERSIDADE FEDERAL DO ALAGOAS, MACEIO, AL, BRASIL. e-mail:carolcaguiar@yahoo.com.br

The resistance of malaria parasites to most drugs, require novel compounds and those based on the Chloroquine (CQ) structure, seem ideal to generate novel and less expensive drugs, with no toxicity. The ex-vivo activity against fresh human parasites isolates of new compounds was evaluated. Those compounds were highly active (against a CQ-resistant W2-clone Plasmodium falciparum), as shown in our previous work (Aguiar et al PlosOne 2012; Penna-Coutinho et al PlosOne 2011). Fresh plasmodia isolates from patients with P.vivax (PV; n=47), or P. falciparum (PF; n=9), maintained in short time blood, were used to test the susceptibility to BAQ, MAQ and Posaconazole®. After incubation (37^0C, 48 to 72h), the schizont maturation was evaluated in thick blood smears. When 40% parasites in the drug-free control wells reached the schizont stage incubation was stopped. As controls, the CQ, mefloquine, artesunate, were used in serial dilutions. The IC50 was calculated in comparison to parasites in free-drug cultures. The parasite fresh isolates were also characterized by sequence analysis of the genes MDR1 and CRT. All PF human isolates had an adequate growth in cultures; whereas in PV isolates only 68% reached an adequate growth. The analogues MAQ and BAQ were active against PF at nanomolar dose, respectiively, (IC50=113 and 84 nM); and against PV (IC50=166 and 169 nM). Posaconazole® was less active ex-vivo against PF and PV (IC50=1,6 and 5 µM). All PF isolates were CQ-resistant; whereas the PV isolates were CQ-sensitive. The PF isolates carried non-synonimous mutations in the PfMDR1 gene, positions 184, 1042 and 1246; and 84% at position 1034; none at codon 86. For the PfCRT gene, all PF isolates had not synonymous mutations at positions 72 and 76. One fresh PF isolate from Angola, studied in parallel, was sensitive to all antimalarials and had no mutations in the resistance genes. Both groups might be useful to help controling human malaria in areas of CQ-resistance. **Supported by:**CNPq/Ministério da Saúde/Fapemig.

PV14 - HEME MODULATES MITOCHONDRIAL BIOENERGETICS IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

SARAIVA, F.M.S.[1]; NOGUEIRA, N.P.A.[1]; LARANJA, G.A.T.[1]; DE OLIVEIRA, M.F.[2]; PAES, M.C.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:francismoniquesaraiva@gmail.com

Trypanosoma cruzi epimastigotes proliferate inside triatomine insect in the presence of heme, which induces reactive oxygen species (ROS) formation. Mitochondria are implicated in cellular redox balance, representing the major source of ROS. Then, we evaluated heme effect on mitochondrial ROS production and mitochondrial membrane potential (ΔΨm). For that, epimastigotes were incubated with DHE or TMRM with or without heme for 30 minutes and FCCP and antymicin A (AA) were added. Mitochondrial ROS production and ΔΨm were analyzed by flow cytometry. Our results showed that heme induced the double of ROS production and induced 4-fold increase of ΔΨm. FCCP addition reversed heme effect on ROS generation. Also, AA induced 2-fold increase of ROS production, but did not altered ΔΨm. The co-incubation with heme and AA presented 3-fold increase on ROS formation and increased ΔΨm by 16%. To eliminate FCCP interference on plasmatic membrane potential (ΔΨp), epimastigotes were incubated with DiBAC4(3) for 10 minutes. Then, FCCP were added. The ΔΨp were analyzed by flow cytometry. Results shows that FCCP did not alter ΔΨp, indicating that its effects are exclusively mitochondrial. To confirm heme effects on mitochondrial ROS production, confocal microscopy and flow cytometry were performed using epimastigotes incubated with DHE with or without mitoTEMPO, a mitochondrial antioxidant, for 30 minutes. Thus, cells were washed and incubated with heme for 30 minutes. MitoTEMPO addition totally eliminated heme-induced ROS. We also evaluated epimastigotes proliferation with or without heme, mitoTEMPO, FCCP or AA. FCCP and mitoTEMPO also reversed heme-induced proliferation and AA promoted a tripanostatic effect. Taken together, our results suggest that *T. cruzi* epimastigotes mitochondrial physiology might be modulated by heme since it modulates ΔΨm and mitochondrial ROS, indicating a mechanism whereby parasites maintain high density cell population inside de vector. **Supported by:**PIBIC-UERJ, INCT-EM, CNPq and FAPERJ

PV15 - KINETOPLAST ASSOCIATED PROTEINS IN SYMBIONT BEARING TRYPANOSOMATIDS

SOUZA, S.S.; PENHA, L.; CATTA-PRETA, C.M.C.; DE SOUZA, W.; SILVA, R.; MOTTA, M.C.M.
UFRJ-RJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:silsouza@biof.ufrj.br

The Kinetoplast Associated Proteins (KAPs) are basic proteins similar to histone H1 of eukaryotes. They have been characterized in different trypanosomatid species considering their role in the mitochondrial metabolism and kDNA topology. In the Trypanosomatidae family there are six monoxenic species, belonging to the genera Angomonas and Strigomonas, that co-evolve in a mutualistic relationship with an endosymbiotic bacterium. The presence of the symbiont has been associated to ultrastructural changes in the host, as the looser arrangement of kDNA network. Our recent analysis of genome revealed that A. deanei contains genes KAPs 3 and 4 and S. culicis KAPs 2 and 4. In this work, we intend to study kaps genes in symbiont bearing trypanosomatids, comparing protozoa from both genera. All kaps genes were amplified by PCR in the monoxenic species. By transcriptome the gene expression of strains of A. deanei the kap3 gene is 3,6 more expressed in aposymbiotic strain than wild type. Data by qPCR indicated the kap4 transcripts are more abundant in the aposymbiotic strain of S. culicis than wild-type. It is well know that KAP 4 is present in all trypanosomatids species and can be considered as universal marker for this family. Since KAP 3 is present in all Angomonas species, it can be used as a molecular marker for this genus, whereas KAP 2 represents a marker for Strigomonas genus. Immunolocalization assays using antibody against KAP4 labelled homogeneously the kinetoplast in all species. Ultrastructural analysis by electron tomography showed that species from the genera present differences in kinetoplast format. In A. deanei present trapezoid shape kinetoplast than S. culicis present a bow shaped kinetoplast. In both species the DNA fibrils are more compact in the kinetoplast region that faces the basal body, this is more evident in A. deanei. Our next goal is to immunolocalize KAPs by ultrastructural immunocytochemistry to verify the distribution in the kinetoplast. **Supported by:**CNPq and FAPERJ

PV16 - QUANTITATIVE UBIQUITINOME OF TRYPANOSOMA CRUZI METACYCLOGENESIS

DE PAULA LIMA, C.V.; KRIEGER, M.A.; MARCHINI, F.K.
ICC, CURITIBA, PR, BRASIL. e-mail:cvplima@gmail.com

Trypanosoma cruzi, the etiologic agent of Chagas disease, alternate between distinct morphological and functional forms during its life cycle. In *T.cruzi* gene expression is mainly post-transcriptionally regulated. This regulation may occur at protein level by modulation of amount, activity, and sub-cellular localization of stage-specific proteins, involving a complex combination of signaling systems, in which ubiquitination – modification of target-proteins by ubiquitin - plays an important role. However, this system is still poorly characterized in *T.cruzi*, and the identification of target proteins may elucidate how this system controls cellular mechanisms. Aiming the identification of the ubiquitination sites, they were enriched from total cell extract of *T.cruzi* using immunoprecipitation approachs. SILAC methodology was used, together with the newly defined medium LM14B, to perform high accuracy large-scale protein and ubiquitination site quantification from different points of metacyclogenesis. In the present work we identified 138 ubiquitination sites in 107 proteins after the enrichment, proteins related to different cellular processes. Quantitative analysis of whole proteome during the differentiation was also performed aiming to compare total proteins levels with ubiquitination levels. Thus, it was possible to quantify 3 132 proteins over the differentiation, in which 819 proteins showed differential expression. 68 ubiquitination sites were quantified during differentiation, revealing interesting profiles. We also identified the ubiquitin modification in all lysine residues of ubiquitin itself, indicating a vast repertoire of poli-ubiquitination in *T.cruzi*. The present work has initiated investigations on the molecular and cellular mechanisms regulated by ubiquitination in this organism. **Supported by:**ICC/FIOCRUZ, PDTIS, PAPES and CAPES

PV17 - STABLE ISOTOPE LABELING BY AMINO ACIDS IN CELL CULTURE (SILAC) IMPLEMENTATION FOR *TRYPANOSOMA CRUZI*

MARCHINI, F.K.; DE PAULA LIMA, C.V.; BATISTA, M.; LUCENA, A.C.R.; KRIEGER, M.A.
ICC / FIOCRUZ-PR, CURITIBA, PR, BRASIL.
e-mail:marchinifk@fiocruz.br

Quantitative proteomics-based approaches are extremely useful to study global protein expression changes in organisms. The stable isotope labeling by amino acids (SILAC) method yields highly accurate protein quantification and has been successfully applied to perform comparative and time-resolved analysis of biological systems. In this work, we successfully apply SILAC (Stable Isotope Label by Amino acids in Cell culture) methodology to perform large-scale protein quantification of Trypanosoma cruzi parasite after describing a new defined medium, which enabled comparison of protein content between epimastigotes forms and stressed epimastigotes forms. A high amino acid incorporation rate (>95%) in epimastigotes proteome was obtained after seven days of cultivation in the heavy defined medium (LM14B/Arg10+Lys8). High resolution mass spectrometry analysis of parasites cultivated in LITB, LM14 and LM14B media, submitted or not to nutritional stress in TAU medium, yield 3,606 quantified proteins, enabling the identification of differential expressed proteins under these conditions. Establishment of SILAC methodology for T. cruzi cells provides an important advance for proteomic analysis of this organism. **Supported by:**Instituto Carlos Chagas/Fiocruz-PR, Program for Technological Development in Tools for Health-PDTIS-

PV18 - DERIVATIVES FROM MEVALONATE PATHWAY INTERFERE WITH *LEISHMANIA AMAZONENSIS* GROWTH *IN VITRO*

PINHEIRO, L.S.; CANTO-CAVALHEIRO, M.M.; TORRES-SANTOS, E.C.
INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:liliane.senapinheiro@yahoo.com.br

Several studies have shown that farnesol (FOH) has essential role in processes of cell growth and differentiation. This compound is produced by an alternative pathway from the sterol biosynthetic intermediate farnesyl pyrophosphate (FPP). The objective of this study is to evaluate the FOH effects on Leishmania amazonensis cultures. Our results showed that FOH inhibits the growth of promastigotes after 24, 48 and 72 h of incubation (IC50 of 46, 34 and 36 µM, respectively). The analysis of the membrane integrity and mitochondrial membrane polarization of the parasite showed that only with the highest concentration of FOH (92µM) these parameters were affected. To evaluate the effect of FOH in Leishmania cell division, promastigotes of L. amazonensis were grown in a defined lipid-free medium and labeled with CFSE. FOH caused the diferentiation in two subpopulations based in CFSE intensity. The first formed by 38% of the total population, with reduced proliferative capacity and the other (48%) with proliferative capacity similar to the untreated culture. Considering that FOH is an endogenous metabolite of Leishmania, we evaluated whether FOH produced by Leishmania could be present in the supernatant of the culture and take part in the regulation of the growth of this parasite in vitro. For this study, the parasites were cultivated in the defined lipid-free medium and the lipophilic metabolites of the culture supernatants were extracted and analyzed by gas chromatography-mass spectrometry. Interestingly, we identified farnesyl acetate and FOH in the supernatants and their concentrations increased over the culture time. We also identified α- and β- farnesene in supernatant from stationary phase culture. Taken together, these results suggest that FOH and other derivatives from mevalonate pathway could interfere with the growth of promastigotes of L. amazonensis, may be playing a role in cell density control. **Supported by:**IOC - FIOCRUZ

PV19 - A PUTATIVE LEUCYL, PHENYLALANYL-TRNA-PROTEIN TRANSFERASE HOMOLOGUE IS ESSENTIAL FOR *LEISHMANIA MAJOR*: THE POSSIBLE CRITICAL ROLE OF N-END RULE PATHWAY.

SHARMA, R.; TERRÃO, M.C.; OLIVEIRA, E.B.; CRUZ, A.K.
FMRP -USP, RIBEIRAO PRETO, SP, BRASIL. e-mail:rohitvns.sharma@gmail.com

The successful survival of *Leishmania* parasites in mammalian host depends on their ability to overcome the drastic environmental changes within the phagolysosome of macrophages. In spite of the relevance of post-transcriptional regulatory mechanisms for the completion of *Leishmania* life cycle, the role of post-translational events such as protein stability or modification to stress responses is poorly understood. The derived amino acid sequence of a hypothetical protein coding gene (*LmjF21.0725*) in *L. major* has shown convincing homology with the signature motif of the NAT super family protein Leucyl, Phenylalanyl-tRNA-Protein transferase (LFTR),. LFTR is a key enzyme of N-end rule pathway; a post-translational mechanism modulating gene expression in response to stress in eukaryotes and prokaryotes. We are performing the functional characterization of LmjF21.0725 through reverse genetics. We obtained a heterozygous mutant (*LmjF21.0725 +/-*), but the double knockout (KO) was never achieved even after seven independent experiments. Taking the negative result as a possible indication of the essentiality of the gene, we generated conditional KO by introducing a plasmid with *LmjF21.0725* into *LmjF21.0725 +/-* followed by a second round of transfection to replace the second allele of the endogenous gene. As a read-out of the essentiality, FACS analysis of the episomal GFP indicated that the homozygous null mutants grown in the absence of drug for ten passages has shown persistence of the plasmid in opposition to its fast loss in *LmjF21.0725+/-* cells. From an antigenic peptide of *LmjF21.0725* a specific polyclonal antibody has been raised to define the protein subcellular localization in different life stages of the parasite. **Supported by:**FAPESP

PV20 - A NEW METHOD FOR GENOTYPING OF *TRYPANOSOMA CRUZI* BASED ON MASSIVE PARALLEL SEQUENCING

PROBST, C.M.; NEVES, J.W.L.; PRETI, H.; PAVONI, D.P.; KRIEGER, M.A.
ICC - FIOCRUZ, CURITIBA, PR, BRASIL. e-mail:cprobst@fiocruz.br

Trypanosoma cruzi is a major public health issue in Latin America. It has a significant degree of evolutionary diversification, being at present classified into 6 distinct groups. This genetic diversification has many implications for the biology of the parasite and the clinical manifestation of the disease. Several methods have been created to identify *T. cruzi* at the molecular level and the great majority used on studies relies on few qualitative markers, as presence/absence of PCR band or its size, and few loci (rDNA, spliced leader); also, most collections of *T. cruzi* are not typed into DTUs, in general. Here, we present a new genotyping method based on next generation sequencing (NGS) that is fast, quantitative, robust, highly informative and scalable. We have taken advantage of the *T. cruzi* ORFeome library, that was produced by our group, to select 96 distinct CDSs, whose size is around 1.5kb. After basic PCR, the purified product can be sequenced using any massive parallel technology. Due to the fact that we are evaluating approximately 150,000 genetic positions, this method is quantitative and highly informative (theoretically, distinguishes samples 99.9994% similar); it is robust as 96 distinct reactions are being performed. Due to the high throughput of current sequencers and to the use of multiplexing, it is fast and scalable. One procedure (96-well PCR, electrophoresis, product purification, sample preparation, sequencing, data transfer and analysis) can genotype 96 samples in 24 hours (1 sample/15 minutes/$25); in a simple setup, it can achieve 1 sample/3 minute/$3 (5,000 samples per week). Our implementation can be adjusted for distinct laboratories, sequencers, gene targets etc, maintaining its utility. We have applied this method in 15 strains with known genotypes and we were able to correctly classify them. It distinguishes strains from the same DTUs and can discover new DTUs. We are now searching new samples to test this method in a broader setup **Supported by:**FIOCRUZ

PV21 - BIOLOGICAL ACTIVITY OF *PIPER ADUNCUM* ESSENTIAL OIL AGAINST DIFFERENT *TRYPANOSOMA CRUZ*I DEVELOPMENTAL FORMS

VILLAMIZAR, L.H.[1]; ANDRADE, J.[2]; TEIXEIRA, M.L.[2]; CARDOSO, M.G.[2]; SOARES, M.J.[1]
1.ICC/FIOCRUZ-PR, CURITIBA, PR, BRASIL; 2.UNIVERSIDADE FEDERAL DE LAVRAS, LAVRAS, MG, BRASIL. e-mail:lhsilva@tecpar.br

Treatment of Chagas disease is based on the use benznidazol, marked by low efficacy in the chronic phase of the disease and several side effects. Thus compounds obtained from plants appear as less toxic alternative drugs. Aim of this study was to evaluate the trypanocidal activity of the essential oil of *Piper aduncum* (jack pepper; Piperaceae), using the MTT methodology. In vitro assays were performed with different developmental forms of *Trypanosoma cruzi* clone Dm28c (culture epimastigotes, trypomastigotes and intracellular amastigotes) to determine the IC50/24h value (concentration that kills 50% of the cell population after 24h). While activity against epimastigotes was low (IC50/24h = 84.74 µg/ml), high trypanocidal activity was obtained against cell-derived trypomastigote forms (IC50/24h = 2.8 µg/ml) and intracellular amastigotes (IC50/24h = 9.01 µg/ml) obtained from infected Vero cell cultures. Assays with in vitro derived metacyclic trypomastigotes at 4°C (same temperature condition of blood banks) also demonstrated high trypanocidal activity (IC50/24h = 3.8 µg/ml). Cytoxicity of the essential oil against human erythrocytes was low (CC50/24h > 100 µg/ml), as well as against Vero cells (CC50/24h = 42.8 µg/ml). Flow cytometry analyzes performed with epimastigotes incubated with the IC50/24h value and then probed with the fluorescent dye Rhodamine123 demonstrated that the essential oil of *Piper aduncum* induced loss of mitochondrion membrane potential in 100% of parasites. On the other hand no alterations in life cycle were observed in epimastigotes probed with propidium iodide (PI). Our data indicate that the essential oil of *Piper aduncum* or derivatives of its main constituent appear as potential natural products to be further evaluated against *Trypanosoma cruzi*. **Supported by:**CNPq; Fiocruz

PV22 - TRANSFERRIN UPTAKE BY TRYPANOSOMA CRUZI ISOLATED INTRACELLULAR AMASTIGOTES

BATISTA, C.M.; KESSLER, R.L.; EGER, I.; SOARES, M.J.
ICC/FIOCRUZ-PR, CURITIBA, PR, BRASIL.
e-mail:cassianombatista@gmail.com

Trypanosoma cruzi epimastigotes are able to uptake, internalize and store macromolecules in reservosomes, the end endocytic organelles in this protozoan parasite. Despite the main role of endocytosis in the life cycle of this protozoan, few studies have focused on this crucial biological event in the more clinically relevant intracellular amastigote form, the proliferative stage found in mammalian hosts. Transferrin uptake was analyzed by flow cytometry and fluorescence microscopy, after incubation of cell culture isolated T. cruzi amastigotes for 30 minutes at 4ºC or 37ºC with transferring coupled to AlexaFluor 633, followed by treatment with or without acetic acid or unlabelled transferrin. Here we present quantitative evidence of transferrin uptake by T. cruzi intracellular amastigotes. Analysis by fluorescence microscopy showed that ingested transferrin co-localized with cruzipain, a reservosome marker. Our data indicate that isolated T. cruzi intracellular amastigotes have small endocytic activity, with storage of ingested extracellular macromolecules in reservosome/lysosome-like organelles. **Supported by:**CNPq, FIOCRUZ, CAPES

PV23 - LYSOPHOPHATIDYLCHOLINE (LPC) AND LYSOPHOSPHATIDIC ACID (LPA) EFFECT ON THE PROLIFERATION AND DIFFERENTIATION OF TRYPANOSSOMA CRUZI

CHAGAS-LIMA, A.C.; SILVA-NETO, M.A.C.; ATELLA, G.C.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:catarinalima@msn.com

Trypanosoma cruzi, etiological agent of Chagas disease. Parasite epimastigote, forms replicate differentiate into infective metacyclic trypomastigotes at the Triatominae insect vector midgut. Our experimental models are T. cruzi and Rhodnius prolixus, Chagas disease vector. R. prolixus saliva contains lysophosphatidylcholine (LPC), a lysophosphatidyl lipid resulted of phosphatidylcholine hydrolysis by phospholipase A2 enzyme. Other important lysophospho lipid is Lysophosphatidic acid resulted of LPC hydrolysis by a kind of lysophospholipase D, the Autotaxi (ATX) enzyme. Theses bioactive lysophospholids are multisignaling found in human plasma ingested by the insect during blood feeding. The goal of the work is determining the paper of LPC and LPA in the proliferation and differentiation of T. cruzi. We analyzed the effect of LPC or LPA or ATX-inhibitor with LPC on parasite proliferation grown in medium delipidad fetal bovine serum supplemented, in three days. We observed increasing in LPA and LPC treated parasite growth. However we observed low growth in major concentrations of LPC and ATX inhibitor-LPC treated parasite growth. LPC effect on parasite differentiation was performed by assaying metacyclogenesis. T. cruzi epimastigotes were grown at nutrient-rich medium bovine fetal serum supplemented, then parasites were transferred to nutrient-poor medium in order to induce nutritional stress, to induce metacyclogenesis, in the absence or in the presence of LPC. Metaciclogenesis rate was stipulating for percentage of metacyclic forms in three days after treatment. We observed major percentage of metaciclyc form when incubated with LPC. From now on, we intend to identify the signaling pathways that are activated by LPC and LPA in T. cruzi and that lead to parasite increased proliferation and metaciclogenesis. Therefore, such molecules may have the potential to generate new targets for help develop a drug to combat parasite. **Supported by:**CNPQ

PV24 - *IN VITRO* ACTIVITY OF DIAMINE AND PHENYLOXAZOLONE DERIVATIVES ON *TRYPANOSOMA CRUZI*

AZEREDO, C.M.O.[1]; EGER, I.[2]; BARBOSA, G.[3]; AVILA, E.P.[3]; AMARANTE, G.W.[3]; DE ALMEIDA, M.V.[3]; SOARES, M.J.[4]
1.INSTITUTO CARLOS / FIOCRUZ CHAGAS, CURITIBA, PR, BRASIL; 2.UNIVERSIDADE ESTADUAL DE PONTA GROSSA, PONTA GROSSA, PR, BRASIL; 3.UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA, MG, BRASIL; 4.INSTITUTO CARLOS CHAGAS / FIOCRUZ, CURITIBA, PR, BRASIL. e-mail:mauriliojsoares@gmail.com

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is endemic in Latin America. Its treatment is based on the use of benznidazole, marked by low efficacy in the chronic phase of the disease and several side effects. Thus, synthesized compounds, together with those extracted from plants, appear as potential alternatives. In this work we have screened the activity of 70 different synthetic derivatives against *T. cruzi* Dm28c epimastigotes (IC_{50}/24h) and Vero host cells (CC_{50}/24h), using the MTT colorimetric methodology. One diamine derivative (GIB24: IC_{50}=5.64 µM; CC_{50}=92.6 µM, Selectivity Index SI=16.4) and one phenyloxazolone derivative (EPA35: IC_{50}=33 µM; CC_{50}=222.4 µM; SI=6.74) were the two compounds that displayed the best results against *T. cruzi* epimastigotes, as compared with benznidazole, which showed IC_{50}=56.5 µM and CC_{50}=2720 µM (SI=48.14). On the other hand, incubation of intracellular amastigotes with EPA35 (IC50=2.08 µM) increased the SI to 106.9, while benznidazole showed IC_{50}=15 µM and SI=181.33. Flow cytometry analysis of epimastigotes treated for 24 h with 6 µM GIB24 showed cell enlargement with fragmented DNA. Decrease in mitochondrial membrane potential and loss of plasma membrane integrity were observed after treatment with doses higher than 6 µM, in a dose dependent manner (up to 10 µM). Incubation of epimastigotes for 24h with EPA35 resulted in loss of mitochondrial membrane potential with increasing drug concentrations, but plasma membrane integrity was maintained with all evaluated concentrations (15–50 µM). Taken together, our data indicate that GIB24 and EPA35 are promising compounds in the search for new drugs effective against Trypanosoma cruzi. **Supported by:**CNPq; Fiocruz

PV25 - CARACTERIZATION OF INORGANIC PHOSPHATE TRANSPORT IN TRYPANOSOMA BRUCEI

RUSSO-ABRAHAO, T.[1]; SILVA-RITO, S.[2]; MARINS-LUCENA, T.[1]; KOELLER, C.M.[1]; ALVES-BEZERRA, M.[1]; DE PAULA JUNIOR, I.F.[1]; HEISE, N.[1]; GONDIM, K.C.[1]; MEYER-FERNANDES, J.R.[1]

1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.IFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:thaisabrahao@hotmail.com

INTRODUCTION. *Trypanosoma brucei* is an extracellular protozoan parasite that causes human African trypanosomiasis or "sleeping sickness ". During the different phases of their life cycle, *T. brucei* depends on exogenous inorganic phosphate (P_i), but little is known about the transport of P_i across the plasma membrane in this organism. Pi transporters have been described in *Saccharomyces cerevisiae, Plasmodium, Trypanosoma rangeli, Leishmania infantum , Trypanosoma cruzi* and other microorganisms. OBJECTIVES. Investigate the kinetics of ^{32}Pi transport, pH influence, H^+ and K^+ ionophores; inhibitors influence, H^+:Pi cotransporter gene expression and RNAi of PHO84 gene. METHODOLOGY. Pi transport was measured by ^{32}Pi entry into cells during 1 h. After washing with the same ice-cold buffer, cells were disrupted by adding 0.1% SDS. The mixture containing ^{32}Pi taken up by cells was transferred to a scintillation vial containing 9.0 ml of scintillation liquid. We used tetracycline-inducible RNAi cell lines against Tb11.02.3020 and evaluate Pho84 gene expression and cell growth. Expression of Pho84 was evaluated by Real time PCR. RESULTS. Pi transport is modulated by pH variation, with higher activity at acidic pH; FCCP, nigericin, valinomycin and SCH28080 inhibited the Pi transport, which was not inhibited by bafilomicina A1. Pi transport showed Michaelis- Menten kinetics. A sequence encoding a carrier of phosphate was identified in the genome of *T. brucei*, and expression of the gene TbPho84 was obtained. The RNAi methodology resulted in a decrease in cell growth and gene expression. CONCLUSIONS. These results confirm the presence of a Pi carrier in *T. brucei,* similar to Pho84 described in *S. cerevisiae*, which contributes to the acquisition of inorganic phosphate and may be involved in the growth and survival of procyclic forms of *T. brucei*. This work presents the first description of a Pi transporter - PHO84 in *T. brucei.* **Supported by:**CNPq, CAPES, FAPERJ

PV26 - CROSS-PROTECTIVE IMMUNITY TO LEISHMANIA AMAZONENSIS IS MEDIATED BY CD4+ AND CD8+ EPITOPES OF LEISHMANIA DONOVANI NUCLEOSIDE HYDROLASE TERMINAL DOMAINS

NICO, D.[1]; GOMES, D.C.[1]; DA SILVA, M.V.A.[1]; FREITAS, E.O.[1]; MORROT, A.[1]; BAHIA, D.[2]; PALATNIK, M.[1]; RODRIGUES, M.M.[3]; PALATNIK-DE-SOUSA, C.B[1]

1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL; 3.UNIFESP, SÃO PAULO, SP, BRASIL. e-mail:dirlei@micro.ufrj.br

The nucleoside hydrolase (NH) of Leishmania donovani (NH36) is a phylogenetic marker of high homology among Leishmania parasites. In mice and dog vaccination, NH36 induces a CD4+ T cell-driven protective response against Leishmania chagasi infection directed against its C-terminal domain (F3). The C-terminal and N-terminal domain vaccines also decreased the footpad lesion caused by Leishmania amazonensis. We studied the basis of the crossed immune response using recombinant generated peptides covering the whole NH36 sequence and saponin for mice prophylaxis against L. amazonensis. The F1 (aa 1–103) and F3 peptide (aa 99–314) vaccines enhanced the IgG and IgG2a anti-NH36 antibodies to similar levels. The F3 vaccine induced the strongest DTH response, the highest proportions of NH36-specific CD4+ and CD8+ T cells after challenge and the highest expression of IFN-γ and TNF-α. The F1 vaccine, on the other hand, induced a weaker but significant DTH response and a mild enhancement of IFN-γ and TNF-α levels. The in vivo depletion with anti-CD4 or CD8 monoclonal antibodies disclosed that cross-protection against L. amazonensis infection was mediated by a CD4+ T cell response directed against the C-terminal domain (75% of reduction of the size of footpad lesion) followed by a CD8+ T cell response against the N-terminal domain of NH36 (57% of reduction of footpad lesions). Both vaccines were capable of inducing long-term cross-immunity. The amino acid sequence of NH36 showed 93% identity to the sequence of the NH A34480 of L. amazonensis, which also showed the presence of completely conserved predicted epitopes for CD4+ and CD8+ T cells in F1 domain, and of CD4+ epitopes differing by a single amino acid, in F1 and F3 domains. The identification of the C-terminal and N-terminal domains as the targets of the immune response to NH36 in the model of L. amazonensis infection represents a basis for the rationale development of a bivalent vaccine against leishmaniasis. **Supported by:**CNPQ, CAPES, FAPERJ

PV27 - LEISHMANIA DONOVANI NUCLEOSIDE HYDROLASE TERMINAL DOMAINS IN CROSS-PROTECTIVE IMMUNOTHERAPY AGAINST LEISHMANIA AMAZONENSIS MURINE INFECTION

NICO, D.[1]; GOMES, D.C.[1]; PALATNIK DE SOUSA, I.[2]; MORROT, A.[1]; PALATNIK, M.[1]; PALATNIK-DE-SOUSA, C.B[1] *1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.PUCRJ, RIO DE JANEIRO, RJ, BRASIL.*
e-mail:dirlei@micro.ufrj.br

Nucleoside hydrolases of the Leishmania genus are vital enzymes for the replication of the DNA and conserved phylogenetic markers of the parasites. Leishmania donovani nucleoside hydrolase (NH36) induced a main CD4(+) T cell driven protective response against L. chagasi infection in mice which is directed against its C-terminal domain. In this study, we used the three recombinant domains of NH36: N-terminal domain (F1, amino acids 1-103), central domain (F2 aminoacids 104-198), and C-terminal domain (F3 amino acids 199-314) in combination with saponin and assayed their immunotherapeutic effect on Balb/c mice previously infected with L. amazonensis. We identified that the F1 and F3 peptides determined strong cross-immunotherapeutic effects, reducing the size of footpad lesions to 48 and 64%, and the parasite load in footpads to 82.6 and 81%, respectively. The F3 peptide induced the strongest anti-NH36 antibody response and intradermal response (IDR) against L. amazonenis and a high secretion of IFN-γ and TNF-α with reduced levels of IL-10. The F1 vaccine, induced similar increases of IgG2b antibodies and IFN-γ and TNF-α levels, but no IDR and no reduction of IL-10. The multiparameter flow cytometry analysis was used to assess the immune response after immunotherapy and disclosed that the degree of the immunotherapeutic effect is predicted by the frequencies of the CD4(+) and CD8(+) T cells producing IL-2 or TNF-α or both. Total frequencies and frequencies of double-cytokine CD4 T cell producers were enhanced by F1 and F3 vaccines. Collectively, our multifunctional analysis disclosed that immunotherapeutic protection improved as the CD4 responses progressed from 1+ to 2+, in the case of the F1 and F3 vaccines, and as the CD8 responses changed qualitatively from 1+ to 3+, mainly in the case of the F1 vaccine, providing new correlates of immunotherapeutic protection against cutaneous leishmaniasis in mice based on T-helper TH1 and CD8(+) mediated immune responses **Supported by:**CNPQ, CAPES, FAPERJ

PV28 - MULTIFUNCTIONAL TH1 CELL RESPONSE AGAINST NUCLEOSIDE HYDROLASE (NH) OF LEISHMANIA DONOVANI (NH36) AND ITS EPITOPES IN HUMAN VISCERAL LEISHMANIASIS

BARBOSA SANTOS, M.L.[1]; ALVISI DE OLIVEIRA, F.[1]; SANTOS, P.L.[1]; NICO, D.[2]; CORRÊA, C.B.[1]; ALMEIDA, R.[1]; PALATNIK-DE-SOUSA, C.B[2]
1.UNIVERSIDADE FEDERAL DE SERGIPE, ARACAJU, SE, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:immgcpa@micro.ufrj.br

Visceral leishmaniasis (VL) is a disease caused by systemic infection with protozoa of the genus Leishmania. VL is associated with a marked impairment of Leishmania-specific Th1 response, as evaluated by in vitro Leishmania antigen stimulation of PBMC. The nucleoside hydrolase (NH) of L. (L.) donovani (NH36) is a phylogenetic marker of Leishmania parasites responsible for immunogenicity and protective efficacy against murine VL. The CD4+ T cell differentiation has been shown to be correlated to the progressive development from single-cytokine (IL-2+, or TNF+), to double- (IL-2+-TNF+) and triple-cytokine producers (IL2+-TNF+-IFN-γ+) which are multifunctional and may provide optimal effector function and protection. We studied the T-cell cytokine production in response NH36 and its recombinant peptide domains F1, F2 and F3 using PBMC of human VL patients by multiparameter flow cytometry. Our preliminary results disclosed that patients before specific Leishmania therapy (D0) showed a pronounced decrease in the percentage of CD4 and minor decrease in CD8 T cells compared the patients after treatment (D180). All peptide domains (F1,F2,F3) increased the frequencies of CD4+-TNF+ and CD4+-IL-2+ T cells while only F2 and NH36 increased the levels of the double-producers TNF+-IL-2+. As previously described in the murine model, the predominant response against NH36 peptides is TNF and IL-2 and IFN-γ expression by CD4+ T cells is not detected. The frequencies of single-cytokine (IL-2+, or TNF+) and double-cytokine (IL-2+-TNF+) CD8+ T cell producers were higher than those of CD4+ T cells and mainly increased in response to the F2 peptide in cured individuals and for NH36 and F3 peptides for patients before treatment. Interestingly, CD8 T cells from patients before treatment showed high frequencies of TNF/IL-2/IFN-g cytokines production in response to F1 and F3 peptide response. Our results suggest that recombinant antigens induce protective response by stimulus of TH1 cytokines production. **Supported by:**CNPQ, CAPES, FAPERJ

PV29 - ANTIGENICITY OF F1, F2, F3 AND NH36 IN EXPOSED INDIVIDUALS TO L.INFANTUM IN MADRID (SPAIN)

PALATNIK-DE-SOUSA, C.B[1]; NICO, D.[1]; FERNANDEZ, L.[2]; BOTANA-VEGUILLAS, L.[2]; SANCHEZ, C.[2]; ALMEIDA, V.A.[2]; IBARRA-MENESES, A.V.[2]; CARILLO, E.[2]; MORENO, J.[2]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.INSTITUTO DE SALUD CARLOS III, MADRID, ESPANHA. e-mail:immgcpa@micro.ufrj.br

Leishmania donovani nucleoside hydrolase NH36 has previously demonstrated to induce protection against Leishmania infection and some of its recombinant domains have recently shown an adequate immunotherapeutic profile in the mice model of cutaneous leishmaniasis. Cell-mediated immune response is essential for the control of leishmaniasis, and consequently, the molecules inducing dominant T cell response in in vitro immunological assays are targeted for the vaccine design. At the present work we have studied the antigenicity of F1, F2, F3 domains of NH36 and NH36 antigen in exposed habitants in a post outbreak area of Visceral leishmaniasis of Madrid. In those individuals with a patent lymphoproliferative response to SLA, we have found a positive response to all the studied antigens compared to those without cellular response to SLA. Further, we have found a significant increased response to F1 and NH36 in cured and asymptomatic individuals when compared to controls. Further, NH36 also induced significant levels of stimulation in those patients with active disease. Our results suggest the potential use of these recombinant leishmanial antigens to identify L. (L.) infantum exposed individuals and in extension as potential vaccine candidates. Further analyses to quantify the profile of secreted cytokines into the supernatants after antigen stimulation are in progress. **Supported by:**CNPQ, CAPES, FAPERJ, ISCIII-AES ref P113/00440

PV30 - ANTI *TRYPANOSOMA CRUZI* ACTIVITY OF FERROCENYL DIAMINES DERIVATIVES AND DIFFERENTIAL EXPRESSION OF TRYPAREDOXIN PEROXIDASE AND OLD YELLOW ENZYME

KOHATSU, A.A.N.[1]; SILVA, F.A.J.[1]; FRANCISCO, A.I.[2]; ANDRÉO, B.G.C.[1]; DA SILVA, M.T.A.[3]; ROSA, J.A.[1]; VARGAS, M.D.[2]; ISAAC, V.L.B.[1]; CICARELLI, R.M.B.[1]
1.UNESP, ARARAQUARA, SP, BRASIL; 2.UFF, NITERÓI, RJ, BRASIL; 3.USP, SÃO CARLOS, SP, BRASIL. e-mail:andrea.akiko@yahoo.com.br

Some studies related an increase on the production of anti-oxidative enzymes which probably could be responsible for the benznidazole (BZ) resistance, such as mitochondrial tryparedoxin peroxidase (mTcTXNPx), and cytosolic old yellow enzyme (cTcOYE). This work evaluated the susceptibility of six strains of epimastigote form from *T. cruzi* to three ferrocenyl diamines derivatives (FDD - compounds 4, 7 and 11) and the difference on the expression level of mTcTXNPx and cTcOYE, because these enzymes could be related with the resistance of the parasites to the BZ and others trypanocidal substances. Cytotoxic assays in vitro were performed using MTT to measure the cellular viability of the parasites and HepG2, used as a model of mammal cells. Differences of mTcTXNPx and cTcOYE expression were evaluated by Western blotting. Each strain showed different IC_{50} values and to all strains FDD were more toxic than BZ. Compound 4 showed the best activity, values ranging from 2.21 to 15.20 µM, whereas to BZ they were 27.28 to 105.28 µM. The FDD were toxic for T. cruzi, but not for HepG2 cells. The redox properties of the ferrocenyl group may be involved in the trypanocidal activity. Differences in susceptibility, given the wide genetic diversity, is one of most important factor that difficult searching for trypanocidal substances that affect the majority of the *T. cruzi* population. Then each strain might express different enzymes that act on the anti-oxidative system, rather than depending on only mTcTXNPx or cTcOYE, which explaining why the expression levels of mTcTXNPx and cTcOYE are not always enhanced in the most resistant strain. Hence, FDD could represent potential candidates for more studies, and in vitro and in vivo models are needed to study the integration of the oxidative pathway and enzymes that are expressed in trypomastigotes and amastigotes. **Supported by:**FAPESP (2011/06525-2), Fundunesp and CNPq (130547/2011-5)

PV31 - THE F1 DOMAIN IS THE ANTIBODY TARGET OF L. (L.) DONOVANI NUCLEOSIDE HYDROLASE NH36 IN SUBCLINICAL DTH+ AND CURED HUMAN PATIENTS OF VISCERAL LEISHMANIASIS FROM ARACAJU (SE).

NICO, D.[1]; DA SILVA, M.V.A.[1]; BARBOSA SANTOS, M.L.[2]; ALMEIDA, R.[2]; PALATNIK-DE-SOUSA, C.B[1]

1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFS, ARACAJU, SE, BRASIL.

e-mail:marcus_vet@yahoo.com.br

The nucleoside hydrolase (NH36) is the main antigen of the Leishmune® canine vaccine, which induces in mice a CD4+ T cell-driven protective and antibody response against L. (L.) chagasi infection directed against its C-terminal domain. Aiming to map the NH36-epitopes for B-cells of human visceral leishmaniasis (VL) patients (Aracaju, SE), we used sera of subclinical subjects with DTH positive response to leishmanial antigen (n= 10, DTH+), of VL patients before and 30, 60, 90 and 180 days after treatment (n=10). Sera samples of human heathy blood donors of the HU-CCFFo-UFRJ were used as non-endemic area controls (n=24). Samples were analyzed by a standard ELISA assay using 1 µg of the recombinant NH36, F1, F2 and F3 peptides and anti-human IgG1, IgG2, IgG3 and IgG4 monoclonal antibodies (Invitrogen). The main target of the anti-IgG1 and IgG3 antibodies was the N-terminal domain (F1, amino acids 1-103). The anti-F1 IgG3 antibodies were increased in subclinical asymptomatic DTH+ subjects and cured patients, starting from day 30 and remaining high until 180 days after treatment. On the other hand, IgG3 antibodies against NH36 were only predominant in patients before treatment. The IgG3 subtype was claimed to be related to the protective response or natural resistance to VL. The F1 peptide was also recognized by IgG1 antibodies of cured subject at day 30 after treatment. NH36, on the other hand, was the main antigen of the IgG2 response (all groups) and of IgG4 response of the cured subjects, sharing the predominance with F3, in patients before treatment. Our preliminary results indicate that, as previously described for infected dogs under immunotherapy with the NH36-DNA vaccine, F1 is the domain which concentrates the epitopes for B cells recognized by IgG3 and IgG1 antibodies and related to cure or natural resistance to VL. Furthermore, as described before for canine VL, the NH36 is the IgG4 and IgG2 serological marker for the human disease. **Supported by:**CNPq, CAPES, FAPERJ

PV32 - CROSS-PROTECTIVE EFFECT OF VACCINATION WITH THE RECOMBINANT CHIMERA CONTAINING THE N- AND C- DOMAINS OF NUCLEOSIDE HYDROLASE FROM LEISHMANIA (L.) DONOVANI TERMINALS ON PROPHYLAXIS OF MURINE CUTANEOUS LEISHMANIASIS BY LEISHMANIA (L.) AMAZONENSIS

DA SILVA, M.V.A.; NICO, D.; PALATNIK-DE-SOUSA, C.B

UFRJ, RIO DE JANEIRO, RJ, BRASIL.

e-mail:marcus_vet@yahoo.com.br

The nucleoside hydrolase (NH) of Leishmania donovani (NH36) is a phylogenetic marker of high homology among Leishmania parasites. In mice vaccination, NH36 induces a CD4+ T cell-driven protective response against Leishmania chagasi infection directed against its C-terminal domain (F3). The C-terminal and N-terminal domain vaccines also decreased the footpad lesion caused by Leishmania amazonensis. We studied if a recombinant chimera containing the N- and C-terminals cloned in tandem would optimize the generation of immune prophylaxis above than the addition of the two peptides in the vaccine formulation. The recombinant chimera F1-F3 was obtained in the pET28b expression system and its efficacy (100 µg and 200 µg/dose), was compared to those of the F1 e F3 used as independent vaccines (100 µg), or added in the same vaccine formulation (F1+F3), in combination with saponin. After immunization, all animals were challenged with infective 106 L. (L) amazonensis promastigotes in the right paws. The optimized chimera showed to be the most potent formulation in the generation of IgA and IgG2a anti-NH36 antibodies after challenge ($p<0.05$), IDR, IFN-γ and IL-10 secretion, probably due to its F1 component, and in reduction of parasite load (limiting dilution assay) and sizes of footpad lesions. Our results indicate that the presentation of F1 and F3 as a chimera potentiates the immunogenic protection above the presentation of the peptide as a mixture. We expect that our results will represent a basis for the rational development of a bivalent vaccine against leishmaniasis. **Supported by:**CNPq, CAPES, FAPERJ

PV33 - POSSIBLE SOURCES OF HYDROGEN PEROXIDE GENERATION STIMULATED BY HEME IN *LEISHMANIA AMAZONENSIS*

MACHADO, N.R.; GOMES, D.C.; PAES, L.S.; MEYER-FERNANDES, J.R.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:rocco@bioqmed.ufrj.br

Leishmania amazonensis is a protozoan that occurs in many areas of Brazil and causes cutaneous lesions. Heme is an important biomolecule with a pro-oxidant and signaling capacity. Recently, hydrogen peroxide (H_2O_2) has been considered an important second messenger, being able to stimulate PKC activity in several models (Cosentino-Gomes D, Rocco-Machado N, Meyer-Fernandes JR. Cell Signaling through Protein Kinase C Oxidation and Activation. International Journal of Molecular Science. 2012 13(9):10697-10721). We find that increased concentrations of heme stimulated H_2O_2 generation in a dose dependent manner, reaching its maximum at 2,5 µM and Na^+/K^+ ATPase was stimulated by increasing concentrations of this reactant and reached its maximum at 0.1 µM. Furthermore, we found that H_2O_2 promotes an increase in the Na^+/K^+ ATPase activity through the activation of PKC. Our goal in this work is to investigate the source of heme-dependent hydrogen peroxide in *L. amazonensis*. Our results shows that Mito-TEMPO, a mitochondria-targeted SOD mimetic, that also reduces the mitochondria electron leak and inhibit the production of all ROS, was capable to abolished the increased of H_2O_2 promoted by heme. Furthermore, inhibitors of NADPH oxidase activity, diphenylene iodonium (DPI) and apocynin abolished the effect of heme on H2O2 generation. The inhibition of H_2O_2 generation by its inhibitors suggests the participation of this enzyme on heme stimulatory effect. We are now investigating the presence of a NADPH oxidase in these cells. **Supported by:**CAPES

PV34 - CONSERVATION OF SUBTELOMERIC REGIONS ACROSS DIFFERENT LINEAGES OF TRYPANOSOMA CRUZI AND THE SUBESPECIES TRYPANOSOMA CRUZI MARINKELLEI: IMPLICATIONS FOR THE CHROMOSOME EVOLUTION

ANTONIO, C.R.[1]; MARINI, M.M.[1]; BARROS, R.R.M.[2]; VAN SCHAIK, M.W.[1]; MACHIANO, F.S.[1]; CORTEZ, D.R.[1]; CORTEZ, C.[1]; TEIXEIRA, M.M.[1]; DASILVEIRA, J.F.[1]
1.UNIFESP/EPM, SAO PAULO, SP, BRASIL; 2.LABORATORY OF MALARIA AND VECTOR RESEARCH, NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASE, N, ROCKVILLE, ESTADOS UNIDOS. e-mail:cris.r.antonio@gmail.com

The protozoan parasite Trypanosoma cruzi is the etiologic agent of Chagas disease. T. cruzi subtelomeric regions are enriched in surface protein and retrotransposon hot spot protein genes suggesting that they may have acted as a site for recombination and generation of new variants of surface proteins. Subtelomeric regions of clone CL Brener are highly polymorphic, mainly as a result of large changes in the abundance and organization of these genes. In this work, we analyzed the organization of subtelomeric regions in different T. cruzi lineages (TcI- isolates G strain and clone Dm28c; TcII- Esmeraldo cl3; TcIII – clone TCC2177 clone 4.2; TcIV – clone CanIII TCC2124; TcV- clone SO3-cl5, TcVI- CL Brener) and the subspecies T. c. marinkellei. We identified 33 telomere-specific markers that were hybridized to the chromosomal bands separated by PFGE. Interstitial markers of each chromosome were also hybridized as an internal control. Identical hybridization patterns were observed with the telomere- and interstitial chromosome-specific markers in different isolates suggesting that synteny at chromosomal ends is conserved across T. cruzi lineages. Subtelomeric regions vary between chromosomes of the same isolate but they seems conserved between chromosomes of different isolates. Only two two chromosome rearrangements were identified. One of them was caused by deletion of 3-oxoacyl-ACP reductase gene in the subtelomeric region of clone Esmeraldo and the other was a duplication followed by translocation in clones Esmeraldo and CL Brener. Conserved synteny at the chromosomal ends between lineages is surprising considering that chromosomal polymorphism between T. cruzi lineages is extensive. It is possible that subtelomeric regions play a role in stabilizing replication and copy number of the chromosomes. Trypanosomes have large genomes, yet synteny seems highly conserved suggesting that there is a specific mechanism governing genome evolution within these organisms. **Supported by:**FAPESP

PV35 - THE INFLUENCE OF THE SYMBIOTIC BACTERIUM ON THE ENERGETIC METABOLISM OF THE HOST TRYPANOSOMATID, *STRIGOMONAS CULICIS*

MACHADO, A.C.L.; CATTA-PRETA, C.M.C.; MARTINS, A.C.A.; DE SOUZA, W.; GALINA, A.; MOTTA, M.C.M.
UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:anacarolinalm@yahoo.com.br

The host trypanosomatids harboring a symbiotic bacterium are models for cell evolution and origin of organelles, as *Strigomonas culicis*. The energetic metabolism in wild and aposymbiotic strains of S. culicis was compared. The presence of the symbiont enhances 74% O2 consumption and mitochondria from wild strain occupies a 27% higher volume when compared to aposymbiotic strain. When we induced the inhibition of NADHdesydrogenase we observed a reduction of 60% in the basal respiration of both strains, which could reach a 100% inhibition in the presence of Cytochrome c oxidase inhibitor. Inhibition of F1Fo-ATPsynthase reduced the wild strain O2 consumption to 60% when compared to the basal respiration, while in the aposymbiotic strain it was not affected. Even after maximum respiration rate stimulation by uncoupling, the aposymbiotic strain was less stimulated than the wild strain. When the glycolitic metabolism of both strain were evaluated by quantification of glycerol release we observed that wild strain is 83% less glycolitic than the aposymbiotic strain. After inhibition by KCN, the ATP content of the wild strain suffered a drastic reduction of 60%, while the aposymbiotic strain ATP concentration was 25% reduced. Hence, the aposymbiotic strain has a higher fermentative capacity. The 3D reconstruction of cells showed that the relative volume occupied by the glycosomes of the wild strain is 3,8%, while in aposymbiotic strain it represents 1,5% of the total cell body volume. The glycosomes distribution in the host cytoplasm is affected by the symbiont and It is frequent the visualization of membrane touches of both structures. The symbiont is also occasionally associated with the mitochondrion membrane. So, the bacterium allows the trypanosomatid to achieve higher respiration rates and suggest that the host can supply the symbiont with glycerol and/or ATP. Herein, the symbiotic bacterium influences the energetic metabolism of the host trypanosomatid. **Supported by:**FAPERJ; CNPQ; INBEB

PV36 - CHARACTERIZATION OF THE SPLICED LEADER (MINI-EXON) *LOCUS* IN CLONES OF *TRYPANOSOMA CRUZI* EXPOSED TO IONIZING RADIATION

VAN SCHAIK, M.W.[1]; BARROS, R.R.M.[2]; ANTONIO, C.R.[1]; MACHIANO, F.S.[1]; ROGERO, S.O.[3]; ROGERO, J.R.[3]; MARINI, M.M.[1]; DASILVEIRA, J.F.[1]
1.UNIFESP, SÃO PAULO, SP, BRASIL; 2.NATIONAL INSTITUTE OF HEALTH, ROCKVILLE, ESTADOS UNIDOS; 3.IPEN, SÃO PAULO, SP, BRASIL.
e-mail:matheus.schaik@gmail.com

Trypanosoma cruzi displays a remarkable resistance to ionizing radiation probably due to high DNA repair ability. Following a dose of 500 Gy of gamma radiation, the fragmented genomic DNA is gradually reconstructed and the pattern of chromosomal bands is restored (Regis-Da-Silva et al, Mol Biochem Parasitol v. 149, n. 2, Oct, p. 191-200. 2006). We demonstrate that single cell clones from CL Brener epimastigotes exposed to gamma radiation showed syntenic conservation of 3 large linkage groups mapped in the chromosomal bands XX, XVI and XIII. However, we detected chromosome alterations in the majority of clones in DNA satellite and spliced leader *loci*. The spliced leader or mini-exon gene is present in tandem-arranged multiple copies in *T. cruzi* genome, what makes it more prone to rearrangements during DSBs repair.
In order to characterize the spliced leader *locus* we defined a consensus sequence with four repeats. By restriction and hybridization analyses we confirmed the presence of tandemly repeat organization and the length of the mini-exon monomer repeat was estimated to be 612 bp. We observed some difference in the migration pattern of the monomer repeat between the irradiated clone E5 and the parental CL Brener, a slow-migrating component than the monomer was observed in clone E5. We have also observed rearrangements in the chromosomal bands that harbor the mini-exon *loci*. These rearrangements can be associated to DNA gain or lost represented by slow- (gain) or faster-migrating bands (lost) confirmed by copy number determinations of spliced leader sequences in irradiated clones. Similar results have been found with the satellite DNA tandem repeats in the irradiated clones. **Supported by:**Fapesp, CNPq, CAPES

PV37 - ENDOSYMBIOSIS IN TRYPANOSOMATIDS: CELL CYCLE ANALYSIS INDICATES CO-EVOLUTION DIVERGENCE IN HOST-BACTERIUM RELATIONSHIP

CATTA-PRETA, C.M.C.[1]; BRUM-DA-SILVEIRA, F.L.[1]; DA SILVA, C.C.[1]; ZUMA, A.A.[1]; ELIAS, M.C.[2]; SCHENKMAN, S.[3]; DE SOUZA, W.[1]; MOTTA, M.C.M.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.BUTANTAN, SÃO PAULO, SP, BRASIL; 3.UNIFESP, SÃO PAULO, SP, BRASIL. e-mail:carol.cattapreta@gmail.com

Co-evolution between primitive organisms associated by symbiosis offers valuable information about the eukaryotic cell evolution. Some monoxenic trypanosomatids maintain a mutualistic relationship with a bacterium that divides in synchrony with other host cell structures. Genomic data showed that the symbiont suffered a massive gene loss, including those related to division and cell wall assembly. In this work, we study the coordinated division between the symbiont and the host protozoan by investigating the cell cycle in two symbiont harboring trypanosomatids, which belong to divergent genera, *Angomonas deanei* and *Strigomonas culicis*. Protozoa were treated with cicloheximide that inhibits protein synthesis in eukaryotes and with different cell cycle inhibitors. Then, protozoa were submitted to immunofluorescence and electron microscopy techniques, as well as to cytometry flow analyses. Our results showed that cicloheximide treatment completely blocked trypanosomatids proliferation, but did not impeach symbiont DNA duplication. However, most bacterium maintained the constricted format, indicating that proteins produced by the host cell are required for the prokaryote division. Treatment with aphidicolin promoted protozoa cell cycle arrest in G1/S phase and the bacterium present in each species responded differently: in *A. deanei*, the symbiont duplicated its DNA and presented up to 4 nucleoids, whereas in *S. culicis* the bacterium acquired a filamentous structure with multiple nucleoids. Camptothecin treatment promoted protozoa cell cycle arrest in G2-phase and generated filamentous symbionts in both species. Oryzalin, which disestablish microtubules, induced cell cycle impairment in mitosis and prevented symbiont division. Taking together, our results indicate that each host trypanosomatid control the bacterium division differently. In *A. deanei* the cell cycle coordination is established in G1/S phase, whereas in *S. culicis* it is induced later on, during mitosis. **Supported by:**FAPERJ, CNPq, FAPESP

PV38 - EVALUATION OF THE TRYPANOCIDAL ACTIVITY OF EXTRACTS FROM PIPERACEAE, RUTACEAE AND BIGNONIACEAE IN Y STRAIN OF *TRYPANOSOMA CRUZI*

SILVA, F.A.J.[1]; KOHATSU, A.A.N.[1]; REGASINI, L.O.[2]; YOUNG, M.C.M.[3]; BOLZANI, V.S.[4]; SILVA, D.H.S.[4]; CICARELLI, R.M.B.[1]
1.UNESP - FCFAR, ARARAQUARA, SP, BRASIL; 2.UNESP - IBILCE, SÃO JOSÉ DO RIO PRETO, SP, BRASIL; 3.INSTITUTO DE BOTÂNICA DE SÃO PAULO, SÃO PAULO, SP, BRASIL; 4.UNESP - IQ, ARARAQUARA, SP, BRASIL.
e-mail:flaviaajs@yahoo.com.br

Since its description in 1909, Chagas disease still affects millions of people especially in Latin America where it is endemic and neglected, being a major problem for public health as it prevails in areas rural and poor. Currently only two drugs are available to treat this disease, which are nifurtimox, which is no longer prescribed in Brazil and benznidazole, but both are limited action and with many side effects. Find a new substance that is more effective against the parasite and without as many side effects to the host is a necessity. Since 2008, the team CicarelliRMB in partnership with nubbe and other collaborators conducts tests with plant extracts, natural or synthetic substances and essential oils. Since were tested to date over a thousand samples of which 80 showed an IC_{50} <10 µg/mL and proved promising candidate for the search for new drugs because it has an IC_{50} less than benznidazole, 9.04 µg/mL. This project aimed to evaluate the trypanocidal activity of 45 samples of natural extracts of families Piperaceae, Rutaceae and Bignoniaceae in epimastigote form of Y strain of *Trypanosoma cruzi*. The tests were performed by colorimetric MTT. Of the three extracts from Piperaceae, two showed an IC_{50} less than 500 µg/mL. Of the 32 extracts Bignonaceae 17 showed an IC_{50} less than 500 µg/mL, and two species, Distictella mansoana (IC_{50} = 30.42 µg/mL) and Stizophyllum perforatum (IC_{50} = 20.20 µg/mL) showed the best results so far, probably due to the presence of naphthoquinones with trypanocidal activity already described in the literature. The trypanocidal activity of Rutaceae has been associated with coumarins and alkaloids, which are characteristic structures of this family. In this study, 10 extracts of species of Rutaceae were tested and four showed trypanocidal activity. **Supported by:**Fapesp

PV39 - PROTEIN EXPRESSION AND FUNCTIONAL ANALYSIS OF THE GAMMA-GLUTAMYLCYSTEINE SYNTHETASE IN NEW WORLD *LEISHMANIA* SPECIES

FONSECA, M.S.; SANTI, A.M.M.; MURTA, S.F.
CPQRR/FIOCRUZ-MG, BELO HORIZONTE, MG, BRASIL.
e-mail:maisa.s.fonseca@gmail.com

Gamma-glutamylcysteine synthetase (GSH1) is the first step enzyme of the glutathione biosynthesis pathway in Leishmania spp. Glutathione is an essential component of the trypanotione, an important molecule involved in the detoxification of oxygen reactive species in trypanosomatids. In this study, we investigate the expression level of GSH in lines of Leishmania spp. (L. guyanensis, L. amazonensis, L. braziliensis and L. infantum) susceptible and resistant to trivalent antimony (SbIII). These lines were previously selected in vitro to SbIII and the resistance index varied from 4 to 20-fold higher than of their wild-type counterparts. Anti-LbGSH polyclonal antibodies were produced in rabbits using His-tagged rLbGSH protein as antigen, and the antibodies were employed in Western blot analyses. GSH1 antibody recognized a 78 kDa protein in all Leishmania lines analyzed. The level of expression of this polypeptide was higher in the SbIII-susceptible L. guyanensis and L. amazonensis lines than in their respective SbIII-resistant lines. Similar protein level was observed between susceptible and SbIII-resistant lines from L. braziliensis and L. infantum. Subsequently, functional analysis was conducted to determine whether the overexpression of GSH1 in the susceptible L. guyanensis, L. braziliensis and L. infantum lines would change the SbIII-resistance phenotype of transfected parasites. Western blot analysis showed that the level of GSH1 protein expression was higher in all transfected parasites compared to untransfected ones. Antimony susceptibility test (IC50 assay) revealed that L. guyanensis clones overexpressing GSH1 had a 3-fold increase in resistance to SbIII when compared to the untransfected parental line. In contrast, no difference in SbIII susceptibility was observed in L. infantum and L. braziliensis clones overexpressing GSH1. Our functional analysis revealed that the enzyme GSH1 is involved in the antimony-resistance phenotype in L. guyanensis line analyzed. **Supported by:**CNPq, FAPEMIG, UNICEF/UNDP/World Bank/WHO and PROEP/CNPq/FIOCRUZ

PV40 - DEVELOPMENT OF A DOUBLE FLUORESCENT MUTANT STRAIN OF TOXOPLASMA GONDII TO FOLLOW CYST FORMATION IN VITRO AND IN VIVO.

SANTOS, T.P.[1]; TOMITA, T.[2]; MA, Y.F.[2]; ATTIAS, M.[1]; WEISS, L.[2]; VOMMARO, R.C.[1]
1.*IBCCF/UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.AECOM, NEW YORK, ESTADOS UNIDOS.*
e-mail:tatianacps.uff@gmail.com

Toxoplasma gondii is a protozoan found in 30% of world population, that can infect and replicate in nucleated cells from warm-blooded animals. In an asexual life cycle has two infective stages: the tachyzoite, a fast replication form in an acute infection, and the bradyzoite, a slow growing form inside tissue cysts found in the central nervous system and muscular tissue in a chronic infection. The conversion process is poorly understood. Recently, our group described the biological behavior of the EGS strain, which is able to form cysts spontaneously in tissue culture, constituting a useful tool to study the conversion process. Based on that, we constructed genetically modified EGS parasites that express specific stage proteins and fluorescent tags. The promoter regions for SAG-1 (tachyzoite specific) and BAG-1 (bradyzoite specific) were amplified by PCR and plasmids were constructed with mCherry and sfGFP sequences, respectively. EGS parasites were electroporated. In some transfections a third plasmid that provided chloramphenicol resistance was also added. After 3 passages for drug selection, the parasites were sorted by FACS for mcherry positive colonies followed by a FACS selection for GPF positive parasites. After that, the double fluorescent clones were selected and inoculated i.p. in mice. Tachyzoites and bradyzoites harvested from the mice brain demonstrated stage specific expression of GFP and mCherry. In cell cultures analyzed by fluorescence microscopy, parasitic vacuoles that were DBA positive (a marker for T. gondii cysts) were also demonstrated to be positive for BAG1-GFP and negative for SAG1-mCherry. In conclusion, this new transgenic EGS (redT, greenB) strain is a very promising tool to elucidate the mechanisms of Toxoplasma gondii bradyzoite tachyzoite differentiation both in vitro and in vivo. **Supported by:**CNPQ/CAPES

PV41 - *RHODNIUS PROLIXUS RPFOR* GENE EXPRESSION IS MODULATED BY FEEDING AND TRYPANOSOME INFECTION

GUARNERI, A.A.[1]; LORENZO, M.G.[1]; ESTIVALIS, J.M.L.[1]; LOWENBERGER, C.[2]
1.CPQRR/FIOCURZ, BELO HORIZONTE, MG, BRASIL; 2.SIMON FRASER UNIVERSITY, BURNABY, CANADÁ. e-mail:guarneri@cpqrr.fiocruz.br

The *foraging* gene (FOR: cGMP dependent protein kinase (PKG)) has been associated with the modulation of behavioural responses in different invertebrates. FOR is highly conserved from nematodes to insects and mammals. We recently characterized this gene in *Rhodnius prolixus* (*Rpfor*), a vector of Chagas disease. The expression of *Rpfor* changes in response to feeding status, and by infection with *Trypanosoma cruzi* or *Trypanosoma rangeli*. We evaluated *Rpfor* expression in the brains, fat bodies, and intestines of infected or control nymphs (5th instar). Tissues were dissected, RNA extracted, cDNAs synthesized, and *Rpfor* expression was evaluated using real time PCR. Rpfor was expressed in all tissues evaluated. Significant effects were observed in the brain and the gut when comparing the effect of a blood meal on the expression of *Rpfor* in *T. cruzi* infected and control insects (a two-way ANOVA per tissue). *Rpfor* expression was only affected by feeding in the brain and in the gut. Fed insects showed a decreased *Rpfor* expression regardless of their infection status when compared with starved ones. However, fat body *Rpfor* expression was affected by feeding, as well as infection, as all fed insects showed a decreased *Rpfor* expression, while starved infected ones showed an increased gene expression in relation to starved controls. When *T. rangeli* infection and nutritional status were analyzed together *Rpfor* expression was affected by feeding, and an interaction between feeding and infection was observed in all tissues evaluated. *Rpfor* expression in unfed infected, fed controls and fed infected insects were all decreased compared with that of starved control insects. We will present data relating *RPfor* expression to the locomotory activity patterns of *R. prolixus* under the same physiological conditions. **Supported by:**CPqRR, INCT-EM, FIOCRUZ (PAPES VI), CAPES

PV42 - INFECTION BY TRYPANOSOMES ALTERS THE SEXUAL BEHAVIOR OF *RHODNIUS PROLIXUS*

FELLET, M.R.G.; LORENZO, M.G.; GUARNERI, A.A.
CPQRR/FIOCURZ, BELO HORIZONTE, MG, BRASIL.
e-mail:guarneri@cpqrr.fiocruz.br

Triatomine reproduction depends on partner recognition and acceptance by females. In this study we showed that infection by *Trypanosoma cruzi* or *Trypanosma rangeli* affects the sexual behavior of *Rhodnius prolixus*. Second and third instar nymphs were orally infected by *T. cruzi* or *T. rangeli*, respectively, through feeding on blood containing culture epimastigotes. In the case of *T. rangeli* infections, insects also had parasites inoculated in the coelomatic cavity when they reached the fourth instar. Insects in control groups were fed only blood and inoculated with PBS. Insects were raised and tested at 25ºC except for one group that was infected with *T. cruzi* and raised at 30ºC until the imaginal moult and then transferred to 25ºC (as well as the respective control). Firstly, we evaluated the number and duration of matings in isolated pairs infected by *T. cruzi* (25-25ºC and 30-25ºC) or by *T. rangeli* (25-25ºC). In this assay the frequency and duration of mating was only affected by *T. rangeli* infection. The mating latency was prolonged for about 4 min and the duration of mating was reduced in 7 min in infected pairs in comparison to control ones. Secondly, we evaluated the effect of infection by *T. cruzi* (30-25ºC) and by *T. rangeli* (25-25ºC) when one uninfected adult could choose between one infected and one uninfected partner. Interestingly, in this situation uninfected male and female insects chose significantly more uninfected partners for both *T. cruzi* and *T. rangeli* infections. For both parasites, 80% of uninfected males mated with uninfected females. In the case of uninfected females, 80% mated with uninfected males in *T. cruzi* infections, and 90% of uninfected females mated with uninfected males in *T. rangeli* infections. This is the first study that shows that trypanosome infections affect the sexual behavior of triatomine bugs. **Supported by:**CPqRR, Fapemig, INCT-EM, FIOCRUZ (PAPES VI)

PV43 - *TRYPANOSOMA CRUZI* EXPRESS AN UNUSUAL EIF2 PROTEIN KINASE TO REGULATE OXIDATIVE RESPONSES AND DIFFERENTIATION

AUGUSTO, L.S.; MORETTI, N.S.; DE CASTILHO, B.A.; SCHENKMAN, S.
UNIFESP, SAO PAULO, SP, BRASIL.
e-mail:leonardo05augusto@gmail.com

Trypanosoma cruzi, the causative agent of Chagas' disease, faces different environmental conditions during its life cycle, such as starvation and alterations in temperature and pH, which requires the activation of specific metabolic pathways to allow its survival. Among those, regulation of translation initiation through the phosphorylation of the alpha subunit of translation initiation factor 2 (eIF2alpha) has been demonstrated to play a key role (Tonelli et al. 2011, PlosOne 6:e27904). Here we show that one of the eIF2 kinases (TceIF2-K2) is associated with endosomal organelles called reservosomes in the epimastigote form. This kinase is modulated by the presence of heme, that also inhibits the differentiation of proliferative epimastigotes to infective metacyclic-trypomastigotes. Parasite lines lacking TcK2 lose the differentiation capacity and displayed a growth deficiency due to the accumulation of hydrogen peroxide as consequence of an increase in the superoxide dismutase activity and peroxidase down modulation. In the presence of iron, the accumulated peroxide generates oxygen species that damage the parasite. As these phenotypes could be restored by the wild type but not by a kinase dead mutant, we conclude that this unusual eIF2 kinase is a key factor in controlling growth, responses to oxidative stress and parasite differentiation. **Supported by:**FAPESP

PV44 - EVALUATION OF DIFFERENT FETAL BOVINE SERUM BATCHES TO PREPARE CULTURE MEDIUM FOR *LEISHMANIA (VIANNIA) BRAZILIENSIS.*

DOS SANTOS, J.C.; GOMES, C.M.; AVILA, L.R.; NUNES, T.N.; OLIVEIRA, M.A.P.
UFG-GO, GOIANIA, GO, BRASIL.
e-mail:jessicacristina_24@hotmail.com

Promastigotes form of Leishmania (Viannia) braziliensis are able to grow in culture medium to be used in different studies. The fetal bovine serum (FBS) is one important component in several culture medium. This work was aimed to evaluate the ability of the MTT assay to identify a suitable FBS to be used in leishmania cultures. Five different FBS batches were tested in Grace´s insect medium. The adaptation of parasites to the medium was evaluated by diary counting and by the ability of parasite to metabolize MTT. It was also quantified the percentage of metaciclic leishmania by Bauhinia purpurea agglutination assay and the ability of parasites to produce lesion in BALB/c mice. The substitution of FBS showed minimal interference in the growing of leishmania in the following six days as observed by counting or MTT assay. When parasite where split for the second time in medium with different SBF, it was observed that PPS6m isolated reached 21±5.8 x 106; 12±3.1 x 106 and 15±3.0 x 106 Leishmania/mL in three media. The isolated CSA7 reached lower number of parasites than PPS6m, but the media that favored the best growing was similar to both isolates. The MTT assay was able to discriminate one FBS as the worst to be used for leishmania culture after the second split and discriminate the best FBS after the third split. The percentage of metaciclic recovered was significantly higher in CSA7 culture that used medium that allowed the lower growing. It was not observed differences in the ability of parasites from different FBS to cause lesion in BALB/c mice. These data suggest that MTT assay can be used to select a good SBF to prepare Leishmania growth medium when used after the second split of parasite. Additionally, the SBF that allowed the best parasite growing was not the best to induce metaciclic differentiation or mouse infection.**Supported by:**CNPq CAPES e FAPEG

PV45 - *IN VITRO* ANTIPROLIFERATIVE, PHYSIOLOGICAL, AND ULTRASTRUCTURAL EFFECTS OF RAVUCONAZOLE, A NOVEL TRIAZOLE AGENT, IN *LEISHMANIA AMAZONENSIS*

MACEDO-SILVA, S.T.[1]; URBINA, J.A.[2]; DE SOUZA, W.[3]; RODRIGUES, J.C.F.[4]
1.IBCCF, RJ, BRASIL; 2.INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTÍFICAS, CARACAS, VENEZUELA; 3.INSTITUTO NACIONAL DE METROLOGIA, QUALIDADE E TECNOLOGIA, INMETRO, RJ, BRASIL; 4.NÚCLEO MULTIDISCIPLINAR DE PESQUISA UFRJ-XERÉM, DIVISÃO BIOLOGIA (NUMPEX-BIO), CAMPUS UFRJ-XERÉM, DUQUE DE CAXIAS, RJ, BRASIL. e-mail:sara.teixeiracp2@gmail.com

Leishmaniasis is caused by more than 20 species of protozoan parasites that belongs to Trypanosomatidae family and *Leishmania* genus. Leishmaniasis is spread around the world and is endemic in 98 countries, presenting different clinical manifestations. The treatments include pentavalent antimonials, miltefosine, amphotericin B, and pentamidine. However, these drugs are unsatisfactory due to toxicity, limited efficacy, cost and administration. Thus, there is an urgent need to identify new therapeutic alternatives. Trypanosomatids and fungi have an essential requirement for ergosterol and other 24-alkyl sterols, which are absent in mammalian cells. The azole antifungals inhibit the cytochrome P-450 dependent enzyme lanosterol 14-α-demethylase that is necessary for the conversion of lanosterol to ergosterol. Ravuconazole (RVZ) is a novel triazole agent that display high activity in vitro against *T. cruzi* and has a longer half-life in humans than in murine and canine models for Chagas disease. Thus, the aim of this work was to investigate the effects of RVZ in *L. amazonensis*. In our studies, RVZ produced a reduction in the viability of *L. amazonensis* promastigotes and intracellular amastigotes, with IC50 of 1µM and 4µM after 48h of treatment, respectively. Scanning electron microscopy revealed several alterations on the shape of drug-treated promastigotes that appeared swollen and rounded. Fluorescence microscopy of Nile Red-labelled cells demonstrated an accumulation of lipid bodies in promastigotes after treatment, which was confirmed by transmission electron microscopy. Other important alterations were also observed such as: an intense disorganization and mitochondrial swelling, and the presence of autophagosome-like structures. Furthermore, treatment of promastigotes with RVZ resulted in a marked reduction in the $\Delta\psi_m$ similar to the effect caused by FCCP. Taken together, these results indicate that RVZ is a promising compound against *Leishmania*. **Supported by:**CNPq, CAPES, PPSUS and FAPERJ

PV46 - *IN VITRO* INFECTIVITY OF DIFFERENT STRAINS ISOLATED FROM NATURALLY *LEISHMANIA CHAGASI*-INFECTED DOGS

RESENDE, L.A.[1]; AGUIAR-SOARES, R.D.O.[1]; LANNA, M.F.[1]; MOREIRA, N.D.[1]; CARDOSO, J.M.O.[1]; MATHIAS, F.A.S.[1]; OLIVEIRA, D.A.P.[1]; COURA-VITAL, W.[2]; REIS, A.B.[1]; GIUNCHETTI, R.C.[2] *1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.* e-mail:lucilenearesende@yahoo.com.br

Visceral leishmaniasis (VL) is a serious disease, whose transmission of the L. chagasi in urban environment necessarily occurs with the participation of dogs. Among the strains in the endemic areas for visceral leishmaniasis, we speculate that part of them should present characteristics regarding of high infectivity and virulence during canine infection. In this context, it is necessary to characterize isolates derived from L. chagasi-naturally infected dogs considering the study of in vitro and in vivo experiments aiming to identify patterns of infectivity and virulence. The goal of this study was to compare the in vitro infectivity of different strains isolated from L. chagasi-naturally infected dogs presenting different clinical status. We isolated eight wild strains (571, 610, 614, 616, 591, 619, 592, 593) from dogs that were used for in vitro infection of canines immortalized macrophage (lineage DH82). This analysis were performed using both the flow cytometer by labeling the promastigotes with CFSE and counting by microscopy the number of parasites per 100 macrophages in slides. Our results demonstrated that all isolated strains were L. chagasi, as analyzed previously by PCR-RFLP. Moreover, we showed differences in the pattern of infectivity among the evaluated strains. In this sense, in both techniques used the 614 strain was less infective, in contrast to 616 strain that presented high in vitro infectivity. These results confirm the existence of strains with different degree of in vitro infectivity in an endemic area for VL. We are performing the in vivo analysis to demonstrate the pattern of the infection, considering the different isolated strains. **Supported by:**FAPEMIG, CNPq, CAPES, FIOCRUZ, UFOP, UFMG

PV47 - LEISHMANICIDAL ACTIVITY OF FUROXAN AND BENZOFUROXAN DERIVATIVES

ALMEIDA, L.; DUTRA, L.A.; PASSALACQUA, T.G.; TORRES, F.A.E.; DOS SANTOS, J.L.; GRAMINHA, M.A.S.
UNESP, ARARAQUARA, SP, BRASIL. e-mail:leticia.almeida.le@gmail.com

Leishmaniasis is a neglected tropical diseases caused by several species of parasites of the genus Leishmania[1]. Since the available drugs have shown to be highly toxic and cases of resistance have emerged[2], new therapeutic agents are urgently needed. Furoxans and benzofuroxans compounds have been described for their leishmanicidal potential. The benzofuroxan derivatives action mechanism is not totally understood. It was hypothesized that benzofuroxan could produce oxygen/nitrogen reactive species into the parasite and inhibit mitochondrial dehydrogenases[3,4]. On the other hand, for furoxan derivatives it has been proposed that N-oxide group seems to act as bio reducible group into the parasite generating free radical species such as nitric oxide (NO)[4,5,6]. Moreover, NO-donors compounds can block Leishmania life cycle by inactivating parasite enzymes such as cysteine proteinases[7]. Therefore, this study aimed to evaluate the leishmanicidal potential of new furoxan and benzofuroxan derivatives against intracellular amastigotes of Leishmania amazonensis as well as their NO-donor potential. The experimental procedures were performed according to Dutra et al., 2014. Compounds 8a and 14a (IC50 < 2.16 µM) showed potent anti-amastigote effect when compared to amphotericin B (IC50=4.92 µM); besides, 14a might be acting as prodrug, releasing the active aldehyde, according to our in vitro studies at pH 5.4. Culture supernatants from murine L. amazonensis- infected peritoneal macrophages treated with 0.17 µM of the compounds 8a and 14a were collected after 48 hours incubation for nitrite content determination by Griess reaction procedure. Our results showed that both compounds were able to increase nitrite in the medium at 1.1 mM and 0.75 mM. In summary, we confirm here the leishmanicidal potencial of benzofuroxan and furoxan derivatives and the NO-donor potential of 8a and the prodrug 14a. The next step is to explore the potential proteases inhibition of these compounds. **Supported by:**CNPq

PV48 - TRANSCRIPTOMICS OF TRYPANOSOMA CRUZI IN RESPONSE TO THERMAL STRESS

BOTTARO, T.; PENHA, L.; HOFFMANN, L.; SILVA, R.; URMENYI, T.P.
UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:thayanebottaro@biof.ufrj.br

Trypanosoma cruzi, the causative agent of Chagas disease, is a parasitic protozoan of the family Trypanosomatidae, order Kinetoplastida, which exhibits a complex life cycle characterized by several cell forms and the ability to infect insect and mammalian hosts. During its life cycle the parasite undergoes heat stress, and the structure and expression pattern of several heat shock protein genes have been previously characterized. The aim of this work is to analyze the transcriptome during heat shock on a large scale to identify all genes involved in the response to thermal stress in T. cruzi. Sequencing of RNA transcripts by next-generation sequencing (RNA-seq) has been described as robust and highly reproducible approach to quantify transcript levels. The transcriptome of epimastigotes from clone CL Brener incubated at different temperatures was obtained by deep sequencing. Total RNA was extracted, enriched for poly (A)+ RNA, fragmented, and used for whole transcriptome sequencing with the Ion Total RNA-seq kit (Life Technologies). The RNA fragments were converted to cDNA and amplified by emulsion PCR in an Ion OneTouch System (Life Technologies), and then subjected to DNA sequencing in an Ion Torrent Personal Genome Machine (Life Technologies). Between 2-3 x 106 reads per sample were generated. In a reference-based strategy, reads were mapped to the publicly available genomic sequence of T. cruzi using the CLC Genomics Workbench software package. Differential gene expression analysis of cells incubated at 29ºC, 37ºC and 40ºC is currently underway. **Supported by:**CNPq, FAPERJ and CAPES

PV49 - CHANGES IN MITOCHONDRIAL AND CYTOSOLIC TRYPAREDOXIN PEROXIDASES LOCALIZATION IN H2O2 – TREATED *TRYPANOSOMA CRUZI* EPIMASTIGOTES

PELOSO, E.F.; GADELHA, F.R.
UNICAMP, CAMPINAS, SP, BRASIL. e-mail:eduardofpeloso@yahoo.com.br

Survival of *Trypanosoma cruzi* is directly related to its repertoire of antioxidant enzymes that enables the parasite to live in highly oxidative environments. Among these enzymes, the mitochondrial and cytosolic tryparedoxin peroxidases (TcMPx and TcCPx, respectively), antioxidant enzymes, have been scored as essential to its survival, as well as potential therapeutic targets. Additionally, the cytosolic pathway has been characterized, but not the mitochondrial one. The exact localization of its components, mainly the TcMPx it is still not clear and it is fundamental to understand how it exert its antioxindant function. These enzymes detoxify H_2O_2 among other species, but their localization under oxidative stress conditions has never been evaluated. In this sense, the objective of this study was to treat parasites (Y strain) expressing Histag-TcMPx or overexpressing TcCPx with 200µM H_2O_2 and analyze by confocal microscopy their intracellular localization. H_2O_2 – treatment led to a change in their localization, where the TcCPx was found more dispersed in the cell in comparison to untreated cells, while TcMPx was more concentrated in the posterior region in the control condition and after treatment it concentrated in the anterior region of the parasite. The change in localization observed here opens new perspectives for a better understanding of TcMPx mode of action and show that this enzyme considered until now to be associated to the mitochondrion is widely dispersed throughout the cell. Additional experiments are being conducted in order to unravel the molecular basis of the observed changes in oxidative stress condition. **Supported by:**Fapesp e CNPq

PV50 - *TRYPANOSOMA CRUZI* MITOCHONDRIAL TRYPAREDOXIN PEROXIDASE INTERACTOME: ONE STEP TOWARDS THE UNDERSTANDING OF ITS MECHANISM OF ACTION

PELOSO, E.F.[1]; DIAS, L.S.[1]; QUEIROZ, R.M.L.[2]; CHARNEAU, S.[2]; DE SOUSA, M.V.[2]; RICART, C.[2]; GADELHA, F.R.[1]
1.UNICAMP, CAMPINAS, SP, BRASIL; 2.UNB, BRASILIA, DF, BRASIL.
e-mail:eduardofpeloso@yahoo.com.br

The antioxidant system centered on trypanothione is critical for the survival of *Trypanosoma cruzi*. The mitochondrial pathway in this system has not been characterized and the mitochondrial tryparedoxin peroxidase (TcMPx) belonging to this pathway has been the subject of intense research. It is speculated that this enzyme interacts with components of the cytosolic pathway. Thus, the aim of this study was to evaluate the interactome of this enzyme in parasites subjected or not to H_2O_2 - treatment. Parasites expressing His-tag-TcMPx were treated or not with 200µM H_2O_2 for 2 h and then submitted to a pull-down assay. The protein extracts obtained were trypsin digested and the resulting peptides were subjected to LC-MS/MS analysis using a DIONEX 3000 nanoUPLC system (ThermoScientific, Waltham, USA) coupled online to an LTQ-Orbitrap Elite mass spectrometer (Thermo Scientific, Waltham, USA). Under no treatment the biological function of the proteins identified were response to stimulus (1), cell organization and biogenesis (4), metabolic process (10) and others with unknown functions (1). Under oxidative stress, besides proteins involved in these functions (38), others related to cell homeostasis (1), cellular component movement (1), cell proliferation (1), cell differentiation (2), transport (2), regulation of biological process (2) and defense response (1) were identified. In relation to cellular component, under no treatment the proteins were related to the nucleus (2), chromosome (2), cytoskeleton (1), cytoplasm (2), while for some proteins (8) their intracellular localization could not be established. Under oxidative stress conditions, proteins related to these cellular components (46) were also found as well as to the endoplasmic reticulum (1), mitochondrion (1), golgi (1), ribosome (5), cytosol (3) and membrane (2). The involvement and significance of the proteins identified interacting to TcMPx will be discussed. **Supported by:**Fapesp, FINEP (CT-INFRA), Capes e CNPq

PV51 - GENETIC POLYMORPHISMS IN BRAZILIAN *TOXOPLASMA GONDII* ISOLATES WITH PHENOTYPIC PROFILE OF RESISTANCE AND SUSCEPTIBILITY TO SULFADIAZINE.

SILVA, L.A.; BARTHOLOMEU, D.C.; CUNHA, J.L.R.; VITOR, R.W.A.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:leticiaazs@gmail.com

Failures in the treatment of toxoplasmosis and malaria with sulfadiazine (SDZ) can be attributed to the development of drug resistance in these parasites, caused by mutations on the drug target dihydropteroate synthase gene (*dhps*). The aim of this study was to identify allelic polymorphisms in *dhps* gene of *T. gondii* with different phenotypes of susceptibility to SDZ, and verify if these polymorphisms may result in amino acid changes that may alter the DHPS structure. DNA from the five *T. gondii* isolates obtained from newborns patients was used on analysis: TgCTBr03, TgCTBr07, TgCTBr08, TgCTBr11 e TgCTBr16, where only the TgCTBr11 isolate was resistant to SDZ. Identification of polymorphic sites in the *dhps* gene was carried out by PCR amplification and direct sequencing. Multiple alignment among all *T. gondii* isolates and reference strains (RH88, ME49 and VEG) were performed. Ten single nucleotide polymorphisms (SNPs) were found in the *dhps* exons. Six of them resulted in silent mutations and four resulted in non-synonymous mutations. Among the non-synonymous mutations, three were previously described (mutation at position 558, exon 2, changes glutamate [GAA] to aspartate [GAC]; mutation 644, exon 4, changes arginine [AGG] to lysine [AAG]; mutation 681, exon 5, changes alanine [GCA] to glutamate [GAA]). One mutation was first identified in this study (mutation 691, exon 5, changes alanine [GCA] to proline [CCA]). The N407D and A587V mutations, previously reported as being associated with *T. gondii* resistance to SDZ, were not identified among the isolates. It was not found an exclusive mutation of the SDZ-resistant TgCTBr11 isolate. Therefore, its resistant phenotype could not be explained by the SNPs found. In conclusion, despite the large number of SNPs identified, there was no association between mutations in *T. gondii dhps* gene and the phenotype of susceptibility to SDZ presented in vivo assays. Further studies are needed to clarify the resistance mechanisms. **Supported by:**CNPq

PV52 - PRODUCTION AND ANALYSIS OF EPIMASTIGOTE FORMS OF TRYPANOSOMA CRUZI EXPRESSING ACTIN 1 WITH A FUSION TAG

BORGES, B.S.; FRAGOSO, S.P.; SOARES, M.J.; SOARES MEDEIROS, L.
ICC/FIOCRUZ, CURITIBA, PR, BRASIL.
e-mail:beatrizsborges@hotmail.com

Trypanosoma cruzi is the etiologic agent of Chagas disease, responsible for the infection of millions of people worldwide. Once this parasite possess a differentiated cytoskeleton, based on a sub-pellicular corset of microtubules, the role of others cytoskeleton components, such as actin, still remains poorly understood. Actin is one of the most abundant protein in several other eukaryotic cells and plays an essential role in cell structure and dynamics. In this context, the aim of this study was to identify the genes encoding actin in the genome of *T. cruzi* and produce genetically modified parasites expressing actin 1 with a fusion tag for subsequent immunolocalization. For this, an in silico analysis of the sequences of actin found in the databases was performed, and genes encoding five isoforms of actin were identified. Phylogenetic analysis showed that the actin 1 has more than 50% of homology when compared with the actin of other organisms, showing a protein highly conserved, including in *T. cruzi*. The sequence of the actin 1 was inserted into a cassette, based on Gateway technology, containing the tag for immunolocalization, and the sequence downstream to the gene for integrative recombination. After transfection and selection of parasites, the presence of the cassette was confirmed by Western Blot assay. The subcellular localization of the tag by indirect immunofluorescence, showed that the protein actin 1 was found predominantly in the posterior region of the epimastigote forms of the parasite, unlike what was observed in previous studies using heterologous polyclonal serum, which described a scattered pattern by the body of the parasite. These results indicate that the use of genetically modified parasites allows a more specific evaluation of the location of the different isoforms of the same protein in *T. cruzi*. 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*. **Supported by:**FIOCRUZ

PV53 - NUTRITIONAL AND PH STRESS CONDITIONS INDUCE AUTOPHAGY AND MITOCHONDRIAL DYSFUNCTION IN TRYPANOSOMA CRUZI EPIMASTIGOTES

STIEBLER, R.; FERNANDES, M.C.; MENDES, C.J.O.; SOUZA, N.P.; PEREIRA, L.O.R.; DE CASTRO, S.L.; MENNA BARRETO, R.F.S.
IOC - FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL. e-mail:stiebler@bioqmed.ufrj.br

Despites the efforts to evaluate Trypanosoma cruzi mitochondrial physiology, a functional characterization of this organelle during stress conditions that the parasite is submitted to complete its life cycle has not been performed yet. T. cruzi life cycle includes two hosts: an invertebrate and other vertebrate. In insect, the proliferative epimastigotes differentiate into metacyclic trypomastigotes while in mammals these metacyclic forms invade cells and differentiate into amastigotes in the host cell cytoplasm. These two differentiation processes are crucial for the success of the parasite and involve stress situations such as environmental acidification and nutritional deprivation. In some eukaryotes, the structural and functional mitochondrial remodeling is regulated by autophagic process. Here, we evaluated the influence of nutritional and pH stress in the parasite mitochondrial function as well as in the generation of reactive oxygen species (ROS) and the possible participation of autophagy in this process. Electron microscopy and flow cytometry analysis evidenced the mitochondrial impairment and ROS production after nutritional and pH stresses, being confirmed by qPCR assays that showed an increase in citrate synthase, mitochondrial complexes III and IV transcripts levels. Additionally, ultrastructural data together with fluorescence microscopy analysis using the autophagic marker monodansyl cadaverine pointed to the exacerbation of autophagy after nutritional or pH (acidic and alkaline) conditions. One more time, qPCR data corroborated the morphological observation, presenting an increase in the expression of several autophagic genes, especially ATG 8 and 18 in pH stress conditions. Thus, our results suggest the existence of a positive correlation between the autophagic pathway and mitochondrial dysfunction in stress conditions that T. cruzi is exposed in vertebrate and invertebrate hosts, suggesting a biological implication during the parasite life cycle. **Supported by:**FAPERJ, CNPq, Capes and FIOCRUZ.

PV54 - HISTIDINE AS AN ENERGY SOURCE IN *TRYPANOSOMA CRUZI.*

BARISON, M.J.; MANTILLA, B.A.S.; RAPADO, L.N.; DAMASCENO, F.S.; SILBER, A.M.
ICB-USP, SAO PAULO, SP, BRASIL. e-mail:mariajulia@usp.br

Trypanosoma cruzi is able to catabolize carbohydrates and amino acids. Histidine (His) is one of the most abundant amino acids in the hemolymph and excreted fluids of the insect vector. Due to the high exposition of *T. cruzi* to this metabolite we characterized its uptake and metabolism. His is incorporated trough a specific and active transport system, and is initially metabolized by two enzymes, histidine ammonia-lyase (TcHAL) and urocanate hydratase (TcUH), rendering 4-imidazolone-5-propionate (IPA), which can be converted in α-ketoglutarate in a non-enzymatic way. We also demonstrate that His is converted to CO_2, suggesting that this amino acid can be oxidized by the parasite. In the present work we continue studying the bioenergetic role of His in *T. cruzi*. First, we measured the capacity of His to restore the oxygen consumption after 16 hs of starvation in PBS: parasites recovered in PBS-His shown a respiratory rate approximately 30% higher when compared with starved parasites. Next, we compared its ability to energize the mitochondria: using Rhodamine 123 and FCCP as uncoupling agent we determine the mitochondrial membrane potential ($\Delta\Psi_m$). Differences among non-energized, His-incubated and control media were found. To verify whether His fulfills mitochondrial demands for ATP synthesis, we used starved epimastigotes (16 hs), which were recovered by adding His. After this, we observed a time-dependent effect on the production of intracellular ATP levels. Furthermore, we evaluate the participation of His *in vitro* metacyclogenesis, considering the relevance of His metabolism in epimastigotes: when incubated in TAU-His we observe 24,95% of differentiation to metacyclic trypomastigotes, similar to TAU-3AAG (29,49%), the classic medium to metacyclogenesis. Taken together our data provide evidences regarding the role of His as an efficient energy source and as a differentiation factor. **Supported by:**FAPESP

PV55 - EVALUATION ANTILEISHMANIAL ACTIVITY AND INHIBITORY EFFECT OF PALLADIUM COMPOUNDS ON DNA TOPOISOMERASE (LITOP1B) OF LEISHMANIA INFANTUM

PASSALACQUA, T.G.; ALMEIDA, L.; ARENAS VELÁSQUEZ, Á.M.; DE SOUZA, R.A.; TORRES, F.A.E.; GRAMINHA, M.A.S.
UNESP, ARARAQUARA, SP, BRASIL. e-mail:thaisgp@gmail.com

Leishmaniasis is a widespread disease, affecting 12 million people around the world with about 1-2 million estimated new cases occurring every year 1. The clinical manifestations range from simple cutaneous lesion to progressive disseminated visceral that can be fatal if left untreated. There are a small number of effective drugs available for treatment of leishmaniasis which present several problems including high toxicity and low efficacy due to resistant parasites 2. Thus, searching for new drugs with high and specific antileishmanial activity is very important especially in development countries where these parasitic diseases constitute a serious public health problem. The aim of this work is to investigate cyclopalladated compounds 2 e 4 against Leishmania infantum (syn. L. chagasi) promastigote and intracellular amastigote forms as well as their inhibitory effect on L. infantum topoisomerase IB (LiTOP1B). Both compounds showed antileishmanial activities presenting the half maximum inhibitory concentration (IC50) of 4.0 µM and 5.4 µM, respectively. Although compounds 2 and 4 presented lower activity when compared to amphotericin B (IC50 = 0.92 µM), the Selectivity Index (SI) values revealed that 2 (SI= 126.1) is five times less cytotoxic than the standard drug (SI= 25.1). To assess the effects of 2 and 4 on LiTOPIB activity, a DNA relaxation assay was performed 3 in the presence of different concentrations of the compounds. The results indicate that compounds 2 and 4 inhibit the relaxation activity of LiTOPIB in a dose dependent manner reaching total inhibition at a drug concentration of 100 or 200 µM, respectively. The obtained results indicate that these compounds should be considered as promising molecules for the development of a new drug against L. infantum. **Supported by:**CAPES

PV56 - COMPARATIVE GENOMIC ANALYSIS OF A *TRYPANOSOMA CRUZI* SINGLE CELL DERIVED CLONE AND ITS PARENTAL STRAIN: CHROMOSOMAL MOSAICISM AND ANEUPLOIDY.

CORTEZ, D.R.[1]; LIMA, F.M.[2]; ANTONIO, C.R.[1]; VAN SCHAIK, M.W.[1]; VILLACIS, R.A.R.[3]; ROGATTO, S.[4]; DASILVEIRA, J.F.[1]; MARINI, M.M.[1]
1.UNIFESP, SAO PAULO, SP, BRASIL; 2.RESEARCH AND DEVELOPMENT DEPARTMENT, FLEURY GROUP, SÃO PAULO, SP, BRASIL; 3.INTERNATIONAL RESEARCH CENTER (CIPE) - A. C. CAMARGO CANCER CENTER, SÃO PAULO, SP, BRASIL; 4.GRUPO MULTIDISCIPLINAR DE ESTUDOS EM NEOPLASIAS HUMANAS, FACULDADE DE MEDICINA, UNESP, BOTUCATU, SP, BRASIL. e-mail:dcortez.maldonado@gmail.com

T. cruzi undergoes chromosomal rearrangements that may be involved in the karyotype heterogeneity and genetic variability observed between different strains of this parasite. Recently we showed the occurrence of extensive chromosomal rearrangements in a clone (termed D11) derived from a single cell of strain G (Lima et al 2013, 8|5|e63738). The karyotype of clone D11 differs from strain G in number and size of chromosomal bands. However, most length differences between chromosomes were small and large syntenic groups were conserved.

We employ the comparative genomic hybridization based in microarray (aCGH) in an attempt to map these regions. From a total of 548 chromosomal abnormalities mapped by aCGH with an average size of 19 Kb, 91.4% were smaller than 50 Kb and only six rearrangements larger than 200 Kb were mapped. Recombination events identified by hybridization of the chromosomal bands separated by PFGE with specific markers could be associated to the chromosomal abnormalities identified by aCGH.

For instance, markers from the in silico chromosome TcChr22 hybridized with two different-sized homologous chromosomes in the parental G strain and only one chromosome in clone D11. By aCGH analysis we were able to identify a 148 Kb deletion in one of homologous chromosomes in clone D11. This allow us to propose a recombination with deletion model that gives rise to homologous chromosomes of the same size in clone D11. Integration of data of aCGH analysis with those from hybridization of chromosomal bands provided significant information regarding the frequency and variety of chromosomal abnormalities observed in a multiclonal, mosaic parasite population submitted to frequent DNA amplification/deletion events. Our results suggest that this approach may be a useful tool for comprehensive aneuploidy screening in *T. cruzi*. **Supported by:**FAPESP, CNPq and CAPES

PV57 - LEISHMANIA (VIANNIA) BRAZILIENSIS AMASTIGOTES ISOLATED FROM PATIENTS WITH MUCOSAL LEISHMANIASIS HAS MORE ABILITY TO DISSEMINATE THAN PARASITES FROM CUTANEOUS LESION

GOMES, C.M.[1]; DOS SANTOS, J.C.[2]; TOME, F.D.[2]; AVILA, L.R.[2]; ALVES BRANDÃO, N.A.[2]; PEREIRA, L.I.A.[2]; DORTA, M.L.[2]; DIAS, F.R.[2]; LINO JÚNIOR, R.S.[2]; OLIVEIRA, M.A.P.[2]
1.UFG-GO E PUC-GO, GOIANIA, GO, BRASIL; 2.UFG-GO, GOIANIA, GO, BRASIL.
e-mail:claysonmoura@yahoo.com.br

Mucosal leishmaniasis is associated with strong cell mediated immune response, low number of parasite in the lesion, intense inflammatory infiltrate and tissue necrosis. It is unknown the reason for the mucosal lesions occurrence in infected patient, but the ability of parasite to disseminate may contribute with disease development. The aim of this work was to evaluate the ability of Leishmania (V.) braziliensis amastigotes isolated from mucosal (ML) or cutaneous lesion (CL) to migrate to the secondary lymphoid organs in wild type, IFNg, induced nitric oxide synthase (iNOS) or phagocyte oxidase (Phox) knockout mice. All mice strains were inoculated with amastigotes in the footpad and the lesions were followed weekly and the parasites burden was estimated by limiting dilution of footpad, popliteal lymph node (LN) and spleen (SP). It was observed more parasites in SP and LN of iNOs KO mice and in SP of IFNg KO mice infected with ML than CL parasites (iNOS KO: ML = 2910±120 vs CL = 160±68 parasites/LN; p=0.0002 and ML = 30±47 vs 11±19 parasites/SP; p=0.002); (IFNg KO: ML = 180±390 vs CL 13±20 parasites/SP; p=0.0008). ML parasites were able to disseminate to the SP of Phox KO mice (32±38 parasites/SP), but CL parasites were not detected in SP of these mice. Additionally, it was observed intense necrosis only in the footpad of iNOS KO infected with ML parasites. It was not detected parasites in SP of wild type mice. This work demonstrates that ML parasites are more able to disseminate to other organs than CL parasites and suggest that this characteristic can be important to generate mucosal leishmaniasis. **Supported by:**FAPEG, Cnpq e Capes

PV58 - EFFECTS OF *L. WALLACEI* (TRYPANOSOMATIDAE) INFECTION ON REPRODUCTIVE ASPECTS OF *O. FASCIATUS* (LYGAEIDAE)

GONÇALVES, I.C.; VASCONCELLOS, L.R.C.; LOPES, A.H.C.S.
UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:inescg.bio@gmail.com

The family Trypanosomatidae comprises parasites of all classes of vertebrates, plants and invertebrates, mainly insects. Within the family, *Leptomonas wallacei* is a monoxenic flagellate isolated from the digestive tract of the hemipteran *Oncopeltus fasciatus* (Lygaeidae). The aim of this study is to investigate the effects of *L. wallacei* on reproductive aspects of *O. fasciatus* by analyzing the morphology of male and female reproductive organs. A colony of infected insects was compared to a non-infected one; both groups where feed on peeled sunflower seeds and water and kept under same humidity (60%–70%) and temperature (28oC). Dissected adults were virgins and in the same developmental period. The number of eggs in ovaries as well as its viability was studied in females (n=30). Shape, position and number of testicular tubules were studied in males (n=40). Infected females produced 25% fewer eggs than the non-infected. Reabsorption of eggs, known as atresia, occurred in 2.5% of infected females while only 0.5% of non-infected females exhibited atresia in follicles. As reabsorption of eggs is known to be related to stressful situations, our data suggest that L. wallacei infection causes redirection of energy from reproduction towards maintenance of essential functions, in order to reach physiological equilibrium. Only 20% of the non-infected males had abnormalities in testis while 60% of the infected males had abnormalities. Deformities in size and position were found only in the infected males. Also, five infected males that did not present malformations had visible reduction in the number of trachea irrigating the testis, a condition not seen in the non-infected group. Lower availability of oxygen and morphological deformities in testis could result in diminished production of spermatozoa thus reducing male fertilization competence. As a conclusion, infection by *L. wallacei* has deleterious effects on both male and female reproductive competence in *O. fasciatus.*

PV59 - IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NUCLEOTIDE SUGAR TRANSPORTERS OF *TRYPANOSOMA CRUZI*

BAPTISTA, C.G.[1]; RODRIGUES, E.C.[2]; MORKING, P.[2]; KLINKE, A.[2]; DARDO, M.L.[2]; SOARES, M.J.[2]; GOLDENBERG, S.[2]; RAMOS, A.S.P.[2]
1.ICB - USP, SÃO PAULO, SP, BRASIL; 2.ICC - FIOCRUZ, CURITIBA, PR, BRASIL.
e-mail:aspramos@fiocruz.br

Introduction: 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). Objectives: In this study we have identified and characterized two transporters from *Trypanosoma cruzi*, the etiological agent of Chagas' disease. TcNST1 transports UDP-N acetylglucosamine (UDP-GlcNAc) and TcNST2 transports UDP-galactose (UDP-Gal) Material and Methods: NSTs from *T. cruzi* were identified by complementation (FACS analysis) of yeast *(Kluyverome*s *lactis*) and mammalian (Chinese Hamster Ovary, CHO) mutant cells. Sub cellular localization and expression analyzes were performed by fluorescence microscopy and RT-PCR, respectively. Results and Discussion: We have identified 11 putative NSTs. Heterologous expression of these genes in a *K. lactis* mutant revealed only one UDP-GlcNAc transporter (TcNST1). The UDP-Gal transporter (TcNST2) was identified by complementation of CHO mutant cells (three candidate genes were tested). The sub cellular localization of both transporters was analyzed by fusion proteins with GFP. Our results showed a specific localization at the Golgi apparatus. By RT-PCR, both genes are expressed in all stages of the parasite life cycle. Knockout experiments indicate that the TcNST1 gene is essential. Single knockout alleles are partial impaired in cellular differentiation. Conclusions: We have identified transporters for UDP-GlcNAc and UDP-Gal in *T. cruzi*. Both transporters are localized to the Golgi apparatus. The fact that the TcNST1 gene is essential suggests that TcNST1 is the only transporter of UDP-GlcNAc in T. cruzi and shows the importance of NSTs in these parasites. **Supported by:**Fiocruz, CNPq and Fundação Araucária

PV60 - HYDROGEN PEROXIDE PRETREATMENT OF *TRYPANOSOMA CRUZI* PROMOTES A BETTER OXIDATIVE STRESS RESPONSE IN THE WILD TYPE IN RELATION TO THE CATALASE HETEROLOGOUS EXPRESSOR

FREIRE, A.C.G.; AGUIAR, P.H.N.; ALVES, C.L.; FRANCO, G.R.; MACEDO, A.M.; PENA, S.D.J.; MACHADO, C.R.
UFMG, BELO HORIZONTE, MG, BRASIL. e-mail:annaclaudia.bio@gmail.com

Chagas disease is caused by parasite protozoan *Trypanosoma cruzi*. After more than a century of the *T. cruzi* discovery, there is still no effective vaccine against this disease, and the available treatments have severe effects and low efficacy during the disease chronic phase. Searching for new chemotherapeutic approaches, the antioxidant defense system of this parasite has called the researcher's attention due to its importance in the adaptation to all types of oxidative environment in which this parasite is exposed to. The catalase enzyme, which decomposes hydrogen peroxide (H_2O_2) into water and oxygen, is found in virtually all aerobic organisms, demonstrating its important protective role for the cell. However, a homologous sequence to the catalase gene was not found in *T. cruzi* genome. Our hypothesis is that *T. cruzi* may have suppressed catalase as a strategy to allow the specific enzymes of the parasite antioxidant pathway signalize the oxidative environment. We had showed that T. cruzi epimastigotas of CL Brener strain that was transfected with *E. coli* catalase gene have the same growth rate than the wild type, but it has an increased resistance to H_2O_2 treatment. We have verified that pretreating both parasite types with a low dose of H_2O_2 24 hours before the treatment, make the WT cells as resistant to H_2O_2 as the catalase expressing cells. The increment of resistance to H_2O_2 is not due a selection of resistant cells since this resistant phenotype is lost with the passage of time after the pretreatment. . These results suggest that the pretreatment with a low dose of H_2O_2 could prepare the parasites for the oxidative environment, although this is not observed with the same intensity in the presence of catalase. We are now evaluating the influence of the H_2O_2 pretreatment and treatment on the expression of antioxidant enzymes of both *T. cruzi* cells. **Supported by:**CNPq, CAPES, FAPEMIG

PV61 - HISTONE H4 ACETYLATION IS REQUIRED FOR *TRYPANOSOMA CRUZI* DNA REPLICATION.

NUNES, V.S.[1]; RAMOS, T.C.[1]; NARDELLI, S.C.[2]; SCHENKMAN, S.[1]
1.UNIFESP, SAO PAULO, SP, BRASIL; 2.ICC-FIOCRUZ, CURITIBA, PR, BRASIL.
e-mail:visnunes@gmail.com

Trypanosoma cruzi is the protozoan that causes Chagas disease. As in other organisms, *T. cruzi* chromatin is constituted by DNA wrapped around an octamer composed by two copies of each histone (H2A, H2B, H3 and H4). However, *T. cruzi* histones are distinct from other eukaryotes, mainly in the N-terminal, where post-translational modifications (PMTs) occur. Those posttranslational histone modifications participate in the chromatin replication and are pivotal to the gene expression and for the chromatin architecture of eukaryotic genomes. To understand the role of histone modifications in *T. cruzi*, we expressed the histones H4 mutated in sites that not allows the acetylation in the N-terminus, at lysines 4, 10, and 14. Here, we provide evidence that the expression of H4 mutated affects parasite growth by modifying chromatin assembly. The tagged and mutated histones H4 were expressed in an inducible manner at very lower protein levels. The modified histones were target to the nucleus and incorporated into the chromatin without affecting the general nuclear organization. Parasite growth was significantly reduced when the lysines 10 and 14 of histone H4 were mutated to arginine. These cells incorporated much less a nucleic acid precursor into the DNA when compared with wild type. On the same hand, the mutations of K10 and K14 caused an increase in solubility of endogenous histone H3 and H4. Altogether, these results suggest that acetylation is important for *de novo* histone deposition, probably by affecting the chaperones or the chromatin remodeling machinery. **Supported by:**FAPESP

PV62 - EXPRESSION AND IMMUNOLOCALIZATION OF CALPAINS IN *TRYPANOSOMA CRUZI*

VIDAL, V.E.[1]; MENNA BARRETO, R.F.S.[1]; PITALUGA, A.N.[1]; SANTOS, A.L.S.[2]; BRANQUINHA, M.H.[2]; D'AVILA-LEVY, C.M.[1]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:vidal@ioc.fiocruz.br

The completed genomes of the kinetoplastid parasites allow a comparative *in silico* approach to identify peptidases with low abundance and tricky to detect biochemically. In this sense, up to now, there are few reports on calpains in *Trypanosoma cruzi*. Calpains comprise a family of calcium-regulated cysteine peptidases implicated in physiological processes, such as regulation of gene expression, apoptosis and proliferation. Here, we identified and classified 40 calpain sequences in the genome of *T. cruzi*. Through multiple alignments and phylogenetic analysis of conserved domains in these sequences, calpains were sorted into four distinct groups characterized by the size of the gene and by the presence of classical domains. We decided to focus on the group that has the highest number of conserved domains, and presents domain II, which contains the catalytic active site (either altered or conserved). The comparison of calpain mRNA abundance by real time qPCR in epimastigote, trypomastigote and amastigote forms showed at least five genes with modulated expression among the evolutive forms. Additionally, our ultrastructural immunocytochemistry revealed the presence of calpains in plasma membrane, spread in the cytoplasm and in the flagellum of all *T. cruzi* forms. The study of calpains in trypanosomatids may help to establish the basis for targeted approaches aiming at a better characterization of these molecules. **Supported by:**MCT/CNPq, FAPERJ, CAPES and FIOCRUZ.

PV63 - ANALYSIS OF A CYTOSOLIC COMPLEX INVOLVING A CAPPING ENZYME HOMOLOGUE AND TRANSLATION INITIATION HOMOLOGUES IN *TRYPANOSOMA BRUCEI*

MALVEZZI, A.M.[1]; FREIRE, E.R.[2]; DE MELO NETO, O.P.[1]; STURM, N.R.[2]; CAMPBELL, D.A.[2]
1.CPQAM/FIOCRUZ, RECIFE, PE, BRASIL; 2.UNIVERSITY OF CALIFORNIA AT LOS ANGELES, LOS ANGELES, LOS ANGELES, ESTADOS UNIDOS.
e-mail:opmn@cpqam.fiocruz.br

TbEIF4E6 (TbE6) 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 TbE6 used a PTP-tagged protein to isolate specific constituents which were identified through mass-spectrometry (Mudpit). Two proteins consistently associated with TbE6. The first, TbEIF4G5, is a homologue of the second subunit of the eIF4F complex, eIF4G, which functions as a scaffold for the complex. A 70.3 kDa protein (Tb927.11.14590) annotated as “hypothetical” and referred here as TbG5-IP (TbG5-interacting protein) was also found. The reciprocal Mudpit analysis of these two TbE6 partners confirmed the interactions of the trio and blue native gel migration was also consistent with the formation of a unique complex. To determine the direct interactions within the complex, a yeast two-hybrid assay was used which confirmed the interaction between the TbE6/TbG5. TbG5 also bound to TbG5-IP in both the bait and prey configurations, while both trials were negative for a TbE6/TbG5-IP interaction, indicating that TbG5 is the scaffold for both TbE6 and TbG5-IP. TbG5-IP-PTP displayed a cytosolic localization, mirroring the distribution seen for TbE6-PTP. Bioinformatic analysis of TbG5-IP by PHYRE2 revealed two provocative domains associated with mRNA 5’ cap formation. Its amino half displayed similarity to triphosphatase enzymes involved in the first step in cap 0 formation on primary transcripts, with the carboxyl half consisting almost entirely of a guanylyltransferase domain. These domains indicate a role for the complex in the modulation of gene expression through the modification of mRNA 5’ ends. Coupled with the observation of compromised flagellar attachment upon TbE6 depletion, this complex may represent a gateway for the expression of proteins involved in flagellar attachment to the cell body. **Supported by:**CNPq, CAPES, NIH

PV64 - EFFECTS OF D-GLUCOSAMINE ON VIABILITY, METACYCLOGENESIS PROCESS AND INVASION SUITABILITY OF *TRYPANOSOMA CRUZI*

CRISPIM, M.; DAMASCENO, F.S.; RAPADO, L.N.; MELO, R.F.P.; FURUSHO PRAL, E.M.; SILBER, A.M.
INSTITUTO DE CIÊNCIAS BIOMÉDICAS - USP, SAO PAULO, SP, BRASIL.
e-mail:marcell@usp.br

Trypanosoma cruzi is a parasite with a complex life-cycle. It is well established that *T. cruzi* uses amino acids and carbohydrates as carbon and energy source and are involved in the stress resistance, invasion and differentiation processes. L-glutamine can be a low-toxicity nitrogen carrier, important for the biosynthesis of essential molecules, such as membrane glycoconjugates which are involved in parasite invasion and virulence in both hosts: insect vectors and mammals. In spite of its relevance, little is known about the hexosamine metabolism in *T. cruzi*. This pathway uses L-glutamine as nitrogen source and is critical for the biosynthesis of UDP-GlcNAc, the glycosil donor of N and O-glycosilation. There are two initial key steps within the hexosamine biosynthesis: (i) the conversion of D-fructose-6-P into D-glucosamine 6-P, catalyzed by glutamine fructose-6-phosphate aminotransferase (GF6PA); (ii) the incorporation of extracellular D-glucosamine that is phosphorylated by hexokinase. Hence we investigated the role of free D-glucosamine in different cellular processes of *T. cruzi*. Our results suggest that epimastigotes incubated in PBS + D-glucosamine during 24hs were able to maintain their viability and ATP production, which was 2-fold higher when compared to D-glucose-treated. It was also verified that D-glucosamine has a positive effect in the metacyclogenesis, showing a differentiation ratio around 25%, compatible to the obtained in the standard medium Triatomine Artificial Urine supplemented with 2 mM Asp, 50 mM Glu, 10 mM Pro and 10 mM Glc (TAU3AAG). Furthermore, we demonstrated that the invasion capacity of D-glucosamine-recovered trypomastigotes was increased after metabolic depletion, when compared with non-recovered parasites, glucose or proline-treated. These results reveal that the parasite can use this molecule for essential processes along the life cycle, suggesting its biosynthetic pathway as a promissory therapeutic target. **Supported by:**CAPES

PV65 - INVESTIGATING THE ROLE OF *TRYPANOSOMA CRUZI* ALBA PROTEINS IN THE CONTROL OF AMASTIN GENE EXPRESSION.

PEREZ-DIAZ, L.-.; SILVA, T.C.; TEIXEIRA, S.M.R.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:letperez@gmail.com

Amastins are surface glycoproteins encoded by a multigenic family that constitute potential virulence factors expressed in the amastigote stage of *T. cruzi* and different *Leishmania* spp. Analysis of the *T. cruzi* genome reveals 12 copies of amastin genes belonging to two of the four described amastin subfamilies. In *T. cruzi* and *Leishmania*, amastin differential expression results from regulatory mechanisms involving changes in mRNA stability and/or translational control. Although distinct regulatory elements were identified in the 3′ UTR of *T. cruzi* and *Leishmania* amastin mRNAs, RNA binding proteins involved in amastin gene regulation have only being characterized in *L. infantum*. By RNA affinity chromatography it was demonstrated that an Alba-domain protein (LiAlba20) from *L. infantum* binds to the 3′ UTR of a δ-amastin mRNA and gene knockout experiments further indicate that LiAlba20 contributes to the stage-regulated stability of amastin transcripts. Here we investigated the role of Alba proteins in the post transcriptional regulation of *T. cruzi* amastin genes by analyzing orthologous sequences present in the CL Brener genome, their expression during the parasite life cycle and the effect of genetic manipulating TcAlba genes. Two LiAlba20 orthologs (TcAlba30 and TcAlba40) showing 94% identity were identified in CL Brener. *T. cruzi* transfected cell lines expressing a cmyc tagged TcAlba30 as well as single allele knock out of TcAlba30/40 were generated. These cell lines are being currently used to analyze TcAlba30-amastin RNA interaction, by RNA immunoprecipitation followed by RT-PCR using anti-Myc antibody as well as by gel shift assays using RNA oligonucleotides containing putative conserved cis elements in T. cruzi δ-amastin 3'UTR identified by *in silico* analysis. Changes in parasite transcriptome resulting from altering TcAlba expression, accessed by comparing RNA-seq data derived from TcAlba30+/- knock out and cmycAlba30 overexpressors are also underway. **Supported by:**CNPq

PV66 - *TRYPANOSOMA CRUZI* CHROMOSOMAL COPY NUMBER VARIATION (CCNV) IN DIFFERENT STRAINS BASED ON READ DEPTH COVERAGE ANALYSIS

CUNHA, J.L.R.[1]; RODRIGUES-LUIZ, G.F.[1]; VALDIVIA, H.O.[1]; MENDES, T.A.O.[1]; BAPTISTA, R.P.[1]; MACEDO, A.M.[1]; DE MORAIS, G.L.[2]; VASCONCELOS, A.T.R.[2]; TEIXEIRA, S.M.R.[1]; BARTHOLOMEU, D.C.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.LNCC, PETRÓPOLIS, RJ, BRASIL.
e-mail:jaumlrc@yahoo.com.br

Trypanosoma cruzi isolates are currently divided into six discrete typing units (DTUs), named TcI – TcVI. *T. cruzi* replication is usually clonal; however, there is evidence of hybridization events and genetic exchange among strains. The hybrid nature of *T. cruzi* DTU TcVI was confirmed by sequencing the CL Brener genome, allowing the identification of two haplotypes, named Esmeraldo-like (TcII) and Non-esmeraldo-like (TcIII), each one derived from a CL Brener parental genotype. Although the *T. cruzi* karyotype is not well defined, several studies have demonstrated significant variation in the size and content of chromosomes among different *T. cruzi* strains. To better characterize the *T. cruzi* karyotype, the CL Brener genome has recently been assembled into 41 chromosomes based on scaffold information, synteny maps, and BAC-end sequences. In the present work, we have analyzed Chromosomal Copy Number Variations (CCNV) in *T. cruzi* strains belonging to different DTUs, based on read depth coverage of the CL Brener chromosomes. We have explored the CL Brener Esmeraldo-like and Non-esmeraldo-like 1:1 orthologs to predict CCNV in the aforementioned strains, without the bias from repetitive sequences and internal chromosomal gaps. This analysis led to the identification of a broader extent of CCNV in *T. cruzi* than previously speculated. TcI DTU strains are usually diploid with very few aneuploidies, while the strains from TcII and TcIII DTUs presented a high degree of chromosomal and segmental duplications, which may explain their higher DNA content when compared with TcI. Pairwise Euclidian distances among the predicted ploidy of each *T. cruzi* strain is in agreement with the parasite phylogeny. Increased gene copy number due to chromosome amplification may contribute to alterations in gene expression, a strategy that may be crucial for parasites that mainly depend on post-transcriptional mechanisms to control gene expression. **Supported by:**FAPEMIG, CNPq, INCTV, CAPES, FAPERJ

PV67 - INVOLVEMENT OF A NUCLEOCYTOPLASMIC SHUTTLING RNA HELICASE IN AN MRNA EXPORT PATHWAY OF TRYPANOSOMES

INOUE, A.H.[1]; SERPELONI, M.[1]; HIRAIWA, P.M.[1]; VIDAL, N.M.[2]; YAMADA-OGATTA, S.F.[3]; MOTTA, M.C.M.[4]; MARCHINI, F.K.[1]; SOARES MEDEIROS, L.[1]; SOARES, M.J.[1]; MORKING, P.[1]; GOLDENBERG, S.[1]; AVILA, A.R.[1]

1.ICC/FIOCRUZ-PR, CURITIBA, PR, BRASIL; 2.NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION, BETHESDA, ESTADOS UNIDOS; 3.UNIVERSIDADE ESTADUAL DE LONDRINA, LONDRINA, PR, BRASIL; 4.UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ, BRASIL. e-mail:haruobiomed@gmail.com

Gene expression in trypanosomes is controlled mostly by post-transcriptional pathways. Little is known about the components of mRNA nucleocytoplasmic export routes in these parasites. Comparative genomics has shown that the mRNA transport pathway is the least conserved pathway among eukaryotes. Nonetheless, this pathway contains a conserved RNA helicase, the Trypanosoma cruzi Hel45, which is similar to the DEAD-box protein Dbp5/DDX19 and conserved across eukaryotes. Hel45 was present in both the nucleus and the cytoplasm. Electron microscopy showed that Hel45 is clustered close to the cytoplasmic side of nuclear pore complexes, and is also present in the nucleus where it is associated with peripheral compact chromatin. Deletion of a predicted Nuclear Export Signal motif led to the accumulation of Hel45ΔNES in the nucleus. The export of Hel45 was dependent on active transcription but did not depend on the exportin Crm1. Knockdown of Mex67 in T. brucei caused the nuclear accumulation of the T. brucei ortholog of Hel45. This strongly suggests that Hel45 is involved in an mRNA export pathway in trypanosomes, in which Mex67 acts as the nuclear receptor. The Hel45 knockout affected growth rate and the cell differentiation, but did not kill the parasite. The knockout also resulted in lower translation levels and smaller numbers of polysomes, but no nuclear accumulation of poly(A)-mRNA was observed. Proteomic analyses revealed modulation of specific protein expression levels in knockout parasites, suggesting a role for Hel45 in posttranscriptional control. It is still necessary to confirm the precise function of Hel45 however, our data so far indicate that this RNA helicase is associated with mRNA metabolism and its nucleocytoplasmic shuttling is dependent on an mRNA export route involving Mex67 receptor. **Supported by:**CNPq, CAPES, FIOCRUZ eFundação Araucária-PR.

PV68 - OVEREXPRESSION OF HUS1 TO STUDY REPLICATIVE STRESS IN *TRYPANOSOMA CRUZI*

SILVA, H.M.C.; ALVES, C.L.; FRANCO, G.R.; MACEDO, A.M.; PENA, S.D.J.; MACHADO, C.R.

UFMG, BELO HORIZONTE, MG, BRASIL. e-mail:hellidamarina@yahoo.com.br

Trypanosoma cruzi is the parasite protozoan that causes Chagas disease. Checkpoints are cell signaling and survival pathways that coordinate DNA repair, chromosome metabolism and cell cycle transitions. Hus1 is a widely conserved gene and encodes a protein involved in the checkpoint process that forms a trimeric complex with Rad1 and Rad9 (the 9-1-1 complex) in response to stopping replication. Studies involving *Leishmania major* suggest an association between Hus1 and an increased capacity of this parasite in dealing with replicative stress. Once the mechanisms controlling this process remain unclear in *T. cruzi*, this work aims to study the importance of the Hus1 gene in the replicative stress of this parasite. We performed transfection of *T. cruzi* epimastigotes of the CL Brener strain with TcHus1 (TriTryp: TcCLB.466823.10) using the pROCK expression vector and generated TcHus1 overexpressing cells (pROCK-TcHus1). The transfection was confirmed by qRT-PCR and showed that the strain pROCK-Hus1 expresses about two times more than the strain transfected with the empty vector (pROCK). We have verified that the pROCK-TcHus1 and pROCK parasites have the same growth rate in the absence of genotoxic agents. However, pROCK-TcHus1 cells have an increased resistance when treated with MMS. This result suggests that TcHus1 operates in stalled DNA replication forks. Ongoing trials aim to evaluating the effect of Hus1 overexpression during the treatment with other drugs such as camptothecin, benznidazole and gamma radiation, to elucidate the function of Hus1 in *T. cruzi*. **Supported by:**CNPq, CAPES, FAPEMIG

PV69 - 3' PROCESSING OF SL RNA IN *TRYPANOSOMA BRUCEI*

SOUZA, R.T.[1]; STURM, N.R.[2]; CARDOSO, T.O.[1]; LANGOUSIS, G.[2]; VASHISHT, A.A.[2]; HILL, K.[2]; WOHLSCHLEGEL, J.A.[2]; CAMPBELL, D.A.[2]
1.UNIFESP, SAO PAULO, SP, BRASIL; 2.UCLA, LOS ANGELES, ESTADOS UNIDOS.
e-mail:renataepm@hotmail.com

Before being added to the 5' end of all kinetoplastids mRNAs by trans-splicing, Spliced Leader (SL) sequence is hypermethylated at the cap4 (5' end) and the 3'-extended tail is removed by two or more exonucleases. Disruption in these processes affect transcripts stability and transcription rate. The final step in the 3'-polishing is performed by an exonuclease named SNIP. The enzyme responsible for the first step is unknown but is known that exossomal machinery is not responsible for this trimming. It has been described that enzymes in a common pathway are often in the same compartment, associated with one another in the same multimeric enzyme or assembled on a common protein complex structure. Our proposal is to check if SNIP is part of a complex responsible for 3'-end processing. To achieve this goal, one SNIP allele was knockout and second allele was tagged with PTP-epitope to ensure that all SNIP copies in the cell were have been tagged with PTP. The success of the double transfectant cell line was demonstrated by the correct genomic integration and by the fusion protein expression. We showed that SNIP is assembled into a higher molecular weight complex using non-denaturing conditions for PAGE- Blue Native gels. The complex was purified using tandem affinity chromatography in mild and native conditions and the identity of these additional proteins has been determined by mass spectrometry. MudPIT result did not find any exonuclease in the SNIP core complex, which suggests that the initial 3'-end trimming occurs separately from the removal of the last uridine in the 3'-end. In addition, MudPIT suggests that SNIP is a dimer or a trimer. Yeast and bacterial two-hybrid interaction techniques are underway in our lab to prove interaction between SNIP molecules. The characterization of all steps involved in the 3'-end processing of this small and essential RNA is imperative in understanding the trypanosome biology. **Supported by:**FAPESP, CNPq, CAPES and NIH

PV70 - IDENTIFICATION AND CHARACTERIZATION OF TWO ALPHA-AMASTINS IN *TRYPANOSOMA CRUZI*

LEMOS, L.[1]; KANGUSSU MARCOLINO, M.M.[1]; TEIXEIRA, S.M.R.[2]; DAROCHA, W.D.[1]
1.UFPR, CURITIBA, PR, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:laiane.lemos.bio@gmail.com

Trypanosoma cruzi express stage-specific proteins whose functions can be related to its survival and multiplication in the mammalian or insect host. Amastins are surface proteins from Trypanosomatids first described in *T. cruzi* and are related to its virulence. These proteins were recently classified in alpha-, beta-, gamma- and delta-amastins subfamilies, wherein *T. cruzi* (CL Brener clone) contains a total of 14 amastin genes stratified as two beta-amastins (upregulated in epimastigotes) and twelve delta-amastins (upregulated in amastigotes). Based on gene prediction data available at TriTrypDB for *T.cruzi* CL Brener genome, this parasite has a reduction in number of amastin subfamilies compared to *Leishmania* species. To confirm this, *in silico* analysis using BLAST were carried out, showing the presence of new amastin members related to alpha-amastin subfamily, named as alpha1- and alpha2-amastins. RFLP and PFGE assays using genomic DNA from different *T. cruzi* strains confirmed its predicted genomic organization and allelic variation in CL Brener. In order to assess the mRNA expression and subcellular localization of alpha-amastins, northern blot and expression of GFP fusion proteins were performed. Intriguingly, both approaches failed to detect any alpha-amastin expression, however additional experiments are underway to confirm these data. In parallel to alpha-amastin characterization, constructs to delete beta-amastin alleles were created, and transfected into epimastigotes. Southern blot analysis has shown that one copy of both beta-amastins was removed from *T. cruzi* genome, and in spite of its upregulation in epimastigotes the beta-amastins +/- cell line is viable. The deletion of the second allele is underway to better characterize beta-amastins function. **Supported by:**CAPES, CNPQ, Fundação Araucária.

PV71 - EFFECT OF THE EXTRACT OF *CECROPIA PACHYSTACHYA, ENTEROLOBIUM CONTORTISILIQUUM* AND *TABEBUIA IMPETIGINOSA* IN THE VIABILITY OF SHAPE PROMASTIGOTE LEISHMANIA AMAZONENSIS

SARAIVA DE LIRA SILVA, N.S.; BOCALETTO FRARE, A.; GONÇALVES ANGELUCI, C.H.; DE CASTILHOS, P.
IFG, FORMOSA, GO, BRASIL. e-mail:nad_saraiva@hotmail.com

This current study aimed at testing 3 Cerrado plants, *Cecropia pachystachya, Enterolobium contortisiliquum* and *Tabebuia impetiginosa,* for evaluated the action of those plants against the viability of promastigotes forms *Leishmania L. amazonesis*. The aqueous extracts at 50% of *Cecropia pachystachya, Tabebuia impetiginosa* and *Enterolobium contortisiliquum* were obtained by decoction, filtered (0,20mm), and stored at -20º C. Firstly, it was used the viability cell assays, after that it were added 1 x 105 promastigotes/well of the *L. amazonensis*, and treated with 50% aqueous extract with a end concentration of 25%. The extracts were lyophilized, diluted in PBS at concentrations of 1 mg; 0.5 mg; 0.25 mg and 0.125 mg for cell viability assay. Parasites, in the same culture conditions, were also treated with 1mg; 0.5 mg; 0.25 mg and 0.125 mg of each extract and incubated for 24 to 48 hours. Viability was determined by trypan blue added and read in light microscopy. The data showed that aqueous extracts at 25% have a great effect on the viability of parasites, since *Cecropia pachystachya*, *Enterolobium contortisiliquum* and *Tabebuia impetiginosa* inhibited the growth of *Leishmania amazonensis* in 94%, 86%, 80% at 24 hours, and 100%, 98% and 96% in 48 hours. After lyophilization, the extracts of Cecropia pachystachya and Tabebuia impetiginosa showed inhibition in 98% and 40% the parasite growth at 24 hours and 100% and 50% of concentrations of 1 mg and 0.5 mg in 48 hours , respectively. However, *Enterolobium contortisiliquum* showed effect of change in viability of 30% in 1 mg concentration in 24 and 48 hours. Our results showed that the use of 25% aqueous extract of *Cecropia pachystachya, Enterolobium contortisiliquum* and *Tabebuia impetiginosa* presented changes in cell viability of Leishmania, reaching up to 98% inhibition. In concentrations of 1 mg the extract *Cecropia pachystachya* showed inhibition of viability 100%.

PV72 - ANALYSIS OF THE METALLOPEPTIDASE ACTIVITY IN *LEISHMANIA TARENTOLAE*: SEARCH FOR GP63

LOPES., C.C.S.; PEREIRA, T.A.; DA COSTA REGO, T.A.N.; MARTINS-ALVES, D.; VIDAL, V.E.; D'AVILA-LEVY, C.M.
FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL. e-mail:carolinecslopes@gmail.com

The GP63 of the protozoan parasite *Leishmania* is a highly abundant zinc metallopeptidase, mainly glycosylphosphatidylinositol-anchored to the parasite surface, which contributes to well-established functions for the parasite interaction with the hosts. In the lizard parasite *Leishmania tarentolae*, GP63 is highly expanded with 49 putative genes as compared to other *Leishmania* species. Interestingly, it was somewhat surprising that no GP63 proteolytic activity was reported in *L. tarentolae* to date. Therefore, based on the bulk of information produced by our research group about GP63 in insect and plant trypanosomatids, which indicates GP63 ubiquity in this family we decided to investigate the proteolytic activity of this molecule in several experimental conditions. We observed a proteolytic degradation at a molecular mass of approximately 63 kDa from three distinct strain of *L. tarentolae* by SDS-page zymography. These activity were detected in a wide range of pH and temperature of crude extracts *L. tarentolae* promastigotes obtained in the logarithmic phase, showing maximum activity at pH 5,5 at 37 °C for 72 h. Additionally, the protease was capable of degrading a wide range of substrates like gelatin, casein, ovalbumin and bovine serum albumin. The proteolytic degradation was also inhibited by metallopeptidase inhibitors (10 mM EDTA or 1,10-phenanthroline). Furthermore, immunoblotting analysis with anti-gp63 antibody revealed a polypeptide band migrating at approximately the same molecular mass of the proteolytic activity, 63 kDa. Similar results were observed in *L. braziliensis* extract used as a positive control. These data suggest that the proteolytic activity observed in the crude extracts of *L. tarentolae* may be related to the GP63, although more studies are necessary to confirm its identity. The determination of the functions and characterization of GP63 of these parasites can contribute to a broader view of GP63 function in whole Trypanosomatidae family. **Supported by:**MCT/CNPq, FAPERJ, FIOCRUZ.

PV73 - ROLE OF MITOCHONDRIA ON *TRYPANOSOMA CRUZI* SURVIVAL TREATED WITH H2O2 OR METHYL METHANE SULFONATE AT CONCENTRATIONS THAT INDUCE DNA DAMAGE

MARCO ANTONIO, T.[1]; PELOSO, E.F.[1]; MACHADO, C.R.[2]; GADELHA, F.R.[1]
1.UNICAMP, CAMPINAS, SP, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:frgad@unicamp.br

In DNA, reactive oxygen species can lead to single- and double-strand breaks, base excision and oxidation. Like most living organisms, *Trypanosoma cruzi* is susceptible to oxidative stress, therefore, DNA repair is essential for their survival and establishment of infection. *T. cruzi* has only one mitochondrion that plays an important role beyond the production of ATP, and is a source of signaling molecules. In this sense, the integrity of this organelle is essential for parasite survival. In this study, it was evaluated the mitochondrial involvement during the repair of nuclear and mitochondrial DNA in cells treated with H_2O_2 or methyl methane sulfonate (MMS) under conditions that induced DNA damage but allowed cell to repair it. For this purpose, *T. cruzi* epimastigotes (CL Brener strain) were incubated in the presence of H_2O_2 (200µM, 15 min) or MMS (1.5 mM, 60 min). After treatment, cells were collected by centrifugation and incubated in "Old LIT medium" for 0, 1, 3, 4, 6, 18 and 24h. The biochemical parameters analyzed were oxygen uptake, ATP levels and H_2O_2 release, and also the proliferation curve, and the determination of IC_{50} for H_2O_2. Overall, it was observed an increase in oxygen consumption, no significant variations in the respiratory control, maintenance of the ATP levels and an increase in H_2O_2 release in H_2O_2 or MMS-treated cells compared to control. Our results demonstrated a situation of oxidative stress after both treatments highlighting the importance of mitochondria to the process of DNA repair, since this organelle is functionally mobilized for the maintenance of the ATP levels required for DNA repair. Herein, the importance of mitochondria for parasite survival, as well as its potential as a therapeutic target has been enhanced. **Supported by:**FAPESP, CNPq

PV74 - OXIDATIVE STRESS IN LEISHMANIA (L.) AMAZONENSIS PROMASTIGOTES CAUSES TELOMERE SHORTENING AND DISPLACEMENT OF LARPA-1 FROM 3' G-OVERHANG

DA SILVA, M.S.[1]; SEGATTO, M.[2]; RODRIGUEZ, F.G.[3]; VIVIESCAS, M.A.[2]; CALADO, R.T.[3]; CANO, M.I.N.[2]
1.UNICAMP, SAO PAULO, SP, BRASIL; 2.UNESP, BOTUCATU, SP, BRASIL; 3.USP, RIBEIRÃO PRETO, SP, BRASIL. e-mail:mamasantos2003@yahoo.com.br

Leishmaniasis is a spectrum of diseases caused by parasites of the genus Leishmania affecting million people around the world. During infection, parasites use different strategies to survive host defenses including overcoming exposure to Reactive Oxygen Species (ROS), mainly responsible for causing DNA damage, especially at telomeres. Telomeres are nucleoprotein structures whose function is to protect chromosome ends of nucleolytic degradation and fusion. Here, we induced acute oxidative stress in promastigotes of Leishmania amazonensis by treating parasites with 2mM hydrogen peroxide for 1 hour, which increased intracellular ROS as demonstrated by CM-H2DCFDA reaction. In addition, oxidative stress induced DNA damage, as confirmed by quantitative analysis of 8-oxodG and TUNEL-positive nuclei. We also observed using different methodologies (Southern blot, telomere-PCR and flow-FISH) that oxidative stress induced telomere shortening. The analysis of protein:DNA co-localization and interaction by FISH-IIF and ChIP assays, show that oxidative stress induced erosion of the 3'G overhang. This, coupled with DNA damage in telomeric internal regions, induces a displacement of LaRPA-1 from its interaction site to the telomeric C-rich ssDNA, probably generated as a consequence of Mismatch repair, suggesting the participation of LaRPA-1 in this pathway. Analysis of growth curves and EdU incorporation showed that oxidative stress induced a decrease in the number of parasites in culture, while the survivors continued proliferating and replicating DNA. Moreover, part of the survivors showed a G2/M cell cycle arrest. Taken together, these results suggest the presence of an altruistic mechanism that facilitates the selection of the fittest parasites that are able to repair efficiently oxidative damaged telomeric DNA, leading to genome stability even in a stressor environment. **Supported by:**FAPESP

PV75 - RELATIONSHIP BETWEEN MAST CELLS AND ALTERATIONS IN MATRIX EXTRACELLULAR IN THE EAR SKIN OF DOGS NATURALLY INFECTED WITH LEISHMANIA INFANTUM

CARDOSO, J.M.O.[1]; VIEIRA, P.M.A.[1]; MOREIRA, N.D.[1]; MATHIAS, F.A.S.[1]; COURA-VITAL, W.[2]; SACRAMENTO, N.A.[1]; OLIVEIRA, D.A.P.[1]; DANIEL, M.C.M.[1]; GIUNCHETTI, R.C.[2]; REIS, A.B.[1]; CARNEIRO, C.M.[1]
1.UFOP, OURO PRETO, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:ja_mirelle@yahoo.com.br

Canine visceral leishmaniasis (CVL) is a zoonosis of major public health impact. All manifestations of the disease must be known to assess the role of dogs in the infection including pathological features in various organs affected. The skin is the first point of contact with organisms of the genus Leishmania for sandy fly vectors and skin lesions are the most usual manifestation of CVL. Thus, the aim this study was to determine histophatologic features in skin of dogs naturally infected with Leishmania infantum through of mast cells and the extracellular matrix analysis. For this, 35 dogs were categorised as asymptomatic (AD, n=11), oligoymptomatic (OD, n=12) and symptomatic dogs (SD, n=12) as well as low (LP, n=12), median (MP, n=11) and high (12, n=12) parasitism and these were compared to control dogs (CD, n = 10). These animals were euthanized and ear skin samples were collected for analysis of mast cells and extracellular matrix. We observed greater quantification of mast cells in the OD group compared to the CD group and a greater number in LP and MD groups compared to the CD group. After staining with Picrossirius red, observed reduction of collagen fibers type I in OD and SD group compared to the CD group and increased collagen fibers type III in the CO group compared the CD and SD groups. Still, decreased type I collagen fibers was observed in the MP group compared to CD group and increased collagen type III in the BP group than in the CD group. The number of mast cells was larger in the OD and LP groups showing that these cells may be involved in tissue remodeling, since the quantification of type III collagen was increased in these groups, with a reduction of type I collagen. Some studies show that chymases and tryptases, present in the granules of these cells seem to have important role in damaged tissues. In this sense, these results add new insights about the pathogenesis of CVL, being a indicator for the severity of clinical disease in dogs. **Supported by:**CAPES, CNPq, FAPEMIG, UFOP

PV76 - STUDY OF FREQUENT SEQUENCES IN *T. CRUZI* GENOME STRAINS

ARAUJO, C.B.; NISHIYAMA-JR, M.Y.; CALDERANO, S.G.; REIS, M.S.; ELIAS, M.C.
INSTITUTO BUTANTAN, SAO PAULO, SP, BRASIL. e-mail:chris.biologia@gmail.com

The *Trypanosoma cruzi* diploid genome size varies around 100 Mb among different strains and about 50% of this genome is composed of repetitive elements. Satellite DNA, for instance, is a 195bp sequence tandemly repeated located at almost all chromosomes. There are also interspersed repeats through the *T. cruzi* genome as the 189 repeats at the subtelomeric regions and many other ranging from 172 bp to 1400 bp, besides the Long Terminal Repeat (2300bp), Short Interspersed Nuclear Elements (260-428bp) and Long Interspersed Nuclear Elements (5000-7219bp). Here we asked how the frequency of fragments shorter than 260bp is in the whole genome of *T. cruzi*. For that purpose, each chromosome of CL Brener Esmeraldo-like and non-Esmeraldo-like obtained from TryTripDB sequenced genome was screened by a 150 bp window over the chromosome sequence and between different strains. During this process, we counted the number of occurrences of each subsequence that appeared during the window screening. The assessment of short sequences was carried out through an in-house Perl program. The results were then summarized through the join of every chromosome counting and the subsequences were grouped by its number of occurrences. We show that 76% of the possible subsequences from the Esmeraldo-like genome are unique, that is, they appear only once in the whole genome. While in the non-Esmeraldo-like the unique subsequences was 81%. However, when discarding subsequences composed only by non-determined nucleotide, the proportion of unique subsequences increases to more than 95% in both groups. In addition, we found that the number of sequences that match more than twice in the genome is higher in CL-Brener when compared to other strains that are not yet assembled. We are now identifying the most frequent sequences in the genome in order to determine the number of copies of each repeated sequence. Also, we intend to present an overview of the frequent sequences in different *T. cruzi* strains.

PV77 - STUDIES ABOUT THE HOMOLOGOUS RECOMBINATION IN DIFFERENT STRAINS OF TRYPANOSOMA CRUZI.

REPOLÊS, B.M.; SANTOS, S.S.; ALVES, C.L.; MACEDO, A.M.; FRANCO, G.R.; PENA, S.D.J.; MACHADO, C.R.
UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE, MG, BRASIL.
e-mail:marcalrepoles@yahoo.com.br

Philogeny studies recently subdivided T. cruzi into six discrete taxonomic units, named T. cruzi I to T. cruzi VI. The parasite presents a complex life cycle which includes an obligate intracellular stage in a mammalian host so it does not face high doses of radiation on his life, but it can resist to doses 100x higher than humans. When the parasite is exposed to doses as high as 500Gy, occurs DNA fragmentation, and it is repaired 48 hours later. IR generates double strand breaks (DSB's) and in T. cruzi, homologous recombination repair (HRR) is the responsible for processing these DSB's. In this process a central protein, Rad51, polymerizes into single-stranded DNA and acts in the search for homology in the other DNA molecule. The function of Rad51 is regulated and depends on his interaction with the BRCA2, which has a domain, named BRC, crucial for it function. To verify the response to IR in the T. cruzi DTU's I, II and VI, the strains CL Brener (T. cruzi VI), Sylvio (T. cruzi I) and Esmeraldo (T. cruzi II) were exposed to IR. It was demonstrated that CL Brener strain resume its growth faster than Sylvio and Esmeraldo . Two hypothesis emerges to explain this phenotype: a difference between the protein interactions involved on DSB repair in each strain; an differential expression of this proteins by each strain. The analyses of the proteins involved in DSB response showed the most relevant difference is on the BRCA2 protein, while Rad51 is equal for all strains. The predicted models of the BRCA2 proteins shows that the position and accessibility of the BRC domain is different for each DTU. Using the HADDOCK WebServer, models were generated showing the interaction is more stable for the Cl Brener complex than for the other DTU's, when analysed for the first BRC domain. In this work we showed that a hybrid strain (CL Brener) responds better to a genotoxic treatment that needs the HRR, which could suggest that recombination is key process to hybrid generation. **Supported by:**CNPq, FAPEMIG

PV78 - EFFECTS OF DEPRIVATION OF WATER OR FOOD ON *ONCOPELTUS FASCIATUS* SURVIVAL, COMPARING NON-INFECTED INSECTS AND NATURALLY INFECTED WITH *LEPTOMONAS WALLACEI*

REIS, L.A.; VASCONCELLOS, L.R.C.; LOPES, A.H.C.S.
INSTITUTO DE MICROBIOLOGIA UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:reisleonan@hotmail.com

The hemipteran insects of the species *Oncopeltus fasciatus* are naturally infecte d by many trypanosomatids, including species of the genus *Leptomonas* . Furthermore, many authors have shown that this phytophag ous insect can be used as an effective experimental host for other trypanosomatids, including *Phytomonas serpens* . *Leptomonas* speci es, such as *L. wallacei* and *L. onco pelt i* , ar e commonly found in the midgut and foregut of *O. fasciatus* . Th e cystic forms of *L. wallacei* are ingested by *O. fasciatus* (coprophagy). Then the cysts transform into the vegetative form of the parasite (promastigote) and bind to the midgut of the insect. The parasites are released into the environment through the feces and urine of the insect. The goal of the present study was to show the effects of deprivation of water or food on *O. fasciatus* survival, comparing non-infected and naturally infected insects. Non-infected bugs are apparently more resistant than infected bugs to both deprivation of water and food. Curiously, under both starvation and water deprivation, female bugs present higher survival rates as compared to male bugs. These data suggest that infected and non-infected male insects are more susceptible to environmental stress than female insects. Our findings may encourage other studies using invertebrate vectors that carry pathogens or insects that are a threat to agricultural crops. **Supported by:**CNPq

PV79 - COMPARATIVE ANALYSIS OF PRIMERS-QPCR USED IN DETECTION OF *LEISHMANIA (L.) INFANTUM* IN BIOLOGICAL SAMPLES OF DOGS NATURALLY INFECTED

REIS, C.B.[1]; SOARES REIS, L.E.[2]; COURA-VITAL, W.[2]; ROATT, B.M.[2]; REIS, A.B.[2]; MARQUES, M.J.[1]; COLOMBO, F.A.[1]
1.UNIFAL-MG, ALFENAS, MG, BRASIL; 2.UFOP, OURO PRETO, MG, BRASIL.
e-mail:colombofabio@uol.com.br

Leishmania is a protozoan that causes a wide spectrum of human diseases, as the visceral form (VL), where the dog is the principal domestic reservoir. In Latin America is caused by *Leishmania (Leishmania) infantum*, transmitted to the dog and eventually to humans by the bite of female sandflies (Psychodidae). The early diagnosis is important to avoid serious damage that can lead to death, and alternative diagnostics techniques has been necessary considering the limitations of conventional methods. This study aimed to compare primers used in detection of *Leishmania (L.) infantum* DNA by real time PCR (qPCR). Were collected forty-eight DNA sample from the skin and spleen of clinically and serologically positive dogs, naturally infected with *L. (L.) infantum*. The qPCR experiments were performed by following primer sets: LinJ31, Ldon and DNA pol, which amplified a fragment of *L. (L.) infantum* hypothetical protein, actin and DNA polymerase, respectively. The results achieved by DNA pol were superior to the other primers sets, both in the skin and in the spleen, reaching with 100% positivity in both tissues. The spleen samples were positive in 91.63% to Ldon and 81.21% to LinJ31, and 87.50 and 72.92% in relation to the skin with the same primers. Although the positivity achieved by Ldon was superior to the LinJ31 statistical difference between the primers was not verified ($p > 0,05$). The DNA pol primers set has better performance than the others on skin ($p<0,05$), shows the same performance as Ldon at spleen, and is statistically superior only to LinJ31. Thus, DNA pol it was noted as best primer for the diagnosis of VL, but due to its significant results the Ldon primers set proved an alternative tool for diagnosis, especially in spleen samples.**Supported by:**FAPEMIG, CAPES, UNIFAL

PV80 - IDENTIFICATION OF *TRYPANOSOMA CRUZI* IN TRIATOMINE FECES COLLECTED IN SOUTH OF MINAS GERAIS

MENDES, F.R.T.; SOUZA, R.V.; MARTINS, V.G.; SIQUEIRA, R.V.; CALDAS, I.S.; MARQUES, M.J.; COLOMBO, F.A.
UNIFAL-MG, ALFENAS, MG, BRASIL.
e-mail:colombofabio@uol.com.br

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 their 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 Federal University of Alfenas (UNIFAL-MG). From 324 insects received, eleven were classified as predators, two as phytophagous and 311 hematophagous, all of them 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 the others 276 feces samples were collected. The triatomines´s intestinal contents were removed by compression and observed through direct examination followed by Giemsa staining to observation of flagellated forms. Part of such contents were collected in 300 µl of saline solution (0.85%) and stored by freezing for use in molecular tests. All the samples were processed according to the protocol for genomic DNA extraction by phenol/chloroform/isoamyl. Detection of *T. cruzi* DNA from feces was performed by conventional PCR using TCZ1/TCZ2 primers, which amplified a 188 base pairs product. The TCZ real-time PCR assay was performed with a probe FAM labeled. From the 276 feces samples, only fourteen specimens (5.07%) showed flagellated protozoa morphological similar to *Trypanosoma cruzi* in intestinal contents, and 262 samples (94.93%) were negative. In molecular analysis, 45 (16.3%) samples were positive for conventional TCZ1/TCZ2 primers and 118 (45%) samples were positive for qPCR. These data shows a superior performance of the qPCR and a high rate of *Trypanosoma cruzi* in intestinal contents from the collected insects, suggesting a possible contribution to the spread of the disease. **Supported by:**CAPES, UNIFAL

PV81 - RPA-1 IS EXPORTED FROM THE NUCLEUS IN T. CRUZI.

PAVANI, R.S.[1]; LAZARIM, L.E.[1]; CRISPIM, M.[2]; SILBER, A.M.[2]; CANO, M.I.N.[3]; ELIAS, M.C.[1]
1.INSTITUTO BUTANTAN, SAO PAULO, SP, BRASIL; 2.USP-ICB, SÃO PAULO, SP, BRASIL; 3.UNESP, BOTUCATU, SP, BRASIL.
e-mail:rapavani@gmail.com

Trypanosoma cruzi is the etiologic agent of Chagas' disease. In the lifecycle of this protozoan, there are different lifeforms, Epimastigotes and Amastigotes (Replicative forms) and Trypomastigotes (Non Replicative form). Replication protein A (RPA), the major eukaryotic single-stranded binding protein, is a heterotrimeric complex formed by three subunits RPA-1, RPA-2 and RPA-3. The heterotrimer participates in various vital functions in DNA metabolism, being a fundamental player during replication, repair, recombination and checkpoint signaling. In higher eukaryotes, RPA-1 is the major subunit that interacts with single stranded DNA via its OBfold domains and with checkpoint and replication proteins such as ATRIP, Rad9 and Polymerase α via its 70N OBfold domain. Curiously, RPA-1 from trypanossomatids lacks the 70N OB fold domain and has some other relevant structural differences that raise doubts about RPA trimerization in these organisms. In immunofluorescence assays using epimastigotes, RPA-1 has nuclear localization, as described for higher eukaryotes. However, in the non-replicative tripomastigote lifeform, most of RPA-1 appears to be exported from the nucleus, with strong cytoplasmic localization and a weak nuclear localization. In amastigotes, part of RPA-1 localizes in the nucleus, but we also observed cytoplasmic localization. RPA exportation has never been described in eukaryotes, so we decided to search some Nuclear Exportation Signal (NES) in RPA-1 amino acid sequence. Utilizing ValidNESs software, we do not observed nuclear exportation signal in RPA-1, but when we made a search of nuclear exportation signal in RPA-2, we found a putative NES with a probability of 67%. Now, we are working on this amino acid sequence to verify if this is a real NES. These results suggests that RPA may act as an heterotrimer and its exportation can be one of the mechanisms involved in the control of replication arrest in T. cruzi. **Supported by:**FAPESP

PV82 - TRYPANOSOMA CRUZI EPIMASTIGOTES STERIFY CHOLESTEROL AND STORE INTO LIPID DROPLETS

PEREIRA, M.G.[1]; DE CICCO, N.N.T.[1]; SALGADO, L.T.[2]; GODINHO, J.L.P.[1]; ATELLA, G.C.[1]; DE SOUZA, W.[1]; CUNHA-E-SILVA, N.L.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.JBRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:miria@biof.ufrj.br

Trypanosoma cruzi epimastigotes do not synthesize cholesterol, but accumulate high amounts of cholesterol and cholesterol esters, obtained by endocytosis of lipoproteins, in reservosomes. Besides reservosomes, many non-characterized lipid droplets are observed in the cytoplasm, suggesting that they can coordinate two lipid stocks. In this work, we investigated the parasite ability to use these two lipid stocks in periods of nutritional lipid stress. Control or starved parasites were kept in medium supplemented with normal or delipidated FCS (dFCS), respectively, for different times and processed for electron microscopy. The reduction of the area of reservosomes (and their lipid inclusions) and lipid droplets was determined by morphometric analysis. After 72h, the area of reservosome lipid inclusions reduced almost 72%, but the area occupied by lipid droplets did not change. Additionally, lipid analysis by HPTLC revealed a decrease of almost 100% of cholesterol and cholesterol esters content. Together, the data suggest that parasite uses exogenous sterol stored in reservosomes as the first source of lipids during lipid starvation. Aiming to follow cholesterol intracellular traffic, we incubated control or starved parasites with LDL-NBD-cholesterol for 0, 30 min, 1h and 2h. After 30 min, the amount of NBD-Chol in plasma membrane was 2.5 fold higher in starved cells, whereas in control cells it raised 76% only after 2h. Epimastigotes were able to synthesize cholesteryl esters from cholesterol-3H, suggesting an active TcACAT (acyl-CoA:cholesterol O-acyltransferase). Avasimibe inhibited TcACAT in a dose-dependent manner, but Sandoz stimulated it, differing from the mammal enzyme. BSA-cholesterol-3H in reservosomes were recovered as 3H-cholesteryl esters in isolated lipid droplets. Together, our data demonstrated that epimastigotes are able to insert cholesterol into membranes and store in cytoplasmic lipid droplets by a yet undescribed TcACAT. **Supported by:**FAPERJ

PV83 - ULTRASTRUCTURAL CHANGES OF THE CYTOSTOME-CYTOPHARINX COMPLEX DURING TRYPANOSOMA CRUZI METACYCLOGENESIS

VIDAL, J.C.; ALCANTARA, C.L.; DE SOUZA, W.; CUNHA-E-SILVA, N.L.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:vidal.ju@gmail.com

Trypanosoma cruzi epimastigotes uptake nutrients by endocytosis via the cytostome-cytopharynx complex. The cytostome consists in an opening of the epimastigote membrane at the anterior region, continuous with a funnel-shaped invagination called cytopharinx. The long and thin invagination is sustained by two microtubule sets, a triplet that extends from underneath the cytostome membrane and a quartet originated close to the flagellar-pocket that follows underneath the preoral ridge membrane (Alcantara et al. J Cell Sci. 127:2227, 2014). At the end of metacyclogenesis the entire complex had disappeared and trypomastigotes lost the endocytic ability. There is no detailed studies about how this process occurs. In this work, we have used advanced electron microscopy techniques, as tomography and dual beam scanning electron microscopy (FIB) and 3D reconstruction to examine the three described morphotypes of intermediates (Ia, Ib, Ic) between epimastigotes and trypomastigotes (Ferreira et al. An Acad Bras Cienc 80:157, 2008). We found that Ib forms presented a shorter cytopharinx compared with epimastigotes, and the preoral ridge displaced, with the cytostome aperture placed at the middle of the cell body. Parasites in Ic phase showed a short and enlarged cytopharynx (~150nm of diameter, the same measure of the cytostome) accompanied by microtubules. Nevertheless, Ib and Ic forms were still able to uptake gold labeled transferrin. In our observations, we found “late Ic” forms devoid of the cytopharinx membrane invagination, but with preserved microtubules, and without endocytic tracer. We concluded that during metacyclogenesis the migration of kinetoplast/flagellar pocket to the posterior region drags the cytostome, the invagination shortens from the end to the beginning and the cytopharynx microtubules are the last to disassemble. **Supported by:**CNPq,CAPES, FAPERJ, INBEB

PV84 - IDENTIFICATION OF GENES IMPLICATED IN RESISTANCE TO BENZNIDAZOL IN *TRYPANOSOMA CRUZI*

GARCIA HUERTAS, P.A.[1]; MEJÍA JARAMILLO, A.M.[1]; MACHADO, C.R.[2]; TRIANA CHAVEZ, O.[1]
1.UNIVERSIDAD DE ANTIOQUE, MEDELLIN, COLÔMBIA; 2.UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE, MG, BRASIL.
e-mail:paolaalexandrag@gmail.com

The resistance to benznidazole (Bz) in *Trypanosoma cruzi* is one of the main causes of the inefficiency of the treatments for Chagas disease. However, such resistance mechanisms remain unknown. To investigate genes that may be related in T. cruzi Bz resistance, we analyse the transcriptome of sensitive and resistant parasites to this drug. The sensitive and resistant to benznidazole *T. cruzi* (TCI) transcriptome was sequenced by 454 pyrosequencing at the National Center for Genome Sequencing of Colombia (CNSG). Genes differentially expressed between both phenotypes were identified using R statistical. We selected 23 genes based on the results of transcriptome, proteome previous results, real-time PCR and literature reviews. The cloning of some of these genes into pTREX and pTEX vectors were performed and sensitive parasites were transfected by electroporation. Overexpression of some of these genes in the transfected parasites was verified by realtime PCR and northern blot and MTT assays were performed to verify any change in the IC50 of benznidazole. Sensitive parasites transfected with prostaglandin F2alpha synthase and alcohol dehydrogenase genes acquired drug resistance while parasites transfected with adenine phosphoribosyltransferase and L-threonine dehydrogenase genes become more sensitive. These genes are mainly involved in the process of nucleic acid metabolism and antioxidant defence. Additionally, preliminary results with parasites overexpressing the L-threonine dehydrogenase gene show an apparent protection to damaged DNA compared to wild type parasites. Our results show that using new sequencing methodologies and experimental validation is possible to identify genes that may be involved in resistance or susceptible to Bz in the *T. cruzi*. **Supported by:**Colciencias – Colombia

PV85 - PARTICIPATION OF ACTIN AND MYOSIN CYTOSKELETON ON ENDOCYTOSIS IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

ALVES, A.A.; BASTOS, L.S.S.; CUNHA-E-SILVA, N.L.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:lu.ssbastos@gmail.com

Epimastigote form of *Trypanosoma cruzi* is a very interesting model to study cell biology due to its peculiar ultrastructure and proteins function. These cells present a high endocytic activity that occurs through two specific domains located in its anterior region: the flagellar pocket and the cytostome-cytopharynx domain. Moreover, the function of cytoskeleton on this process is not clear. We are interested in actin and myosin participation during endocytosis in this model. Corrêa and coworkers (Exp. Parasitol. 119:58, 2008) have shown a drastic reduction in transferrin uptake and alterations in cytopharynx structure after treatment with Cytochalasin, a drug that destabilizes actin filaments. However, the Cytochalasin target on epimastigotes is discussed. We show that the effects of Cytochalasin D and Latrunculin B, a drug that blocks the actin polymerization, on epimastigotes are similar, indicating that actin is the target of both drugs. Cytometry flow analyses show that both drugs are able to block transferrin uptake. The arrest of endocytic vesicles traffic is observed by fluorescence microscopy. The actin immunolocalization at cytostome-cytopharynx complex was determined by transmission electron microscopy using both commercial anti-actin antibody and polyclonal anti-Tc actin produced by Cevallos and coworkers (Exp. Parasitol. 127:249, 2011). The immunolocalization of myosin at the anterior region of the parasite was achieved using anti TcMYO1 antibody produced by Aviva. Myosin concentration was dispersed when epimastigotes were treated with Cytochalasin D, suggesting an interaction between this protein and actin filaments. Furthermore, myosin localization accompanied the kinetoplast during cell division. Our results suggest a relationship among actin, myosin and the endocytic sites, indicating these proteins participation on the initial steps of endocytosis. **Supported by:**PIBIC/CNPq, CAPES, FAPERJ, CNPq

PV86 - CAMPTOTHECIN, A TOPOISOMERASE I INHIBITOR, AS A TOOL TO STUDY THE SYMBIOSIS IN TRYPANOSOMATIDS

DA SILVA, C.C.; ZUMA, A.A.; ATELLA, G.C.; DE SOUZA, W.; MOTTA, M.C.M.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:mylacrys@ig.com.br

Trypanosomatids are intensively studied since they are pathogenic to men and animals. However, most protozoa of this family are monoxenics and inhabits a single invertebrate host during all its life cycle. Some of them, as Strigomonas culicis, co-evolve in a mutualistic relationship with a symbiotic bacterium, thus representing an interesting model to study cell evolution. The symbiont divides in synchronicity with other host structures, thus at the end of the cell cycle, each protozoa contains a single bacterium. DNA topoisomerases regulate the topological state of DNA and play essential roles in replication, transcription and DNA repair. Camptothecin is a topoisomerase I inhibitor that affects trypanosomatid proliferation, promoting cell cycle arrest in G2 phase. In this work we used camptothecin as a tool to interfere in the symbiotic relationship of S. culicis and its symbiotic bacterium. Thus, protozoa were treated with different concentrations of this compound for 72 hours. After each 12 hours, samples were collected for counting on Neubauer's chamber and for processing to fluorescence and transmission electron microscopy, as well as to staining with Nile Red. Our results showed that camptothecin caused a strong inhibition of S. culicis proliferation. Ultrastructural alterations were also observed, as the unpacking of nuclear heterochromatin, the appearance of filamentous symbionts and accumulation of lipidid bodies, as revealed by the osmium-imidazole cytochemistry technique. Data showed that camptothecin enhanced lipid quantity in a concentration dependent manner along the treatment. Biochemical analysis showed that lipid composition was affected, since increased amounts of phospholipids were observed in treated cells. Taking together, our results showed that topoisomerase I inhibition promotes cell cycle arrest resulting in impeachment of symbiont division and interfering in lipid metabolism. **Supported by:**CNPq and FAPERJ

PV87 - SORTING OF THE P-TYPE H+-ATPASE IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

QUINTAL, S.N.; CUNHA-E-SILVA, N.L.; LIMA, A.P.C.A.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:snquintal@gmail.com

Acidification of the endocytic pathway is important in a variety of cellular processes, such as the dissociation of ligands from their receptors in early endosomes and for the activity of hydrolases inside lysosomes. In *Trypanosoma cruzi*, this pathway is acidified by a P-type H^+-ATPase unlike most eukaryotic cells, whose acidification depends on V-type H+-ATPase activity. Previous studies identified two genes encoding different P-H^+-ATPases in *T. cruzi* Y strain, called TcHA1 and TcHA2, which are differently distributed in the cell. TcHA1 was found in the plasma membrane and endocytic pathway, while TcHA2 was localized exclusively in reservosomes (lysosome-like organelles). Since the main difference between the enzymes is the predicted insertion of 50 amino acids at the N terminal region of TcHA2, we asked if this region could be responsible for the targeting of TcHA2 to reservosomes. To investigate this hypothesis in Dm28c *T. cruzi*, we constructed transfection cassettes containing distinct domains of TcHA2 in fusion with eGFP in pTEX. Surprisingly, the fusion with the 50 amino acids from the N terminal region of TcHA2 did not alter the cytoplasmic dispersion of eGFP, indicating that this domain does not contain a signal peptide. However, the first 363 amino acids of TcHA2 are sufficient to deliver the protein to the secretory pathway both in *T. cruzi* epimastigotes and in *Leishmania major* promastigotes. Immunoelectron microscopy using anti-GFP also revealed gold labeling in reservosomes and in the cytostome-cytopharinx complex. In addition, we transfected epimastigotes with full TcHA1 or TcHA2 genes in order to raise the expression of these proteins and search for phenotypes induced by a change in acidification of the endocytic pathway. Kinetics assays of endocytosis using fluorescent tracers are ongoing and the results analyzed by fluorescence microscopy and flow cytometry. **Supported by:**CNPq and FAPERJ

PV88 - DIFFERENCES IN ENDOPEPTIDASE GENES EXPRESSION AMONG SUBPOPULATIONS OF A SAME *LEISHMANIA (VIANNIA) BRAZILIENSIS* STRAIN: EVIDENCES OF A HETEROGENEOUS POPULATION

SOUZA, R.S.; ALMEIDA, M.S.; PEREIRA, B.A.S.; SILVA, F.S.; GUIMARAES, M.L.R.; CYSNE-FINKELSTEIN, L.; ALVES, C.R.
IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:bpereira@ioc.fiocruz.br

Leishmania (Viannia) braziliensis is an etiologic agent of tegumentary leishmaniasis in Brazil, presenting an important impact over Public Health. Currently, there is the need to develop new treatments for this infection, as the available drugs present many toxic side effects and resistant strains are emerging. To this end, it is necessary to understand the characteristics of this parasite, including how populations of a same species are composed. Our group is presently conducting a study where many distinct features of 18 subpopulations isolated from a well-studied *L. (V.) braziliensis* strain (Thor) are under scrutiny and a cluster analysis based on these data will be performed. Thus, our objective is to visualize how this population is composed: whether by a set of homogenous clones or by a range of organisms with distinct characteristics. One of the features under study is the levels of endopeptidase genes expression, as many reports indicate that these enzymes play important roles in Leishmania spp. life cycle and are relevant virulence factors during mammalian hosts' infection. Therefore, we have selected one representative gene of each endopeptidase class known to be expressed in Leishmania: cysteine-protease (CP), serine-protease (SP), aspartic-protease (AP) and metalloprotease (MP), and analyzed their expression levels in promastigote and amastigote forms by the ΔΔCt method using real time PCR technique. The genes elected for analysis were: cysteine-protease B (for CP), subtlisin (for SP), presilin-like (for AP) and gp63 (for MP). Our preliminary results indicate that, although harvested from a single strain, not all subpopulations express these genes in the same levels, hence suggesting that parasite populations may actually be composed of a mixture of organisms with distinct features. Other characteristics of these subpopulations are now being examined to subsequently be applied in the cluster analysis and further confirm our current results. **Supported by:**CAPES, Faperj e CNPq

PV89 - LEISHMANIA AMAZONENSIS PINX1 ORTHOLOG, A NATURAL INHIBITOR OF TELOMERASE ACTIVITY, PRESENTS CONSERVED G-PATCH AND TID DOMAINS AND NUCLEAR LOCALIZATION

STORTI, C.B.; CANO, M.I.N.
UNESP, BOTUCATU, SP, BRASIL. e-mail:camilabaldin.s@gmail.com

Protozoan parasites of the genus Leishmania cause leishmaniasis, a disease that threaten million people and is expressed by different clinical forms. The drugs used to treat leishmaniasis are highly toxic and present low efficiency and high cost. The study of parasite telomeres is an important way to obtain molecular information that can be used for the development of new anti-parasitic drugs. There are several proteins associated with telomeres that are responsible for ensuring their stability and also work to regulate the access of telomerase, the enzyme responsible for telomere length maintenance. PinX1 was described in humans and other eukaryotes, as a natural inhibitor of telomerase since it interacts directly with the TRBD (Telomerase RNA Binding Domain) domain of telomerase (TERT, telomerase reverse transcriptase) through its small TID domain (telomerase inhibitory domain). Genomic DNA of *Leishmania amazonensis* was used as template for the amplification of the LaPinX1 gene ortholog using PCR. The amplicon (LaPinX1) was cloned into pCR2.1-TOPO cloning vector and subcloned into the bacterial expression vector pET-28a (+) to allow the recombinant protein LaPinX1 to be expressed with an N-terminal 6xHis-tag. DNA from the recombinant LaPinX1-pET-28a (+) clone was subjected to automated sequencing which showed that the predicted amino acid sequence of LaPinX1 shares 25%identity and 43% similarity with human PinX1and contains the G-patch and the TID domains that characterize it as an ortholog of eukaryotes PinX1. Recombinant LaPinX1 was expressed at 25ºC in *E. coli* BL21 DE3 codon plus and purification tests of the protein are being conducted with the aim to perform protein: protein interaction assays. Preliminary results using indirect immunofluorescence and western blot revealed with anti-LaPinX1 immune serum showed that the protein is predominantly nuclear in *L. amazonensis.* **Supported by:**CNPQ-PIBIC, FAPESP

PV90 - IDENTIFICATION OF GENES INVOLVED IN BIOSYNTHESIS OF BIOACTIVE PHOSPHOLIPIDS IN *TRYPANOSOMA CRUZI*

COELHO, F.S.; OLIVEIRA, M.M.; PINHEIRO, L.S.; MOREIRA, I.C.F.; MACRAE, A.; LAZOSKI, C.V.; LOPES, A.H.C.S.
UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:felipesoares59@yahoo.com.br

The phospholipids lysophosphatidylcholine (1-acyl-2-hydroxy-sn-glycero-3-phosphorylcholine; LPC) and platelet-activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine; PAF) are structurally very similar. These lipid mediators have been implicated in experimental models of Chagas disease. LPC is present in the saliva of at least one of the insect vectors of Chagas disease, the hemipteran *Rhodnius prolixus*, acting as an anti-hemostatic molecule and immunomodulator of *T. cruzi* infection in a mammalian model. PAF induces numerous physiological and pathophysiological effects, such as cell differentiation, inflammation, and allergy. *T. cruzi* synthesizes a lipid with platelet-aggregating properties similar to PAF, a bioactive C18:1-LPC, which aggregates platelets via PAF receptor (PAFR). In the present study, enzymes implicated in the synthesis of PAF and LPC were selected from the KEGG pathway database and a search for their gene sequences was performed on genomic data bank TritrypDB. The genes that were not found in *T. cruzi* genome were searched for in other species of the Excavata group, and their sequences were used for finding protein coding regions in *T. cruzi*. To confirm the existence of these genes in *T. cruzi*, we performed PCRs and the amplified regions were sequenced. Out of the 10 genes involved in the biosynthesis of PAF, only 2 were found in *T. cruzi*. Out of the 46 genes involved in the biosynthesis of LPC, 23 of them were found in the *T. cruzi* genomes, 19 were found in other organisms and 4 were not found in the databases used. We constructed maps indicating the possible biosynthetic pathways for both PAF and LPC. So far, 8 of the 10 genes that code for enzymes from the LPC biosynthetic pathway in *T. cruzi* were amplified by PCR. The products of these amplifications were then sequenced, as the biosynthesis of LPC could eventually be exploited as a potential target for Chagas disease control. **Supported by:**CNPQ

PV91 - FUNCTIONAL ANALYSIS OF THE ORNITHINE DECARBOXYLASE IN THE THREE NEW WORLD *LEISHMANIA* SPECIES

FONSECA, M.S.[1]; RESENDE, B.V.[2]; MURTA, S.F.[1]
1.CPQRR, BELO HORIZONTE, MG, BRASIL; 2.UNIVERSIDADE FUMEC, BELO HORIZONTE, MG, BRASIL. e-mail:bethaniavazr@hotmail.com

Ornithine decarboxylase (ODC) is responsible to convert L-ornithine in putrescine in the polyamine biosynthetic pathway. In this study, to investigate a possible involvement of the ODC in the potassium antimony tartrate (SbIII) resistance phenotype, three New World Leishmania species, L. (V.) guyanensis, L. (V.) braziliensis and L. (L.) infantum were transfected with expression constructs containing ODC gene (pIR1-BSD-ODC). The vectors were linearized and electroporated into promastigote forms. Linearization allows integration of the construct into the ribosomal small subunit locus. Clonal lines resistant to blasticidin (BSD) were selected and analyzed by PCR and western blotting to confirm the presence of the BSD gene and overexpression of ODC. Western blotting analysis revealed that the polyclonal antibody anti-ODC from L. major recognized a 83-kDa polypeptide in all tested Leishmania lines. Densitometry of the ODC band using an anti-α tubulin antibody as reference showed that the level of ODC expression was higher in transfected clones than in the untransfected controls. Antimony susceptibility test (IC50 assay) revealed that L. guyanensis clones overexpressing ODC had a 3-fold increase in resistance to SbIII when compared to the untransfected parental line. The SbIII IC50 of the non-transfected wild-type LgWTS line was 0.011 mg/mL; in contrast, the SbIII IC50 of ODC overexpressing clones 1 and 2 was 0.037 mg/mL and 0.034 mg/mL, respectively. In contrast, no significant difference in SbIII-resistance was observed for ODC overexpressing clones from two Leishmania species analyzed L. (V.) braziliensis and L. (L.) infantum. Our data revealed that the enzyme ornithine decarboxylase is involved in the antimony-resistance phenotype in L. (V.) guyanensis. **Supported by:**CNPq, FAPEMIG, UNICEF/UNDP/World Bank/WHO and PROEP/CNPq/FIOCRUZ

PV92 - TESTING ZINC FINGERS NUCLEASES TO IMPROVE GENETIC MANIPULATION IN TRYPANOSOMATIDS

CALDAS, G.A.B.[1]; BURKARD, G.S.[2]; MARCOLINO, M.M.K.[3]; DAROCHA, W.D.[3]; RODITI, I.[2]; TEIXEIRA, S.M.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UNIVERSIDADE DE BERNA, BERN, SUÍÇA; 3.UFPR, CURITIBA, PR, BRASIL. e-mail:gabrielaburle@hotmail.com

Stable transfection of T. brucei and T. cruzi relies in the integration, by homologous recombination (HR), of linear plasmids into the parasite genomes. However integration events seem to occur at low rates and therefore, overall efficiency for generating stable transfectants by conventional electroporation is generally low. Zinc finger nucleases (ZFNs) 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, which make their activity highly specific. Because the resulting double-strand DNA break is restricted to a single site in a genome and can be repaired by non-homologous end joining or HR, foreign DNA fragments can be inserted with high efficiency in a defined genomic locus. To test the use of ZFNs as a tool to facilitate genomic DNA integration in T. brucei and T. cruzi we transfected both parasites expressing enhanced GFP (eGFP) with ZFNs that were designed to target this sequence. Surprisingly, neither GFP disruption nor ZFN expression was observed in T. cruzi. Considering that the expression of ZFNs might be toxic, we decided to generate transfected parasites expressing the nuclease under the control of the tetracycline (tet) operon. Indeed, tet induction in T. brucei confirms that ZFN expression causes cell growth inhibition but also resulted in a 3-5 fold increase in transfection efficiency of bloodstream forms. To be able to control the expression of ZFN in T. cruzi, a vector designed to express T7 RNA polymerase and the tetracycline repressor carrying proper mRNA processing signals are being generated. We also showed that it is possible to improve transfection efficiency of T. cruzi epimastigotes using the nucleofector technology: higher transfection efficiency was obtained using 5 ug of circular pTREXGFP, compared to the conventional electroporation protocol which requires 100 ug of plasmid. **Supported by:**Cnpq

PV93 - FUNCTIONAL CHARACTERIZATION OF L. AMAZONENSIS RPA-1 OVEREXPRESSING PARASITES

DIAS DOS SANTOS, G.A.G.[1]; NUNES, V.S.[2]; DA SILVA, M.S.[3]; CANO, M.I.N.[1]
1.UNESP- CAMPUS BOTUCATU, BOTUCATU, SP, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL; 3.UNICAMP, CAMPINAS, SP, BRASIL. e-mail:arantes_gabriel@hotmail.com

Leishmania spp. are protozoa parasites among which are species that cause leishmaniasis, a disease that occurs in different clinical forms (cutaneous, muco- cutaneous and visceral) and is endemic in many countries around the globe. Disease control and treatment are still inefficient and parasite drug resistance is a challenge since it may involve DNA amplification. 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 roles played by Replication Protein A subunit 1 (RPA-1), a single-stranded DNA-binding protein involved in many eukaryotic DNA processing pathways, including telomere maintenance and DNA damage signaling, present in most eukaryotes including Leishmania spp. LaRPA-1 overexpressing parasites were obtained by transfecting promastigotes with a recombinant Leishmania expression vector. Preliminary results with a non cloned population showed that LaRPA-1 overexpression increased parasite resistance to genotoxic agents, compared to wild type and to the population transfect with vector only. Individual clones of LaRPA-1 overexpressing parasites obtained by serial dilution are actually being tested in growth curves, protein expression and subcellular localization using western blot and indirect immunofluorescence, and telomere length by southern blot. Our goal is to understand the roles played by Leishmania amazonensis RPA-1 (LaRPA-1) in telomere maintenance. **Supported by:**FAPESP

PV94 - COULD THE TC*RAD51* PROTEIN BE RESPONSIBLE FOR THE EVENTS OF GENE EXCHANGE IN *TRYPANOSOMA CRUZI*?

ALVES, C.L.[1]; MACEDO, A.M.[1]; FRANCO, G.R.[1]; PENA, S.D.J.[1]; GUARNERI, A.A.[2]; ANDRADE, L.O.[1]; MACHADO, C.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.FIOCRUZ, BELO HORIZONTE, MG, BRASIL.
e-mail:ceres_luciana@yahoo.com.br

The genetic exchange has been described in several kinetoplastid parasites, however, how this mechanism happens remains unclear. *Trypanosoma cruzi*, the causative agent of Chagas disease, exhibits high levels of genetic diversity. The molecular characterization of *T*. *cruzi* has demonstrated the existence of homologous recombination and nuclear hybridization, as well as the presence of six main genetic clusters or “discrete typing units” (DTUs). TcRAD51 protein is a component of the DNA repair pathway and plays a pivotal role in the genetic variability processes. This pathway promotes homologous recombination and repair of DNA double-strand breaks. In order to investigate the importance of this gene in events of genetic exchange in *T*. *cruzi*, parasites that overexpress Rad51 (CL Brener strain) and carry the hygromycin resistance gene and parasites transfected with the empty vector carrying the G418 resistance gene were used. Thus, all parasites were passaged singly or together in mammalian cell cultures or axenic epimastigote cultures. After co-passage, recovered cells were placed in culture with hygromycin and G418 to select resistant populations to both drugs. Preliminary results show that a mixture of Tc*RAD51*-hyg and pROCK-neo epimastigotes yielded one population of cells resistant to both drugs, and four clones were obtained from this population. No double-drug-resistant populations were recovered from mammalian cell cultures infected with the mixture. If these results are confirmed, the recovery of hybrids from axenic epimastigote cultures implies that genetic exchange probably occurs in *T*. *cruzi* invertebrate hosts, and it is mostly dependent of homologous recombination. Preliminary experiments using triatomine bugs did not yet recovered double-drug-resistant populations. New experiments are ongoing to try recovering double-drug-resistant populations from triatomine bugs. **Supported by:**CNPq, FAPEMIG, CAPES

PV95 - SPHINGOSINE KINASE (SK) ACTIVITY IN DIGESTIVE TRACT OF RHODNIUS PROLIXUS

LIMA, M.S.; SILVA-NETO, M.A.C.; ATELLA, G.C.
UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:limamiche@gmail.com

Rhodnius prolixus is a blood-sucking bug widespread in the Southern Cone countries of South America where it vectors Chagas' disease. An adult R. prolixus will ingest from two to three times its own weight of blood at a single meal. We have previously reported the presence of bioactive lipids in R. prolixus salivary glands and digestive tract. Such molecules play a major role at insect feeding, parasite proliferation and differentiation. Sphingosine kinase (SK) is a member of a growing class of lipid kinases that participate in cell signaling. This enzyme phosphorylates sphingosine generating sphingosine 1 phosphate (S1P). Many studies have implicated SK activation and S1P generation as mediators of angiogenesis, tumorigenicity, metastasis, cell proliferation, motility, lymphocyte trafficking, endocytosis, and survival. In our lab we are investigating the role of these bioactive lipids in digestive tract of R. prolixus. To determine the SK activity, we have used 15-NBD-Sph fluorescent as substrate and the enzymatic reaction was followed by fluorimetry. We collected the digestive tract of 10 insects in the presence of saline and total protein was measured. The sample was subjected to the fluorimetric SK assay at different protein concentrations. Our results demonstrated that SK activity in digestive tract was both time and concentration dependent. These results confirm the presence of SK activity in the digestive tract. We are currently searching for the eventual role of such molecules in R. prolixus digestive tract both as a cell-signaling mediator as well as its role in any parasite transmission. **Supported by:**CNPq, CAPES, FAPERJ

PV96 - XPC AND CSB GENES HAVE DIFFERENTIAL ROLES IN DNA REPAIR AND CELL CYCLE IN TRYPANOSOMES

MENDES, I.C.[1]; CALDERANO, S.G.[2]; ELIAS, M.C.[2]; MCCULLOCH, R.[3]; MACHADO, C.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.INSTITUTO BUTANTAN, SAO PAULO, SP, BRASIL; 3.UNIVERSITY OF GLASGOW, GLASGOW, ESCOCIA.
e-mail:isacecilia@gmail.com

XPC and CSB proteins participate in the initial steps of the Nucleotide Excision Repair (NER) pathway, responsible for detecting and repairing lesions that alter DNA conformation. XPC performs distortion detection in Global Genome-NER, while CSB is recruited by stalled RNA polymerase II during Transcription-Coupled-NER. The extent of the genome of trypanosomatids that is transcribed is highly unusual, since most genes are co-transcribed in multigene transcription units, each from a single promoter. The consequences of this genetic organisation for trypanosomatid genome stability have been little explored. For instance, DNA repair and cell cycle control mechanisms are intimately related. Checkpoint activation is normally the first cell response during DNA damage detection and signalling. The aim of this study was to evaluate the roles of XPC and CSB in T. cruzi and T. brucei cell cycle and DNA repair. With this purpose, we generated T. cruzi single knockouts strains for XPC (XPC+/-) and CSB (CSB+/-), and the same genes were silenced by RNA interference in T. brucei. Currently, our data show that T. cruzi XPC and CSB single knockouts strains present altered cell cycles and resistance to genotoxic agents. While XPC+/- cells shows delayed cell cycle progression and multinucleated cells, CBS+/- cells show a faster cell cycle and no changes in DNA content. CSB+/- cells were more sensitive to MMS and UV treatments, unlike XPC+/- parasites. RNAi of CSB in T. brucei led to increased sensitivity and altered mRNA levels after treatment with UV and cisplatin, while RNAi of XPC was found to be lethal. This lethality may be because XPC acts in inter-strand crosslink repair, since RNAi caused elevated sensitivity to cyclophoshamide. These findings reinforcing the hypothesis that DNA repair genes are involved in cell cycle progression and demonstrate substantial divergence of NER in trypanosmatids, suggesting this may be the best example of any genome repair machinery being a potential drug target against trypanosomatid parasites. **Supported by:**CNPq CAPES FAPEMIG

PV97 - *TRYPANOSOMA CRUZI* TRANSCIPTOME DURING EPIMASTIGOTE GROWTH

SANTOS, C.M.B.[1]; RAMPAZZO, R.C.P.[2]; KRIEGER, M.A.[2]; PROBST, C.M.[2]; PAVONI, D.P.[2]
1.ICC/FIOCRUZ-PR E UFPR, CURITBA, PR, BRASIL; 2.ICC/FIOCRUZ-PR, CURITBA, PR, BRASIL. e-mail:cyndiamara@hotmail.com

Trypanosoma cruzi is the etiological agent of Chagas disease. In culture, the epimastigote form growth can be divided in two phases: exponential, when the parasite multiplies constantly, and stationary, when they respond to starvation and high density entering into a nonproliferative state. Few cells from the stationary phase may undergo metacyclogenesis, giving rise to non-dividing, infective metacyclic trypomastigotes. During the growth curve, many changes occur in the cell, and understanding these is critical to the comprehension of the *T. cruzi* biology. Our goal was to perform transcriptomics analysis of *T. cruzi* epimastigote growth curve by RNA-seq, as verifying mRNAs relative abundance during the whole growth curve will contribute to expand the knowledge on the metabolic activity and may indicated possible genes involved in the metacyclogenesis. Three biological replicates of Dm28c strain epimastigotes were followed for 10 days, harvesting each day 10^8 and 10^9 cells for total and polysomal RNA extraction, respectively. For SOLiD sequencing we prepared two libraries, for total and polysomal RNAs. The number of reads generated from total and polysomal RNA libraries was 455 and 412 millions and the average number per sample was 15.7 millions and 19.6 millions, respectively. We identified 2695 and 3085 differentially expressed genes (DEG) at 10% FDR. As expected, a large fraction of DEGs has a metabolic function. Interestingly, several functional gene categories (kinases, RNA binding proteins, unknown function proteins) show distinct transcript level modulation during the growth curve, enabling the classification of these broad, interesting categories into smaller, more cohesive subgroups. Deeper bioinformatics analysis, including clustering of DEG, Gene Ontology (GO) terms enrichment and KEGG metabolic pathway identification are underway. This work represents the first assessment of gene expression modulation in the growth curve of a kinetoplastid protozoa. **Supported by:**CAPES , Fiocruz

PV98 - FUNCTIONAL EVALUATION OF *LEISHMANIA MAJOR* ARGININE METHYLTRANSFERASE 6 (*LMJ*PRMT6)

LORENZON, L.B.; FERREIRA, T.R.; RODRIGUES, M.A.; CRUZ, A.K.
DEPTO. DE BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGÊNICOS, FMRP-USP, RIBEIRAO PRETO, SP, BRASIL. e-mail:lucas_lorenzon@hotmail.com

Gene expression regulation in *Leishmania* parasites occurs mostly at post-transcriptional level. In this context, RNA binding proteins (RBPs) are relevant protagonists that modify transcript stability and/or translation rates. Recent studies show that posttranslational modifications such as arginine methylation are relevant in several pathways in other organisms and different classes of Arginine Methyltransferases (PRMTs) have been shown to not only modify histones but also many RBPs. Therefore, proteins that modify RBPs and consequently their interaction with RNA and/or partner proteins become interesting objects of study in *Leishmania* spp. In this work we report our studies with *Lmj*PRMT6 (LmjF.16.0030). We produced and purified this protein in a heterologous system (*Escherichia coli*) to conduct *in vitro* methylation assays and to raise antibodies against it. As soon as we get *Lmj*PRMT6 antiserum, we expect to determine its subcellular localization and its expression pattern during *L. major* development. We obtained an overexpressor and a heterozygous line for *Lmj*PRMT6 in *L. major* LV39; the second round of transfection to obtain the null mutant is in course. *L. major* transfectants overexpressing HA-tagged PRMT6 allowed us to investigate possible *Lmj*PRMT6 targets using immunoprecipitation assays and the effect of its expression levels on virulence in Balb/c mice. Among other proteins, immunoprecipitation assays recovered tryparedoxin peroxidase, which has been already described as a PRMT ligand in *Trypanosoma brucei*. *L. major* tryparedoxin peroxidase (LmjF.15.1040) was, thus, expressed in *E. coli* and purified to be evaluated as *Lmj*PRMT6 substrate in methylation assays. The *Lmj*PRMT6 transfectants will be important tools to evaluate whether this protein affects *L. major* degree of virulence. **Supported by:**FAPESP (2013/13237-9)

PV99 - HEME DEGRADATION PATHWAY IN *TRYPANOSOMA CRUZI*: NEW INSIGHTS INTO HEME FATE IN THIS CHAGAS DISEASE PROTOZOAN

CUPELLO, M.P.[1]; FERREIRA, A.T.S.[2]; SOUZA, C.F.[2]; NOGUEIRA, N.P.A.[1]; VIEIRA, C.S.D.[1]; LARANJA, G.A.T.[1]; VALENTE, R.H.[2]; PAES, M.C.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:mauricio.cupello@yahoo.com.br

Trypanosoma cruzi, the causal agent of Chagas disease, must cope with diverse environmental conditions during its life cycle and is dependent on the host for some nutritional needs. Among these dependencies is the heme compound, Fe-protoporphyrin IX, a crucial growth factor that the parasite cannot synthesize on its own. On the other hand, it is well-established that this heme compound, in its free form, induces cytotoxic activity; hence, there must be a still unknown degradation pathway for this porphyrin, since *T. cruzi* effectively metabolizes free heme. In this work we have initially shown that epimastigotes, cultivated with heme, yielded the compounds verdoheme and biliverdin, as determined by HPLC with diode array detector. Furthermore, we observed ion species of *m/z* 583.4 and *m/z* 619.4 from epimastigote extracts, as detected by direct infusion on an LTQ Orbitrap mass spectrometer. Typical biliverdin and verdoheme daughter-ion species were generated from *m/z* 583.4 and *m/z* 619.4 fragmentations, respectively. Finally, through immunoblotting assays of epimastigote extracts, we observed recognition by an antibody against mammalian heme oxygenase-1 (HO-1). Taken together, these results reinforce the existence of an active heme degradation pathway in this parasite. Although HO-1 has not been described in the parasite's genome, our results offer new insights into heme metabolism in *T. cruzi*, which could eventually unveil potential therapeutic targets. **Supported by:**INCT-EM, FAPERJ, PIBIC-UERJ

PV100 - TELOMERES NATURALLY SHORTEN DURING LEISHMANIA AMAZONENSIS DEVELOPMENT CYCLE

SEGATTO, M.[1]; DA SILVA, M.S.[2]; NUNES, V.S.[3]; RODRIGUEZ, F.G.[4]; SANTANA-LEMOS, B.A.A.[4]; GIORGIO, S.[2]; CALADO, R.T.[4]; CANO, M.I.N.[1]
1.UNESP, BOTUCATU, SP, BRASIL; 2.UNICAMP, SAO PAULO, SP, BRASIL; 3.UNIFESP, SÃO PAULO, SP, BRASIL; 4.USP, RIBEIRÃO PRETO, SP, BRASIL.
e-mail:mamasantos2003@yahoo.com.br

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 two hosts: an insect and other mammalian. Inside the insect vector, Leishmania promastigote forms 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 preliminary results showed that, no matter the assay performed, amastigotes telomeres are shorter than promastigotes telomeres, with a difference of about 500 bp. This difference may be attributed to the fact that amastigotes lives and multiplies in a very stressor environment and in a temperature (37oC) which is perhaps non-permissive for telomerase activity. We intend to compare telomerase activity in both Leishmania developmental forms in order to confirm this hypothesis. **Supported by:**FAPESP

PV101 - METACYCLOGENESIS IN L. GUYANENSIS: PURIFICATION OF METACYCLIC PROMASTIGOTES USING BAUHINIA PURPUREA LECTIN.

MENDES, B.P.[1]; SILVA-JR, I.[2]; DA MATA, J.P.[1]; VIEIRA, L.Q.[1]; RIBEIRO-DIAS, F.[2]; HORTA, M.F.M.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFG, GOIÂNIA, GO, BRASIL.
e-mail:phorta@icb.ufmg.br

Parasites of the genus *Leishmania* undergo several modifications to become infective for the mammalian host. This process, called metacyclogenesis, takes place in the gut of sandfly vectors, but can also occur in axenic cultures of promastigotes. This differentiation renders the parasite adapted to the hostile host macrophages environment, where they overcome the immune response and succeed. This process is characterized by morphological changes of promastigotes, biochemical alterations in their lipophosphoglycan (LPG) and variation in gene expression. Metacyclic forms are small and thin forms with the flagellum at least twice the body size. They are more resistant to complement and more infective than their precursor procyclic forms. To date, there are no data whatsoever about metacyclogenesis in *Leishmania guyanensis*. In the present work, we have defined metacyclogenesis in *L. guyanensis* in axenic culture developing a method to purify metacyclic forms. Using *Bauhinia purpurea* lectin (BPL), which binds to *L. braziliensis* LPG of procyclic forms, agglutinating them, and has been used for negative selection of metacyclic forms, we were able to purify and study various features of BPL-negative (BPL-) forms. By flow cytometry and optical microscopy we have shown that parasites change their morphology during culture, reducing the body size and elongating the flagellum at the beginning of the stationary phase (4-5 days). The BPL- parasites were also more resistant to complement-mediated lysis and more infective to macrophages, features already described for other species of *Leishmania*. In conclusion, *L. guyanensis* also undergo metacyclogenesis in axenic culture and the metacyclic forms share the features already described for other species, including the loss of their ability to bind to BPL, as described for *L. braziliensis*. This is the first systematic study of metacyclogenesis in *L. guyanensis* and the first described method to purify metacyclic forms from this species. **Supported by:**FAPEMIG, CNPq

PV102 - SEARCHING FOR GENES POTENTIALLY INVOLVED IN VIRULENCE CONTROL IN *LEISHMANIA MAJOR*

LONDOÑO, P.A.C.; FERREIRA, T.R.; TERRÃO, M.C.; PINZAN, C.F.; CRUZ, A.K.
UNIVERSIDADE DE SÃO PAULO, RIBEIRAO PRETO, SP, BRASIL.
e-mail:pauliscast@gmail.com

Human infections with *Leishmania spp*. give rise to a spectrum of clinical manifestations that may vary from mild cutaneous, to disfiguring and diffuse tegumentary lesions or to the severe visceral disease. The progress and clinical manifestations of the disease is closely related to the infecting parasite species and mammalian host response to infection. In this context, the search for and characterization of genes involved in the parasite virulence is relevant to improve understanding of pathogenicity. In previous studies conducted in the laboratory it has been shown that *L. braziliensis* and *L. major* parasites overexpressing the Spliced Leader RNA (SL RNA) lost the strain original level of virulence in *in vivo* infection. The proteome and transcriptome profiles of the attenuated parasites were examined and led to the identification of over- or under-expressed genes that might individually contribute to the observed loss of virulence. With the aim of identifying novel genes correlated with virulence, six of the differentially expressed genes were selected and their coding sequences were cloned in pSPBT1YNeoαIR , transfected in *L. major* and maintained under drug pressure. Two strains of *L. major* were transfected: an avirulent line (LT252) and a virulent one (LV39). Growth behavior of axenic promastigotes was determined to be similar to parental and control transfectants. A pilot experiment of infection of susceptible mice model (BALB/c) with the six transfectants and control was conducted; overexpression of 3 genes (LmjF.12.0660; LmjF.36.5640; LmjF.36.4740) decreased virulence of *L. major* LV39 in BALB/c (P <0.05) when compared to control transfectant. Preliminary results suggest that the proteins produced by these genes are all cytoplasmic. Another *in vivo* infection experiment will be conducted and the ability of these parasites to infect macrophages *in vitro* will be evaluated. **Supported by:**FAPESP, CNPq

PV103 - THE *IN VITRO* TRANSMISSION BLOCKING POTENTIAL OF MEFAS, A HYBRID COMPOUND DERIVED FROM MEFLOQUINE AND ARTESUNATE

PENNA-COUTINHO, J.[1]; ALMELA, M.J.[2]; HERREROS-AVILES, M.E.[2]; BOECHAT, N.[3]; KRETTLI, A.U.[1]

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.GLAXOSMITHKLINE, MADRID, ESPANHA; 3.FARMANGUINHOS, RIO DE JANEIRO, RJ, BRASIL.

e-mail:juliapenna@cpqrr.fiocruz.br

Mature gametocytes of *Plasmodium sp* are responsible for parasite transmission from the mammalian host to the mosquito, and represent specific targets for transmission-blocking interventions. Both male and female *P. falciparum* gametocytes are required fo ite transmission, and may show different responses to antimalarial drugs. Se al screening protocols aim at molecules with dual activity against th f the parasites. MEFAS, a synthetic hybrid salt deriv (AS), shown to be more effective against P combination of AS and MQ (de cultured and mature escence assay. Bas ocytes, after 48 h of (IC_{50} = 19.7 ± 5.1 µM), and A of MEFAS on the functional capability o *falciparum* was also investigated and measured fo activate and form gametes expressing the marker *Pf*s25, res male gametocytes were more sensitive to MEFAS (IC_{50} ~ 17 nM) than the ametocytes (IC_{50} = 70 nM). MEFAS was three to four times more active than the original drugs against mature gametocytes and up to twelve times more active than AS against male gametocytes. In conclusion, these data support MEFAS as an antimalarial with a dual-function, which can be used to block the malaria infection and parasite transmission. Now, is necessary to evaluate if MEFAS is more active than the mixture of AS and MQ. **Supported by:**CNPq/Ministério da Saúde

PV104 - PROTEOMIC CHANGES DURING *TRYPANOSOMA RANGELI* DIFFERENTIATION *IN VITRO*

LÜCKEMEYER, D.D.[1]; STOCO, P.H.[1]; WAGNER, G.[2]; MOURA, H.[3]; BARR, J.[3]; STEINDEL, M.[1]; GRISARD, E.C.[1]

1.UFSC, FLORIANÓPOLIS, SC, BRASIL; 2.UNOESC, JOAÇABA, SC, BRASIL; 3.CDC, ATLANTA, ESTADOS UNIDOS. e-mail:edmundo.grisard@ufsc.br

Trypanosoma rangeli is a hemoflagellate parasite widely distributed in Central and South Americas and occurring in sympatry with *Trypanosoma cruzi*. Little is known about the *T. rangeli* life cycle within mammalian hosts since it is considered as non-pathogenic. In the present study we have evaluated the *T. rangeli* protein profiles during *in vitro* cell differentiation towards the infective trypomastigote form, aiming the detection of differentially expressed proteins. Soluble protein extracts were obtained from parasites during the differentiation process starting with epimastigote forms (T0) and every two days (T2, T4, T6) up to the 8th day were trypomastigotes forms are obtained. Three proteomic approaches were used: 1DE and 2DE gel-based following MS analysis (Gel LC-MS/MS) and a direct, gel-free MS analysis (LC-MS/MS). Using biological and technical replicates, proteins revealing differential expression were further analyzed by qPCR and western blotting using antisera obtained with recombinant proteins. A total of 1,455 non-redundant *T. rangeli* proteins were identified (1,410 by 1DE; 182 by 2DE and 716 by gel-free approaches). Among which, 12 were selected for further analysis due their differential expression: spermidine synthase (ES), histidine ammonia-lyase (HAL), glycosomal protein (Gim5A), mitochondrial aspartate aminotransferase (mASAT), apical membrane protein (AMA-1), mitochondrial RNA binding protein (MRP2), kinetoplastid membrane protein 11 (KMP-11), calpain cysteine peptidase (CCP) and four hypothetical proteins (HP). qPCR analysis showed a global reduction of the mRNA levels of these proteins in trypomastigotes rather than epimastigotes. ES, HAL, MRP2 and HP1 showed higher expression in epimastigotes forms (T0). Based on these data, we will carry out functional studies to assess the importance of these proteins in *T. rangeli* biological cycle. **Supported by:**CAPES, CNPq, FINEP, CDC and UFSC

PV105 - INVESTIGATION OF THE INVOLVEMENT OF THE CHECKPOINT PROTEIN LMHUS1 IN *LEISHMANIA* GENOME PLASTICITY

OBONAGA GOMEZ, R.; DAMASCENO, J.D.; ORSINI TOSI, L.R.
FMRP-USP, RIBEIRAO PRETO, SP, BRASIL.
e-mail:obonagar@hotmail.com

Gene copy number variation is a hallmark of genomic plasticity in *Leishmania*. The phenomenon is also commonly observed in tumor cells and mainly results from a deficient cell cycle checkpoint response. In mammals and yeast, a three-protein complex (the 9-1-1 clamp) plays a central role in the recognition of DNA damage, and in the signaling events that lead to cell cycle arrest and DNA damage repair. We have characterized the *Leishmania* homologues of Hus1 and Rad9, which compose the 9-1-1 clamp in other eukaryotes. We found that LmHus1 and LmRad9 not only are recruited to the chromatin upon genotoxic stress, but also forms a DNA damage responsive complex in vivo. We also observed that reduced levels of LmHus1 and LmRad9 affected the parasite ability to cope with the genotoxic stress caused by hydroxyurea, camptothecin or methyl methanesulfonate treatment. Based on the evidence that LmHus1 and LmRad9 are involved in DNA metabolism we speculate on whether these proteins participate in the molecular mechanisms underlying genome plasticity in *Leishmania*. We have used EdU incorporation to monitor DNA replication in individual WT and LmHus1-deficient cells and observed that LmHus1 deficiency led to significant deregulation of DNA replication upon genotoxic stress. We have also compared these cells upon methotrexate (MTX) selection. MTX resistance can be mediated by the amplification of DHFR-TS and/or PTR1 genes. Quantitative PCR and pulse field gel-separated chromosomes were used to analyze DHFR-TS and PTR1 copy number variation in these cell lines. The data revealed that a clear distinction between WT and LmHus1-deficient cells. Genotoxic stress also had an impact on DHFR-TS copy number variation upon MTX selection. The results from these experiments suggest that LmHus1 is involved in the genome plasticity of *Leishmania*. Our current work is focused on the investigation of the molecular mechanisms underlying the role of LmHus1 in gene copy number variation in the parasite. **Supported by:**FAPESP, CAPES, CNPq

PV106 - THE ROLE OF SMYB1 PROTEIN OF SCHISTOSOMA MANSONI IN RESPONSE TO OXIDATIVE STRESS IN TRYPANOSOMATIDS

ROCHA, E.A.[1]; AGUIAR, P.H.N.[1]; DIAS, S.R.C.[1]; HILÁRIO, H.[1]; CERQUEIRA, P.G.[1]; SILVA, V.G.[1]; TEIXEIRA, S.M.R.[1]; MACEDO, A.M.[1]; MCCULLOCH, R.[2]; MACHADO, C.R.[1]; FRANCO, G.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UNIVERSITY OF GLASGOW, GLASGOW, REINO UNIDO. e-mail:almeida.elizangela@gmail.com

Y-box binding proteins (YBP) are considered to be multifunctional regulators of gene expression that present two characteristic domains: a highly conserved nucleic acid binding domain (cold shock domain, CSD) and a less conserved C-terminal domain (tail domain). SMYB1 is a Schistosoma mansoni protein that presents a CSD highly similar to CSDs from other YBP family members. S. mansoni, the etiologic agent of schistosomiasis, is a trematode parasite with a complex life cycle that alternates between two different hosts and also lives in diverse environments. The characterization of proteins involved in the regulation of gene expression is of great importance for the understanding of molecular events that control physiological and morphological changes in such organisms. The human YB1 protein is involved in the mismatch DNA repair in human mitochondria. The present study aims to evaluate if SMYB1 is able to work in the same pathway to protect against oxidative stress. SMYB1 was able to complement the oxidative stress deficiency present by Trypanosoma cruzi MSH2 single knockouts and T. brucei MSH2 double knockout. The functional complementation of the MSH2 deficiency in T. cruzi and T. brucei (bloodstream form) by SMYB1 suggests that this protein also may act in oxidative stress responses. The immunolocalization experiments withT. cruzi, single MSH2 knockout and WT cells, located SMYB1 protein in the cytoplasm and possibly in the mitochondria. These data suggest that SMYB1 protein could work in oxidative stress response in S. mansoni cells, a similar function that is made by MSH2 protein in Trypanosomatids. **Supported by:**Fapemig, capes, cnpq

PV107 - FROM WHOLE *LEISHMANIA INFANTUM* PROTEOME TO VACCINE TARGETS: SELECTION AND CLONING OF POTENTIAL PROTEINS TO VACCINE DEVELOPMENT AGAINST CANINE VISCERAL LEISHMANIASIS USING BIOINFORMATICS

FORTES DE BRITO, R.C.[1]; VELLOSO, J.P.L.[1]; SOARES REIS, L.E.[1]; ALMEIDA FERREIRA, S.[2]; CORRÊA-OLIVEIRA, R.[3]; RUIZ, J.C.[4]; REIS, A.B.[2]; DE MELO RESENDE, D.[1]

1.LABORATÓRIO DE PESQUISAS CLINICAS - CIPHARMA/UFOP, OP, MG, BRASIL; 2.LABORATÓRIO DE IMUNOPATOLOGIA - NUPEB/UFOP, OP, MG, BRASIL; 3.LABORATÓRIO DE IMUNOLOGIA CELULAR E MOLECULAR, CENTRO DE PESQUISAS RENÉ RACHOU – FIOCRUZ MINAS, BH, MG, BRASIL; 4.GRUPO INFORMÁTICA DE BIOSSISTEMAS, CENTRO DE PESQUISAS RENÉ RACHOU – FIOCRUZ MINAS, BH, MG, BRASIL. e-mail:rorybrito@gmail.com

Canine visceral leishmaniasis (CVL) is a zoonosis in Latin America and dogs have a central role in the urban cycle of *Leishmania infantum*. Reverse vaccinology is a post-genomic approach that allows the design of vaccines with reduced time making epitope prediction for T and B cells in silico, which are important for protective immune responses. Previous studies showed the feasibility of epitope prediction in proteins of protozoa using open source algorithms. This work was performed in two steps. Firstly, potential proteins from whole *L. infantum* predicted proteome were selected based on bioinformatics tools as follows: NetCTL and NetMHC for MHC-I binder; NetMHCII for MHC-II binder; BepiPred, AAP12 and BCPred12 for B cells; Sigcleave, TargetP and WoLFPSORT for the prediction of subcellular localization of proteins. Using these algorithms to predict epitopes binders we analyzed 12 human MHC-I alleles and seven mouse MHC-I alleles. In the context of MHC-II, we analyzed 14 human alleles and three mouse alleles. To integrate the results and select the potential proteins, a relational database was created in MySQL language. Secondly, we cloned the genes previously selected in an eukaryotic system to express the potential proteins. The genes were cloned into the expression vector pPICZαA in bacteria (*Escherichia coli*) and the clones were confirmed by PCR and restriction enzyme digestion. After that, the recombinant plasmids were linearized, transfected and integrated to the yeast genome (*Pichia pastoris*). Recombinant clones were selected by PCR. Through bioinformatics tools and some appropriate selection criteria, e.g. proteins with $TCD8^+$/$TCD4^+$ epitopes binders, four candidates proteins considered vital for the parasite biology were selected. The cloning of two genes that encode two of the selected proteins and the recombinant plasmids transfection were successful. The resource developed represents an important tool that can be used to drive vaccine targets against CVL. **Supported by:**CAPES, CNPq, FAPEMIG AND UFOP

PV108 - THE IMPORTANCE OF GLUTAMINE METABOLISM IN THE DIFFERENTIATION AND INFECTIVITY OF TRYPANOSOMA CRUZI

DAMASCENO, F.S.; RAPADO, L.N.; CRISPIM, M.; BARISON, M.J.; FURUSHO PRAL, E.M.; SILBER, A.M. *ICB-USP, SAO PAULO, SP, BRASIL.* e-mail:flaviadamasceno@usp.br

Chagas' Disease is caused by the protozoan parasite Trypanosoma cruzi. The participation of amino acids metabolism in different biological processes in the parasite, such as metacyclogenesis, differentiation, osmoregulation and proliferation is well established. In particular, our group described the role of proline and glutamate in the differentiation between two intracellular stages and in the resistance to stress conditions (thermal, oxidative, pH and metabolic). Currently, our group is approaching the involvement of glutamine, histidine, serine and branched chain amino acids in several biological parasite processes. In the present work the importance of glutamine metabolism in differentiation and infection of T. cruzi was evaluated. Glutamine was able to support the differentiation process from epimastigotes to metacyclic trypomastigotes in TAU medium, at the same level than the traditionally used glucose/proline/aspartate/glutamate mix (3AAG). The infectivity of the obtained trypomastigotes was also evaluated resulting similar to that of metacyclic obtained from 3AAG. Moreover, to evaluate the participation of glutamine in infection, trypomastigotes forms obtained from infected CHO cells, were stressed in PBS for 4 hours and then recovered in different metabolites: glutamine, glutamate, proline or glucose for 1 hour (as control, the parasites were kept in RPMI medium). After that time the capacity of infectivity was evaluated. The parasites recovered in glutamine were able to infected cells similar to the parasites kept in RPMI, and higher than those recovered in glutamate. In conclusion, glutamine was able to provide the necessary requirements for metacyclogenesis and infection. The role of glutamine metabolism makes of the enzymes involved in its biosynthesis a putative drug target. This possibility is presently being explored. **Supported by:**FAPESP

PV109 - INVESTIGATION OF A HIGHLY DIVERGENT RAD1 HOMOLOG IN LEISHMANIA DNA DAMAGE RESPONSE.

SANTOS, E.V.; DAMASCENO, J.D.; ORSINI TOSI, L.R.
FMRP/USP, RIBEIRAO PRETO, SP, BRASIL.
e-mail:elainevs@usp.br

In many eukaryotes, the Rad9-Hus1-Rad1 (9-1-1) checkpoint complex participates in the early steps of the DNA damage response to replicative stress and is a central player in genome homeostasis. We have investigated the existence of homologues of the 9-1-1 components in Leishmania and found that LmRad9 and LmHus1 are phylogenetically related to the 9-1-1 complex subunits from other eukaryotes. We have shown that LmHus1 participates in the DNA damage response and also interacts with LmRad9 homologue in vivo. Both, LmHus1 and LmRad9 also participate in telomere maintenance. LmHus1 is well conserved in eukaryotes while LmRad9 presents a much lower conservation. Interestingly, conventional searches in databases suggested that the parasite genome does not encode a Rad1 homologue. Therefore, we set out to investigate the existence of a Leishmania Rad1 homologue. To investigate the conservation of Rad1 in the parasite, we performed tridimentional structure modelling using the human Rad1 structure and the Protein Homology/AnalogY Recognition Engine V2.0 (Phyre2). We identified the predicted protein Lm362 as a possible Leshmania Rad1. An overexpressed tagged version of Lm362 localized both to the nucleus and cytoplasm. We have also found that Lm362 levels were increased upon exposure to hydroxyurea suggesting the involvement of this protein in the response to genotoxic stress. Moreover, we identified LmRad9 in a pulldown experiment that used in vitro translated Lm362 as bait suggesting a possible interaction between Lm362 and LmRad9 in vivo. Altogether, these preliminary data indicates the participation of Lm362 in the Leishmania DNA damage response possibly as part of a complex homologue to 9-1-1. Our current efforts are focused on the generation of appropriate tools to better investigate the function of Lm362, such as, anti-Lm362 antibodies and a Lm362-deficient cell line. **Supported by:**FAPESP, CNPq, CAPES

PV110 - INFLUENCE OF THE EVOLUTIVE FORM OF *TRYPANOSOMA CRUZI* TCIV IN THE EVOLUTION OF EXPERIMENTAL INFECTION AND SUSCEPTIBILITY TO TREATMENT WITH BENZNIDAZOLE

GRUENDLING, A.P.[1]; DIAS, G.B.M.[1]; TESTON, A.P.M.[2]; MONTEIRO, W.M.[3]; GOMES, M.L.[2]; DE ARAÚJO, S.M.[2]; TOLEDO, M.J.O.[2]
1.UFSC, FLORIANOPOLIS, SC, BRASIL; 2.UEM, MARINGÁ, PR, BRASIL; 3.UEA, MANAUS, AM, BRASIL. e-mail:aninha_gru@bol.com.br

In the absence of clinical trials to evaluate the efficacy of specific chemotherapy of Chagas disease in the Amazon region, where the disease emerges as an important public health problem, experimental in vivo studies may clarify important aspects of the behavior of isolates of Trypanosoma cruzi towards treatment with benznidazole (BZ). The purpose of this study was to perform biological analisys of mice inoculated with blood and metaciclic trypomastigotes of T. cruzi and BZ-treated in the acute phase of infection. In this work, we evaluated four strains belonging to TcIV isolated from pacients in acute phase of Chagas disease from Amazon region. Groups of 26 Swiss mice with 21 to 28 days were inoculated with 1x104 blood tripomastigotes (BT) or 2x106 metacyclic trypomastigotes (MT)/animal with each strain: 13 were treated with BZ 100 mg/kg/day (TBZ), for 20 consecutive days, and 13 constituted the non treated group (NT). Parasitemia was evaluated daily from day 3rd after inoculation. Infectitivity (%INF) and cure rates were determinate through blood fresh examination, hemoculture and pcr. The %INF ranged 57-90% in mice inoculated with BT and BZ-treated and 7-58% in those inoculated with MT and BZ-treated. In NT mice, the %INF was 100% in animals inoculated with BT and ranged 54-80% in those inoculated with MT. The cure rate in mice inoculated with BT and BZ-treated ranged 30-71% and 14-85% in animals inoculated with MT and BZ-treated. The mortality rate in BZ-treated mice ranged 0-7% for BT group and 0-23% for MT group. In NT mice, the mortality ranged 0-100% in animals inoculated with BT and 0-15% in those inoculated with MT. These results show that the evolutive form of Trypanosoma cruzi can have influence the course of infection, the mortality and cure rate in mice experimentally infected. **Supported by:**CNPq Capes Fundação Araucária

PV111 - A TIGHT REGULATION CONTROLLING THE EXPRESSION OF TWO *TRYPANOSOMA BRUCEI* EIF4G HOMOLOGUES

MOURA, D.M.N.[1]; NASCIMENTO, J.F.[2]; CARRINGTON, M.[2]; DE MELO NETO, O.P.[3]
1.CPQAM/UFPE, RECIFE, PE, BRASIL; 2.UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, REINO UNIDO; 3.CPQAM, RECIFE, PE, BRASIL.
e-mail:d.moura@cpqam.fiocruz.br

The initiation stage of protein synthesis in eukaryotes can be divided into several steps which include the attachment of pre-initiation complexes to the mRNA, recruitment of 40S ribosome subunit, scanning of 5'UTR and assembly of elongation-competent 80S ribosomes. The entire process requires several eukaryotic initiation factors (eIFs), including the eIF4F complex. eIF4F comprises the cap-binding protein eIF4E, the RNA helicase eIF4A and eIF4G, which functions as a 'scaffold' for the whole complex. There is an unusual number of homologues of eIF4F components in trypanosomatids and this has led to speculation that this proliferation has arisen to facilitate control of translation initiation. Five eIF4G homologues have been identified, but only two (EIF4G3 and EIF4G4) display properties compatible with a role in translation initiation. Procyclic cells expressing transgenes encoding EIF4G3, EIF4G4 and variants were evaluated and it was noticed that transgene expression can lead to a decrease in the levels of the respective endogenous proteins depending on the functionality of the protein tested. Here, we aimed to investigate how the EIF4G3 and EIF4G4 expression levels are regulated. Time course experiments showed that the decrease of endogenous proteins occurs soon after transgene induction, however, for EIF4G4, no equivalent differences in its mRNA levels were observed, suggesting a co-translational or post-translational regulation. To investigate a regulation mediated by proteasome, we set out to develop a *T. brucei* cell line expressing a reporter protein consisting of an unstable ubiquitin-GFP fusion. The constitutively expressed protein is effectively stabilized when treated with proteasome inhibitors, such as lactacystin. Our aim is to see if the same stabilization effect can be induced by the inhibitor upon the endogenous EIF4G3 and EIF4G4 levels and verify if this mechanism controls the intracellular concentration of the two eIF4G homologues. **Supported by:**CAPES, CNPq, FIOCRUZ and FACEPE

PV112 - ECO-EPIDEMIOLOGICAL RESEARCH AND BIOGEOGRAPHIC FACTORS ANALYSIS OF CHAGAS DISEASE IN 21 SCHOOLS AT VINTO MUNICIPALITY, COCHABAMBA - BOLIVIA

MERCADO, N.M.; DIAZ, J.L.N.; CRESPO, C.K.O.
UNIV. MAYOR DE SAN SIMON, COCHABAMBA, BOLÍVIA.
e-mail:medrano.nora@gmail.com

Specific Objectives : 1) Determine the seroprevalence of Chagas disease in 21 Schools at Vinto Municipality. 2) Apply Geographic Information Systems (GIS) and spatial analysis to map seropositive´s houses. 3) Obtain risk maps through spatial multicriteria analysis based on biogeographical factors.
Method: 1) Analysis by indirect hemagglutination, ELISA and Xenodia
2) Using a GPS the seropositive students houses were geo
processed in order to obtain a map of seropositiv
and 9.3 ARGIS software. 3) Spatial M
map based on a set of biog
Results: From 10
disease
for Chagas
condary level, 4.03%
ale; 12% were xenopositives, from
t
T
an area of Vinto Municipality in an area that has 0.81
to
esence of triatomine, located at an altitude between 2531-2902
me
vel, in a zone with very high population density considering Bolivia standard
100
abs / km (SEDAC, 2005) and are located at a semi-arid environment with xeric bioclimatic characteristics in a zone with high anthropogenic influence and disturbed vegetation.
Conclusions: There is an eco-epidemiological situation that is favorable for the transmission of Chagas disease at Vinto Municipality, due to the presence of environmental characteristics and high population density that contributes for the spread of the disease, despite the vectorial control campaigns **Supported by:**Agencia Sueca de Cooperación para el Desarrollo Internacional (Asdi)

PV113 - ENOLASE EXTERNALIZATION AND APOPTOTIC MIMICRY BY *LEISHMANIA AMAZONENSIS*

PAES, L.S.[1]; GOMES, M.T.[1]; WANDERLEY, J.L.M.[1]; BARCINSKI, M.A.[2]; MEYER-FERNANDES, J.R.[1]; UCKER, D.S.[3]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 3.UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE, CHICAGO, ESTADOS UNIDOS.
e-mail:lisvanesp@yahoo.com.br

Apoptosis facilitates the removal of damaged, aged, and otherwise functionally inappropriate cells without harm to surrounding tissue. Cells undergoing apoptosis acquire new activities that enhance their clearance and modulate the function of viable cells. Externalization of the phospholipid phosphatidylserine (PS) on apoptotic cells promotes their engulfment. Distinct from PS, the externalization of glycolytic enzyme molecules, including enolase, is a common and early aspect of apoptotic cell death. Surface-exposed enolase appears to be responsible for the characteristic immunomodulatory gain-of-function acquired during apoptosis. It has become clear that the subversion of host functions through apoptotic mimicry is a strategy employed by a variety of pathogens. For example, viruses and parasitic protozoa expose PS, enhancing their entry into host cells. In *L. amazonensis*, this apoptotic mimicry co-exists with apoptotic death and is differentially regulated. While PS exposure and apoptotic death are linked normally in a sub-population of parasite promastigotes, the infectious amastigote form of the parasite externalizes PS homogeneously, independent of cell death. We explored enolase exposure by *L. amazonensis*. Enolase externalization was assessed cytofluorimetrically on different forms of the parasite. Our data indicate that enolase is externalized selectively on metacyclic promastigotes. Approximately 30% of viable promastigotes expose enolase, and in those cells, enolase appears to be externalized completely. As with authentic apoptotic cells, externalized enolase molecules on the *L. amazonensis* surface are enzymatically inactive. By comparison, the externalization of another glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), is dissociable and significantly less extensive. We currently are evaluating the functional role of enolase in the immunomodulatory activity of *L. amazonensis* sub-populations.
Supported by:FAPERJ, CAPES e CNPq

PV114 - MIRNAS: AND UNEXPECTED CARGO OF RHODNIUS PROLIXUS LIPOPHORIN ?

CARDOSO, L.S.[1]; LACERDA, L.L.[2]; ALVES, M.R.[2]; SILVA-NETO, M.A.C.[1]; ATELLA, G.C.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:lcardoso@bioqmed.ufrj.br

Rhodnius prolixus is a hematophagous insect, belonging to the family Reduviidae, that transmit Trypanosoma cruzi, agent of Chagas disease. In Latin America, besides malaria, the numbers that historically attract attention are those related to Chagas disease, where 18 million people are infected with T. cruzi. Despite never leave the digestive tract, T. cruzi is able to manipulate the insect metabolism by unknowns' mechanisms, including lipid metabolism that is the focus of our group. miRNAs present in insect vectors were first identified in the mosquito Anopheles gambiae, the vector of Plasmodium sp. During Plasmodium berghei infection, mir-988 is up regulated in insect midgut, supporting that infection modifies miRNA profile and vector manipulation by parasites. In the present work, we showed the presence of miRNAs in salivary glands, crop, midgut, fat body, ovary and hemolymph of Rhodnius prolixus. Besides, we also demonstrated that microRNAs are associated with lipophorin (Lp), the main hemolymphatic lipoprotein. Lp is synthesized and secreted by the insect fat body, a metabolic tissue functionally analogous to the vertebrate liver. Our hypothesis is that Lp circulates in hemolymph carrying microRNAs that could be delivered to target tissues.
Furthermore, we have chosen miRNAs related to lipid metabolism and infection and analyzed the homology between all insect related sequences found on mIRBase database. We have found 14 fourteen conserved sequences using such approach. We designed degenerated primers for all these sequences and by we have evaluated the levels of expression by qPCR of 13 of them (miR-34-5p, miR989-3p, miR-1175-3p, miR-92-3p, miR-92a-3p, miR-92c-3p, miR-970, miR-980-3p, miR989-3p, miR-6, miR-125-5p, miR-133-3p, miR-3804b-3p). The expression of most of them was confirmed in R. prolixus fat body. In conclusion, we are showing for the first time the characterization of microRNAs in R. prolixus and its association with Lp.
Supported by:CNPQ, FAPERJ, INCT-EM

PV115 - HIGH MOLECULAR WEIGHT PROTEINS IN THE FLAGELLUM/FAZ ARCHITECTURE IN *TRYPANOSOMA BRUCEI*

MOREIRA, B.P.; DE CASTRO, C.G.; BAQUI, M.M.A.
FMRP-USP, RIBEIRAO PRETO, SP, BRASIL.
e-mail:bpmbio@gmail.com

The causative agent of sleeping sickness, *Trypanosoma brucei*, has been used as experimental model for cellular, biochemical and molecular studies. The most prominent organelle, the flagellum harbors most of the cytoskeletal content of the cell and is responsible for cell division, motility, morphogenesis and infectivity. It comprises the axoneme and paraflagellar rod and both are connected to the cell body via the flagellar attachment zone (FAZ). In all genera of the Trypanosomatidae family, giant proteins were described as a novel class of high molecular mass phosphoproteins (1000-4000 kDa), which, besides their structural function, might play a role in the organization and regulation of cytoskeleton and its constituents. Here, we identified a giant protein of the *T. brucei* cytoskeleton. SDS-PAGE analysis of detergent-extracted whole cytoskeleton revealed the presence of a high molecular weight protein. After solubilization of the cytoskeleton with 1M NaCl, this giant protein remained in the flagellar fraction. We injected the corresponding band in Balb/C mice in order to generate a polyclonal serum. Indirect immunofluorescence and immunoelectron microscopy revealed the localization at a specific region between the cell body and the flagellum corresponding to the flagellar attachment zone (FAZ). Additionally, this protein did not co-localize with tubulin (axoneme) neither with paraflagellar rod (PFR) suggesting its participation in the FAZ structure. Through MS analysis, we obtained a list with putative candidates most of them with no function described so far. Known candidates included the recently described FLAM3, which is an essential protein in the FAZ architecture. The next steps include endogenous expression of tagged proteins and an RNAi assay to reveal what role they might play in the cell. Also, we want to investigate the giant protein-interacting partners.**Supported by:**FAPESP, CNPq, FAEPA

PV116 - *TRYPANOSOMA CRUZI* CYP51 EXPRESSION IS DEVELOPMENTALLY REGULATED AND LOCALIZED NOT ONLY IN ER BUT ALSO IN LIPID DROPLETS

CALVET, C.M.[1]; ALVAREZ, C.M.[1]; MESQUITA, L.[1]; PEREIRA, M.C.S.[1]; MCKERROW, J.[2]; PODUST, L.[2]
1.FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSITY OF CALIFORNIA SAN FRANCISCO, SAN FRANCISCO, ESTADOS UNIDOS.
e-mail:cmcalvet@ioc.fiocruz.br

CYP51 (sterol 14 alpha demethylase), a monooxygenase from the ergosterol biosynthesis pathway of *Trypanosoma cruzi*, has been stablished as a therapeutic target for treatment of Chagas disease. However, knowledge of its biology, expression and subcellular localization in *T. cruzi*, information that may influence drug design, is sparse. The level os CYP51 expression in the different stages of *T. cruzi* life cycle was assessed by Western blot (WB) analysis of parasites extracts, and the intracellular distribution of this enzyme was detected by indirect immunofluorescence and electron microscopy in trypomastigote, epimastigote and intracellular amastigote forms. WB revealed that amastigote and epimastigote replicative stages presented higher CYP51 expression levels than trypomastigotes. During intracellular cycle, amastigotes' CYP51 level maxed at 72h and then declined through differentiation into trypomastigotes. In all developmental forms, CYP51 was detected distributed in cytoplasm, partially associated with ER, as indicated by co-staining of anti-$CYP51_{TC}$ and anti-BiP (ER marker) antibodies. Localization of CYP51 in ER was also confirmed by CYP51 detection in ER fraction of *T. cruzi* extracts. Another portion of CYP51 was associated with vesicles which were identified as lipid droplets by BODIPY and anti-$CYP51_{TC}$ co-localization. No double staining was visualized with anti-$CYP51_{TC}$ and Mito-Tracker and Lyso-Tracker in all evolutive forms, suggesting the specific localization in ER and lipid droplets. Also, ultra-structural immunocytochemistry analysis of trypomastigotes and epimastigotes revealed staining profile consistent both with ER and vesicle localizations. Our results suggests that CYP51 expression is developmentally regulated, and ergosterol biosynthesis in *T. cruzi* might finish in lipid droplets and not only in the ER, as first expected given that most cytochrome P450 enzymes reside in this organelle. **Supported by:**CNPq, FAPERJ, FIOCRUZ, NIH

PV117 - BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF STRAINS OF *TRYPANOSOMA CRUZI* FROM GROUPS I, II AND III ISOLATED FROM BAHIA, RIO GRANDE DO SUL, SANTA CATARINA AND SAO PAULO.

RIBEIRO, A.R.[1]; ALMEIDA, L.A.[2]; LIMA, L.[3]; MONTEIRO, J.[4]; MORENO, C.J.G.[4]; GRAMINHA, M.A.S.[2]; TEIXEIRA, M.M.G.[3]; SILVA, M.S.[4]; STEINDEL, M.[5]; ROSA, J.A.[2]
1.UNICAMP, ARARAQUARA, SP, BRASIL; 2.UNESP, ARARAQUARA, SP, BRASIL; 3.USP, SÃO PAULO, SP, BRASIL; 4.UNL, LISBOA, PORTUGAL; 5.UFSC, FLORIANÓPOLIS, SC, BRASIL. e-mail:linerimoldi@bol.com.br

Goals: Biological and molecular characterization of ten strains of *Trypanosoma cruzi* isolated from Bahia, Rio Grande do Sul, Santa Catarina and Sao Paulo through the kinetic growth, parasitemia, cellular infection, serological reactivity and sequencing of the gene V7V8-SSU. Methodology: Firstly, the kinetic growth of epimastigoteswas performed by the inoculation of $5x10^6$parasites/mL in 5mL of LIT culture counted for 10 days. Secondly, the number of parasiteswere counted in the blood of Balb/c mice for 60 days to perform the parasitemic curve. Thirdly, the infection of J774 and peritoneal macrophages cultivated in RPMI culture were adjusted to a concentration of $5x10^6$ tripomastigotes/mL and after 72h, it was counted 200 macrophages and their amastigotes.Fourthly, the immunoenzimatics experiments were performed to detect the serological reactivity. Finally, the phylogeny was determined by sequencing of the region V7V8 using the methods of “Maximum Likelihood” and Bayesian. Results:The kinetic growth of group TcI (Bolivia, Tm, Tlenti and SC90) resulted in peaks at 5th-8th day, while the group TcII (Y, SC96 and SI8) resulted at 4th-9th day and TcIII (SI5, QMM3 and QMM5) at 6th-8th day. Moreover, the parasitemic curve demonstrated variability and expected profile since animals are able to modulate the infection; however, the parasitemic profile was similar between TcI, TcII and TcIII. All the strains were able to infect cells, but TcI demonstrated higher capacity of invasion than TcII, and TcII higher than TcIII. The serological reactivity occurred to TcI, TcII and TcIII for IgG (1/12800), IgG1, IgG2a and IgG3 (1/500). Finally, the phylogenetic analysis demonstrated emergence of TcII, TcI and later TcIII. Conclusions: The biological characterization of ten strains of *T. cruzi* demonstrated distinct parameters, which can separate them in different groups. Furthermore, the phylogenetic analysis demonstrated that TcII came first, followed by TcI and TcIII. **Supported by:**CAPES

PV118 - IMMUNIZATION WITH PHAGE DISPLAY PARTICLES PROTECT BALB/C MICE AGAINST *LEISHMANIA AMAZONENSIS* INFECTION

DUARTE, M.C.[1]; ALMEIDA, J.F.[2]; FUMAGALLI, M.C.[1]; COSTA, L.E.[1]; MARTINS, V.T.[1]; LAGE, P.S.[1]; DE JESUS, N.C.[1]; LAGE, D.P.[1]; PEREIRA, C.A.B.[1]; GOULART FILHO, L.R.[2]; COELHO, E.A.F.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFU, UBERLÂNDIA, MG, BRASIL.
e-mail:marianacostaduarte1@gmail.com

Leishmaniasis is a disease with a wide spectrum of clinical manifestations caused by different species of protozoa belonging to the Leishmania genus. The treatment of disease is still based on the use of the parenteral administration of pentavalent antimonial compounds; however, several side effects reported by patients and increased parasite resistance have produced serious problems. Therefore, the development of new strategies to prevent leishmaniasis has become a high priority. In this study, polyclonal antibodies purified of sera from patients with cutaneous leishmaniasis (CL) were used to screening mimotopes in a peptide phage display library, in order to select those that could be recognized and evaluate them in vaccination protocols against Leishmania amazonensis infection. The selected peptide sequences were evaluated based on their frequencies and a bacteriophage clone, namely A5, was selected to test its ability to induce protection against challenge infection. Vaccinated mice with A5 clone plus saponin showed a high and specific production of IFN-□ IL-12, and GM-CSF after in vitro stimulation with the phage clone or L. amazonensis extract. In addition, a decrease in the parasite-mediated IL-4 and IL-10 response was also observed. Immunized and infected mice, as compared to the control groups (saline, saponin and wild-type phage clone plus saponin), showed significant reductions in the infected footpad swelling, as well as in the parasite burden in liver, spleen, bone marrow and in the paws’ draining lymph nodes, in comparison to the control groups. The present study showed that a selected antigen by antibodies present in sera of CL patients by a phage display technique could be employ as a candidate to compose a vaccine against L. amazonensis infection. **Supported by:**CNPq, FAPEMIG CAPES INCTV

PV119 - SYNTHESIS AND EVALUATION OF MUTAGENIC AND TRYPANOCIDAL PROPERTIES OF NITROIMIDAZOLES SUBSTITUTED WITH DIFFERENT TRIAZOLIC RINGS

BOMBAÇA, A.C.S.[1]; SALOMÃO, K.[1]; QUARESMA, B.M.C.[2]; CARVALHO, S.T.[3]; CARVALHO, A.S.[2]; BOECHAT, N.[2]; DE CASTRO, S.L.[1]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 3.UNIVERSIDADE DO GRANDE RIO, RIO DE JANEIRO, RJ, BRASIL.
e-mail:ks@ioc.fiocruz.br

Chagas' disease, caused by *Trypanosoma cruzi*, affects approximately eight million individuals in Latin America and is emerging in non-endemic areas due to the globalization of immigration and non-vector transmission routes. The available therapy for this disease is unsatisfactory, therefore there is an intense effort to find new drugs for treatment of this disease. Megazol (1) is a 5-nitroimidazole highly active against *T. cruzi*, including on drug-resistant strains, but due to its mutagenic and genotoxic properties its pre-clinical development was interrupted. Trying to circumvent the undesired profile of 1, several analogues with different structural modifications were synthesized. In this work, the nitroimidazole moiety was linked to triazole groups and the derivatives were assayed against bloodstream trypomastigotes of *T. cruzi*; their toxicity to cardiac cells was also evaluated. Our results demonstrated the promising activity of one derivative (2), which that was 1.8 more active than 1 on *T. cruzi*, and displayed low toxicity to mammalian cells leading to a selectivity index of 14. The mutagenic potential of 2 is currently under study. Compound 2 will be assayed on intracellular amastigotes and its mode of action and *in vivo* activity will be investigated. Other compounds of this series are being synthesized for trypanocidal activity/chemical structure studies, aiming the design of potential candidates for new drug leads. **Supported by:**CNPq

PV120 - BIOLOGICAL ASPECTS OF EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION IN VERTICAL TRANSMISSION

NOTÁRIO, A.F.O.; RODRIGUES, A.A.; QUINTAL, A.P.N.; ALVES, R.N.; DE CASTILHOS, P.; SILVA, C.V.; FERRO, E.A.V.
UFU, UBERLANDIA, MG, BRASIL. e-mail:flavia.notario@hotmail.com

Introduction: *Trypanosoma cruzi*, a kinetoplastid flagellate, is the causative agent of Chagas disease. This trypanosomiasis has become a global public health problem due to migrations of Latin Americans to non-endemic countries. In Latin America with the successful implementation of control home vector infestation and blood transfusion, the importance of congenital transmission has recently increased. Considering the tight regulation of immune system during gestation, we aimed to investigate the changes in the immune system caused by *T. cruzi* infection in the gestation outcome. Material and Methods: *T. cruzi* G and CL strains were used to infect female BALB/c mice before mating with uninfected male mice. The presence of a vaginal plug was used as indicative of mating. Females were euthanized after 8 days after confirmation of vaginal plug. We used three female control groups, just infected, pregnant not infected and neither one. Two groups were infected before mating and other two were infected 4 days after confirmation of vaginal plug. The uterus and spleen was collected to cytokine analyses, also were observed. Results: Results showed that *T. cruzi* infection impaired normal gestation development, besides levels of IFN-γ and TNF-α were higher in infected compared to control group. Most of females infected with CL strain had problems with embryo implantation. The infection also increased vascularization and induced edema in uterus from just infected females. Conclusion: This work suggests that *T. cruzi* infection impedes embryo implantation and consequence development. Further investigations are necessary to understand the role of infection, as well as the mechanisms of such effects on gestation. **Supported by:**FAPEMIG

PV121 - FUNCTIONAL CHARACTERIZATION OF TUZIN PROTEIN FROM TRYPANOSOMA CRUZI

LAIBIDA, L.A.[1]; FRAGOSO, S.P.[2]; DAROCHA, W.D.[1]
1.UFPR, CURITIBA, PR, BRASIL; 2.INSTITUTO CARLOS CHAGAS, CURITIBA, PR, BRASIL.
e-mail:leticia_laibida@yahoo.com.br

The first genome published for a *Trypanosoma cruzi* strain was CL Brener, in 2005, and after almost ten years, at least 50 % of coding genes remains as genes of unknown function. Tuzin gene is one of them, in spite of being part of a gene cluster, alternating their gene copies with amastigote specific surface proteins (delta-amastins). The major feature of Tuzin protein toward its function is the presence of part of AAA+ domain (ATPases Associated with a wide variety of cellular Activities). *T. cruzi* Tuzin protein shows a higher conservation when compared to *T. brucei*, *T. evansi* and *T. grayi* (>80% identity), however lower conservation is detected with *Leishmania* spp. (~50%). Albeit both delta-amastin and Tuzin genes are transcribed in a single polycistronic RNA, their cellular abundances are not the same, suggesting a different mechanism of post-transcriptional regulation. This distinct mechanism of post-transcription regulation, along with the fact its function and its cellular location still unknown, prompted us to characterize the Tuzin genes from *T. cruzi*. To fulfill this goal, one of our strategies was to construct plasmids to express Tuzin fused at the C-terminus to the green fluorescent protein (GFP) or FLAG peptide in Sylvio X10/cl 1. Tuzin cellular location is scattered all over the parasite body, in a cytoplasmic-like pattern, as shown by GFP fusion fluorescence. Western blot assays showed that this fusion has been expressed at the expected size. Further experiments are underway, such as protein localization by cellular fractioning and confocal microscopy using parasites overexpressing Tuzin::FLAG fusion protein. These parasites will also be used to identify molecular partners by pull down assays followed by mass spectrometry. **Supported by:**CAPES; CNPq; Fundação Araucária

PV122 - THE *RHODNIUS NEGLECTUS* SALIVARY TRANSCRIPTOME

SANTIAGO, P.B.M.; NEGREIROS, R.S.; OLIVEIRA, J.V.A.; BASTOS, I.M.D.; ALENCAR, T.R.; ARAÚJO, C.N.; SANTANA, J.M.
UNIVERSIDADE DE BRASÍLIA, BRASÍLIA, DF, BRASIL.
e-mail:negreiros@unb.br

Hematophagous feeding habit of bloodsucking hemiptera provides the favorable scenario for the transmission of the causative agent of Chagas disease, *Trypanosoma cruzi*, to the vertebrate host. To allow proper feeding, salivary glands release potent pharmacological compounds, counteracting host hemostasis, such as anti-platelet, anti-clotting, vasodilator and anti-inflammatory molecules. *Rhodnius neglectus* bloodsucking can be found in the Cerrado in association with different wild palms. In addition, this specie is found in both intra and peridomestic environments in the state of Goiás, a vector transcriptome would provide a catalog of investigate the salivary transcriptome of *R.* isolated and its integrity was assessed. A library. The sample was sequenced by Illumina MiSeq platform. As the specie has no sequenced genome, assembly was *de novo* assembly using Trinity. A total of 47,948 contigs were annotated. Some contigs code for proteins which have been characterized in other hematophagous, such as apyrase, triabin and antigen-5. Furthermore, transcripts relative to the nitrophorin and rhodniin already described in *Rhodnius prolixus* saliva were also identified. Evaluation of *R. neglectus* transcriptome is essential to understand the biological processes affecting hematophagous feeding. Besides, owing to the different pharmacological properties of hematophagous saliva molecules, they represent a potential source for discovery and development of drugs for the treatment of vascular diseases. Gene Ontology and SignalP analyses will be performed to further analysis of this transcriptome set. **Supported by:**CNPq, CAPES, FAPDF, PRONEX, Finep

PV123 - PROTEINS ASSOCIATED WITH THE TRANSLATION MACHINERY OF TRYPANOSOMA CRUZI

AMORIM, J.C.; ZANCHIN, N.T.; MARCHINI, F.K.
ICC, CURITIBA, PR, BRASIL.
e-mail:julianaamorim-22@hotmail.com

The translation machinery components and regulation of the protein synthesis process are best characterized in higher eukaryotes and in yeast, whereas in protozoan parasites of medical importance like pathogenic trypanosomatids this topic still remains unexplored. Despite of some initiation factors identified in the *Leishmania* and *Trypanosoma brucei* genomes, in addition to a study on translation regulation starting from the 3'-untranslated region in *Leishmania*, there are few data about the translation mechanism for this group of parasites. Therefore, we started a project with the objective of identifying the proteins and translation factors that interact with the translation machinery of *Trypanosoma cruzi*, the protozoan parasite causative of the Chagas disease. To investigate the dynamic association of proteins with the translation machinery, extracts from cells of the epimastigote form were prepared for enrichment with the cytoplasmic fraction, which was fractionated by ultracentrifugation through a sucrose cushion. The pellet was salt-washed with 500 mM sodium chloride and submitted to a new round of ultracentrifugation. The fractions obtained (soluble fraction, ribosome salt wash and post salt wash pellet), were subjected to liquid chromatography coupled with high resolution mass spectrometry (LC-MS/MS). Many of the proteins were common to the three fractions, whereas some were exclusive to a particular fraction. Gene ontology enrichment methods and KEGG were used to classify the proteins according to their function, involvement in biological processes and metabolic pathways. Ribosomal proteins represent for the largest percentage of proteins identified, and the non-ribosomal proteins were of various functional classes like RNA-binding proteins, RNA transport protein, and proteins involved in metabolism and translation process. Altogether these results allowed us to identify the association of different proteins with the translation machinery of *T. cruzi*. **Supported by:**CAPES; PAPES;Instituto Carlos Chagas-Fiocruz-PR

PV124 - EFFECT OF THE IRON CHELATOR 2,2-DIPYRIDYL ON *LEISHMANIA (VIANNIA) BRAZILIENSIS* GROWTH AND IRON UPTAKE

MESQUITA-RODRIGUES, C.[1]; MENNA BARRETO, R.F.S.[1]; CUERVO, P.[1]; DE JESUS, J.B.[2]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFSJ, SÃO JOÃO DEL REY, MG, BRASIL.
e-mail:camilarodrigues87@yahoo.com.br

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, which are essential for the maintenance of the energy metabolism and the redox balance in these parasites. Promastigote and amastigote forms of *Leishmania* can acquire iron from transferrin, lactoferrin, hemoglobin and LIT1, a ferrous iron transporter expressed by parasites cultured in poor iron environments. In the present work we investigate how the intracellular concentration of iron is affected for the treatment with the chelator 2,2-dipyridyl and how it influences the expression of genes involved with the metabolism of iron. Promastigotes were treated with distinct concentrations of the iron chelator and cellular density was determined. Intracellular concentration of iron was quantified by the colorimetric method of ferrozine and the gene expression of LIT1 and FeSOD was analyzed by qPCR after 24 and 48 hours of treatment. It was observed that treatment with 2,2-dipyridyl affected proliferation in a dose and time-dependent manner, but growth was restored after inoculation of the parasites in fresh culture medium. The intracellular concentration of iron in treated parasites was equal or greater than that observed in control ones. Accordingly, gene expression of LIT1 and FeSOD increased in parasites treated with the chelator. The results show that although iron depletion by 2,2-dipyridyl inhibits the growth of *Leishmania* promastigotes, 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

PV125 - EXERCISE IMPROVES THE TH1 RESPONSE AGAINST *LEISHMANIA* MAJOR

TERRA, R.M.S.[1]; ALVES DE ARAUJO, R.L.[1]; ALVES, P.J.F.[1]; LIMA, A.K.C.[1]; SILVA, S.A.G.[1]; SALERNO, V.[2]; DUTRA, P.M.L.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:professorrodrigoterra@gmail.com

The leishmaniasis represent a group of diseases caused by protozoa from the genus *leishmania*. During the disease progression, the celular immune response (Th1) influences the control of infection while the humoral response (Th2) relates to the progression of the disease. Several studies suggest that moderate exercise influences the immune system by stimulating the Th1 response that controls parasitic infections. We evaluated the impact of moderate aerobic exercise on progression of infection by *Leishmania major* in mice. Animals were grouped into cohorts (N=8) according to the following variables: Exercise, Infection and treatment with Glucantime. Infection with *Leishmania* was initiaded by an inoculation with $2x10^7$ promastigotes into plantar cushion. Moderate exercise consisted of swimming with progressive weights related to body mass (25 min., 3 days/week). Exercise intensity was measured by blood lactate levels and TBARS assays. Blood lactate levels after a round of moderate exercise (~3.0 mmol/L) were significantly higher than the resting value (~1.6mmol/L) and lower than levels after intense exercise (~6.0mmol/L). TBARS indicated that lipid peroxidation was not significantly different between the exercise and control groups. Glucantime treatment (5 days/week) commenced six weeks after infection using therapeutic doses (8mg/kg). After 12 weeks the lesions in the exercise and glucantime treated groups were reduced approximately 93%. Only the trained groups presented a DTH response. The parasitic load in the trained groups was ~1,000 fold less than the infected control group and in the group trained from the begining of the infection 12,000 fold less. The cytokines IL-12 and IFN-y also were mensured in infected legs. Their concentrations were significantly higher in exercise groups compared with infected control group. These data suggest that exercise modulates the Th1 immune response in mice infected with L.major providing a protective response. **Supported by:**FAPERJ

PV126 - THE ROLE OF TOXOPLASMA GONDII SIRTUIN TGSIR2A IN EPIGENETIC REGULATION AND PARASITE DIFFERENTIATION

NARDELLI, S.C.[1]; AVILA, A.R.[1]; GOLDENBERG, S.[1]; KIM, K.[2]
1.INSTITUTO CARLOS CHAGAS, CURITIBA, PR, BRASIL; 2.ALBERT EINSTEIN COLLEGE OF MEDICINE, NEW YORK, ESTADOS UNIDOS.
e-mail:shenardelli@gmail.com

Toxoplasma gondii has a complex repertoire of chromatin remodelers that affect gene expression and chromatin assembly. Among those are seven candidate histone deacetylases encoded in the genome (HDAC1-5 and Sir2A and Sir2B). We have characterized sirtuin histone deacetylases containing the Sir2-domain in T. gondii using molecular tools including endogenous tagging and gene knock-out. In other eukaryotes these NAD-dependent enzymes deacetylate histones and other proteins including tubulin and p53. In Plasmodium falciparum sirtuins have an important role in var gene regulation essential for P. falcipaum virulence. We started with TgSir2A, which is similar to Plasmodium PfSir2A. Using the endogenous tagged protein, we observed that TgSir2A-HA is located mainly in the cytoplasm and close to the nuclear periphery, suggesting its involvement in deacetylation of cytoplasmic proteins as well as histones. By chromatin immunoprecipitation followed by microarray hybridization (ChIP-chip), we localized TgSir2A along the chromosomes of the parasite. Although the protein is distributed throughout the genome, a higher enrichment was observed on the centromeric region, as confirmed by co-localization with the centromeric histone variant CenH3. The centromere is known as a silent heterochromatic region, consistent with the general property of deacetylation to decrease intermolecular interactions of DNA, making it more compact and transcriptionally silenced. Next, we disrupted TgSir2A in RH and Pru strains, showing this gene is not essential for Toxoplasma survival. Sir2AKO tachyzoites grow faster, reflecting a phenotype related to parasite replication rather than invasion. In addition, preliminary results show that during differentiation to bradyzoites forms, the cysts are formed faster in the KO parasites. The function of TgSir2A in Toxoplasma differentiation and regulation of gene expression will be investigated by further analysis of gene expression and chromatin in the KO and complemented strains. **Supported by:**CAPES-Ciência sem Fronteiras

PV127 - IMMUNE RESPONSE OF RHODNIUS PROLIXUS TO INFECTION BY TRYPANOSOMA CRUZI.

GOMES, K.B.; FIGUEIREDO, M.B.; DE CASTRO, D.P.
IOC, FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:kate.gomes@ioc.fiocruz.br

Rhodnius prolixus is a triatomine vector of the etiologic agent of Chagas disease, Trypanosoma cruzi. The transmission of this parasite to vertebrate hosts occurs after the complete development of parasite in the invertebrate host. The study of insect immune response allows us to understand the process of parasite cycle development and opens new perspectives for insect control and disease dissemination. The immune responses that the parasite encounter in the digestive tract are the induction of antimicrobial factors, activation of prophenoloxidase cascade and production of reactive oxygen and nitrogen species. These molecules peroxidases lipids, proteins and DNA and can act as signaling molecules. The objective of this study is to investigate the action of insect immune responses by reactive nitrogen species (RNS) and antimicrobial factors in the success of T. cruzi infection. Therefore, treated fifth instar nymphs of R. prolixus with L-arginine (2mg/mL blood) and L-NAME (2mg/ml blood), which induces and inhibits the production of RNS respectively and infected with T. cruzi (epimastigote 1x107/ml blood) were analyzed. The anterior and posterior midgut were dissected in different days after feeding to count the numbers of parasite infected and the samples were quantified for RNS by colorimetric assay kit (Griess Reagent System, Promega ®) and for antimicrobial activity by turbidometric assay. We observed that L-arginine treated insects had as expected higher production of RNS, low number of parasites and increase antibacterial activity possibly as a result of increased signaling molecules. However, the L-NAME treated insects presented same results as control insects. Our work suggest that RNS is involved in the T. cruzi infection in the insect vector R. prolixus and can interfere also in other immune responses such as antibacterial activity. **Supported by:**CAPES, CNPq, FIOCRUZ, INCT-EM.

PV128 - CRISPR/CAS9-INDUCED DISRUPTION OF PARAFLAGELLAR ROD PROTEINS 1 AND 2 GENES IN *TRYPANOSOMA CRUZI* REVEALS THEIR ROLE IN FLAGELLAR ADHESION

LANDER, N.[1]; LI, Z.[2]; NIYOGI, S.[2]; DOCAMPO, R.[1]
1.UNICAMP/UNIVERSITY OF GEORGIA, CAMPINAS, SP, BRASIL; 2.UNIVERSITY OF GEORGIA, ATHENS, GEORGIA. e-mail:noelia2309@hotmail.com

Current methods for the genetic manipulation of *Trypanosoma cruzi* have been highly inefficient. We report here the use of the CRISPR/Cas9 system for disrupting genes in the parasite. To investigate whether the CRISPR/Cas9 system could be used to silence genes of *T. cruzi*, we used a genetically modified version of the type II CRISPR system from *Streptococcus pyogenes*, consisting of the endonuclease Cas9 and an engineered RNA chimera or single guide RNA (sgRNA) that conform a ribonucleoprotein complex able to recognize the target gene and produce double strand breaks. Double strand breaks generated by Cas9 can be repaired by homologous recombination using donor DNA or by error-prone, nonhomologous end-joining (NHEJ). Trypanosomatids are deficient in canonical NHEJ, but they have a more recently characterized repair mechanism, named microhomology-mediated end joining (MMEJ). MMEJ repairs DNA breaks via the use of substantial microhomology and always results in deletions. Using the CRISPR/Cas9 method we were able to rapidly generate mutant cell lines in which paraflagellar rod proteins 1 (*PFR1*) and 2 (*PFR2*), and *gp72* genes have been silenced. Assessment of their phenotypes revealed that all these proteins contribute to flagellar attachment to the cell body and motility of the parasites. Therefore, CRISPR/Cas9 allows efficient genome editing in an almost genetically intractable parasite and suggest that this method will improve the functional analyses of its genome. **Supported by:**Work funded by NIH (AI108222) and FAPESP (13/50624-0)

PV129 - IMPROVED T. CRUZI COMPARATIVE GENOME SEQUENCING REVEALS MECHANISMS OF GENETIC EXCHANGE AND THE FULL SURFACE ANTIGEN REPERTOIRE

ANDERSSON, B.; LOPEZ, C.T.
KAROLINSKA INSTITUTET, STOCKHOLM, SUÉCIA.
e-mail:bjorn.andersson@ki.se

The protozoan parasite Trypanosoma cruzi is the causative agent of Chagas disease, a devastating neglected disease that affects more than 10 million people, mainly in Latin America but it is spreading to other parts of the world. As a part of a large project to investigate T. cruzi strain diversity and epidemiology, we have performed comparative whole genome sequencing of several T. cruzi strains. These include the first sequence of a strain, Sylvio X10/1, from the TcI clade, which is the dominating clade north of the Amazon, and a clone of the bat-specific subspecies T. cruzi marinkellei. I will here present the genome sequences of several TcIV strains from Venezuela and Brazil that have revealed the hybrid nature of this clade. While TcIV originates from a hybrid formation event, most of the allelic polymorphism has been eliminated over time. The origin of each gene, as well as the polymorphism pattern provides insights into the processes that control genetic exchange in this parasite. I will, in addition, present expanded comparisons of TcI strain genomes from multiple geographic locations, in order to explore the extensive genome-wide variation within TcI. As a part of this project, an improved TcI reference genome has been produced using long read single molecule sequencing. The resulting assembly has shown that we are able to resolve complex repetitive regions for the first time and clarify the complete chromosome structure of the parasite. I will discuss how to utilize these data for the study of surface molecule genes and their importance for immune evasion and pathogenesis.