BM001 - Small RNAs as microvesicle cargo in *T. cruzi* are linked to cell-to-cell communication, metacyclogenesis and susceptibility to infection.

GARCIA-SILVA, M.R.[*1]; SANGUINETTI, J.[1]; CABRERA-CABRERA, F.[1]; GUIDA, M.C.[1]; FERREIRA CURA DAS NEVES, R.[2]; MEDEIROS, L.[2]; SOUTO-PADRÓN, T.C.[2]; DE SOUZA, W.[2]; FERNANDEZ, T.[1]; PENA, A.[1]; NAYA, H.[1]; ROBELLO, C.[1]; CAYOTA, A.[1]
1.IPMONT, MONTEVIDEO, URUGUAI; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:rgarcia@pasteur.edu.uy

The protozoan *Trypanosoma cruzi* has a complex life cycle including diverse cellular forms which alternate between invertebrate and mammalian hosts. To cope with these changes T.cruzi undergoes rapid modifications in gene expression which are achieved essentially at post-transcriptional level. However the precise mechanisms of this fine tune regulation remain to be elucidated. At present, the families of small no-coding regulatory RNAs (sRNAs: ie. microRNAs and siRNAs) are recognized as key players in post-transcriptional gene regulation in most eukaryotes; nonetheless, T.cruzi lacks canonical sRNA pathways. In a recent effort of our group aimed to identify the presence of alternate sRNA pathways in T.cruzi, we reported the presence of a homogeneous population of sRNAs derived from mature tRNAs (mini-tRNAs) and other non-coding RNAs including rRNAs, snoRNAs, CDS and intergenic sRNAs and the presence of an Argonaute protein distinctive of trypanosomatids that were recruited to particular cytoplasmic organelles in stressed T.cruzi epimastigotes. Using high-throughput sequencing and transmission electron microscopy, we demonstrated that stressed epimastigotes shed high levels of microvesicles to the extracellular medium which carry selective populations of intracellular sRNAs as cargo; we also showed that mini-tRNAs were recruited to several vesicular organelles suggesting that mini-tRNAs biogenesis is part of endocytic/exocytic routes. This cargo was transferred between parasites and to mammalian susceptible cells but not to non-susceptible cells which became susceptible with the artificial incorporation of microvesicles cargo. In addition, microvesicles shed by T.cruzi drive to life cycle transition of epimastigotes toward infective metacyclic forms. To the best of our knowledge this is the first evidence of a cross-kingdom transfer of genetic material from protists to mammalian cells which could conduct us to rethink some concepts in host-pathogen biology.**Supported by:**:ANII CNPq

BM002 - Structural implications of the *Plasmodium falciparum* pyridoxal kinase

KRONENBERGER, T.[*]; WRENGER, C.
USP, SÃO PAULO, SP, BRASIL.
e-mail:kronenberger7@gmail.com

Malaria is a devastating infectious disease and the most fatal form, Malaria tropica, is caused by *Plasmodium falciparum*. Due to the spreading degree of resistance against the current chemotherapeutic treatments there is an urgent need for the discovery of novel drugs which interfere selective with the proliferation of the human pathogen. Previously our group showed that the parasite possesses a specific mechanism for the provision of vitamin B6: (i) The de novo biosynthesis of pyridoxal phosphate and (ii) the inter-conversion pathway facilitated by the pyridoxal kinase (PdxK). The druggability of the latter has already been validated by our group. In order to gain structural insights into the functionality of the protein we applied in silico homology modelling of the plasmodial protein combined with normal mode analysis (NMA) after energy minimisation and identified the respective regions involved in active site formation and the dimerisation region of the protein. NMA reveals that interfering with the dimerisation process of PdxK is not just leading to a loss in dimmer formation but also in instability of the individual monomers. Molecular dynamic analyses show additionally energetic and conformational differences in the lid formation of the ATP binding site in the presence or absence of the respective substrate. Furthermore ATP binding results in an increased mobility of the entire subunit of PfPdxK. In order to validate the respective mutagenic sites we performed site directed mutagenesis experiments. **Supported by:**:FAPESP

BM003 - DNA replication analysis of a 347kb fragment from *T. brucei* chromosome 1

CALDERANO, S.G.[*1]; DROSOPOULOS, W.C.[2]; KOSIYATRAKUL, S.T.[2]; SCHILDKRAUT, C.L.[2]; ELIAS, M.C.[3]
1.IB-CAT, SÃO PAULO, SP, BRASIL; 2.AECOM, BRONX, ESTADOS UNIDOS; 3.IB - CAT, SÃO PAULO, SP, BRASIL.
e-mail:siguedes@usp.br

In order to duplicate the whole genome in a short space of time during S phase of the cell cycle, many start points of DNA replication (the origins of replication) are needed. In human cells is known that there are about 30 to 50 thousands origins of replication activated at S phase. In *Trypanosoma brucei* nothing is described about the numbers of origins, the distance between them and the speed rate of replication. Using the SMARD (Single Molecule Analysis of Replicated DNA) technique we were able to analyze the DNA replication pattern of a 347 kb fragment from chromosome 1 of *T. brucei* that is 1 Mb in length. We found that the 347 kb fragment from chromosome 1 can be replicated by at least three different origins of replication (named Ori 1, Ori 2 e Ori 3); two of them outside and one origin of replication inside this analyzed fragment. Ori 1 is found inside the 347 kb fragment from chromosome 1 while Ori 2 is downstream and Ori 3 upstream to Ori 1. These three origins are not always activated at S phase and we could observe different combinations of activation of these three origins in order to replicate this fragment. Also based on the molecules analyzed we could suggest that the replication speed rate is about 5 kb/min. These results are just the beginning to understand the replication profile of chromosome 1. However we already can conclude that the *T. brucei* DNA replication is similar to human cells where there are many origins, but not all of them are activated at S phase. And the speed rate replication in *T. brucei* (5kb/min) is similar to mammalian cells (2-3 kb/min). **Supported by:** FAPESP.

BM004 - MOLECULAR ANALYSIS OF VIRULENCE OF *Toxoplasma gondii* ISOLATES FROM ANIMALS AND HUMANS OBTAINED IN MINAS GERAIS STATE, BRAZIL.

SILVA, L.A.[*]; VITOR, R.W.A.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:leticiaazs@gmail.com

Virulence of *T. gondii* isolates has been defined in experimental mouse model; however, this method is laborious. CS3 marker (VIIa chromosome) has been previously proposed as an alternative parameter to determine *T. gondii* virulence, but it is not clear if CS3 is reliable to reproduce it. The aim of this study was verify if CS3 marker can be used to predict virulence of *T. gondii* isolates in mice. 49 *T. gondii* isolates from Minas Gerais, Brazil (eight from dogs, 11 from chickens and 30 from congenital toxoplasmosis cases in humans) were genotyped by PCR-RFLP at CS3 locus. PCR products were digested by restriction enzymes NlaIII and MboI. DNA banding pattern was resolved in polyacrylamide gels and compared with reference strains type I, II and III. Chi square or Fisher's exact tests were used to verify associations between CS3 allele type and the virulence phenotype in mice previously defined. 49% of *T. gondii* isolates showed presence of allele I, 27.5% had allele II and 17.6% had allele III. Two isolates had unusual alleles (u-1 and u-2) and one had the combination of alleles I/III (mixed infection). 83% of the isolates that had virulent or intermediately virulent phenotype for mice (38/46) harbored alleles I or II. 80% of avirulent isolates harbored alleles III. The difference was statistically significant between alleles I and III (P = 0.0002) and between alleles II and III (P = 0.0462), but not between alleles I and II (P = 0.0958). These results suggest that CS3 marker can be used to predict virulence of *T. gondii* strains in mice. Furthermore, it corroborates that isolates with the alleles I or II are associated with mortality in infected mice, whilst isolates with allele III are associated with surviving. It is important to know the virulence of Brazilian strains, because it allows its association with *T. gondii* genotypes and severity of toxoplasmosis in humans. These studies can provide subsidies for the employment of an appropriate treatment in population. **Supported by:**::CNPq and FAPEMIG

BM005 - Role of the Orc1/Cdc6 ATP binding domain and ATPase activity for the stability of the pre-replication complex in *Trypanosoma brucei*

SOARES, D.R.[*1]; SILVA, E.M.[1]; DE JESUS, T.C.[2]; ELIAS, M.C.[1]
1.IB, SÃO PAULO, SP, BRASIL; 2.USP - INSTITUO DE FISICA, SÃO PAULO, SP, BRASIL.
e-mail:daiane.bio@hotmail.com

Chromosomal replication starts with the formation of the pre-replication complex (pre-RC) onto DNA regions named origins of replication. In yeast, the pre-RC is composed by the ORC complex (Orc 1-6), Cdc6, Cdt1 and MCM complex (Mcm 2-7), which presents helicase activity. Trypanosomas present a gene homologous to Cdc6 and Orc1 called Orc1/Cdc6. Silencing of TbOrc1/Cdc6 by RNAi blocked DNA replication, evidencing its role in *T. brucei* DNA replication. In yeast cells, ORC – ATP interaction is essential for binding of ORC to replication origins. The ATP hydrolysis by Cdc6 and ORC ensures the specific interaction between DNA and ORC and Cdc6 as well as the stability of MCM complex. The primary sequence of *T. brucei* Orc1/Cdc6 presents a site of interaction to ATP / GTP and also the sensor regions 1 and 2 that are essential for ATPase activity. Our laboratory has demonstrated that TbOrc1/Cdc6 recombinant protein binds and hydrolyzes ATP "in vitro". The objective of this study is to evaluate the importance of TbOrc1/Cdc6 - ATP binding and hydrolysis for formation and stability of pre-RC. Therefore, we generated recombinant proteins of *T. brucei* Orc1/Cdc6 mutated at ATP binding site (TbOrc1/Cdc6K65T) or at sensor 2 region (TbOrc1/Cdc6R265,266E). TbOrc1/Cdc6K65T was expressed and will be analyzed concerning its ability to bind ATP. TbOrc1/Cdc6R265,266E was expressed and its ATP hydrolysis activity was drastically reduced compared with wild type TbOrc1/Cdc6. Both genes were cloned into transfection vector and *T. brucei* procyclic forms were transfected and are now under selection. The formation and stability of the pre-RC as well as DNA replication and survival of parasites will be analyzed concerning its ability to bind ATP. TbOrc1/Cdc6R265,266E was expressed and its ATP hydrolysis activity was drastically reduced compared with wild type TbOrc1/Cdc6. Both genes were cloned into transfection vector and *T. brucei* procyclic forms were transfected and are now under selection. **Supported by:**:Fapesp

BM006 - FUNCTIONAL CHARACTERIZATION OF MYH OF *Trypanosoma cruzi*

LIMA, M.K.[*]; FURTADO, C.; RAJÃO, M.A.; MENDES, I.C.; MACEDO, A.M.; FRANCO, G.R.; PENA, S.D.J.; MACHADO, C.R.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:kunrathlimam@gmail.com

The maintenance of the integrity of the genetic material is essential for the survival of living organisms. One of the biggest threats to genome's integrity is the oxidation of nitrogenous bases, being 8-oxoguanine the most frequent of them. The base excision repair is the most important cellular process in the repair of 8-oxoguanine. This lesion is removed by 8-oxoguanine DNA glycosylase (OGG1 or Fpg), enabling other enzymes to insert a normal guanine in the place of the oxidized guanine, whereas the MutY DNA glycosylase (MutY or MYH) removes the adenine that is wrongly paired with 8-oxoguanine during DNA replication. *Trypanosoma cruzi* is a flagellated protozoan that is the causative agent of Chagas disease. Like most living organisms, it is susceptible to oxidative stress, and needs to adapt to distinct environments. Hence, DNA repair is essential for its survival and improvement of infection. Given the importance of DNA repair to T. cruzi, we decided to study if this protozoan has homologous of the enzyme MYH (TcMYH), through the performance of heterologue complementation assay. TcMYH complemented the bacterial MutY- strain, reducing the mutation frequencies to a level similar to the wild type's frequencies. This result shows that TcMYH probably has a MutY DNA glycosylase activity. At this moment, *T. cruzi* strain that overexpresses TcMYH is being constructed to further characterize this enzyme. **Supported by:**:CAPES, CNPq, FAPEMIG

BM007 - Evaluation of the susceptibility of epimastigote strains from *T. cruzi* to ferrocenyl diamines

KOHATSU, A.A.N.[*1]; SILVA, F.A.J.[1]; ARENAS VELÁSQUEZ, Á.M.[1]; RODRIGUES, D.F.[1]; CHIARI, B.G.[1]; DE ALMEIDA, M.G.J.[1]; FRANCISCO, A.I.[2]; 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

Studies have searched potential targets for trypanocidal substances, some reporting an increase on the production of enzymes involved in the oxidative stress metabolism which probably could be responsible for the benznidazole resistance, only drug available in Brazil for Chagas disease. Such enzymes have important roles in the survival and growth of the parasites: Peroxiredoxin (peroxidase and peroxynitrite reductase activity); Superoxide Dismutase (reduces superoxide radicals) and Old Yellow Enzyme (involved in the reduction of some trypanocidal substances and PGF2α synthase activity). This work aims to evaluate the susceptibility of epimastigote strains from *T. cruzi* to ferrocenyl diamines and analyze the difference in their expression level which would be related with the resistance of parasites. Cytotoxic assay was performed using MTT, our results showed that all three substances tested (AAC04, AAC09 and AAC10) had trypanocidal activity higher and in less concentration than the benznidazole. AAC09 showed the best activity against *T.cruzi* and IC_{50} values obtained were: Y = 2.20 µM, SI1 and SI8 = 10.80 µM, QMII = 15.20 µM, Bolívia and SIGR3 = 13.40 µM; whereas for benznidazole were: Y = 63.78 µM, SI1 = 34.62 µM, SI8 = 58.40 µM, QMII = 63.78 µM, Bolívia = 96.06 µM and SIGR3 = 105.28µM. In order to characterize the differences in the expression levels of those proteins, after treatment with the ferrocenyl diamines, polyclonal antibodies in rabbits were produced. Next steps will involve: i) Western blot analysis to determine the oxidative response from parasites and ii) the testing of the cytotoxicity of these ferrocene diamine derivatives in HepG2, used like a model for human cell liver.**Supported by:**:FAPESP; FUNDUNESP; CNPQ

BM008 - Development of tools for systematic analyses of parasites genes involved in mRNA nucleocytoplasmic export

AVILA, A.R.[*1]; MEISSNER, M.[2]
1.INSTITUTO CARLOS CHAGAS - FIOCRUZ -PR, CURITIBA, PR, BRASIL; 2.UNIVERSIDADE DE GLASGOW, GLASGOW, ESCOCIA.
e-mail:aravila@fiocruz.br

RNA is exported by specialized pathways in eukaryotes. Previous results indicate that several key proteins involved in RNA export pathways are well-conserved across eukaryotes. However, mRNA export pathways are less conserved, suggesting a divergence during evolution. The most conserved protein, a specific component of mRNA transcription/export complex (TREX), is UAP56. This protein is involved in transcription and export of mRNA in trypanosomes. However, its function (and that of other components of mRNA export pathway) remain largely unknown in Apicomplexa. To analyse the function of parasite genes involved in the mRNA export pathway, we developed a system to follow the nucleocytoplasmic transport of mRNA using fluorescence microscopy. The previous establishment of inducible systems that allow rapid and systematic characterisation of essential factors makes T. gondii a useful biological model for this purpose. Here, we aim to generate a T. gondii stable cell line for knockout of genes where a specific mRNA is labelled with yellow fluorescent protein (YFP) fused to a MS2 protein tag. We have obtained a stable cell line for inducible expression of the MS2 protein that was enriched in the nucleus by addition of an N-terminal nuclear localization signal which does not interfere with mRNA export. The results show that low expression of the protein resulted in specific labelling of mRNA in the cytoplasm. The overexpression of the homologous gene of UAP56 in T.gondii (TgUAP56) is deleterious for the parasite, indicating an essential role of this protein. We intend now to prove that this approach is useful for studying gene function, using TgUAP56 as target. **Supported by:**:CNPq, Fiocruz, CAPES, Wellcome Trust

BM009 - MOLECULAR DIFFERENTIATION AMONG *LEISHMANIA* COMPLEXES: WHAT ASSAY SHOULD BE PERFORMED?

QUINTAL, A.P.N.[*1]; MACHADO, F.C.[1]; RIBEIRO, E.S.[2]; RODRIGUES, F.P.[3]; FLOETER-WINTER, L.M.[4]; ZAMPIERI, R.A.[4]; SILVA, C.V.[1]; NUNES, C.M.[2]
1.UFU, UBERLÂNDIA, MG, BRASIL; 2.UNESP, ARAÇATUBA, SP, BRASIL; 3.UNB, BRASILIA, DF, BRASIL; 4.USP, SÃO PAULO, SP, BRASIL.
e-mail:apnquintal@yahoo.com.br

*Leishmania*ses are parasitic diseases with vector transmission, caused by protozoa of the genus *Leishmania*. Species discrimination is important not only for epidemiological but also for clinical reasons and there is a variety of assays available and its choice should be cautious, since not all experimental methodology overcomes the challenge that samples may present, such as low parasite load, small amount of available biological samples and storage time. We evaluated RFLP-PCR with kDNA, RFLP-PCR SSUrDNA, nested and semi-nested SSUrDNA PCR, G6PD-nested PCR, dissociation temperature (T melting) by quantitative PCR (qPCR) with degenerated kDNA primers and SL - RNA mini exon multiplex PCR assay for the identification of *Leishmania* species in different biological samples from naturally infected animals. The major problems were the low DNA concentration and low available samples volume. RFLP PCR showed to be useful in samples with high parasite load and should be used for screening because it is less expensive than the others assays. SSurDNA nested PCR and sequencing or G6PD semi-nested PCR achieved the same positivity. Real time PCR T melting was not effective for species differentiation. SL-RNa multiplex PCR was able to make the *Leishmania* complexes differential diagnosis, which may be applicable for the laboratory diagnosis. **Supported by:**:FAPESP and CAPES

BM010 - Nutrient Acquisition and endosome formation in the human malaria parasite *Plasmodium falciparum*

LINDNER, J.[*1]; WRENGER, C.[1]; MÜLLER, I.[2]
1.USP, SÃO PAULO, SP, BRASIL; 2.BERNHARD-NOCHT-INSTITUT, HAMBURGO, ALEMANHA.
e-mail:jasminlindner@hotmail.de

Apicomplexan parasites have a significant impact on health of human and livestock, and chemotherapy remains a problem. The most severe of these parasites is Plasmodium, the pathogenic agent of malaria *Plasmodium falciparum* is proliferating within the human red blood cells. Thereby the parasite depends on the host metabolism in terms of carbohydrate, cofactor and amino acids (etc.) supply which have to be imported. Uptake of these metabolites is either facilitated by transporters or by pinocytosis. The latter is mediated by cytostome formation followed by lysosomal transport towards the digestive vacuole. The mechanism which triggers this is unknown in the malaria parasite; however in other organisms it has been shown that phosphorylation of membrane anchored proteins play a role. In this sense we focussed on protein kinases which were identified in the plasmodial genome database. 18 were cloned and analysed for their recombinant expression profile. Five of them were found to be expressed in a soluble manner which was analysed by SDS-PAGE and subsequently by Western blotting using a Strep-Tag specific antibody. In order to verify substrate acceptance of the kinase a protein kinase activity test has been established using the cytosolic localised peptide of the plasmodial secreted acid phosphatase as substrate. So far one kinase was analysed for its activity and found not to be accepting the peptide as substrate. Currently the other four are investigated for their substrate acceptance. This analysis to discover proteins involved in this lysosomal vesicle transport is promising target for the discovery of novel drugs, taking into account that the parasite relies on metabolic environment its host. **Supported by:**:PROMOS

BM011 - THE NOVEL *LEISHMANIA* EIF4E4 N-TERMINUS IS A TARGET FOR MULTIPLE PHOSPHORYLATION EVENTS AND IS REQUIRED FOR PROPER PROTEIN FUNCTION

DE MELO NETO, O.P.[*1]; DA COSTA LIMA, T.C.[1]; ROMÃO, T.P.[1]; PAPADOPOULOU, B.[2]
1.CPQAM/FIOCRUZ, RECIFE, PE, BRASIL; 2.CRI/UNIVERSITE LAVAL, QUEBEC, CANADÁ.
e-mail:opmn@cpqam.fiocruz.br

Both the 5' and 3' ends of eukaryotic mRNAs are required for translation through their binding to eIF4E, the cap binding protein, and the poly-A binding protein (PABP). EIF4E is part of the translation initiation complex eIF4F, which through interactions of the eIF4G subunit with other translation factors and PABP facilitates ribosome binding to the mRNA. In trypanosomatids, several eIF4E homologues have been described, with the EIF4E4 homologue being the most likely candidate to perform the functions ascribed for eIF4E in translation. EIF4E4 has recently been seen by us to be differentially phosphorylated during different phases of *Leishmania* cell cultures. Here we show that in L. infantum this phosphorylation is associated with the exponential phase of cell growth, both in promastigotes and amastigotes and that is not associated with the differentiation process. The same pattern of phosphorylation is observed when the full-length protein tagged at the C-terminal with HA is overexpressed in L. infantum. Phosphorylation is not affected by single mutations on residues previously implicated in eIF4E4 binding to mRNA or to its partner eIF4G3. These various phosphorylation events are targeted to multiple serine-proline or threonine-proline motifs in the N-terminus region of the protein since mutations of most of these motifs lead to a progressively lower number of phosphorylated isoforms. However, the episomally-encoded mutant proteins are still able to complement a knockout mutant lacking both endogenous eIF4E gene copies. In contrast, mutations in three related motifs also found within the eIF4E4 N-terminus, which do not significantly disrupt phosphorylation, prevent its ability to complement the loss of both endogenous alleles. Overall, this unique eIF4E4 N-terminus seems to participate in critical interactions required for protein function and regulation of translation and further highlights unique aspects of translation initiation in *Leishmania*. **Supported by:**:Canadian Institutes of Health Research (CIHR); CAPES; CNPq; Agence Universitaire de la Francophonie

BM012 - A new species of trypanosome infecting African bats: taxonomic appraisal based on morphological, behavioral and molecular features and phylogenetic inferences

LIMA, L.[*1]; ESPINOSA-ALVAREZ, O.[1]; CAMPANER, M.[1]; TAKATA, C.S.[1]; ATTIAS, M.[2]; DE SOUZA, W.[2]; CAMARGO, E.P.[1]; TEIXEIRA, M.M.[1]
1.USP - ICB II, SAO PAULO, SP, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:lumassaro@ig.com.br

We characterised morphologically, biologically and phylogenetically 12 trypanosomes isolates from microbats captured in Mozambique, Southeastern Africa; 11 from *Rhinolophus landeri* (Rhinolophidae) and one from *Hipposideros caffer* (Hipposideridae).The large trypomastigotes in the blood of bats morphologically resemble to trypanosomes of the subgenus *Megatrypanum*, a controversial taxa. Culture and biological behavior and morphology of the isolates clearly separated them from the species of *Schizotrypanum*, which comprises almost all bat trypanosomes maintained in culture and molecularly characterized. Barcoding through V7V8 SSUrRNA sequences and phylogenetic analyses using SSUrRNA and gGAPDH genes demonstrated that the new bat isolates formed a homogeneous clade separated from all other trypanosomes by genetic distance sufficient to be considered a new species, not nesting in any known subgenera. Analyses of polymorphic ITSrDNA and SL gene sequences disclosed unique molecular markers for this new species, which was placed basal to the large assemblage called *T. cruzi* clade that comprises all bat trypanosomes from Africa, Europe and South America, besides some trypanosomes from terrestrial mammals, so far included in phylogenetic trees. Positioning of this new species in the phylogeny of *Trypanosoma* supports a common and ancient shared evolutionary history of bat trypanosomes and lends further support to the bat seeding hypothesis for the origin of *Trypanosoma cruzi* and allied species. **Supported by:**:Capes

BM013 - Phylogenetic analyses of insect trypanosomatids support the monophyly of the genus Herpetomonas, its strong association with flies and the description of five new species

BORGHESAN, T.C.[*1]; FERREIRA, R.C.[1]; TAKATA, C.S.[1]; CAMPANER, M.[1]; BORDA, C.C.[1]; PAIVA, F.[2]; MILDER, R.V.[1]; TEIXEIRA, M.M.[1]; CAMARGO, E.P.[1]
1.USP - ICBII, SÃO PAULO, SP, BRASIL; 2.UFMS, CAMPO GRANDE, MS, BRASIL.
e-mail:tarcillacb@usp.br

In order to review the taxonomy of genus *Herpetomonas* through phylogenetic and morphological analyses we barcoded 527 insect trypanosomatids by sequencing the V7V8 region of SSUrRNA. Fifty two flagellates, 90% from Diptera, were shown to be related to known species of *Herpetomonas*. In phylogenetic analyses based on entire gGAPDH and SSUrRNA including representatives of all genera of Trypanosomatidae, the 52 selected flagellates clustered into a monophyletic assemblage that we are considering as the redefined genus *Herpetomonas*. ITS1rDNA sequences and putative secondary structures of this region were compared for evaluation of inter- and intraspecific variability. The flagellates were classified in six already known species and five new species. In addition, two *Leptomonas* spp. were moved to *Herpetomonas*, comprising 13 valid species, while four species were excluded from the genus. Light and electron microscopy revealed the extreme polymorphism of *Herpetomonas*. Our findings also showed that some species of *Herpetomonas* are generalist parasites of flies and appear to be as cosmopolitan as their hosts. **Supported by:**:CNPq

BM014 - Characterization of a new family of membrane proteins of *Trypanosoma cruzi* that shares similarity with the *Trypanosoma brucei* procyclic-form surface glycoproteins

MARTINS, N.O.[*1]; SOUZA, R.T.[1]; BAYER-SANTOS, E.[1]; ALMEIDA, I.C.[2]; DASILVEIRA, J.F.[1]
1.UNIFESP, SÃO PAULO, SP, BRASIL; 2.UTET, EL PASO, ESTADOS UNIDOS.
e-mail:nadini.oliveira@gmail.com

We identified a new family of *Trypanosoma cruzi* membrane proteins (Tc-MP) that shares 40% identity with the procyclic-form surface glycoproteins (PSSA-2) from *Trypanosoma brucei*. PSSA-2 gene encodes a stage-specific surface antigen with properties of type I integral membrane proteins, suggesting that the polypeptide is attached to the bilayer by means of a stable transmembrane anchor unlike the GPI-lipid anchor. PSSA-2 protein requires phosphorylation of a cytoplasmic threonine residue (T305) in order to exit the endoplasmic reticulum and go to surface membrane. We identified eight Tc-MP genes in *Trypanosoma cruzi* databases that encode 400-450 amino acid proteins with 2-3 conserved transmembrane domains. By cDNA cloning we identified three additional members of Tc-MP family that were not deposited on Genbank. Chromoblot hybridization analysis showed that Tc-MP genes were located in a single chromosomal band of clone CL Brener. Northern blot hybridization revealed the presence of single 1.6-kilobase Tc-MP transcript in epimastigote and two 1,5 and 2,0- kb Tc-MP transcripts in the other developmental stages of the parasite. Tc-MP transcripts were identified by RT-PCR one step using specific primers in all stages of parasite life. Western blot analysis revealed the presence of Tc-MP in epimastigotes and trypomastigotes. The localization of Tc-MP protein could be provided by immunofluorescence with Tc-MP polyclonal antibody that localized this protein in the cytoplasmic vesicles around the nucleus. Peptides that displayed high similarity (95%) with Tc-MP sequences were identified by mass spectrometry in vesicle preparation secreted by epimastigote forms, suggesting that Tc-MP proteins are released by the parasite. Since the genes copies in *T. cruzi* lack the T305 amino acid that is important to the correct addressing of PSSA-2 to the *T. brucei* surface membrane, we may suggest that in *T. cruzi* this protein is not at the surface membrane, but in intracellular membranes. **Supported by:**:FAPESP, CNPq and CAPES

BM015 - Insights into the transfer of *Trypanosoma cruzi* kDNA minicircle sequence and its role in genotype modifications and autoimmunity in Chagas heart disease

GUIMARO, M.C.*; HECHT, M.M.; ROSE, E.C.P.; NITZ, N.; ALVES, R.M.; ANDRADE, R.R.; VITAL, T.E.; SOUSA, A.O.; TEIXEIRA, A.R.L.C.
LMPDC - UNB, BRASILIA, DF, BRASIL.
e-mail:maroldiniz@gmail.com

To eliminate any role played by parasite persistence in the pathogenesis of Chagas disease, we used the congenic birds of Prague to determine further the origin of the autoimmune rejection of the heart in Chagas disease. Although chickens are refractory to the *T. cruzi* infection, the inoculation of virulent trypomastigotes into the air chamber of fertile egg generates parasite-free chickens at hatching, which retain the parasite kDNA into the genome. Herein we describe the integrations of kDNA into cyclin M2 (CNNM2) and tetraspanin 18 (tspan18) *loci*. CNNM2 appears to mediate Mg^{2+} desorption by kidney cells. Clinical symptoms such as tetany, seizures, and cardiac arrhythmias have been associated with mutations in this gene. Tetraspanins comprise a gene family associated with regulation of intracellular signalling, trafficking, cell fusion, viral infection, and cancer. The demonstration of kDNA mutations in the tspan18 NW_001471707.1 and in the CNNM2 NW_001471720.1 *loci* suggested the use of host-specific primers sets to disclose intrinsic features of these kDNA mutations that might be fixed in the parental chicken and progeny. We found the tpTAIL PCR amplification pattern in parents is usually different from that seen in progeny. Southern blot hybridization showed bands with migration-pattern alterations, possibly, due to recombination and deletion occurring over time. The kDNA mutations in CNNM2 and in tspan18 *loci* in parents and progeny showed topology differences stemming from the kDNA variable regions. Moreover, observed differences in the kDNA mutations in two gene *loci* in parents and progeny result from the kDNA minicircle diversity, and mosaicisms arising from semi-conservative, non-Mendelian sexual reproduction. In conclusion, rupture of CNNM2 and tspan18 genes in avian parents and progeny have been associated with inflammatory cardiomyopathy. The next step in this research is to analyse if there are alterations in gene expression. **Supported by:**:CNPq and CAPES

BM016 - Rates of integration of minicircle sequences of kDNA from *Trypanosoma cruzi* in a human cohort never exposed to triatomines

HAGSTRÖM, L.*; MARQUES, A.L.; ALMEIDA, A.J.B.; BRITTO, M.M.; NITZ, N.; TEIXEIRA, A.R.L.C.
LMPDC - UNB, BRASILIA, DF, BRASIL.
e-mail:loubex@hotmail.com

Trypanosoma cruzi infections and the endemic Chagas disease are long lasting in Latin America. Recent studies have demonstrated lateral transfer of minicircle DNA (kDNA) sequences from *T. cruzi* to the human genome, which can be vertically (via germ line) transmitted to progeny. Considering that one century encloses four generation, it is expected that four centuries is sufficient time for inheritance and fixation of exogenous mutations. The Federal District in Brazil has been considered triatomine-free for over 50 years. Thus, the aim of this study is to identify the rates of kDNA mutations in a cohort of the population living in the Federal District who do not show specific anti-*T. cruzi* antibodies. The DNA extracted from each healthy individual was subjected to PCR procedures using specific kDNA and nuclear DNA (nDNA) primer sets. The results showed the parasite nDNA in 5 out of 143 individuals tested. On the other hand, the kDNA primer sets revealed 90% of these individuals yielded amplifications that hybridized with a specific radio labeled probe. The prevalence of *T. cruzi* kDNA in the genome of individuals never exposed to triatomine bugs is explained, possibly, by its integration and fixation in the human genome. Of great interest, the demonstration of the parasite nDNA in five cases in the absence of specific antibodies suggests that the hosts are tolerant of the infections: possibly, congenitally acquired. These studies are carried out in order to demonstrate that lateral and vertical transfer of eukaryote kDNA to man is an environmental pressure-dependent natural phenomenon associated with evolution of species rather than of pathology over time. **Supported by:**:CAPES/CNPq/FAPDF

BM017 - Systematic analysis of FKBP inducible degradation domain tagging strategies for the human malaria parasite *Plasmodium falciparum*

DE AZEVEDO, M.F.[*1]; GILSON, P.R.[2]; GABRIEL, H.B.[1]; SIMÕES, R.F.[1]; ANGRISANO, F.[3]; BAUM, J.[3]; CRABB, B.[2]; WUNDERLICH, G.[1]
1.INSTITUTO DE CIENCIAS BIOMEDICAS, SÃO PAULO, SP, BRASIL; 2.MCFARLANE BURNET INSTITUTE, MELBOURNE, AUSTRÁLIA; 3.WEHI, MELBOURNE, AUSTRÁLIA.
e-mail:gwunder@usp.br

Targeted regulation of protein levels is an important tool to gain insights into the role of proteins essential to cell function and development. In recent years, a method based on mutated forms of the human FKBP12 has been established and used to great effect in various cell types to explore protein function. The mutated FKBP protein, referred to as destabilization domain (DD) tag when fused with a native protein at the N- or C-terminus targets the protein for proteosomal degradation. Regulated expression is achieved via addition of a compound, Shld-1, that stabilizes the protein and prevents degradation. A limited number of studies have used this system to provide powerful insight into protein function in the human malaria parasite *Plasmodium falciparum*. In order to better understand the DD inducible system in *P. falciparum*, we studied the effect of Shld-1 on parasite growth, demonstrating that although development is not impaired, it is delayed, requiring the appropriate controls for phenotype interpretation. We explored the quantified regulation of reporter Green Fluorescent Protein (GFP) and luciferase constructs fused to three DD variants in parasite cells either via transient or stable transfection. The regulation obtained with the original FKBP derived DD domain was compared to two triple mutants DD24 and DD29, which had been described to provide better regulation for C-terminal tagging in other cell types. When cloned to the C-terminal of reporter proteins, DD24 provided the strongest regulation allowing reporter activity to be reduced to lower levels than DD and to restore the activity of stabilised proteins to higher levels than DD29. Importantly, DD24 has not previously been applied to regulate proteins in P. falciparum. The possibility of regulating an exported protein was addressed by targeting the Ring-Infected Erythrocyte Surface Antigen (RESA) at its C-terminus. The tagged protein demonstrated an important modulation of its expression. **Supported by:**:FAPESP, CNPQ, CAPES, PRONEX

BM018 - *Trypanosoma cruzi* kDNA minicircle integrates in the dystrophin gene of parasite-free chickens

HECHT, M.M.[*]; NITZ, N.; GUIMARO, M.C.; ROSE, E.C.P.; NUNES, C.; TEIXEIRA, A.R.L.C.
LMPDC - UNB, BRASILIA, DF, BRASIL.
e-mail:marianahecht@gmail.com

Genetically driven autoimmune rejection of target heart cells by the immune system effector lymphocytes is involved in the pathogenesis of Chagas disease. Transfer of mitochondrial DNA (kDNA) minicircle sequences to the genome of chagasic rabbits, humans, and chickens, has been documented. It is well-known that birds are refractory to *T. cruzi*, but the infection can be established early in the chicken embryo during the first week of incubation. Infection-free chicks that hatched from *T. cruzi* inoculated eggs, retaining the kDNA in their genome, develop Chagas heart disease similar to that in humans. In this work, the tpTAIL-PCR methodology was used to disclose chimera kDNA-dystrophin sequences in a chicken family. Chickens showing the kDNA minicircle sequence integrated in the dystrophin gene developed skeletal muscle weakness and heart disease. Interestingly, parental and progeny kDNA mutations inserted in single-gene cluster base pair at the *locus* NW_001471534.1. However, the kDNA mutation fixed in the progeny shows topology differences between parents and progeny and among progeny. Southern blot hybridizations revealed specific bands with migration pattern differences, thus suggesting recombination, deletion, and hitchhiking, therefore increasing genetic diversity. Furthermore, observed features of the kDNA mutations in the dystrophin gene suggest a semi-conservative, non-Mendelian pattern of inheritance. These findings also suggest that kDNA mutation-induced rupture of dystrophin gene in avian parents and progeny can be associated with the pathogenesis of Chagas-like heart disease. Further studies are necessary to disclose specific alterations of gene expression. **Supported by:**:CNPq and CAPES

BM019 - CHARACTERIZATION OF THE GENE PTERIDINE REDUCTASE 1 (ptr1) IN *Leishmania* spp. LINES SUSCEPTIBLE AND RESISTANT TO POTASSIUM ANTIMONY TARTRATE

FERREIRA, R.F.*; MURTA, S.F.
CPQRR, BELO HORIZONTE, MG, BRASIL.
e-mail:rafaelfernandesf@yahoo.com.br

Pteridine reductase is a NADPH-dependent short-chain reductase that participates in the salvage of pterins in trypanosomatid protozoans, converting biopterin to tetrahydrobiopterin. In this study, we performed the molecular characterization of ptr1 gene in susceptible and antimony-resistant lines of 4 *Leishmania* species, L. guyanensis, *L. amazonensis*, *L. braziliensis* and L. infantum chagasi. PFGE analysis showed that the ptr1 gene is located in a chromosomal band of 797kb in all species of *Leishmania* analyzed. An additional chromosomal band of 1070kb was observed only in SbIII-resistant *L. braziliensis* line. Southern blot analysis showed that the ptr1 gene is approximately 11 to 14-fold more amplified in SbIII-resistant *L. braziliensis* line than its susceptible pair. This ptr1 gene amplification reflected in the level of ptr1 mRNA in this line, since that Northern and qPCR results showed that the levels of ptr1 mRNA is 5 to 6-fold higher in the SbIII-resistant *L. braziliensis* line than its susceptible pair. PTR1 protein expression evaluated by Western blot assays showed that PTR1 is approximately 3, 4 and 10-fold more expressed in the SbIII-resistant L. guyanensis, *L. amazonensis* and *L. braziliensis* lines than their respective susceptible counterparts. Functional analysis of PTR1 was performed to determine whether the overexpression of LbPTR1 gene would change the SbIII-resistance phenotype of transfected parasites. The SbIII IC50 analysis showed that the overexpression of this gene in the susceptible *L. braziliensis* and SbIII-resistant L. infantum chagasi increased the SbIII-resistance phenotype compared to non-transfected lines. In contrast, the overexpression of PTR1 in the resistant *L. braziliensis* line turned these parasites more sensitive to SbIII. The overexpression of PTR1 in susceptible L. infantum chagasi line did not change the SbIII-resistance phenotype. The results suggest that the PTR1 may be related to SbIII-resistance phenotype in *L. braziliensis*. **Supported by:**:CNPq, FAPEMIG, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR

BM020 - Molecular karyotype of *Trypanosoma cruzi*: integration of computational chromosome assemblies (TcChr) with the chromosomal bands separated by pulsed field gel electrophoresis

CORTEZ, D.R.*; SOUZA, R.T.; LIMA, F.M.; DASILVEIRA, J.F.
UNIFESP, SAO PAULO, SP, BRASIL.
e-mail:dcortez.maldonado@gmail.com

The definition of the linear sequence of *T. cruzi* chromosomes is a critical step in the construction of physical and genetic maps of this parasite. The increasing production of sequence data in *T. cruzi* genome projects has opened the possibility to link information from mapping studies to the underlying sequences. Recently, the genome sequences of clone CL Brener were grouped into 41 computational chromosome assemblies designated as *T. cruzi* chromosomes (TcChr). To integrate the in silico chromosomes TcChr to the molecular karyotype, we hybridized chromosome-specific markers with chromosomal bands separated by PFGE from clone CL Brener (group TcVI) and strain G (group TcI). Chromosome specific markers hybridized with two chromosomal bands in clone CL Brener, confirming the presence of the haplotypes Esmeraldo (S) and non Esmeraldo (P) in this hybrid strain. In the G strain, a genetically homogeneous isolate, 46% of the chromosome specific markers hybridized with only a single chromosomal band, while 34% of TcChr chromosomes hybridized with two chromosomal bands. The results suggest that the frequency of size-different homologous chromosomes is higher in the hybrid strain. The distribution of in silico chromosomes TcChr into the chromosomal bands is consistent with the hypothesis that *T. cruzi* is diploid for most of its chromosomes, although there is evidence of aneuploidy for some chromosomes.We found that two or more in silico chromosomes TcChr hybridized with the same chromosomal band indicating that they are heterologous chromosomes of similar size. In several cases it was possible to integrate several TcChr chromosomes mapped to the same chromosomal band into a single platform that represents the linear sequence of a single chromosome. These results indicate that the combination of molecular cytogenetics and computational approaches can be used to filling gaps in computational chromosome sequence assemblies. **Supported by:**:CNPq/ FAPESP/ Capes

BM021 - GENOTYPIC DETERMINATION OF ERYTHROCYTE BINDING ANTIGEN 175 (EBA-175) OF *Plasmodium falciparum* IN INDIVIDUALS NATURALLY EXPOSED IN BRAZILIAN ENDEMIC AREA

PERCE-DA-SILVA, D.S.[*1]; PRATT-RICCIO, L.R.[1]; DÔRES, T.L.[1]; LIMA-JÚNIOR, J.C.[1]; SANTOS, F.[2]; OLIVEIRA-FERREIRA, J.[1]; BANIC, D.M.[1]
1.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.LACEN, PORTO VELHO, RO, BRASIL.
e-mail:daiana@ioc.fiocruz.br

Introduction: The EBA-175 of *P. falciparum* plays a central role in erythrocytes invasion being considered, therefore, a target for malaria vaccine development. This antigen present 2 well characterized regions: *the region II* is conserved, immunogenic and contains 2 cysteine-rich segments (F1 and F2), which are involved in binding to glycophorin-A of erythrocytes; and the *region III* contains mutually exclusive C (strain CAMP) and F (strain FCR3) fragments, which defines the 2 allelic families of EBA-175. Studies performed in high endemicity malaria areas have shown the influence of this dimorphism on clinical disease and outcome. Objective: Evaluate the genetic diversity of regions II and III of EBA-175 in *P. falciparum* isolates from Porto Velho (RO) and the influence of this diversity on malaria morbidity and exposure variables. Metodology: Samples were collected in 3 time points between 1994-2007 (PV94, n=101;PV02,n=57;PV07,n=30). The genetic polymorphism was analyzed by PCR and sequencing. Results: We observed in the region II only 1 type of fragment with 926 bp. In the region III we observed the classic dimorphism with a higher frequency of the C-fragment (84.3%). The mixed infection was observed in 1.6% of isolates. There were no differences in the frequency of fragments C and F among the 3 groups. Sequencing of region II revealed 5 nucleotide changes in 3 of 15 isolates, leading to 2 amino acids replacements. Sequencing of region III revealed that: in the C-fragment there were 8 nucleotide changes in 3 of 45 samples, leading to 7 amino acids replacements; in the fragment-F there were 2 nucleotide changes, in 2 of 11 samples, leading to 2 amino acids replacements. Conclusion: We observed: reduced genetic diversity in *P. falciparum* EBA-175 isolates circulating in Porto Velho; predominance of C-fragment and temporal stability of allelic dimorphism of EBA-175. No association was observed between the EBA-175 dimorphism and malaria morbidity and exposure variables. **Supported by:**:IOC, CNPq, FAPERJ

BM022 - CLONING AND EXPRESSION OF IMIDAZOLONEPROPIONASE FROM *TRYPANOSOMA CRUZI*

MELO, R.F.P.[*]; BARISON, M.J.; SILBER, A.M.
USP, SÃO PAULO, SP, BRASIL.
e-mail:raissamelo@usp.br

Trypanosoma cruzi, the etiological agent of Chagas' disease, is able to catabolize both amino acids and carbohydrates as carbon and energy sources. It is well established that, in trypanosomatids, amino acids can act in several biological processes, such as differentiation, resistance to different kinds of stress and adhesion to the host-cells. Studies performed in other organisms show that histidine acts as an anti-inflammatory, a physiological antioxidant and confers resistance to the accumulation of different divalent metal ions. It is also known that histidine, together with sulfured amino acids, is involved in ovothiol-A formation, a compound with antioxidant properties. Despite these characteristics, little is known about histidine metabolism in *T. cruzi*. An analysis of *T. cruzi* genome suggested us that this organism is able to metabolize histidine to glutamate by a four-enzymatic-step pathway. In the present work, we describe the experimental conditions for expression and purification of the third enzyme, imidazolonepropionase (*Tc*IP, EC: 3.5.2.7), which converts urocanate in 4-imidazolone-5-propionate. The recombinant *Tc*IP was cloned in pGEM T-easy® and expressed in *Escherichia coli* BL21 strain using pET28a(+) vector. As expected, the apparent molecular mass for *Tc*IP fused to a 6-histidine tag (N-terminus), was 47 kDa as verified by SDS-PAGE. The recombinant protein was purified by affinity chromatography using NTA-Ni2+ resin, and it will be used for kinetic characterization. In order to test the effect of some compounds in the parasite biology, we choose 4-Imidazole Acetic Acid, an substrate analogue and 3-(2,5-dioxoimidazolidin-4-yl) propionic acid, an enzyme inhibitor. It was verified that these compounds do not affect the epimastigotes growth and now we are looking for new inhibitory compounds. Furthermore, we will obtain specific antibodies against *Tc*IP to evaluate the expression and cellular localization of the enzyme along the parasite's life cycle. **Supported by:**:CAPES; FAPESP; USP

BM023 - CHARACTERIZATION OF ONE ABC TRANSPORTER GENE OF *TRYPANOSOMA CRUZI* POTENTIALLY INVOLVED IN BENZNIDAZOLE RESISTANCE

FRANCO, J.F.C.J.[*1]; FERREIRA, R.C.[2]; BRIONES, M.R.S.[2]; ZINGALES, B.[1]
1.USP, SÃO PAULO, SP, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL.
e-mail:jaquesfranco@yahoo.com.br

ATP-binding cassette transporters (ABC transporters) are members of a transmembrane protein superfamily that uses ATP energy to translocate various substrates across membranes. Previous data from our group indicate that one ABC transporter gene (TcABCG1) is overexpressed in benznidazole (BZ)-resistant *T. cruzi* strains, as compared to susceptible strains. The major goal of this study was to characterize TcABCG1 single-copy gene in the two groups of strains and search for phenotype-associated characteristics. TcABCG1 gene sequence (2 Kb) of 17 strains belonging to different DTUs and with characterized BZ-susceptibility was determined. Sequences were aligned using ClustalX and BioEdit sequence editor and the presence of SNPs was visually assessed. Few non-synonymous amino acid substitutions were detected in the ATP binding domain of the ABC transporter in BZ-resistant TcI strains. The relevance of this finding is under investigation (Nunes, S.L. et al. this meeting). Network genealogy of TcABCG1 shows three distinct clades corresponding to TcI, TcII and Hybrids (H clade). All TcIII haplotypes as well as one TcV and one TcVI haplotype belong to clade H. The other TcV and TcVI haplotype is closer to TcII. Tcbat gene is a sister group of TcI clade. Two scenarios have been proposed for the origin of *T. cruzi* hybrid strains assuming two (TcI and TcII) or three (TcI, TcII and TcIII) ancestrals. To understand the origin of TcABCG1 hybrid haplotypes, potential recombination was inferred using the bootscan/RDP analysis. Intragenic recombination in TcIII supports the proposition that it is a hybrid between TcI and TcII whereas TcV haplotype sequence patterns indicate recombination between TcII and TcIII. One CL Brener haplotype is in its full length closer to TcII while the other is closer to TcIII. In the Tcbat gene some regions of recombination between TcI and TcIII were observed which could indicate a recombination event not previously described. **Supported by:**:FAPESP, CNPq

BM024 - Taxonomic identification and diagnostic evaluation of natural infection by *Leishmania* spp. in the sandfly fauna (Diptera: Psychodidae) of Rio Branco municipality (Acre, Brazil) using multiplex PCR and PCR-RFLP.

PEREIRA, T.A.[*]; DA COSTA REGO, T.A.N.; RODRIGUES, A.F.; MENDES, C.V.; BRAZIL, R.P.; BRITTO, C.; PEREIRA, D.P.
FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:pereirathata@gmail.com

In the state of Acre, American Cutaneous *Leishmania*sis (ACL) notifications have increased in the recent years without records of Visceral *Leishmania*sis (VL). In 2010, in the municipality of Rio Branco, there were 81.7 cases of ACL per 100,000 inhabitants, a very high prevalence identified by the Ministry of Health and local Secretariat. The studies on the sandfly fauna in Acre are still limited. The main objectives of the present investigation are related to the application of a multiplex PCR assay for the molecular diagnosis of female phlebotomines captured in Rio Branco and their taxonomic identification. Sand flies are being collected with the support of the Municipal Surveillance and Health board since April 2011. The captures were performed during 12 hours each, using HP light traps distributed in 6 areas: 5 residential areas and 1 recreation area. Sand flies collected were identified according to the methodology proposed by Galati et al. 2003. For the detection of natural infection by *Leishmania* spp., the phlebotomines were submitted to a multiplex PCR assay directed to the kDNA molecular target, followed by non-isotopic hybridization with a specific probe for the *Viannia* subgenus. Up to now, the captured sand flies were identified and distributed by species and by sex as individual samples. Three collections were performed, with 549 specimens collected. From these, 173/237♀ were individually submitted to molecular diagnosis until the time and 312 (193♂ and 119♀) were placed on slides for the correct taxonomic identification. We found positive results for infection with parasites from the *Viannia* subgenus in 12 out of 173 ♀ corresponding to 6.9% of this total. The implementation of this study in the state will bring useful information for supporting the development of epidemiological indicators in order to contribute to the assessment of risk infection, and further, generating more effective prevention and control measures for *Leishmania*s.**Supported by:**:FAPERJ

BM025 - CHARACTERIZATION OF THE MRPA GENE IN FOUR *Leishmania* LINES SUSCEPTIBLE AND RESISTANT TO POTASSIUM ANTIMONY TARTRATE

MOREIRA, D.S.[*1]; MONTE NETO, R.L.[2]; ANDRADE, J.M.[1]; FREZARD, F.[2]; MURTA, S.F.[1]
1.FIOCRUZ-MINAS, BELO HORIZONTE, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:silvane@cpqrr.fiocruz.br

ATP-binding cassette (ABC) transporters have been associated with drug resistance in various diseases. The MRPA gene, a transporter of ABCC subfamily, is involved in the resistance by sequestering metal-thiol conjugate in vesicles close to the flagellar pocket of *Leishmania* parasite. In this study, susceptible and resistant lines of four *Leishmania* species, L. guyanensis, *L. amazonensis*, *L. braziliensis* and L. infantum chagasi, were analyzed for: chromosomal location, presence of extrachromosomal amplification, analysis of amplification and mRNA levels of MRPA gene and Pgp protein expression. These lines were selected in vitro to potassium antimony tartrate (SbIII) and the resistance index varied from 4 to 20-fold higher than of their wild-type counterparts. Pulsed field gel electrophoresis (PFGE) analysis indicated an association of chromosomal amplification of MRPA gene with the drug resistance phenotype in SbIII-resistant *L. amazonensis*, *L. braziliensis* and L. infantum chagasi lines. The results obtained by alkaline lysis of these *Leishmania* samples showed the presence of extrachromosomal amplification only in the antimony-resistant *L. braziliensis* line. Southern blot assays with the BamHI endonuclease indicated a fragment with intensity ten-fold higher in the SbIII-resistant *L. braziliensis* line when compared to its susceptible counterpart. Levels of mRNA MRPA gene determined by real-time quantitative RT-PCR showed an increased expression of two fold in antimony-resistant lines of *L. amazonensis* and *L. braziliensis* compared to their respective susceptible counterparts. Western blot analysis revealed that the C219 anti-Pgp monoclonal antibody (Abcam) recognized the Pgp protein of 170 kDa. This polypeptide is more expressed in the SbIII-resistant L. guyanensis and *L. amazonensis* lines than in their susceptible counterparts. Our results indicate that the mechanisms of antimony-resistance of the MRPA gene are different among species of *Leishmania* analyzed. **Supported by:**:CNPq, FAPEMIG, CPqRR, PDTIS/CPqRR and UNICEF/UNDP/World Bank/WHO/TDR

BM026 - Functional analysis of ornithine decarboxylase (ODC) in *Leishmania* guyanensis and L. infantum chagasi lines susceptible and resistant to antimony

ZÓBOLI, A.P.C.[*]; MURTA, S.F.
FIOCRUZ-MINAS, BELO HORIZONTE, MG, BRASIL.
e-mail:silvane@cpqrr.fiocruz.br

Ornithine decarboxylase (ODC) is the first enzyme that initiates synthesis of polyamines putrescine, spermidine and spermine in the cells. The polyamines are necessary for a variety of biological events such as protein synthesis, DNA replication and cell division. Initially in this study the levels of ODC enzyme expression were analyzed in lines of *L. amazonensis*, *L. braziliensis*, L. infantum chagasi and L. guyanensis susceptible and resistant to antimony. Protein expression was determined by Western blotting experiments using a polyclonal antibody anti-LdODC. The results showed that the enzyme ODC is 4 and 40-fold more expressed in resistant lines of *L. amazonensis* and L. guyanensis than in their respective susceptible lines. On the other hand, this enzyme is 11-fold less expressed in the resistant *L. braziliensis* line than in its susceptible counterpart. No difference in the ODC enzyme expression level was observed between susceptible and resistant L. infantum chagasi lines. Functional analysis of ODC gene was performed to determine whether overexpression of ODC in susceptible and resistant L. guyanensis and L. infantum chagasi lines would alter the antimony-resistance phenotype of transfected parasites. Analysis by Western blotting showed that the level of expression of the ODC enzyme was higher in transfected parasites when compared to non-transfected ones. IC 50 analysis showed that susceptible L. guyanensis line that overexpress ODC protein are approximately 5-fold more resistant to SbIII compared to its parental non-transfected line. On the other hand, overexpression of the ODC in resistant L. infantum chagasi line reversed the SbIII-resistance phenotype. However, overexpression of ODC in the susceptible L. infantum chagasi line did not alter the SbIII-resistance phenotype. In conclusion, our results suggest that the ornithine decarboxylase may be involved in the antimony-resistance phenotype in L. guyanensis. **Supported by:**:CNPq, CAPES, FAPEMIG, PIDTIS/FIOCRUZ, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR.

BM027 - Killer cell immunoglobulin-like receptor gene diversity in population naturally exposed to malaria in Porto Velho, Northern Brazil

SILVA, L.A.[*1]; PERCE-DA-SILVA, D.S.[1]; LIMA-JÚNIOR, J.C.[1]; CARDOSO-OLIVEIRA, J.[2]; SANTOS, F.[3]; PORTO, L.C.[2]; OLIVEIRA-FERREIRA, J.[1]; BANIC, D.M.[1]

1.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UERJ, RIO DE JANEIRO, RJ, BRASIL; 3.LACEN, PORTO VELHO, RO, BRASIL.

e-mail:daiana@ioc.fiocruz.br

Introduction: Killer immunoglobulin-like receptors (KIR) regulate the activity of natural killer and T cells through interactions with human leucocyte antigen (HLA) class I ligands. Some studies have been associated KIR genes and genotypes KIR/HLA ligands with incidence and progression of various infectious diseases. Objective: Characterize the genetic frequency of KIR receptors and their ligands HLA-I in subjects (n=377) naturally exposed to malaria (Porto Velho-RO). Methodology: Genotyping of the population was performed by PCR-SSO and Luminex equipment for reading. The data were analyzed by chi-square test (χ2) with Yates' correction or Fisher's exact test. Results: We observed a higher frequency of the genes KIR2DL1, 3DL1, 2DS4 and 2DL3 (>89% in all), HLA-C1,-Bw4 and-C2 (>66% in all) and pairs KIR2DL2_3/C1, KIR3DL1/Bw4 and KIR2DL1/C2 (>66% in all) in the population of Porto Velho, which is similar to other Brazilian regions. In our study, we identified 48 KIR genotypes being the most frequent genotypes 1 (30.8%) and 2 (15.2%). Individuals in the group of natives of Porto Velho presented a greater genotypic variability (43/48) and a higher frequency of genotype 2 compared to migrants (P<0.01). The natives group presented a large number of KIR genes that were present only in this group (27/43). The natives with genotypic profile exclusive reported less malarias in the past and longer time since the last infection. Conclusion: it was not observed any influence on the KIR genes and their HLA class I ligands on susceptibility to malaria. Group of natives with exclusives genotypes are associated with Amerindians population and such genotypes have a higher gradient gene activating. Our data suggest that the genotypes of natives exclusives confer added protection against malaria. The data obtained in this work may contribute to future studies on the functional impact of these genes in regulating the immune response in malaria and other infectious diseases. **Supported by:**:IOC, CNPq

BM028 - New trick for a conventional enzyme: mevalonate kinase, a glycosomal enzyme, is secreted by *Trypanosoma cruzi*, and can modulate cell invasion and signaling. Another moonlighting enzyme?

FERREIRA, E.R.[*1]; CORTEZ, C.[1]; CRUZ, M.C.[1]; REBOREDO, E.H.[2]; SILVA, C.V.[3]; LIMA, F.M.[1]; DASILVEIRA, J.F.[1]; YOSHIDA, N.[1]; MORTARA, R.A.[1]; BAHIA, D.[1]

1.UNIFESP, SAO PAULO, SP, BRASIL; 2.USP, SAO CARLOS, SP, BRASIL; 3.UFU, UBERLÂNDIA, MG, BRASIL.

e-mail:edendearaujo@gmail.com

Mevalonate kinase (MK) is an essential enzyme in the mevalonate pathway. This pathway converts mevalonate into sterol isoprenoids, such as cholesterol in humans or ergosterol in trypanosomatids. MK, which is conserved from bacteria to mammals, localizes to glycosomes in trypanosomatids. Bioinformatics tools demonstrated that *T. cruzi* has two MK isoforms (TcMK1 and TcMK2). TcMK2 has a well-defined signal peptide at N terminus suggesting that it could be secreted. To evaluate the role of secreted TcMK in host cell interaction, recombinant TcMK was produced and anti-TcMK antibodies were raised. Secreted TcMK isoform bound in dose-dependently fashion to HeLa cells and positively modulated internalization of *T. cruzi* extracellular amastigotes (EA) whereas inhibited invasion by metacyclic trypomastigotes (MTs). TcMK was found in the supernatant of MTs and EAs by Western blot analysis using anti-TcMK antibodies. Immunofluorescence of MTs, assessed by confocal microscopy, revealed partial colocalization of TcMK and BiP, a constitutive endoplasmic reticulum protein. HeLa cell signaling experiments showed that TcMK induced phosphorylation of both MAPK pathway components and proteins related to actin cytoskeleton modifications. The results suggested that TcMK is another moonlighting enzyme given its unexpected behavior. Moonlighting enzymes are proteins can that perform more than one function; the second one is usually an unexpected one. Many moonlighting enzymes are involved in glycolysis, an ancient metabolic pathway. In addition to playing a classical role in isoprenoid synthesis in glycosomes, TcMK is secreted and may modulate host cell signaling required for *T. cruzi* invasion. This is a unique feature for a *T. cruzi* enzyme. These observations open new questions as to be how moonlighting proteins could have evolved and what would be the advantage for an intracellular parasite of having distinct functions performed by a single protein. **Supported by:**:FAPESP, CAPES E CNPQ

BM029 - EPITOPE MAPPING OF CENTRAL DOMAIN OF SAP (SERINE-, ALANINE-, AND PROLINE-RICH PROTEIN) AND ITS ROLE IN HOST CELL ADHESION, LYSOSOME EXOCYTOSIS AND INVASION BY *TRYPANOSOMA CRUZI* METACYCLIC FORMS

ZANFORLIN, T.[*1]; BAYER-SANTOS, E.[1]; ALMEIDA, I.C.[2]; CORTEZ, C.[1]; YOSHIDA, N.[1]; DASILVEIRA, J.F.[1]

1.UNIFESP, SAO PAULO, SP, BRASIL; 2.UNIVERSITY OF TEXAS, EL PASO, ESTADOS UNIDOS.

e-mail:tamiris.zanforlin@gmail.com

SAPs peptides are constituted by high content of serine, alanine and proline residues and present a 155-aa conserved central domain (SAP-CD). Using overlapping sequences from SAP-CD, a 54-aa peptide named SAP-CE (residues 60-113 of SAP-CD) was identified by anti-SAP murine monoclonal antibody (MAb-SAP). The recombinant protein SAP-CE was able to adhere to HeLa cells in a dose-dependent and saturable manner, suggesting a ligant-receptor interaction. The recombinant protein SAP-CE reduced in 50% the host cell invasion by *T. cruzi* metacyclic forms (CL strain). This phenomenon could not be seen in HeLa cells incubated concomitantly with SAP-CE and metacyclic forms. The peptide SAP-CE induced a 130% increase in host cell lysosome exocytosis, which was also confirmed by immunofluorescence assays that showed the lysosome mobilization from a typical perinuclear distribution to host cell plasmatic membrane. Transcription of SAP genes was investigated by RT-PCR. A 135-bp fragment was amplified in epimastigotes, metacyclic trypomastigotes and amastigotes, suggesting that family SAP is expressed in all developmental forms. SAP1 transcripts encoding signal peptide and GPI anchor site were also isolated from epimastigotes by RT-PCR. Real time PCR analysis demonstrated that metacyclic forms present a transcriptional level of SAP family 1.86 ± 0.12 times higher compared with epimastigotas. Immunoblotting assays with MAb-SAP identified a 55-kDa SAP protein in soluble protein fraction secreted by epimastigotes (Dm28c strain) and metacyclic trypomastigotes (G strain). A peptide (TRLPVTPPKK) that displayed high similarity with SAP sequence (Tc00.1047053503973.70) was identified by mass spectrometry in vesicle preparation secreted by metacyclic forms (Dm28c strain), suggesting that two variants of SAP proteins are released by the parasite. Indirect immunofluorescence assays using anti-SAP polyclonal antibody and MAb-SAP showed reactivity with the flagellar pocket and cytoplasm. **Supported by:**:FAPESP, CNPq and CAPES

BM030 - Molecular characterization of a specific L-proline permease from *Trypanosoma cruzi*

SILBER, A.M.[*1]; MARTÍNEZ SAYÉ, M.[2]; MIRANDA, M.R.[2]; CÁMARA, M.L.M.[2]; PEREIRA, C.A.[2]

1.ICB-USP, SAO PAULO, SP, BRASIL; 2.LBMTC-CONICET-UBA, BUENOS AIRES, ARGENTINA.

e-mail:asilber@usp.br

In trypanosomatids, proline is a key amino acid since constitutes a main carbon and energy source. Recently, new roles in the parasite biology have been assigned to this metabolite. In *Trypanosoma cruzi*, proline also provides the energy to support the host-cell invasion process in metacyclic trypomastigotes and the infection progression, particularly participating in the differentiation from intracellular epimastigotes to trypomastigotes. It is also noticeable that the accumulation of free proline constitutes a defense mechanism against oxidative stress. This accumulation is determined mainly by the rate three independent events: degradation, biosynthesis and transport. In this work we identified a proline permease called TcAAP1, which belongs to the Amino Acids/Auxin Permeases superfamily (TcAAAP). This protein of 476 aa, has 9-10 transmembrane spanners, with an N-terminal domain of 90 aa completely variable in terms of primary structure. This feature constitutes a characteristic of all the TcAAAP family. Using deletion mutant yeast strains, lacking the general amino acid permease (GAP1) and the proline permease (PUT4), and the plasmid pDR196-TcAAP1, complementation assays were performed. Yeasts transformed with TcAAP1 were able to grow in minimal medium using proline as the single nitrogen source. In addition, yeast transformed with TcAAP1 showed a proline transport rate up to 10-folds higher than controls. Finally, a TcAAP1 overexpression model was constructed in *T. cruzi* epimastigotes. In this model the subcellular localization of TcAAP1 will be established using the endogenous tri-FLAG tag, and also the parasites will be tested for in vitro differentiation assays and for oxidative stress resistance. **Supported by:**:FAPESP-CNPq-INBEQMEDI-CONICET-ANPCyT

BM031 - The genome sequencing of *Leishmania* (*Leishmania*) amazonensis predicted expanded subfamilies of amastin-like surface proteins unique to L. (L.) mexicana complex

REAL, F.[*1]; VIDAL, R.O.; CARAZZOLLE, M.F.[2]; MONDEGO, J.M.C.; HERAI, R.H.[3]; CARVALHO, L.M.D.[2]; FERREIRA, R.C.[1]; MORTARA, R.A.[1]; BARBIéRI, C.L.[1]; CARVALHO, R.P.[4]; DASILVEIRA, J.F.[1]; BRIONES, M.R.S.[1]; PEREIRA, G.A.G.[2]; BAHIA, D.[1]
1.UNIFESP, SÃO PAULO, SP, BRASIL; 2.UNICAMP, CAMPINAS, SP, BRASIL; 3.UC, SAN DIEGO, ESTADOS UNIDOS; 4.UNC, CHAPEL HILL, ESTADOS UNIDOS.
e-mail:fernando.real@unifesp.br

Here we report the genome sequence and gene model annotations of *Leishmania (L.) amazonensis* that belongs to the *L. (L.) mexicana* complex. This protozoan parasite is responsible for simple and diffuse forms of cutaneous *Leishmania*sis in humans. The diffuse form is characterized by lesions that do not heal spontaneously and are unresponsiveness to current treatment. The genome assembly of *L. (L.) amazonensis* resulted in a genome length of 29.6 Mb similar to that of other *Leishmania* species. The in silico construction of gene models based on genome open reading frames suggested that *L. (L.) amazonensis* has an estimated number of 8,100 genes comparable to that of other *Leishmania* species. The predicted proteins encoded by *L. (L.) amazonensis* genes were searched for conserved domains by use of CDD-PFAM databanks. Transferase and chaperone-related were among the most prevalent conserved domains expanded in *L. (L.) amazonensis* and *L. (L.) mexicana*. Some conserved domains, such as viral protein, ATPase and thioredoxin, were identified exclusively in these two species, confirming that few species-specific genes may exist despite the striking conservation at gene content level among *Leishmania* species. The comparison of *L. (L.) amazonensis* orthologous gene families with other *Leishmania* species revealed an expansion of carboxypeptidades, branched-chain-amino acid aminotransferase and amastin-like subfamilies in both *L. (L.) amazonensis* and *L. (L.) mexicana*. Amastin multi-gene family belongs to one of the largest families of surface proteins in *Leishmania* and is only found in trypanosomatids. The phylogeny of *Leishmania* amastins suggested that *L. (L.) amazonensis* and *L. (L.) mexicana* share a subfamily of amastin proteins unique to the genus. These results contribute to the wealth of the now available genomic data related to trypanosomatids that may represent an important tool for future clinical studies. **Supported by:**:FAPESP

BM032 - Development of a semisolid medium for *Trypanosoma cruzi* and *Trypanosoma rangeli* cultivation

FAJARDO, E.F.[*]; FERREIRA, K.A.M.; RAMÍREZ, L.E.; SANTOS, M.C.; PEDROSA, A.L.
UFTM, UBERABA, MG, BRASIL.
e-mail:emanuella_fajardo@yahoo.com.br

LIT (Liver Infusion Tryptose) medium is used for in vitro cultivation of *Trypanosoma cruzi*, the causative of Chagas' disease, and *Trypanosoma rangeli*, a parasite that causes only temporary manifestations in humans and in other mammals, but it is pathogenic for triatomine vector. One of the major drawbacks in the genetic characterization and manipulation of these parasites is the lack of conditions to securely isolate parasite clones. Thus, the objective of this work was to establish conditions to isolate clones of *T. cruzi* and *T. rangeli* by plating them on semisolid medium. Three *T. cruzi* strains and two *T. rangeli* strains were used in this work. Epimastigote forms were plated in LIT semisolid medium prepared with 1% (w/v) noble agar supplemented with sterile HU (human urine) and 10% (v/v) Fetal Calf Serum (FCS). Colonies obtained were transferred to liquid medium for DNA analysis by Polymerase Chain Reaction (PCR). The supplementation of LIT/1% (w/v) noble agar plates with 3% (v/v) HU and the ressuspension of the parasites in LIT with 10% (v/v) FCS (Fetal Calf Serum) at the moment of spreading the inocula allowed observation of parasite clones at approximately 10 days after incubation at 28^{o}C. The semisolid medium developed provides the growth of *T. cruzi* and *T. rangeli* homogeneus populations with plating efficiencies between 1 and 1,5%. When these parasites were mixed and cultivated on the same plate only *T. cruzi* could be isolated. This procedure allows future studies of genetic manipulation in order to understand different biological aspects of these parasites.

BM033 - Analyses of *Trypanosoma cruzi* and yeast mutants for genes involved in the glycosylphosphatidylinositol biosynthetic pathway

CARDOSO, M.S.[*1]; MACEDO, I.B.[1]; TRIGUEIRO, R.C.[1]; SHAMS-ELDIN, H.[2]; SCHWARZ, R.T.[2]; GOMES, D.A.[1]; MARTINELLI, P.M.[1]; MENDONÇA-PREVIATO, L.[3]; PREVIATO, J.O.[3]; GAZZINELLI, R.T.[1]; TEIXEIRA, S.M.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.PHILIPPS-UNIVERSITY, MARBURG, ALEMANHA; 3.UFRJ, RIO DE JANEIRO, RJ, BRASIL. **e-mail**:marianascardoso@yahoo.com.br

Glycosylphosphatidylinositol (GPI) is an important structure *Trypanosoma cruzi* uses to anchor proteins on its surface, many of them essential for the infectivity of the parasite or its escape from the host immune response. *T. cruzi* GPI anchors are also strong proinflammatory molecules, being critical in the modulation of the host immune response against this parasite. Thus, the *T. cruzi* GPI biosynthetic pathway offers potential new targets for drug development against Chagas disease. We identified, through in silico analyses, several *T. cruzi* genes encoding enzymes of the GPI biosynthetic pathway. Sequences corresponding to DPM1, GPI3 and GPI12 genes, expressed in epimastigotes as GFP-fusion proteins, showed a cellular localization compatible with endoplasmic reticulum. Analyses of mRNA levels of TcGPI8 and TcGPI10 showed increased expression in epimastigotes and amastigotes, the two proliferative stages of the parasite. We transformed yeast mutants defective in each GPI gene and showed that TcDPM1, TcGPI10 and TcGPI12 restore the growth of yeast mutants in non-permissive conditions. The presence of complete GPI anchors synthesized by yeast mutants complemented with the *T. cruzi* genes was confirmed by thin layer chromatography (TLC) and SDS-PAGE analyses of yeast cell extracts containing [2-^{3}H] *myo*-inositol. The complementation of yeast mutants with the *T. cruzi* orthologs constitute a valuable new tool that can be used in high throughput screening of drugs against the parasite enzymes. To investigate the role of GPI anchored proteins in *T. cruzi*, we disrupt the GPI8 gene, which encodes the catalytic subunit of the GPI:protein transamidase complex. mRNA expression analyses showed that GPI8 single knockout parasites have decreased mRNA levels. However, attempts to delete the GPI8 second allele resulted in double resistant parasites presenting gene rearrangement of GPI8 sequences. Functional and morphological characterizations of these parasites are in progress. **Supported by:**:CNPq, HHMI, INCTV, FAPEMIG

BM034 - Species-specific polymorphisms in *Trypanosoma rangeli*

FERREIRA, K.A.M.[*]; FAJARDO, E.F.; LAGES-SILVA, E.; RAMÍREZ, L.E.; PEDROSA, A.L.
UFTM, UBERABA, MG, BRASIL.
e-mail:keiladri@yahoo.com.br

The high prevalence of *Trypanosoma rangeli* in humans, mixed or not with *Trypanosoma cruzi*, has been reported in several countries of Latin America. DNA-based detection methods can be useful tools for determining intraspecific polymorphisms and an alternative for the differential diagnosis of infections caused by these parasites. The aim of this work is to study polymorphisms in specific sequences of *T. rangeli* by analysis of microsatellites and single nucleotide polymorphisms. *T. rangeli*-specific primer pairs ALPTr1/2 and ALPTr5/6 designed from both a *T. rangeli* hypothetic protein and one anonymous sequence and DNA samples obtained from axenic cultures of *T. cruzi*, *T. rangeli* and *Leishmania* spp. were used in PCR. The amplification products of P07, Cas4, P18, and P19 strains were subjected to DNA sequencing. We obtained specific products of DNA from *T. rangeli* in PCR and no amplification of unwanted fragments of other trypanosomes. Additionally, primers ALPTr5/6 allowed the observation of a differential amplification pattern (280 and 250bp amplicons). Sequencing of the PCR products revealed the presence of polymorphic dinucleotides repeats (20 AT repeats in KP1 (-) and five AT repeats in KP1 (+) strains) entirely coincident with *T. rangeli* main lineages determined by kDNA classification. Furthermore, it was also possible to determine the presence of SNPs, some of which coincide with the genotypes KP1(+) e KP1(-) and others unrelated to this genotypic classification. Both sequences totalize 363bp and of these, 322 (88.7%) are constant sites and 41bp (11.3%), parsimony informative sites, with 8 transitions (19.5%), three transversions (7.3%) and 30 (73.2%) insertion/deletion (INDEL) events. The use of the ALPTr5/6 primers opens the perspective of identifying different genotypes of *T. rangeli* and could be used in further analyses to test the identification of *T. rangeli* and *T. cruzi* in humans and other biological fluids. **Supported by:** FAPEMIG and CNPQ. **Supported by:**:FAPEMIG E CNPQ

BM035 - Involvement of a DNA Mismatch repair protein in the oxidative stress response in Trypanosmatids

SILVA, V.G.*; MIRANDA, J.B.; CAMPOS, P.C.; MACHADO, C.R.; TEIXEIRA, S.M.R.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:juju_bmiranda@hotmail.com

DNA repair mechanisms are crucial for the genetic maintenance and can also contribute to generating genetic variability in many species, including members of the Trypanosomatidae family, which are causative agents of important human diseases. In one of these mechanisms, the Mismatch Repair Pathway (MMR), the MSH2 protein takes part in all heterodimers that can be formed and recognizes DNA mismatches that occur during DNA replication. Recent evidences have suggested that, in human fibroblast and may be also in trypanosomatids, MSH2 acts in an alternative pathway that responds to oxidative DNA damages. Our previous studies have shown that there are three different isoforms for MSH2, based on nucleotide polymorphisms, named TcMSH2 A, B and C, in the *T. cruzi* population. Functional characterization of isoforms A, B and C using different strains of the parasite provided evidences indicating that they respond differently to oxidative stress after treatment with genotoxic agents. Our data suggest that strains presenting TcMSH2A have a more efficient MMR when compared to strains presenting TcMSH2 B or C. In order to better evaluate the function o TcMSH2 as well as other components of MMR gene knockouts were performed in the CL Brener clone. Tcmsh2 single knockouts (KO) are more susceptible to hydrogen peroxide treatment and accumulate more oxidized guanine in the kinetoplast DNA when compared with wild type parasites. We have recently generated double knockouts for Tcmsh2 through homologous recombination, which were analyzed by PCR and Northern Blot. Single and double KO msh2 clones are currently being evaluated for their ability to resist to a highly oxidative environment such as the one inside infected macrophages. In addition, we will evaluate the involvement of other MMR proteins in the oxidative stress response, by analysing single KO of TcMSH6, one of the proteins that forms heterodimers with TcMSH2. **Supported by:**:CNPq, FAPEMIG, HHMI

BM036 - Isolation and characterization of novel polymorphic microsatellite loci in different chromosomal bands from *Trypanosoma cruzi* genome

LACERDA, A.G.*[1]; LOPES, D.O.[1]; MACEDO, A.M.[2]; VALADARES, H.M.S.[1]
1.UFSJ, DIVINÓPOLIS, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:alineguerralacerda@gmail.com

Since its discovery in the *T. cruzi* genome, the microsatellite markers proved to be extremely useful in several analyzes of genetic variability, population structure and phylogeny for this parasite. Previous studies performed by Oliveira et al. (1998) and Valadares et al. (2008) allowed the isolation of microsatellite loci composed by di, tri and tetranucleotide motifs. However, these two studies present some limitations, such as short flanking regions to the repeats and microsatellite loci restricted to chromosome 3 from T. cruzi. After the complete sequencing of *T. cruzi* genome, an extensive number of lengthy DNA sequences and the chromosomal rearrangement for the CL Brener clone genome are available on TritrypDB database. Based on these data, our major goal in this study was to isolate new microsatellite loci presenting great flanking regions and allocated in different chromosomal bands. Initially for identification of microsatellite loci, the DNA sequences corresponding to Esmeraldo like haplotype from chromosomes 1 to 11 (except chromosome 3) were retrieved from TritrypDB and submitted to Tandem Repeats Finder software. After this analysis, 8 novel microsatellite loci composed by di, tri and tetranucleotide motifs were selected: TcCAA9, TcAT13, TcTTA15, TcCAG8, TcAAAT9, TcTG14, TcAC13 and TcCTTT7 from chromosomes 1, 2, 4, 5, 6, 7, 8 and 11, respectively. The specific primers for each microsatellite loci were designed and the PCR amplifications assays were standardized employing 1 ng of genomic DNA from CL Brener clone. To evaluate the polymorphism of these 8 microsatellite loci, was performed a preliminary screening involving 12 *T. cruzi* strains belonging to six philogenetic lineages and they all proved to be polymorphic loci. Subsequently, a great number of *T. cruzi* strains and genetic population parameters will be analyzed for to further characterize these novel microsatellite loci. **Supported by:** FAPEMIG/UFSJ

BM037 - **IMMUNE RESPONSE TO RIBONUCLEOPROTEIC ANTIGENS CONTAINING REPEATED AMINO ACID SEQUENCES FROM *TRYPANOSOMA CRUZI***

CALDAS, G.A.B.[*1]; VALENTE, B.M.[1]; DE MELO, M.F.[1]; FILHO, B.G.[1]; DE ALMEIDA, R.M.[1]; DA ROCHA, W.D.[2]; GAZZINELLI, R.T.[1]; TEIXEIRA, S.M.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFPR, CURITIBA, PR, BRASIL.
e-mail:gabrielaburle@hotmail.com

Using an immunoscreening approach, we isolated several antigens derived from a *Trypanosoma cruzi* amastigote cDNA library that react with sera from chronic Chagas disease patients. Sequence analyses of these clones indicate that ribonucleoproteins and proteins containing tandem amino acids repeats are among the most prominent antigens expressed in *T. cruzi* amastigotes. We characterized one such antigen presenting homology to the eukaryotic L7a ribosomal protein and containing an Ala-Lys-Pro-rich repetitive domain at its N-terminus. To evaluate the role, during infection, of amino acid repeats present in *T. cruzi* antigens, we generated recombinant version of the complete antigen (TcRpL7a) as well as a version containing only its repetitive domain (TcRpL7aRep) and used them to immunize mice. Whereas mice immunized with TcRpL7a produced IgG antibodies against the complete protein and against the repeats, mice immunized with TcRpL7aRep did not develop antibodies. Moreover, levels of IFN-γ were higher in animals immunized with TcRpL7a compared to TcRpL7aRep. After challenging immunized animals with *T. cruzi* trypomastigotes, we observed that, whereas immunization with the complete TcRpL7a antigen confers partial protection when compared to control groups, animals immunized with TcRpL7aRep showed incresead parasitemia. Thus, our results suggest that the repetitive domain present in TcRpL7a may be used by the parasite to modulate the host immune response in a way that allows parasite persistence. To further investigate this hypothesis, we have produced a truncated version of the recombinant protein in which the repetitive domain has been deleted (TcRpL7aΔRep) and also inserted the repetitive domain into a non related protein (GST-Rep). These new antigens are being tested in immunization protocols, in which we will use *T. cruzi* expressing luciferase as a challenging strain so that we can perform luciferase assays to evaluate parasitemia. **Supported by:**::CAPES, CNPq and INCTV.

BM038 - Characterization of Kinetoplast Associated Proteins (KAPs) of symbiont containing trypanosomatids

SOUZA, S.S.[*1]; VASCONCELOS, A.T.R.[2]; DASILVEIRA, J.F.[3]; DE SOUZA, W.[4]; MOTTA, M.C.M.[4]; SILVA, R.[4]
1.UFRJ-RJ, RIO DE JANEIRO, RJ, BRASIL; 2.LNCC, PETRÓPOLIS, RJ, BRASIL; 3.UNIFESP, SÃO PAULO, SP, BRASIL; 4.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:silsouza@biof.ufrj.br

The mitochondrial DNA of trypanosomatids is localized in a structure named kinetoplast, is composed by a network of circular and catenated DNA, usually organized into a disc structure. The Kinetoplast Associated Proteins (KAPs) are basic proteins similar to histone H1-like proteins, which may take part in the topology and segregation of the kDNA network. In the family Trypanosomatidae, six monoxenic species, as Angomonas deanei and Strigomonas culicis, harbor a symbiotic bacterium in the cytoplasm, which maintains an obligatory association with the host protozoan, thus constituting an excellent model to studying the origin of organelles. Interestingly, the kinetoplast in symbiont bearing species, present differences in the ultrastructure and kDNA topology when compared with other trypanosomatid protozoa. In this work, our main goal was to identify and characterize the KAPs in symbiont containing trypanosomatids. For this purpose, we performed Blast searches on the genome databases of A. deanei and S. culicis aiming to identify orthologous genes to KAPs in other trypanosomatids. Thus, we found two well-known KAPs with high content of basic amino acids lysine and alanine in A. deanei, which are predicted to be KAP3 and KAP4. As expected, they showed some features of KAPs such as small size (16.1 and 13.6kDa) and basic nature (pI 12.0 and 11.4), share 52% and 62% amino acid identity with Crithidia fasciculata and *Leishmania* major, respectively. In S. culicis, there are three KAPs which have high content of amino acids lysine and alanine, named ScKAP4, ScKAP2 and ScKAP-like. The ScKAP4 has molecular weight of 14.5 kDa, pI 11.57 and 62% of identity with KAP of *L. major*. The ScKAP2 has 16.5 kDa, pI 11.8 and 50% of identity with KAP2 of *L. major*. Finally the ScKAP-like has 21.5 kDa, pI 11.8, with 38% identity with KAP from *Trypanosoma cruzi*. Further characterization of these proteins includes the immune localization using specific antibodies against KAPs. **Supported by:**::CNPQ AND FAPERJ

BM039 - Histopathological and molecular evaluation of Trypanosoma vivax infection in the ovaries of goats experimentally infected with an isolate from Brazilian semi-arid region

RODRIGUES, C.M.F.[*1]; OLINDA, R.G.[2]; SILVA, T.M.[2]; VALE, R.G.[2]; LIMA, G.L.[2]; GARCIA, H.A.[1]; TEIXEIRA, M.M.[1]; BATISTA, J.S.[2]
1.USP, SÃO PAULO, SP, BRASIL; 2.UFERSA, MOSSORÓ, RN, BRASIL.
e-mail:cmonadeli@hotmail.com

Infection by T. vivax and other African trypanosomes strongly compromises the productive and reproductive performance of livestock and causes severe economic losses. Outbreaks of T. vivax in the semi-arid region of northeastern Brazil are characterized by wasting disease in cattle, sheep and goats with hematological, cardiac and nervous compromises in addition to reproductive failures. Similar to reports from Africa, we previously observed a reduction in fertility rates and severe testicular degeneration and epididymitis in male sheep infected with T. vivax from this region. Although anestrus is frequently reported in goats and sheep infected with T. vivax, the effects of this infection on the female reproductive organs need clarification. In this study, we addressed this issue through a histopathological evaluation of ovarian follicular morphology and classification in goats experimentally infected with a T. vivax isolate from the Brazilian semi-arid region. The infected animals presented typical clinical signs of trypanosomosis by T. vivax, including anemia, hyperthermia, pallor of the mucous membranes, enlarged lymph nodes, apathy and progressive loss of weight. All the infected goats remained anestrus throughout the experimental period, and we demonstrated important disturbances in the ovary evidenced anestrus and atrophy, abnormal ovary follicular development and, through PCR, we detected T. vivax DNA in the ovarian tissues of the infected goats. Our findings greatly contributed to the understanding of the reproductive failure of female ruminants associated with trypanosomosis by T. vivax. **Supported by:**:CNPQ

BM040 - Genomic characterization and functional studies of the amastin gene family of *Leishmania* braziliensis

PAIVA, R.M.C.[*]; NAKAGAKI, B.N.L.; MELO, M.N.; TEIXEIRA, S.M.R.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:ritamarciabqi@yahoo.com.br

*Leishmania*sis constitutes an important global health problem for which there is no vaccine and the available drugs for treatment are inadequate. *L. brasiliensis* is the human protozoan parasite that causes cutaneous *Leishmania*sis. Its genome has been sequenced recently and post-genomic studies on *L. brasiliensis* can be largely benefited from the discovery of a functional RNAi pathway. Amastins are surface glycoproteins initially described in *Trypanosoma cruzi* and subsequently found to be encoded by large gene families also present in the genomes of several species of the genus *Leishmania* as well as in other trypanosomatids. Although amastins have been found to be highly expressed in the intracellular amastigote stage of several *Leishmania* species and are among the most immunogenic of all *Leishmania*l surface antigens, their function remains unknown. Here we described the characterization of different members of the amastin gene family present in the genome of *L. braziliensis*. In silico analyses revealed 47 amastin genes located on eight different chromosomes and belonging to all of the four previously described amastin subfamilies: 2 copies of alfa- and beta-amastins respectively, about 4 copies of gama-amastins and 41 copies of delta-amastins. Only delta-amastin genes are organized in clusters with alternating copies of tuzin genes. Northern blot analysis with RNA obtained from axennically grown amastigote and promastigotes showed that these genes are up-regulated in *L. brasiliensis* amastigotes. To investigate their function, we overexpressed two members of the amastin gene family and are attempting to reduce their expression by RNAi, using the pIR1-Phleo-GFP+(a) vector (gently provided by Dr. Stephen M. Beverley). Real Time RT-PCR analyses indicated that we have succeeded in altering expression levels of these two amastin genes in transfected *L. brasiliensis* promastigotes, which are currently being characterized in in vitro as well as in in vivo infection assays. **Supported by:**:CAPES, FAPEMIG, INCTV

BM041 - Oxidative stress alters the interaction of *Leishmania amazonensis* RPA-1 with telomeres

DA SILVA, M.S.[*1]; PEREZ, A.M.[1]; CANO, M.I.N.[2]
1.UNICAMP, BOTUCATU, SP, BRASIL; 2.UNESP, BOTUCATU, SP, BRASIL.
e-mail:mamasantos2003@yahoo.com.br

*Leishmania*sis is a spectrum of diseases caused by parasites of the genus *Leishmania*. During infection, parasites use different mechanisms to survive host defenses including the exposition to reactive oxygen species (ROS). To overcome these barriers they usually increase the expression of specific proteins, which are able to protect their genome from damage. However, it is still unknown how these parasites protect telomeres from ROS attack. Telomere integrity plays a crucial role in continuous cell proliferation and is the main target of oxidative damage. Replication Protein A (RPA) is an important player in DNA metabolic pathways including DNA repair and telomere maintenance. *Leishmania* RPA-1 was well characterized as a telomeric protein and is probably also involved in damage response, by participating directly in DNA replication and telomere protection during damage. Differential oxidation of deoxyribose in genomic DNA from parasites treated with H2O2 and from non-treated controls was estimated by the quantification of 8-oxodG using high-performance liquid chromatography (HPLC). The extent of DNA damage was detected by TUNEL assay. Parasites treated in the same conditions as above were analysed by telomeric FISH combined with IF and by Western blot using anti-LaRPA-1, anti-Ogg1 and anti-AP endonuclease sera. In vivo ChIP assays of BrdU incorporated to DNA was combined with Western blot probed with anti-BrdU and Southern blot using a C-rich telomeric probe. Protein:protein interaction assays were also performed using nuclear extracts from parasites treated with H2O2 and recombinant proteins. Preliminary results showed alterations in the interactions among LaRPA-1 and telomeres and/or with proteins partners after H2O2 treatment, suggesting that this protein is involved with the oxidative stress response and telomere protection. **Supported by:**:FAPESP, CNPq

BM042 - Organization and evolution of *Trypanosoma cruzi* telomeres

MARINI, M.M.[*1]; ANTONIO, C.R.[1]; BARROS, R.R.M.[1]; CORTEZ, D.R.[1]; CHIURILLO, M.A.[1]; BARTHOLOMEU, D.C.[2]; DASILVEIRA, J.F.[1]
1.UNIFESP, SÃO PAULO, SP, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:marjoriemm@globo.com

The subtelomeres of many protozoa are enriched in genes with roles in niche adaptation. Genes located in these regions can be submitted to high rates of mitotic recombination. The *T. cruzi* telomere is composed by tandem repeats of TTAGGG followed by a species-specific telomeric junction. *T. cruzi* subtelomere is enriched in trans-sialidase, DGF-1, RHS, retrotransposons, RNA-helicase and N-acetyltransferase genes and its structure varies widely, mainly as a result of large differences in abundance and organization of these genes. The lack of synteny observed in subtelomeric regions suggests the occurrence of recombination between chromosomal ends. Our aim is integrate the chromosomal extremities of *T. cruzi* in silico chromosomes to the chromosomal bands of clone CL Brener separated by PFGE. We analyzed 49 chromosomal ends defined in silico to identify genes located only at one chromosome or in the homologous pair that can be used as telomere specific markers. We were able to identify 32 telomeres specific markers distributed in 23 different in silico chromosomes. Interstitial markers of each chromosome were also hybridized as a reference. Some markers were mapped on homologous chromosomes with same size whereas others were mapped on sized-different homologous. Our results indicate that subtelomeric region assemblies seem to be very accurate. We also hybridized the telomere specific markers with the chromosomal bands of isolates from different *T. cruzi* lineages (clone CL Brener, Esmeraldo cl3, Dm28 and G strain) separated by PFGE. Despite the subtelomeric regions of *T. cruzi* are highly diverse in gene content, organization and size the same hybridization pattern was observed. **Supported by:**:fapesp

BM043 - PCR-RFLP MULTILOCUS ANALYSIS OF *Toxoplasma gondii* GENOTYPES CIRCULATING BETWEEN ANIMALS AND HUMANS IN MINAS GERAIS STATE, BRAZIL.

SILVA, L.A.*; VITOR, R.W.A.; ANDRADE, R.O.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:leticiaazs@gmail.com

Toxoplasma gondii may be transmitted directly or indirectly from animals to humans. It is important to investigate the genotypes of *T. gondii* isolates, because it allows studying its correlation with the parasite biology, as well molecular epidemiology and sources of infection or transmission routes. The aim of this study was to verify if genotypes of *T. gondii* circulating in animals from Minas Gerais (Brazil) are equivalent to genotypes circulating in humans from the same region. For this, 24 *T. gondii* isolates previously obtained in Minas Gerais (8 from dogs, 12 from free range chickens and 4 from congenital toxoplasmosis in humans) were genotyped by PCR-RFLP in 11 loci (SAG1,5'+3'SAG2, SAG2 alt, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico). PCR products were digested by specific restriction enzymes and type I, II and III strains were used as references. The genotypes identified were compared with genotypes of 24 isolates previously obtained from human cases of congenital toxoplasmosis in the same state (Carneiro, 2011). The complete genotyping was achieved in 100% of isolates. We observed a high genetic variability and 12 different genotypes were identified. Two isolates had genotype Type BrI, 6 had Type BrII and 2 had Type BrIII. These genotypes are common in Brazil. One isolate had archetypal genotype Type III. Four isolates belonged to ToxoDB genotype #108 and 2 had ToxoDB genotype #19. Five new genotypes were identified among 5 isolates. Two isolates had an atypical genotype that has been previously described by Carneiro (2011). This author also found BrII, BrIII and #108 genotypes among isolates from newborns. By a descriptive analysis we can conclude, despite the existence of unique genotypes, there is a high degree of similarity between genotypes circulating in humans and animals in Minas Gerais. *T. gondii* genotypes that commonly circulate among these species in this region are: BrII, BrIII, #108 and one atypical described by Carneiro (2011). **Supported by:**::CNPq and FAPEMIG

BM044 - A phosphatidylinositol kinase (PIK) gene identified in trypanosomatids exhibits a unique architecture containing a FYVE domain at N terminal.

OLIVEIRA, P.*[1]; LIMA, F.M.[1]; SANCHEZ-FLORES, A.[2]; FERREIRA, E.R.[1]; MORTARA, R.A.[1]; DASILVEIRA, J.F.[1]; BAHIA, D.[1]
1.UNIFESP, SAO PAULO, SP, BRASIL; 2.SANGER, CAMBRIDGE, INGLATERRA.
e-mail:edendearaujo@gmail.com

Phosphatidylinositol (PI) kinases are at the heart of one of the major pathways of intracellular signal transduction. Recently, we reported a survey for conventional PIK and PIK-related kinases made by similarity searches against five human pathogenic trypanosomatids. Conventional PIK and PIK-related subtypes were classified into five models (I-V). The phylogenetic inference of trypanosomatid PIK has generated a panel which we called the TriPIKinome. During the mining steps for identifying PIK genes a *Trypanosoma cruzi* gene presented a particular domain architecture. This gene contains a FYVE domain at the N-terminal position in addition to the phosphatidylinositol kinase domain. There is no evidence in databases of eukaryotic genes carrying this architecture. Multiple alignment of this protein revealed orthologs in all trypanosomatid genomes available. The FYVE-PIK proteins of trypanosomatids are phylogenetically distant from the groups containing exclusively the FYVE or PIK domain. We have identified this same architecture in Acanthamoeba polyphaga mimivirus virus species but the identity was very low when compared to the trypanosomatid proteins. In order to study the genomic organization of this gene family on *Trypanosoma cruzi* chromosomes, a specific fragment of FYVE-PIK gene was cloned. Chromosomal bands from G strain (TcI) and CL Brener (TcVI) clone were separated by pulsed-field gel electrophoresis (PFGE) and hybridized with FYVE-PIK gene probe. This probe hybridized with bands of 2.95 Mb and 1.35 Mb in CL Brener clone and with a band of 1.43 Mb in G strain, suggesting a recurrence of chromosomal polymorphism between *T. cruzi* isolates. This hybridization pattern was different from that observed for other PIK family members. We concluded that we have identified a phosphatidylinositol kinase with unique architecture that is distant to other kinases proposed in our classification. **Supported by:**::CAPES, CNPQ, FAPESP

BM045 - PRESENCE OF GROUP II TRANS-SIALIDASE GENES IS A COMMON FEATURE OF MEMBERS OF THE *TRYPANOSOMA CRUZI* CLADE

CHIURILLO, M.A.[*1]; RAMIREZ, J.L.[2]; LIMA, F.M.[3]; DASILVEIRA, J.F.[3]
1.UCLA/UNIFESP, BARQUISIMETO, VENEZUELA; 2.IDEA, CARACAS, VENEZUELA; 3.UNIFESP, SAO PAULO, SP, BRASIL.
e-mail:mchiurillo@ucla.edu.ve

Trans-sialidase/trans-sialidase-like (TS) constitutes a polymorphic protein superfamily, classically classified into four groups, that has been described in several members of genus Trypanosoma. Whereas members of TS group I (some with catalytic activity) are present both in *T. brucei* and *T. cruzi* taxonomic clades, TS group II, which in *T. cruzi* comprise members of the gp85 surface glycoproteins, have been described only in species included in *T. cruzi* clade (*T. cruzi*, *T. rangeli* and *T. dionisii)*. This group has been implicated in *T. cruzi* in host attachment and invasion. In this work we cloned and performed sequences analysis of TS group II of some American trypanosomas (*T. dionisii*, *T. conorhini*, *T. cruzi marinkelli* and *T. saimiri*) utilizing a PCR-based approach with degenerated primers based on ATG and VTVxNVxLYNR motif, which are characteristics of all members of the gp85/trans-sialidade superfamily. We compared TS sequences obtained with those deposited in databases and constructed a phylogenetic tree with MEGA5. TS genes from *T. dionisii*, *T. conorhini* and *T. saimiri* exhibited the conserved asp-box and VTVxNVxLYNR motifs, but lacks FRIP motif (conserved in TS group I), being sequences obtained from *T. conorhini* the most divergent. Moreover, phylogenetic analysis grouped these sequences with TS group II. In conclusion, our results suggest that TS group II members are presents in all members of *T. cruzi* clade, which might mean that the expansion of this TS subgroup occurred after separation of both clades of genus Trypanosoma. **Supported by:** CDCHT-UCLA 007-ME-2007 grant , FAPESP, CNPq and CAPES. MAC is a fellowship from FAPESP at UNIFESP.

BM046 - GENERATION OF *TRYPANOSOMA CRUZI* MUTANT CELL LINES TO INDUCT DNA DOUBLE-STRANDED BREAK IN THE SUBTELOMERIC REGION OF PARASITE CHROMOSOMES

SOUZA, R.T.[*1]; BARROS, R.R.M.[1]; CHIURILLO, M.A.[2]; MARINI, M.M.[1]; ANTONIO, C.R.[1]; LIMA, F.M.[1]; DASILVEIRA, J.F.[1]
1.UNIFESP, SAO PAULO, SP, BRASIL; 2.UCLA, BARQUISIMETO, VENEZUELA.
e-mail:mchiurillo@ucla.edu.ve

The subtelomeric sequence of *Trypanosoma cruzi* presents a high abundance of genes and pseudogenes of surface proteins (Trans-Sialidase –TS– and Dispersed Gene Family-1) adjacent to repetitive sequences considered remnants of mobile elements. Recently, we showed a lack of synteny at subtelomeric region of several homologous chromosomes, suggesting that genes located at these regions are subjected to recombination, which could participate in increasing TS variants. The aim of this work is to test this hypothesis through the generation of *T. cruzi* mutant cell lines and the induction of a DNA double-stranded break in the subtelomeric region of *T. cruzi* to increase the frequency of recombination events. An I-SceI restriction site was introduced adjacent to a gp85 pseudogene in the subtelomeric region of *T. cruzi*, and subsequently this fragment was cloned into the pTAC vector (Trypanosoma Artificial Chromosome). The heterologous expression of yeast endonuclease I-SceI was tested initially using the Tetracycline-inducible pLEW13/pTcINDEX system. However, although in previous results we showed luciferase expression induced by tetracycline in pLEW13/pT7LUC plasmids transfected parasites, problems encountered in drug selection of double mutant cell lines after transfection with pTcINDEX-(I-SceI) led us to design a second approach using *T. cruzi* expression vector pTREX to obtain constitutive expression of I-SceI nuclease. Moreover, we made molecular constructs to test two nuclear localization signals (from SV40 Large T-antigen and *T. cruzi* Histone H2B) fused to the N-terminus of I-SceI protein to target it to the parasite nucleus. I-SceI nuclease location will be assessed using the GFP reporter. Rearrangements of *T. cruzi* genome will be followed by PFGE and Southern blot. Here we show molecular designs and constructs used in this work, as well as, preliminary results. This work was **Supported by:** FAPESP, CNPq and CAPES. MAC is a fellowship from FAPESP at UNIFESP.

BM047 - Production, expression and characterization of recombinant protein SAG1 of *Toxoplasma gondii* by gene selection by PCR.

COSTA, A.[*1]; SUMITA, L.M.[2]; GALISTEO JUNIOR, A.J.[2]; OZAKI, C.Y.[2]; ZORGI, N.E.[1]; RODRIGUES, J.P.[2]; ANDRADE JUNIOR, H.F.[2]
1.ICBUSP, SÃO PAULO, SP, BRASIL; 2.IMTSP, SÃO PAULO, SP, BRASIL.
e-mail:andreacosta@usp.br

Toxoplasma gondii is a protozoan, obligate intracellular parasite that infects felids as definitive hosts and a wide variety of warm blood vertebrates including man as intermediate hosts. Toxoplasmosis is a usually asymptomatic zoonosis of worldwide distribution affecting both human and animal populations, but casus economic losses e social burden in congenital and ocular disease. Specific antibody detection is the key to the diagnosis of toxoplasmosis, requiring high sensitivity and specificity. Most assays uses parasite extracts, with some cross reactions and the use of purified recombinant proteins expressed on parasite surface such as SAG1, SAG2, p26, p35 and SAG3 were proposed and used as alternatives. Most studies produced recombinant proteins by mRNA library but we developed the production of recombinant protein SAG1 using the DNA sequence of *T. gondii* recovered from PCR with specific probes, from ToxoDb database. After insertion of the sequence on plasmid pET28a (+), the recombined plasmid was amplified, purified and inserted in *Escherichia coli* and rSAG1 was expressed by IPTG induction. After purifying the protein by His-Tag tail using nickel resin, rSAG1 was characterized by SDS-PAGE and Western Blot, using anti SAG1 mab. rSAG1 showed strong recognition of specific antibodies derived from mice chronically infected with ME49 strain by Western blot. Used as solid phase sorbent in ELISA, rSAG1 provide high sensitivity and specificity, compared to the total protein extract. The surface antigen SAG1 is highly specific and very well maintained in the different strains of *T. gondii*, as ME-49, RH or VEG. This protein could be also used in cell based assays for detection the efficiency of vaccine protocol or schedules, a approach that are inadequate the use of whole parasite extracts, due to low epitope prevalence. **Supported by:**:LIMHCFMUSP & CAPES

BM048 - In the race of the mitochondrial Eve of the *Trypanosoma cruzi*

VALLE, Í.F.D.[*]; BAPTISTA, R.P.; FRANCO, G.R.; MACHADO, C.R.; PENA, S.D.J.; MACEDO, A.M.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:italodovalle@gmail.com

The genome of the *Trypanosoma cruzi*, etiologic agent of Chagas disease, was completed sequenced in 2005, but many aspects of its populacional structure have not been elucidated yet. Currently, *T. cruzi* strains are classified into six DTUs: TcI-TcVI. However, there is not a consensus about the phylogeny of these groups. While one hypothesis postulates that TcI and TcII are the ancestral lineages from which all the others were originated, the other postulates the existence of at least three ancestral lineages: TcI, TcII and TcIII. So, which are the parental lineages and which are hybrids remains to be clarified. The *T. cruzi* has a single mitochondria that contains its extra nuclear DNA, kinetoplast DNA (kDNA), constituted of a concatenated network of two circular DNA types (minicircle and maxicircle). Many studies have demonstrated the usefulness of the kDNA as target to access the *T. cruzi* diversity. For example, by using the polymorphism of the subunit two of the Cytochrome Oxidase gene the parasite populations can be set into three haplotypes - A, B and C - corresponding to TcI, TcIII-VI and TcII respectively. The aim of this study was to reconstruct the ancestral history of the *T. cruzi* taxon by looking for the more ancient mitochondrial genotype (the Mitochondrial Eve) among the *T. cruzi* lineages. For that, we complete sequenced the maxicircle DNA of the 231 *T. cruzi* strain (TcIII) and compared its 14.4 kb coding region with the sequences already obtained for TcI, TcII and TcVI strains and available in the GenBank. Analysis of genetic similarity showed that TcI and TcII are equally distant from TcIII supporting the hypothesis that TcIII is also a parental *T. cruzi* DTU. Phylogenetic trees built by genetic distance methods and maximum parsimony algorithms, and **Supported by:** high bootstrap values, indicate that TcII is the first lineage to differentiate from the common ancestor of the *T. cruzi* taxon, namely, that the Mitochondrial Eve of *T. cruzi* is a TcII strain. **Supported by:**:FAPEMIG, CNPq e Capes

BM049 - The involvement of RPA complex and Orc1/Cdc6 protein in the replication of chromosomal ends in trypanosomes.

PAVANI, R.S.[*1]; LLANOS, R.P.[1]; SOARES, D.R.[1]; CANO, M.I.N.[2]; ELIAS, M.C.[1]
1.IBU- CAT, SÃO PAULO, SP, BRASIL; 2.UNESP, BOTUCATU, SP, BRASIL.
e-mail:rapavani@gmail.com

Trypanosoma cruzi is the etiologic agent of Chaga´s disease, the second highest illness burden among neglected tropical diseases. Nowadays, Chagas disease still causes 40,000 new infections per year. Thus, improving the knowledge about the molecular biology of this parasite may facilitate the discovery of new therapies and the development of antiparasitic drugs. Our group study the pre-replication and replication machinery in trypanosomes and in this work we propose that some of these components play a role in telomere complex maintenance in trypanosomes. Telomeres are formed by the interaction of DNA with protein complexes which are responsible for maintaining these terminals. The ORC complex is part of the pre-replication machinery and during replication is found along the chromosome, including the telomeric ends. Replication Protein A (RPA) comprises a trimeric complex formed by three subunits, that performs, alone or together with other proteins, various vital functions in DNA metabolism, being a fundamental player during replication and also in telomere maintenance. In trypanosomes, such as *Leishmania*, only subunit 1 of the RPA is found associated to telomeric DNA. In trypanosomes Orc1/Cdc6 is a member of the pre-replication machinery but the role played by the trypanosome RPA in this context has not yet been characterized. This study aims to characterize the *T. cruzi* RPA, as well as analyze the interaction of this protein and Orc1/Cdc6 with parasite telomeres. We have already cloned and expressed recombinant RPA and now we are purifying this protein. Orc1/Cdc6 has already been purified, and now we are standardizing EMSA assays to study the relations among both proteins and parasite telomeres. **Supported by:**:Fapesp

BM050 - Characterizing the interactions among the conserved LaRbp38-TRFH binding motif with the *L. amazonensis* TTAGGG repeat binding factor (LaTRF)

PEREZ, A.M.[*]; RIBEIRO, J.A.; DIAS GONÇALVES, D.; DA SILVA, M.S.; MADEIRA, P.V.M.; CANO, M.I.N.
UNESP, BOTUCATU, SP, BRASIL.
e-mail:arinabio@gmail.com

The ends of the chromosomes are characterized by arrays of telomeric repeats which interact with a variety of proteins forming high order complexes whose function is to ensure proper telomere maintenance. Rbp38 is a protein exclusively expressed in trypanosomatid parasites, including *L. amazonensis*, the etiologic agent of Tegumentar *Leishmania*sis in the Americas. LaRbp38 interacts in vivo with GT-rich DNAs (e.g. kDNA and telomeres) and is involved with nuclear and kDNA replication. LaRbp38 contains a non-conserved DNA binding domain and a short conserved TRFH-interaction motif (FKLAP), which in mammals, is found in proteins recruited to the telomeric complex by TRF proteins. This link, contributes to the stabilization of the TRFs on telomeres and to telomere length regulation. Homologues of the TRF proteins were also characterized in other eukaryotes, including *Leishmania*. TRF proteins present a Myb-like DNA binding domain and a dimerization and protein-interacting TRFH domain which accepts [FxLxP] peptide motifs. Here we show the partial characterization of the interaction among LaRbp38 and LaTRF with the aim to contextualize this interaction in the dynamics of the telomeric chromatin in *Leishmania* spp. Our preliminary results show that both proteins co-immunoprecipitate and their physical interaction was confirmed by pull-down capture assays. We are currently testing this interaction using the yeast-two hybrid system. **Supported by:**:FAPESP, CNPq

BM051 - Vertical transfer of mitochondrial DNA (kDNA) minicircle sequences from *Trypanosoma cruzi* to chicken progeny

ROSE, E.C.P.*; HECHT, M.M.; NITZ, N.; GUIMARO, M.C.; VITAL, T.E.; ANDRADE, R.R.; ALVES, R.M.; TEIXEIRA, A.R.L.C.
LMPDC - UNB, BRASÍLIA, DF, BRASIL.
e-mail:estercprose@gmail.com

The lateral transfer of kDNA from *T. cruzi* in Chagas patients and progeny was shown, which can be possibly be associated to the pathogenesis of the human disease (Hecht e cols. 2010). In order to eliminate the persistence of cryptic infections in mammals, it was used the transkingdom chicken model refractory to the *T. cruzi* infections. It was shown that chiks hatched from *T. cruzi*-infected eggs were parasite-free, but they retained the parasite kDNA in the genome. Moreover, adult kDNA-positive chickens develop dilated inflammatory cardiomyopathy like that of Chagas disease (Teixeira e cols. 2011). This study was undertaken to determine the ratio of inheritance and fixation of kDNA mutations in chiks hatched from eggs inseminated with sperm from kDNA positive roosters. The birds that hatched and became roosters (sperm) and hens (non-fertile ova) yielded germ line cell DNA for analysis. The PCR made with the *T. cruzi* nDNA primers Tcz1/2 revealed absence of the live infections in germ line cells. However, the PCR exams run with kDNA primer sets S35/36 showed 330 bp band and its catamers, which were documented by Southern hybridizations with specific radio labeled probe. A tpTAIL-PCR technique used yielded amplicons, which were cloned and sequenced and revealed chimera minicircle sequences-host DNA. Interestingly, kDNA mutations were disclosed in specific genomic *loci* of bird's in the study families. In one family, the kDNA mutation entered in a unique hotspot of the parental and progeny. Although the mutation entered at the same nucleotide in the *locus* of the NADP-dependent malic enzyme, the kDNA variable regions were different in these birds. This may occur primarily due to the minicircle shear-mass sequences in the parasite mitochondria, and also by recombination events. **Supported by:**:Capes, CNPq

BM052 - Is cytochrome oxidase I a good barcode for monoxenic trypanosomatids?

MARTINS, C.B.*; PEREIRA, S.S.; RIBEIRO, C.M.; MORELLI, K.A.; MONTEIRO, F.A.; D`AVILA-LEVY, C.M.
FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:carolbmartins85@gmail.com

Trypanosomatids are parasitic flagellated protozoa comprised of two distinct groups: (1) the monoxenics, which live mainly in an invertebrate host, and (2) the heteroxenics, whose life cycle alternates between an invertebrate host and a second host (which may be a vertebrate or a plant). The DNA barcoding methodology has been successfully used for species identification. It is based on the premise that a short standardized DNA sequence can distinguish individuals of a species whenever the magnitude of the genetic variation seen between species exceeds that observed within species, creating a "barcoding gap". Here, we evaluated the usefulness of the DNA barcoding method in the taxonomic identification of monoxenic trypanosomatids deposited in the Protozoa Collection (COLPROT), Fiocruz by means of cytochrome oxidase I mitochondrial gene (COI) sequencing. Degenerate primers were designed based on public sequences retrieved from GenBank. We have obtained sequences for nine species belonging to four genera: Phytomonas, Crithidia, Leptomonas, and Strigomonas (formerly referred to as Blastocrithidia). Curiously, Leptomonas samueli and L. wallacei displayed identical sequences, which may be an indication that they represent the same species. Furthermore, the results showed a sister-taxon relationship between Blastocrithidia culicis and Crithidia oncopelti, further corroborating the proposition that they should compose the new genus Strigomonas. The results obtained so far indicate that COI has power to discriminate the species analyzed; however, a larger number of taxa must be studied to validate COI as a trypanosomatid barcode. The observation that an unidentified choanomastigote-shaped trypanosomatid presented complete COI sequence identity with Crithdia hutneri, suggesting co-specificity, seems to point that way. ***Agência Financiadora :*** *MCT/CNPq, FAPERJ, CAPES, and FIOCRUZ.*

BM053 - Sub2 protein is related with pre-mRNA processing in trypanosomatids

HIRAIWA, P.M.[*]; SERPELONI, M.; INOUE, A.H.; PAVONI, D.P.; GOLDENBERG, S.; DALLAGIOVANNA, B.; AVILA, A.R.
INSTITUTO CARLOS CHAGAS, CURITIBA, PR, BRASIL.
e-mail:prihiraiwa@yahoo.com.br

The mRNA export is a point of gene expression regulation in eukaryotic cells; however, this pathway is poorly understood in several species of parasites. Recently, our group analyzed the conservation of proteins involved with RNA export pathway through different lineages of eukaryotes. The Sub2/UAP56 RNA helicase is essential for mRNA export and for pre-mRNA processing by cis splicing in mammalian cells. It have been demonstrated that Sub2 is also essential for mRNA export in trypanosomatids, which processes the pre-mRNA by trans splicing. Thus, the goal of this study was to evaluate if Sub2 protein could be related with trans splicing in trypanosomatids using *Trypanosoma brucei* as a model. The knockdown of TbSub2 expression by RNAi reduced the level of Sub2 coding RNA, causing a decrease of protein level. It has been observed that SL RNA accumulated and decreased Y structure (a trans splicing subproduct) levels after RNAi induction, indicating that Sub2 is related to the first steps of trans splicing. The knockdown of TbSub2 neither affected the metilation of SL RNA cap nor the general transcription levels, including pre-RNAs and SL RNA, suggesting that the SL RNA accumulation is a consequence of the TbSub2 involvement in trans splicing and not in transcription. The interaction of TbSub2 with snRNP has been analyzed. However, TbSub2 does not interact with SmD1 (a snRNP protein) or U snRNAs, at least in the conditions analyzed. The silence of TbSub2 has altered the ratio of pre-mRNA/mRNA from PAP and GAPDH. Interestingly, the level of mRNAs increased after TbSub2 RNAi, suggesting that processed mRNA might be accumulating in the nucleus, since it has been already demonstrated that silencing of TbSub2 prevents the export of mRNA to cytoplasm. Our data suggests that Sub2 is related to pre-mRNA processing by trans splicing and, similar to mammalian cells, there is a close association between the splicing machinery and the mRNA export pathway in tripanosomatids. **Supported by:**:Capes, CNPq, FIOCRUZ

BM054 - Mining downstream of phosphatidylinositol kinase family in trypanosomatids: the architectures of Akt/PKB and PDK1, two PH (Pleckstrin Homology) domain containing kinases, indicate a particular evolution of protein domains in kinetoplastid parasites

BAHIA, D.[*1]; SANCHEZ-FLORES, A.[2]
1.UNIFESP, SÃO PAULO, SP, BRASIL; 2.SANGER, CAMBRIDGE, INGLATERRA.
e-mail:dianabahia@hotmail.com

Akt/PKB (Protein Kinase B) and PDK1 (Phosphoinositide-dependent kinase-1) are downstream effectors of PI3 kinase and belong to the cAMP-dependent protein kinase AGC superfamily. Akt/PKB is arguably one of the most important cell signalling molecules and is involved in critical cellular processes, such as survival via the inhibition of apoptosis. Akt/PKB proteins share an architecture consisting of a N-terminal pleckstrin homology (PH) domain for binding phosphoinositides, a central kinase domain, and a C-terminal regulatory domain. PDK1 is crucial for the activation of Akt/PKB and other AGC kinases. In humans and other mammals, the roles of these enzymes have been described, but little knowledge is available for other organisms. In this study, we searched for Akt/PKB and PDK1 proteins among all sequenced and annotated trypanosomatid genomes. We used the profiles and signatures that characterize each protein familiy in all translated CDS of four *Leishmania* and seven *Trypanosoma* genomes obtained from TriTrypDB v3.3. We identified 12 Akt/PKBs and 73 PDK1 proteins. Except for *T. cruzi*, only one Akt/PKB gene is present in each genome and several copies of PDK1 genes in all genomes. Akt/PKB with a PH domain at the N-terminus has not been identified in fungi or plants, but occurs both in very primitive eukaryotes such as *Dictyostelium* and in higher eukaryotes, and now we found it in kinetoplastids. This suggests that the ancestral Akt/PKB kinase possessed a PH domain that was lost in other species. The origin of PDK1 proteins in kinetoplastids seems to be the result of convergent evolution since no relationship to other PDK1 proteins in other species was detected. Occurrence of organism specific novel domain combinations suggests functional diversity achieved by protein kinases in order to regulate a variety of biological processes. In addition, the domain architecture of protein kinases can suggest their emerging roles in the signaling of eukaryotic organisms. **Supported by:**:FAPESP, CNPq

BM055 - Polymorphism analysis of four new linear B cell epitopes in *Trypanosoma cruzi* lineages and their potential use for serodiagnostic and serotyping of Chagas disease

MENDES, T.A.O.[*1]; CUNHA, J.L.R.[1]; ROCHA DOS SANTOS, A.R.[1]; LOURDES, R.A.[1]; LEMOS, L.C.D.[1]; CÂMARA, A.C.J.[2]; GALVÃO, L.M.[2]; GILMAN, R.[3]; FUJIWARA, R.T.[1]; GAZZINELLI, R.T.[1]; BARTHOLOMEU, D.C.[1]

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFRN, NATAL, RN, BRASIL; 3.UCH, LIMA, PERU.

e-mail:tiagomgmendes@yahoo.com.br

The factors influencing the variation in the clinical manifestation of Chagas disease have not been elucidated, but it is likely that genetic of the host and parasite is involved. Several studies trying to correlate the *T. cruzi* strain involved in the infection and the clinical manifestation of the disease have used hemoculture and/or PCR-based parasite genotyping from infected human tissues, but both techniques have limitations. Previously, we identified a conserved epitope (C6_30_cons) with potential use for sorodiagnostic and three polymorphic (A6_30_col, B2_30_Y and B9_30_cl) epitopes differentially recognized by sera of mice chronically infected with distinct parasite strains. Here, we validated the use of these peptides using sera from chagasic patients monoinfected with TcI and TcII. Furthermore, we analyzed the polymorphisms of the epitopes and predicted their reactivity with sera from individuals infected with *T.cruzi* strains representative of each lineage (TcI to TcVI). To this end, first we used the AlaScan technique to identify which amino acids are important for differential recognition of Colombiana, Y or CL Brener infection. Next, we sequenced the genomic DNA encoding the four epitopes in Colombiana (TcI), Y (TcII), 231 (TcIII), CanIII (TcIV), 115 (TcV) and CL Brener (TcVI). We predict that the conserved peptide C6_30 would be able to identify the infection caused by five of the six *T.cruzi* strains/clones. We also confirmed that the peptides A6_30_col and B2_30_Y have potential to identify patients infected only with TcI and TcII, respectively. The B9_30_cl was predicted to identify patients infected with TcIII or TcVI. ELISA experiments using human sera confirmed the predictive reactivity of A6_30_col and B2_30_y, since the first peptide was able to identify 100% of the patients infected with TcI (p=0.0006) and the second one, 80% of the infected with TcII (p=0.0009), confirming the potential use of this peptide set for serotyping of Chagas disease. **Supported by:**::INCTV, FAPEMIG, CNPq, CAPES

BM056 - A novel strategy to develop a vaccine against Chagas disease based on the generation of a *Trypanosoma cruzi* line induced by the cell reprogramming technique

NOVAES, R.[*]; LIMA, A.P.C.A.; REHEN, S.; MORROT, A.

UFRJ, RIO DE JANEIRO, RJ, BRASIL.

e-mail:renateenha@gmail.com

Trypanosoma cruzi is the agent of Chagas disease, an illness present from southern Argentina to the southern United States. It is estimated that nearly 20 million people are infected in the Americas, and about 100 million people are still at risk of infection. The drugs currently available for treatment have considerable side effects and there is no vaccine to prevent this disease. Protection against infection through experimental vaccination can be achieved by CD8(+) T-cell mediated immune responses in mice infected with *T. cruzi* or vaccinated with recombinant parasite antigens derived from the vertebrate stages of the parasite. The present study aims at expressing the Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc genes) in *Trypanosoma cruzi* epimastigotes. The principle is based on the somatic cell reprogramming technique through expression of the Yamanaka factors, used so far only in mammalian eukaryotic cells, to generate induced pluripotent stem cells. We hypothesize that a reprogrammed parasite could express genes specific for different life stages, possibly involved in the protective host immune response. Currently, we have generated *T. cruzi* epimastigote lines transfected with the Oct4 and Sox2 genes. We are analyzing the effect of the expression of those genes in expression of the stage-specific surface markers and their influence on metacyclogenesis and host adaptive immunity. **Supported by:**::CNPq, CAPES, FAPERJ, INCTv

BM057 - Congenital Transmission Appears to Account for a Majority of *Trypanosoma cruzi* Infections in the Amazon

ARAUJO, P.F.*; ALMEIDA, A.J.B.; HECHT, M.M.; BRITTO, M.M.; ROSA, A.C.; NITZ, N.; TEIXEIRA, A.R.L.C.
LMPDC-UNB, BRASÍLIA, DF, BRASIL.
e-mail:manuela.mba@gmail.com

Outbreaks of acute Chagas disease have been described in the Pará State, Brazil. Having undertaken clinical exams of *T. cruzi*-infected patients in the Counties of Barcarena and of Breves, we carried on a genetic study. 109 individuals in four families (A, B, and C, Barcarena; and D, Breves) were studied. The infections were identified by anti-*T. cruzi* antibodies (IF and ELISA tests), and by PCR amplification of the parasite nuclear (nDNA) and mitochondrial (kDNA) DNAs. Each case showing kDNA amplifications were further investigated by nested PCR, combining host and kDNA specific primers sets. The anti-*T. cruzi* antibodies were demonstrated in 35,7% (39/109) individuals: A, 29.5% (13/44); B, 26.6% (4/15); C, 20.6% (6/29); and, D, 76.1% (16/21). The nDNA PCR tests revealed 76.1% (83/109) cases had live *T. cruzi* infections: A, 77.2% (34/44); B, 100% (15/15); C, 75.8% (22/29); and, D 57.1% (12/21). The kDNA PCR revealed minicircle sequences were retained in 101 out of 109 individuals (92.6%): A, 84% (37/44); B 100% (15/15); C, 96.5% (28/29); and, D 100% (21/21). Morever, the tpTAIL-PCR products that were cloned and sequenced revealed kDNA-host DNA chimeras in total 101 cases. The study revealed that over twice as many individuals in the families had the *T. cruzi* infections that could not be disclosed by the search of anti-parasite antibodies (83 - 39 = 44 cases). Moreover, the study showed kDNA was retained in the genome of 101 individual in four families. Each of these kDNA-positive cases had the kDNA integrated into the genome. The absence of specific antibodies in immune tolerant cases means a majority of those infections are congenitally acquired. **Supported by:**:CAPES/CNPq/FAPDF

BM058 - SCREENING OF CALPAIN-LIKE PROTEINS IN *TRYPANOSOMA CRUZI* AND *LEISHMANIA* BRAZILIENSIS

VIDAL, V.E.*[1]; VITORIO, B.S.[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

Peptidases are enzymes that are crucial in relevant steps of parasites-host cells interaction, and therefore, have been extensively studied as drug targets candidates. The complete mapping of the genome of trypanosomatids prompted the identification and analysis of peptidases either with low abundance or tricky to detect biochemically. In this sense, up to now, there are few reports on calpains in *Trypanosoma cruzi* and *Leishmania braziliensis*. Calpains comprise a large family of calcium-regulated cysteine peptidases present in a broad range of organisms, and it is implicated on a variety of physiological processes. Here, we identified and classified sequences of calpain-like proteins in the genome of *T. cruzi* and *L. braziliensis* through *in silico* analysis. After that, we evaluated the mRNA expression of calpains in the different life cycle forms of these parasites. The *in silico* analysis revealed 55 sequences in the *T. cruzi* genome and 34 sequences in *L. braziliensis*. Through multiple alignments, and phylogenetic analysis of conserved domains in the sequences obtained from both species, we sorted the calpains in four distinct groups characterized by the size of the gene, and by the presence of classical domains of this multigenic family. We decided to focuse 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): 20 sequences in *L. braziliensis* and 16 in *T. cruzi*. The analysis of calpain mRNA abundance in epimastigote, trypomastigote and amastigote forms of *T. cruzi* revealed at least five genes with modulated expression among the life stage forms. In conclusion, a comparative study of calpains expression in trypanosomatids with different life cycles, such as *T. cruzi* and *Leishmania* spp., may help to determine the general functions of these molecules in the Trypanosomatidae family, as well as its specific role for each parasite. **Supported by:**:MCT/CNPq, FUJB, CEPG/UFRJ, FAPERJ, CAPES, FIOCRUZ

BM059 - *LEISHMANIA VIANNIA* PHYLOGENY AS REVELEAD BY MITOCHONDRIAL GENES

ANDRADE, I.F.*; VALLE, Í.F.D.; FRANCO, G.R.; MACHADO, C.R.; PENA, S.D.J.; MELO, M.N.; MACEDO, A.M.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:isa88_andrade@yahoo.com.br

*Leishmania*sis is widely considered a neglected and remerged disease in many tropical and subtropical countries. Species of the genus *Leishmania*, as other kinetoplastida, have two genomes, represented by the nuclearand the kinetoplast DNA, this last formed by two types of circular molecules: maxicircles and minicircles. Maxicircles are the functional homologues of mitochondrial DNA in other eukaryotes. Different studies have investigated the usefulness of the kDNA as taxonomic markers due to its great copy number and genetic variability among species. In Brazil, *L. braziliensis* is the main cause of cutaneous *Leishmania*sis, but their evolutionary relationship with other *LEISHMANIA VIANNIA* species is not well defined. Once this information is essential to understand mechanisms of pathogenesis and transmission, we aim to study the phylogeny of *LEISHMANIA VIANNIA* species, by analysing mitochondrial gene sequences. Initially, Cytochrome b (Cyb) gene sequences from different *Leishmaniae* species available in GenBank were used to construct phylogenetic trees by PHYLIP (Phylogeny Inference Package). By this strategy, all *L. braziliensis* analysed strains were clustered together with strains of *L. guyanensis* complex in a major branch **Supported by:** high bootstrap values (> 90%). However *L. equatoriensis*, which is nowadays classified as belonging to the *L. braziliensis* complex, were set apart in a distinct branch. Complete sequencing of mitochondrial genomes of *L. braziliensis* and *L. guyanensis* is ongoing to better understand how these parasites have evolved from their common ancestor, in order to identify more appropriated taxonomic markers for both complexes.
Supported by::CAPES, CNPq, Fapemig

BM060 - *Trypanosoma cruzi* G strain metacyclic trypomastigotes: 1-D phosphopeptide mapping of a sub-set of high molecular weight proteins

CORTEZ, C.*[1]; LEON, I.R.[2]; VIDAL, R.O.[3]; MORTARA, R.A.[1]; YOSHIDA, N.[1]; BAHIA, D.[1]
1.UNIFESP-EPM, SAO PAULO, SP, BRASIL; 2.UNIVERSITY OF SOUTHERN DENMARK, COPENHAGEN, DINAMARCA; 3.UNICAMP, CAMPINAS, SP, BRASIL.
e-mail:ramortara@unifesp.br

Phosphorylation and/or dephosphorylation of serine, threonine, and tyrosine residues is a major mechanism that cells use to regulate protein function. Phosphopeptide mapping of these residues allows investigation into the positive and negative regulatory roles these sites may play in vivo. Here we search for the presence of phosphopeptides in a sub-set of proteins from metacyclic trypomastigotes protein extract of *Trypanosoma cruzi* G strain with apparent high molecular weight in SDS-PAGE gels. 1-D-bands were in-gel digested with trypsin. Peptide mixture was enriched for phosphopeptide by TiO2 affinity chromatography and analysed on a LTQ Orbitrap Velos by selecting the top-three most intense ions for low-resolution CID-MS/MS using MSA. Data analysis was performed with Sequest server and Proteome Discoverer (v1.2, Thermo Fisher- Waltham, USA). We developed some Perl scripts to summarize the data, such as number of unique proteins, number and type of modifications, density of each phosphopeptide, protein molecular weight and finally to retrieve the FASTA sequences of each unique protein for annotation propose. The annotation was done with blast2go and groups were formed using biological process GO (Gene Ontology) terms with at least more than four proteins. Putative protein kinases phosphorylating peptides were found with NetPhosK. In total, 166 phophoproteins have been detected, with an average of 130 kDa in MW; the frequency of phospho-serine, threonine and tyrosine residues was 71, 16 and 1%, respectively. The more abundant proteins included CAP family of transcription factors; fusaric acid resistance protein; IQ calmodulin-binding motif protein family and a number of unknown proteins. Protein modification, catabolism, response to stress and external stimulus are among the frequent biological processes in which these phosphoproteins are involved. We found 60 phosphoproteins that have not been reported in previous published studies. **Supported by:**:FAPESP, CNPq, Capes

BM061 - Inheritance and fixation of *Trypanosoma cruzi* mitochondrial minicircle (kDNA) in a chagasic family

BRITTO, M.M.*; HAGSTRÖM, L.; ARAUJO, P.F.; HECHT, M.M.; NITZ, N.; TEIXEIRA, A.R.L.C.
LMPDC-UNB, BRASÍLIA, DF, BRASIL.
e-mail:manuela.mba@gmail.com

Previous studies (Simões-Barbosa *et al*. 2006; Hecht *et al*. 2010; Teixeira *et al*. 2011) have shown lateral transfer of mitochondrial DNA (kDNA) minicircle sequences from *Trypanosoma cruzi* to human hosts. In this study we aim at the fixation of the kDNA mutations in parental Chagas patients and progeny. The parental had live *T. cruzi* infection disclosed by PCR with nDNA primer Tcz1/2, whereas the progeny had only kDNA revealed by specific primer sets s35/s36. The mapping of the kDNA integrations in parental and progeny was obtained by a target primer TAIL-PCR technique (Hetch *et al.* 2010), cloning amplicons, and sequencing. In view of the diversity of sequences of the kDNA variable region, frequently observed location of the kDNA in retrotransposon LINE-1 at several chromosomes presents a major difficult towards understanding the patterns of heritability of the kDNA mutations. However, it was shown that a main hotspot for chimera kDNA-host sequences was the LINE-1 on the X chromosome in parental and progeny. At this hotspot, it was observed that chimera sequences alignment, which were made using algorithm BLASTn, and DNAMAN, and BIOEDIT, did not show frequently expected Mendelian inheritance among siblings. Instead, we observed that kDNA mutations in three siblings were present at two different *loci*. Siblings A and B shared same minicircle sequence variable region inserted at *locus* AC012596.4 on chromosome 7, whereas B and C shared another minicircle sequence inserted at *locus* AC084364.20 on chromosome 12. This finding suggests that in addition to occasional conservative Mendelian inheritance, a semi-conservative pattern of inheritance (diverse minicircle variable region) is most frequently observed in a chagasic family. **Supported by:**::CAPES/CNPq/FAPDF

BM062 - Differential MASP expression profile in tissue culture and bloodstream trypomastigotes of *Trypanosoma cruzi*

SANTOS, S.L.*; OLIVEIRA, A.C.S.; FREITAS, L.M.; LOBO, F.P.; RODRIGUES-LUIZ, G.F.; MENDES, T.A.O.; ANDRADE, L.O.; CHIARI, E.; GAZZINELLI, R.T.; TEIXEIRA, S.M.R.; FUJIWARA, R.T.; BARTHOLOMEU, D.C.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:ninalarrude@gmail.com

A key *Trypanosoma cruzi* strategy to survive in a mammalian host is its ability to invade several non-phagocytic host cells. Therefore, an important aspect to understand *T.cruzi* infection is the identification of molecular components of both parasite and host cells that play a role in the infection of a variety of cell types. Although several trypomastigote surface proteins have been implicated in host-cell invasion, so far there is no clear association between a *T.cruzi* expression profile and its ability to invade/proliferate in a given host cell. MASP is the most polymorphic *T.cruzi* gene family, mainly expressed on the surface of the trypomastigotes, thus contributing to a large polypeptide repertoire that could be exposed to the host. As an attempt to investigate whether MASP could be implicated in interactions with specific host cells, we investigated the MASP expression profile in tissue culture trypomastigotes derived from epithelial and myoblast cell lines and in bloodstream trypomastigotes after sequential passages in mice. We did not detected significant changes in the MASP expression profile between the two host cells after 4 passages in tissue culture. However, differential expression of MASP genes was detected by sequencing and by qRT-PCR between trypomastigotes derived from the two host cells after a larger number of tissue culture passages. This is an indirect evidence that different MASP genes may be implicated in the interaction with distinct cell types. In fact, an association between the selection of a MASP profile and the infectivity of myoblast-derived parasites is suggested by cell invasion assays. We also found striking differences in MASP expression profile comparing bloodstream and tissue-culture trypomastigotes and between bloodstream forms from sequential passages in infected mice. Taken together, these results indicate that tissue culture and in vivo infection may selectively configure a distinct MASP expression profile in trypomastigotes. **Supported by:**::FAPEMIG, CNPq, CAPES

BM063 - Genomic analysis of sequence-dependent DNA curvature in *Leishmania*

SMIRCICH, P.[*1]; FORTEZA, D.[1]; EL-SAYED, N.M.[2]; GARAT, B.[1]
1.FACULTAD DE CIENCIAS, MONTEVIDEO, URUGUAI; 2.UNIVERSITY OF MARYLAND, MARYLAND, ESTADOS UNIDOS.
e-mail:psmircich@fcien.edu.uy

Leishmania major is a flagellated protozoan parasite of medical importance. Like other members of the Trypanosomatidae family, it possesses unique mechanisms of gene expression such as constitutive polycistronic transcription of directional gene clusters, gene amplification, mRNA trans-splicing, and extensive editing of mitochondrial transcripts. The molecular signals underlying most of these processes remain under investigation. In order to seek for a role for DNA secondary structure signals in gene expression, we carried out a genome-wide *in silico* analysis of the intrinsic DNA curvature. *L. major* genome revealed a lower frequency of high intrinsic curvature regions as well as inter- and intra- chromosomal distribution heterogeneity, when compared to prokaryotic and eukaryotic organisms. Using a novel method aimed at detecting region-integrated intrinsic curvature (RIIC), high DNA curvature was found to be associated with regions implicated in transcription initiation. Those include divergent strand-switch regions between directional gene clusters and regions linked to markers of active transcription initiation such as acetylated H3 histone, TRF4 and SNAP50. These findings suggest a role for DNA curvature signals in transcription initiation in *Leishmania* supporting the relevance of DNA secondary structures signals. The characterization and mapping of regions with distinctive intrinsic curvature provided by the method here presented, could be of general application to study different cells and processes.

BM064 - SEARCHING FOR MOLECULAR MARKERS CANDIDATES FOR *TRYPANOSOMA CRUZI* DTUS CHARACTERIZATION

SANTOS, V.C.[*]; PEDROSO, J.S.; BAPTISTA, R.P.; FRANCO, G.R.; MACHADO, C.R.; MACEDO, A.M.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:vivicorreasantos@gmail.com

Achieving a consensus in the scientific community regarding the subdivision of *T. cruzi* in six lineages or DTUs represented a considerable jump for sharing information among different research groups improving investigations about possible association between parasite diversity and biological features and clinical aspects of Chagas disease. However, it remains to be established a simple and applicable standard protocol, suitable for molecular characterization in different laboratories, to be used to classify natural *T. cruzi* populations into these six lineages. In this context, the TcIII and TcIV remains as the least studied DTUs, few markers are useful to differentiate these two lineages and their phylogenetic relationships remain poorly known. To allow a correct identification and appropriate resolution of the evolutionary aspects of these lineages, this study seeks to find new molecular markers capable to identify and separate TcIII and TcIV lineages. To achieve that, we initially performed data mining in GenBank, NCBI, looking for gene sequences available for all six *T. cruzi* lineages. These sequences were used for phylogenetic reconstructions by maximum likelihood and distance methods. Genes whose trees' topology allowed to distinguish TcIII and TcIV lineages from the others were chosen for the search for lineages specific SNPs. From the sequences so far analyzed the mitochondrial genes COII and ND1 did not presents appropriate SNPs suitable to distinguish among TcIII-VI lineages, but presents SNPs capable to distinguish these from the others DTUs. Among the nuclear genes, GPI presented SNPs that allowed differentiating specifically TcIV from the other DTUs. These markers were selected as potentially new molecular marker candidates for *T. cruzi* DTUs identification. **Supported by:**:CAPES, CNPq and FAPEMIG

BM065 - EARLY DIAGNOSIS OF CHAGAS DISEASE REACTIVATION AND *TRYPANOSOMA CRUZI* GENOTYPING BY PCR ANALYSES DIRECTLY IN TISSUES OF PATIENTS SUBMITTED TO HEART TRANSPLANTATION

SEGATTO, M.*; ANDRADE, S.A.; GELAPE, C.L.; JUNQUEIRA, L.L.; FRANCO, G.R.; MACHADO, C.R.; PENA, S.D.J.; CHIARI, E.; MOREIRA, M.C.V.; BRASILEIRO FILHO, G.; MACEDO, A.M.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:marcelasegatto@yahoo.com.br

Chagas disease has a variable clinical course with cardiac involvement being the most serious and frequent manifestation. Heart transplantation (HTx) is an useful therapy for end-stage Chagas heart disease (CHD), although Chagas reactivation remains as a major complication. In the last six years, 112 HTx were carried out at the Clinical Hospital of the UFMG, from which 50 were from patients suffering from CHD. After HTx, patients are submitted to periodic endomyocardial biopsies to monitor transplant rejection and Chagas reactivation. On average 50% of the transplanted patients developed infection reactivation. Since amastigotes are rarely found in histopathological analyses of the biopsies, it is very difficult to get the differential diagnosis between inflammatory process resulting from allograft rejection and from infection reactivation. The aim of this study was to investigate the usefulness of PCR strategies for early identification of *Trypanosoma cruzi* DNA in the follow up endomyocardial biopsies and also to genotype the parasites presented in explanted tissues obtained from the transplanted patients. Diagnoses were conducted by PCR targeted to the kDNA and qPCR to the 24Sα rRNA gene. From 35 patients and 250 samples so far analyzed, *T. cruzi* DNA was detected in 70 samples. In 66 samples it was detected the presence of TcII, one case of TcVI, one case partially identified as TcV/VI, one mixed infection of TcII and TcVI, and a case of TcI. This last may represent the first reported case of TcI detected directly in heart of a CHD patient in Brazil. In a retrospective study with 4 patients who presented reactivation of infection, positive results were detected in the firsts held endomyocardial biopsies, 1–18 months earlier than the clinical reactivation. These results indicate that PCR is a good strategy to the early diagnosis of Chagas disease reactivation with potential to assist physicians in treatment decisions before onset of reactivation. **Supported by:**:FAPEMIG, CNPq e CAPES

BM066 - Natural *Leishmania* Infection of *Lutzomyia auraensis* in Madre de Dios, Peru, Detected by a FRET Based Real-time PCR Assay

VALDIVIA, H.O.*; DE LOS SANTOS, M.B.; FERNANDEZ, R.; BALDEVIANO, C.G.; ZORRILLA, V.O.; VERA, H.; LUCAS, C.M.; EDGEL, K.A.; LESCANO, A.G.; MUNDAL, K.D.; GRAF, P.C.
NAMRU-6, LIMA, PERU.
e-mail:hvalrod@hotmail.com

Leishmania species of the *Viannia* subgenus are responsible for most cases of New World *Leishmania*sis (NWL) in South America. Studying the prevalence and distribution of *Leishmania*-infected sand flies is critical to understanding the dynamics of disease transmission and predicting the emergence of new endemic areas. Despite this issue, little is known regarding the vectors involved in *Leishmania*sis transmission in Amazonic endemic regions. In this study, we used a novel real-time PCR assay to detect natural *Leishmania* infections in phlebotomines collected in ten households from a jungle community in Madre de Dios, Peru. A total of 1,299 non-blood fed female sand flies belonging to 33 species were captured using miniature CDC light traps. *Lutzomyia auraensis* was the most abundant species (63%) in this area. Seven out of 164 sand fly pools (4.3%) were positive for *Leishmania* by kinetoplastid-DNA PCR. The real-time PCR assay identified four *Lu. auraensis* pools positive for *Leishmania (V.) lainsoni* and *Leishmania (V.) braziliensis*. Further studies are needed to assess the importance of *Lu. auraensis* in the transmission of NWL in endemic areas of Peru. **Supported by:**:NIH/AFHSC/GEIS

BM067 - MULTIPLEX PCR DESIGN FOR *LEISHMANIA* SPECIE-SPECIFIC IDENTIFICATION

ROCHA DOS SANTOS, A.R.*; RODRIGUES-LUIZ, G.F.; LOURDES, R.A.; CUNHA, J.L.R.; FUJIWARA, R.T.; BARTHOLOMEU, D.C.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:nanarochabh@yahoo.com.br

*Leishmania*sis, caused by protozoan parasites from the genus *Leishmania*, affects mainly people of low socioeconomic status from tropical and subtropical countries. This disease is classified into three clinical manifestations: visceral, cutaneous and mucocutaneous. To date, there is no simple methodology for specie-specific *Leishmania* identification, and there are few studies investigating whether distinct species coexist into the same vertebrate host. Here we have performed an *in silico* analysis searching for species-specific microsatellites in the genomes of *L. major*, *L. braziliensis* and *L. infantum* to design set of primers for genotyping using multiplex PCR. Thirty pairs of primers were designed their specificity was checked by e-PCR. So far, seventeen pairs of primers were tested by PCR using as template the corresponding genomic DNA, and eight pairs of primers out of that were also tested with DNA from the other two species. Based on these analyses, we have selected three pairs of primers to compose a multiplex PCR assay, but as not all primers were tested, we have not performed this PCR yet. Additional bands were detected in some systems and it is likely due to the polymorphism in the pairs of alleles targeted in the PCR reaction. Since only one haploid content is represented in the assembled genome sequences, the e-PCR analysis would not identify the other allele. Nevertheless, *a priori*, the amplification profile allowed us the discrimination of genomic DNA samples from the three species. After optimization of the PCR conditions, the best combination of primers will be applied in a multiplex PCR assay using artificial mixtures of genomic DNA from the three species and samples of infected patients to evaluate the occurrence of co-infections. This study may help design a specie-specific diagnosis and evaluate the prevalence of mixed infections caused by different *Leishmania* species. **Supported by:**:CNPq; CAPES; FAPEMIG.

BM068 - Identification of a *Leishmania (Leishmania) major* protein related with Tubercidin resistance

AOKI, J.I.*; YAMASHIRO-KANASHIRO, E.H.; COTRIM, P.C.
IMT, SAO PAULO, SP, BRASIL.
e-mail:juaoki@gmail.com

Molecular aspects of drug resistance are a promising field for better understanding the mechanisms of action of antiparasitic drugs.Tubercidin (TUB),a purine toxic compound,can be considered a potential antiparasite agent by DNA synthesis inhibition.Using a transfection-overexpression selection strategy,we isolated a 31kb *locus* of *L.(L.)major* (cosTUB2) capable to render wild type cells 4 fold resistant.A set of deletions of the original *locus* yielded a 3kb fragment (pSNBR/3kb*Cla*I-*Eco*RI),able to make wild type cells 2 fold resistant.Analysis of the *L.(L.)major* genome,found within 3kb fragment the presence of gene that codifies for a hypothetical protein of unknown function.Aiming to understand the role of this hypothetical protein in the TUB resistance,a series of experiments was performed.First,a recombinant of 70kDa from the protein was obtained and its level of expression is under analysis.The expression level of the hypothetical protein mRNA,analyzed by RT-PCR,showed 70 fold increase when compared with wild type cells.We generated,by drug pressure,resistant mutants from *L.(L.)major* and *L.(L.)amazonensis* maintained in 5µM of TUB: LmTUB^{r}5 and LaTUB^{r}5.When compared with wild type cells,these mutants showed a great resistance ratio (30 and 40 times,respectively),indicating that both mutants were well adapted to high TUB concentrations.We believe this resistance can be related with gene amplification of this protein,since the TUB resistance was reversed in the absence of TUB.Analyses of cross-resistance were carried out with all the cell lines described herein.No cross-resistance was observed with two drugs used in the treatment of *Leishmania*sis as Glucantime and Allopurinol,the latter linked to the purine metabolism.Functional assays with Pentamidine (PEN),a third drug used in the treatment of *Leishmania*sis,showed discrete levels of cross-resistance between cosTUB2 and pSNBR/3kb*Cla*I-*Eco*RI (1.6 and 2.4, respectively).Mutants did not present cross-resistance with PEN. **Supported by:**:CAPES, FAPESP, CNPq, LIM48-FMUSP

BM069 - *Trypanosoma cruzi* LINES LACKING THE CYSTEINE PEPTIDASE INHIBITOR CHAGASIN AS A TOOL TO STUDY THE BIOLOGICAL ROLES OF CRUZIPAIN

DOS REIS, F.C.G.*; AZEVEDO, R.S.P.; PINTO, L.M.M.V.; LIMA, A.P.C.A.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:coelho@biof.ufrj.br

Chagasin was the first identified member of a family of natural tight binding inhibitors of cysteine peptidases. It was primarily found in *T. cruzi* and inactivates enzymes belonging to family C1 of cysteine peptidases, like cruzipain and cathepsin L. The generation of *T. cruzi* Dm28 lines overexpressing chagasin helped to assess the biological role of this inhibitor. We reported that parasites overexpressing chagasin have fourfold higher peptidase inhibitory activity. Those parasites displayed reduced metacyclogenesis and tissue culture trypomastigotes were less infective in vitro. Analysis of the levels of cruzipain and chagasin in different *T. cruzi* strains revealed that their molar ratios are conserved (50:1 of cruzipain:chagasin), with exception of the G strain, which is poorly infective. This strain displays lower cruzipain:chagasin molar ratio (5:1) and significantly reduced cruzipain activity. We hypothesized that low cruzipain activity resulting from excess chagasin could contribute to the poor infectivity of G. In an effort to confirm the roles attributed to chagasin, we aimed at generating chagasin-deficient mutants in two strains - Dm28 and G. We constructed a recombination cassette containing the 5' and 3' untranslated regions of chagasin flanking the selectable markers hygromycin or puromycin.The cassestes were excised from the plasmid, gel purified and transfected into epimastigotes by the Amaxa method. The populations were selected for antibiotic resistance and then cloned in Agarose-LIT plates. Deletion of the first allele by homologous recombination was PCR-checked and confirmed by Southern blot. Tests with a G-strain heterozygote line revealed 40% increase in peptidase activity as compared to WT parasites. Hygromycin resistant heterozygote lines were transfected to generate null mutants, and two clones were selected. We are currently investigating their cruzipain and chagasin contents and further addressing the biological behaviour of the mutants. **Supported by:**:FAPERJ; CNPq; CAPES

BM070 - Role of Rad51 protein in *Trypanosoma cruzi*

SANTOS, S.S.*[1]; AGUIAR, P.H.N.[1]; MURTA, S.F.[2]; ANDRADE, L.O.[1]; MACHADO, C.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.FIOCRUZ, BELO HORIZONTE, MG, BRASIL.
e-mail:selmasantosilva@yahoo.com.br

The main consequence of oxidative stress is the formation of DNA lesions, which might result in genomic instability and lead to cell death. Guanine is the base that is most susceptible to oxidation due to its low redox potential, and 8-oxoguanine (8-oxoG) is the most abundant. This characteristic makes 8-oxoG a good cellular biomarker to indicate the extent of oxidative stress. When 8-oxoG is not removed from DNA or nucleotide pool, G-C to T-A transversion mutations can occur, which makes this lesion particularly deleterious. Trypanosoma cruzi needs to deal with various oxidative stress situations that it is exposed to. Previous study performed by our group demonstrated that T. cruzi Rad51 gene presents an important function in the DNA repair of double strand breaks and oxidative lesions. In order to investigate the importance of homologous recombination pathway during parasite cell infection, we used T.cruzi mutants generated from wild type CL Brener T.cruzi clone, that either overexpress Rad51 gene or present only one copy of this gene (hemi-knockout mutant Rad51+/-). Tissue culture trypomastigotes forms from wild type and mutants parasites were used in infection assays to study parasite invasion and intracellular replication in immortalized murine embryonic fibroblasts. Infection assays were performed in 24-well plates containing round glass coverslips seeded with murine fibroblasts. These cells were infected with parasites and fixed at 24, 48 and 96 hours intervals. Invasion as well as parasite intracellular multiplication was evaluated by immunofluorescence. The results obtained show that the mutant strain overexpressing RAD51 present increased intracellular growth after 48 and 96 hours of infection when compared to wild type cells ($p<0.001$), while no differences were observed between wild-type and the hemi-knockout mutant strains. These results indicate that Rad51 overexpression may confer an advantage to T.cruzi during parasite intracellular replication.
Supported by::CAPES and CNPq

BM071 - Identification and molecular characterization of ubiquitin-like genes of *Trypanosoma cruzi*

MALUCELLI, L.C.*; DE PAULA LIMA, C.V.; SAMPAIO, F.; ROMANCINI, N.T.; KALB, A.L.; KUGERATSKI, F.G.; BATISTA, M.; KRIEGER, M.A.; MARCHINI, F.K.
ICC-FIOCRUZ, CURITIBA, PR, BRASIL.
e-mail:caprichabou@yahoo.com.br

Our group's present work is based on studies related to several ubiquitin-like proteins. The ubiquitin-like proteins, in turn, are proteins that can be identified as post-translational modification (PTM), as ubiquitin. Its name is related to its sharing of similar residues of ubiquitin domain, as well as its functional similarity, which can result in a proteolytic effect on the target protein, changes in protein stability, modification of protein function or subcellular localization. The relevance of our study is based in the formation of protein complexes, which involves the modified protein, the ubiquitin-like protein and in some cases, other proteins. The identification of the modified targets is therefore essential in order to determinate its action and importance to the organism. The present work is based on the identification of proteins that are related to ubiquitin in similarity of protein sequence, determinated by BLAST and ubiquitin related domains, determinated by PFAM. The work is restricted in 8 different genes coding ubiquitin-like proteins, with length varying from 237 bp to 1443 bp, all of these suitable to the Gateway cloning system. The genes coding the ubiquitin-like proteins used in this work were urm1, ufm1, three kinds of SUMO-like genes (SUMO-like, SUMO-like3 and RAD-60 SUMO-like), two kinds of atg8 (atg8-like1 and atg8-like2) and APG-like3. Among these, we managed to produce antibodies to atg8-like1 and atg8-like2. Other 2 genes are already in pDEST vector, waiting to be sequenced and subsequently expressed, purified and inoculated in mice to the production of specific antibodies (SUMO-like and SUMO-like3). The remaining proteins (RAD-60 SUMO-like, APG-like3, urm1 and ufm1) are still in the amplification stage, seeking for excelence in future clonings. Our studies aims to help the comprehension concerned in the ubiquitin-like pathways, as well to determinate subcellular localization and identify other proteins involved in the process.

BM072 - CHARACTERIZATION OF THE RNA BINDING PROTEINS DRBD3 AND NRBD1 OF *Trypanosoma cruzi*

ANTES, C.O.*; ALVES, L.R.; GOLDENBERG, S.
ICC-FIOCRUZ, CURITIBA, PR, BRASIL.
e-mail:coliveira@tecpar.br

The regulation of gene expression in trypanosomatids occurs mainly at the post-transcriptional level, and as yet few RNA binding proteins have been characterized. The RRM domain (RNA Recognition Motif) is one of the most abundant in RNA binding proteins in higher eukaryotes. RRM domain-containing proteins are involved in the majority of post-transcriptional processes, such as transport, splicing, and stability. Two proteins with RRM domains were chosen for characterization, DRBD3 and NRBD1. Genes encoding these proteins have been cloned and the recombinant proteins were used to obtain polyclonal antibodies. In silico analysis showed that both proteins have RRM in their structure. Western blot assays were performed and demonstrated that both proteins are expressed throughout the life cycle. Immunofluorescence was also carried out, showing that the NRBD1 protein has a possible mitochondrial localization while DRBD3 presents a slightly granular dispersion throughout the cytoplasm. The sucrose gradient polysome profile of these proteins showed that DRBD3 is not associated with polysomes while NRBD1 is associated with polysomes, migrating to heavier fractions. Ribonomic assays and analysis of mRNAs associated with both proteins was made using the CLC Genomics Workbench software. NRBD1 was more associated with heat shock protein 70 (hsp70) and small nucleolar RNAs (snoRNA) in stressed epimastigotes. DRBD3 was associated with kinase and delta tubulin in stress conditions. Immunoprecipitation assays with DRBD3 and NRBD1 is being conducted to characterize the protein partners in epimastigote parasites and epimastigotes under nutritional stress. **Supported by:**:Capes - Fiocruz

BM073 - Influence of MAP kinases depletion on phosphoproteome and proteome of *Trypanosoma brucei*

BATISTA, M.*; KUGERATSKI, F.G.; DE GODOY, L.M.F.; KRIEGER, M.A.; MARCHINI, F.K.
ICC-FIOCRUZ, CURITIBA, PR, BRASIL.
e-mail:batista.mic@gmail.com

Cellular signaling has been the focus of study in biological and medical areas. This process allows the interaction between cells and their environment. Cellular signaling is driven mainly by protein phosphorylation and dephosphorylation events, controlled by protein kinases and protein phosphatases, respectively. Important steps to elucidate the cellular signaling pathways are the matching of kinases and phosphatases with their respective substrates and the evaluation of global influence of phosphorylation on the cells. Here we focused in *Trypanosoma brucei* mitogen-activated protein kinases (MAPKs), a remarkable family that participates in the signal transduction through phosphorylation cascades, coordinating many cellular processes, such as cellular division, stress response, cellular differentiation, and other. An intriguing issue about this family in trypanosomatids is how the MAPKs coordinate cellular processes in an organism without transcriptional control of gene expression? In order to respond this and other questions, we evaluated the influence of MAPK family (15 members) on the *T. brucei* phosphoproteome and proteome combining RNAi with high accuracy MS-based proteomics. We used SILAC to improve the phosphoproteomic relative quantification by MS analysis. For this, the knockdowns were metabolic labeled, induced for RNAi and mixed with their respective non-induced/non-labeled counterparts. Then, phosphopeptides were enriched by titanium dioxide and proteome counterparts were SDS-PAGE pre-fractioned prior to LC-MS/MS analysis. Cellular growth evaluation of knockdowns revealed a decrease in the cellular replication after depletion of specific MAPKs, as has been reported for other organisms. In preliminary MS results, few hundreds of phosphorylation sites were quantified for two knockdowns. This is the first step towards a comprehensive analysis about the influence of system perturbations on the dynamics of *T. brucei* phosphoproteome and proteome.

BM074 - Assessment of parasite load in chronic chagasic cardiomyopathy patients from the BENEFIT study by Real-Time PCR

MOREIRA, O.C.*[1]; MELO, M.[2]; FERREIRA, C.L.[2]; BRITTO, C.[2]
1.FIOCRUZ/ IFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:otacilio@ioc.fiocruz.br

The *Benznidazole Evaluation for Interrupting Trypanosomiasis* (BENEFIT) study is an international multicenter, randomized, double-blinded, placebo-controlled clinical trial to evaluate the efficacy of benznidazole (BZ) treatment in patients with chronic Chagas' cardiomyopathy (CCC). Here, we describe the evaluation of distinct procedures for DNA extraction and qPCR-based protocols to establish a consensus methodology for the absolute quantification of *Trypanosoma cruzi* DNA in Guanidine-EDTA blood (GEB) samples. A panel of five primer sets directed to the *T. cruzi* nuclear satellite DNA (Sat-DNA) and to the minicircle DNA conserved regions (kDNA) was compared in either SYBR Green or TaqMan systems. Standard curve parameters such as, amplification efficiency, coefficient of determination and intercept were evaluated, as well as different procedures to generate the standard samples containing pre-established concentrations of *T. cruzi* DNA. The results achieved from Bayesian transmutability analysis elected primers Cruzi1/Cruzi2 (p=0.0031) and Diaz7/Diaz8 (p=0.0023) to be used together with the QIAamp DNA Kit extraction protocol (silica gel column) for monitoring parasitemia in chronic infection. A comparison between the parasite burden of 150 GEB samples from BENEFIT patients inhabitant in Argentina, Brazil and Colombia, prior to drug/placebo administration was performed. The median parasitemia found in patients from Argentina and Colombia (1.93 and 2.31 parasite equivalents/mL, respectively) was around 20 times higher than the one estimated for the Brazilian patients (0.1 parasite equivalents/mL). This difference could be in part due to the complexity of the *T. cruzi* genetic diversity, one factor possibly implicated in the different clinical presentations of Chagas disease and/or influencing the parasitemia levels in infected individuals from different Latin America regions.**Supported by:**:CNPq/ FAPERJ/ FIOCRUZ

BM075 - Construction and assessment of heterologous expression vectors for the non-pathogenic trypanosomatid Angomonas deanei

LAIBIDA, L.A.[*1]; FRAGOSO, S.P.[2]; DA ROCHA, W.D.[1]
1.UNIVERSIDADE FEDERAL DO PARANÁ, CURITIBA, PR, BRASIL; 2.INSTITUTO CARLOS CHAGAS, CURITIBA, PR, BRASIL.
e-mail:leticia_laibida@yahoo.com.br

The Trypanosomatidae family comprises ancient parasitic protozoa with peculiar genetic features and many of them cause severe human diseases, such as Chagas disease, sleeping sickness, and *Leishmania*sis. One strategy to drug development against these diseases depends on protein expression and genetic manipulation in these protozoa. However, biosafety and time consuming methods are needed when manipulating these parasites, since some of them are pathogenic to humans. In order to reduce these difficulties, the trypanosomatid *Angomonas deanei* was tested as a model organism for heterologous expression and gene manipulation in trypanosomatids. This protozoan grows in minimal medium to high cell density and represents no risk of infection for mammals, and it is also able to realize posttranslational modification of target proteins, such as glycosylation. To fulfill our goals, we developed two vectors for protein expression: one of them for integration in the α-tubulin *locus* (pCDXTub) and the other one to integrate into the rDNA *locus* (pCDXRibo). The southern blot data of stable transfected parasites indicate that both vectors can integrate in *A. deanei* genome, however, expression assays for the reporter genes GFP and luciferase failed to show any activity. Despite the lack of fluorescence detection, cells carrying the pCDXTub vector were able to express a transcript compatible with GFP and neomycin resistance mature mRNAs. The reasons for the lack of reporter genes expression are under investigation. **Supported by:**:CNPq; Fundação Araucária; PPSUS

BM076 - DEVELOPMENT OF A SEMI-AUTOMATED KNOCKOUT SYSTEM IN *Trypanosoma cruzi*

LORUSSO, A.B.[*]; PRETI, H.; FRAGOSO, S.P.; PAVONI, D.P.; MARCHINI, F.K.; PROBST, C.M.
ICC, CURITIBA, PR, BRASIL.
e-mail:andrelorusso@gmail.com

Trypanosoma cruzi has ~9,000 distinct protein coding genes, mostly characterized just in silico, being several of unknown function (~50%) and very few were studied through reverse genetics. This is due to the absence of a fast and highly effective method. In this context, we are developing a semi-automated system for gene knockouts (KO) in T. cruzi, which will allow large scale functional characterization of multiple genes. The system design consists of three parts: development of primer prediction software, construction of KO cassettes and sequential gene KO. The primer prediction software, originally aimed at gene replacement, has gained new features for genetic modifications, such as gene disruption and gene tagging. The primers designed were used in the fast and efficient construction of KO cassettes, using a simple two-step fusion PCR, and the products obtained were transfected into *T. cruzi* cells through electroporation. Different genes (n=21) were selected for the first trial, and their null mutants are being evaluated regarding phenotypical changes. For different simultaneous KO, distinct drug/drug resistance gene pairs would have to be used, which is of limited application. To solve this problem, we are applying strategies for unlimited multiple gene knockout, based on the use of recyclable cassettes through the Cre/lox system. At first, we intended to build a vector using a drug resistance gene for positive selection and the gene pyr6-5 as a negative selection marker, whose expression is toxic to *Trypanosoma brucei* in the presence of the compound 5-FOA; however, 5-FOA showed very low toxicity in T. cruzi. We are now looking for other metabolic selective markers, as well as using fluorescent proteins instead of selection markers, and flow cytometry assays to sort the null mutants, without selective pressure. Taken together, this effort represents the first initiative trying to incorporate reverse genetics techniques in *T. cruzi* in a systematic way.

BM077 - Role of the Chromatin Remodeling Enzyme HDAC1 in *Leishmania amazonensis* Infection: Implications for Host Transcription Repression

CALEGARI-SILVA, T.C.[*]; VIVARINI, Á.C.; SILVA, G.M.C.; LOPES, U.G.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:teresa@biof.ufrj.br

Leishmania parasites subvert important host cell signaling pathways involved in the control of the infection. NF-kappaB is an transcriptional factor which modulates the expression of immune response genes. Our group demonstrated the activation of NF-kappaB transcriptional repressor homodimer (p50/p50), in *L. amazonensis*- infected macrophages. As a result of this complex activation, we observed the down-regulation of the expression of nitric oxide synthase (iNOS) in infected macrophages treated with gamma Interferon. Besides the activation of transcriptional factors, chromatin epigenetic modifications are pivotal regulators of gene transcription. Chromatin remodeling proteins such as deacetylase histones (HDAC) are involved with transcriptional repression and may be associated with transcriptional factors, forming large repressor complexes. In this work, we have studied in details the iNOS transcriptional repression during *L. amazonensis* infection through the analysis of iNOS promoter occupancy by p50/p50 NF-kappaB complex and the participation of HDAC 1 in these events. We have found that the increased occupancy of p50/p50 iNOS promoter depends on PI3K/Akt pathway in *L. amazonensis* infected cells. Consistent with transcription repression, we have detected an increase in HDAC1 mRNA and protein levels, as well as an increased activity of total histone deacetylase in infected macrophages. We have verified a relevant reduction of *L. amazonensis* amastigote growth in macrophages silenced for HDAC1 expression. We also verified the mRNA iNOS increased levels in infected macrophages during HDAC1 silecing, showing the participation of this deacetylase in iNOS promoter regulation. In fact, we have observed an increased occupancy of HDAC1 in NF-kappaB promoter - binding site and a decreased occupancy of acetylated histone 3 (Lys 9). These results indicate that epigenetic modifications associated with p50/p50 NF-kappaB homodimer are taking place in infected macrophages. **Supported by:**:CNPq, CAPES

BM078 - MAPPING FUNCTIONAL DOMAINS INVOLVED IN NOVEL AND KNOWN INTERACTIONS IN THE *Leishmania* PABP1 HOMOLOGUE

DA COSTA LIMA, T.C.[*1]; XAVIER, C.C.[2]; DE MELO NETO, O.P.[2]; REIS, C.R.S.[2]
1.UFPE / FIOCRUZ PE, RECIFE, PE, BRASIL; 2.FIOCRUZ PE, RECIFE, PE, BRASIL.
e-mail:chreis@cpqam.fiocruz.br

In eukaryotes, the poly(A) binding protein (PABP) binds to the 3'end poly-A tail of most mRNAs and participates in an extensive range of cellular functions associated with mRNA metabolism including biogenesis, processing, degradation and during the various stages of protein synthesis. In the latter PABP acts through a critical interaction with the eIF4G subunit of the translation initiation complex eIF4F, responsible for recruiting the mRNA to translation through another subunit, eIF4E. Multiple PABP, eIF4G and eIF4E homologues have been identified in *Leishmania* with the PABP1, EIF4G3 and EIF4E4 homologues performing equivalent functions in translation to those described for other eukaryotes. Roles for the PABP2, PABP3, EIF4G4 and EIF4E3 homologues have also been postulated but have not been well defined yet. A unique aspect for this parasite is a recently identified direct interaction observed between PABP1 and EIF4E4. Here we have investigated through pull-down assays putative binding domains within PABP1 and EIF4G3 which might be involved in mediating these interactions. First we investigated within the three known domains of EIF4G3 (N-terminus, MIF4G and C-terminus) which would be involved in the interaction with PABP1 and found out that both N and C-terminal domains seem to be required. As for the PABP1 both its N-terminal half, which binds poly(A), and its C-terminal end, involved in protein-protein interaction, seem to be required for its interaction with EIF4G3, suggesting two interacting interfaces between the two proteins. We then investigated possible interactions between PABP1, 2 and 3 and the EIF4E3 and 4 homologues and found that PABP1, and to a minor extent PABP3 but not PABP2, binds directly to both. Within PABP1 this novel interaction requires its C-terminal end. So far our results highlight the occurrence of novel interactions which mediate critical steps required for efficient translation initiation in *Leishmania*. **Supported by:**:CAPES, UFPE, FIOCRUZ, FACEPE.

BM079 - Functional analysis of the tryparedoxin peroxidase in *Leishmania* braziliensis and L. infantum chagasi lines susceptible and resistant to antimony

ANDRADE, J.M.*; MURTA, S.F.
CPQRR- FIOCRUZMG, BELO HORIZONTE, MG, BRASIL.
e-mail:juvana.moreira@bol.com.br

Tryparedoxin peroxidase (TxP) participates of antioxidant defense by metabolizing hydrogen peroxide in water molecules. Literature data have reported that drug-resistant parasites may increase the levels of TxP along with other enzymes, protecting them against oxidative stress. Initially in this study, we analyzed the TxP mRNA levels and protein expression levels in lines of *L. amazonensis*, *L. braziliensis*, *L. Infantum chagasi* and *L. guyanensis* susceptible and resistant to SbIII. These lines exhibit index of resistance to SbIII 4 to 20-fold higher compared to their respective counterparts susceptible. The level of cTxP mRNA, determined by northern blot and/or qRT-PCR, was higher in the resistant *L. guyanensis, L. amazonensis* and *L. braziliensis* lines, than in their respective susceptible lines. Southern blot analyzes showed that the TxP gene is not amplified in the genome of SbIII-resistant *Leishmania* spp. lines analyzed. Western blotting assays using polyclonal antibody against the TxP recombinant protein from T. cruzi, showed that TxP protein is more expressed in all SbIII-resistant *Leishmania* spp. lines analyzed. Functional analysis of TxP was performed to determine whether overexpression of LbTxP in the susceptible and resistant *L. braziliensis* and *L. infantum chagasi* lines would change the resistance phenotype of transfected parasites to antimony SbIII. SbIII IC 50 analysis showed that susceptible *L. braziliensis* line that overexpress of TxP protein are 2-fold more resistant to SbIII compared to its parental non-transfected line. On the other hand, overexpression of TxP in the resistant *L. braziliensis* line caused inversion of resistance phenotype. In addition, overexpression of TxP enzyme in the susceptible and resistant *L. infantum chagasi* lines did not alter the resistance phenotype to SbIII. In conclusion, our functional anaysis results suggest that the enzyme tryparedoxin peroxidase may be involved in the antimony-resistance phenotype in *L. braziliensis*. **Supported by:**::FAPEMIG, CNPq, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR

BM080 - Functional analysis of eukaryotic initiation factor 5A in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole.

DUARTE, A.P.M.*[1]; GONÇALVES, A.T.S.[1]; SCHENKMAN, S.[2]; SILVA-PEREIRA, R.A.[1]; MURTA, S.F.[1]; ROMANHA, A.J.[1]
1.CPQRR/FIOCRUZ, BELO HORIZONTE, MG, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL.
e-mail:apduarte@cpqrr.fiocruz.br

Eukaryotic initiation factor 5A (eIF-5A) is a protein of 18 kDa, acidic (pI 4.5), highly conserved. Although it was identified as a translation initiator factor, its functional role in eukaryotic cells is not defined. This protein possesses a characteristic that consists in the addition of a hypusine residue, originated from spermidine, to a lysine residue in the protein. eIF5A and deoxyhypusine/hypusine modifications are essential for growth of eukaryotic cells. We have previously observed by proteomic analysis that in *Trypanosoma cruzi* the eIF-5A expression was decreased after an in vivo selection for Benznidazole (BZ)-resistance in a *T. cruzi* population (BZR) when compared to a BZ-susceptible *T. cruzi* population (BRS). Functional analysis of eIF-5A was performed to determine whether the overexpression of TceIF-5A gene in the susceptible (BZS and 17 WTS) and BZ-resistant (BZR and 17LER) *T. cruzi* populations would change the BZ-resistance phenotype of transfected parasites. We generated *T. cruzi* mutants for each population that either overexpress the wild-type TceIF5A sequence or TceIF5A mutant sequence (aa position 2: Serine to Alanina). (mutTceIF5A). Western blotting analysis using anti-TceIF5A polyclonal antibody showed that the level of TceIF5A protein expression was approximately 2 to 3-fold higher in transfected parasites compared to non-transfected ones. The BZ IC50 analysis showed that the overexpression of the wild-type TceIF-5A gene in the BZ-susceptible (BZS and 17 WTS) and in the BZ-resistant (BZR) *T. cruzi* populations increased 2-fold the BZ-resistance phenotype compared to non-transfected parasites. On the other hand, the overexpression of mutTceIF5A sequence in the four *T. cruzi* populations analyzed did not alter the BZ-resistance phenotype. These data suggest that the overexpression of TceIF-5A gene may be involved in the BZ-resistance phenotype in T. cruzi. **Supported by:**::CNPq, FAPEMIG, CPqRR and PDTIS/FIOCRUZ

BM081 - Neospora caninum developed resistance against pyrimethamine with transfection of mutated DHFR-TS gene from N. caninum, *Toxoplasma gondii* or "hybrid" T. gondii/N.caninum

PEREIRA, L.M.[*]; YATSUDA, A.P.
FCFRP-USP, RIBEIRAO PRETO, SP, BRASIL.
e-mail:miguepereira@yahoo.com.br

Neospora caninum is an Apicomplexa, obligate intracellular organism, with high economic impact in the veterinary area, especially in the cattle industry due to reproductive losses. Genetic manipulation is one of the methods to describe the key pathways related to invasion and replication of Apicomplexa. One of the tools to insert genes in *Toxoplasma gondii* is the cassette based on the expression of mutated DHFR-TS (Dihydrofolate reductase-Thymidylate synthetase), which confers to parasite resistance against pyrimethamine. This method is well tolerated by the parasite and allows multiple insertions in the genome by homologue recombination. Based on the original description of *Plasmodium* pyrimethamine resistant strains and adapted for *T. gondii*, we aimed to develop the same model for *N. caninum*. The NcDHFR-TS gene was point-mutated in two aminoacids: serine 36 to arginine (M2) and tyrosine 83 to aspartic acid (M3), generating DHFRM2M3. Three different constructions have been tested: NcDHFRM2M3 flanked by the promoter and 3' UTR Ncdhfr (Ncdhfr-DHFRM2M3), Tgdhfr-DHFRM2M3 (*T. gondii* DHFR-TS mutated) and a "hybrid" composed by the promoter dhfr and the DHFRM2M3 domain from *T. gondii* and the TS domain and 3'UTR from *N. caninum*. All three transfections conferred resistance against pyrimethamine, but resistance of the "hybrid" was at a similar level when compared to NcDHFRM2M3 and the Tgdhfr-DHFRM2M3 was lower. We also investigated the effect of TS (Thymidylate synthetase) domain by removing it from the cassete Ncdhfr-DHFRM2M3 with *Xho*I treatment, generating Ncdhfr-DHFRM2M3-truncated. This cassette also conferred resistance against pyrimetamine. The resistance against pyrimethamine was conferred by the four cassettes: mutated DHFR-TS from *N. caninum*, from *T. gondii*, the "hybrid" form and the construction without the TS domain, indicating that this enzyme is adaptable and functionally conserved among Apicomplexa. **Supported by:**:FAPESP

BM082 - Analysis of the ecto-NTPDase I expression in *Trypanosoma cruzi*: correlation with the parasite infectivity

GOMES, N.L.S.[*1]; FERREIRA, J.C.[2]; VIDAL, V.E.[1]; MOREIRA, O.C.[3]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.FIOCRUZ /IFRJ, RIO DE JANEIRO, RJ, BRASIL; 3.FIOCRUZ / IFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:natalylsg@yahoo.com.br

The distinct *T. cruzi* strains involved in infection, in conjunction with the regional distribution, have been suggested to cause different clinical manifestations and therapeutic efficiency in Chagas Disease. The ecto-NTPDase I is an apyrase located on the outer surface of *T. cruzi* plasma membrane. Previous studies related this enzyme to the infectivity and virulence of the parasite. In this study, we evaluate the mRNA levels for the Ecto-NTPDase I by RT-qPCR in distinct *T. cruzi* strains (Dm28c- *T. cruzi* I; Y- *T. cruzi* II; 3663- *T. cruzi* III; 4167- *T. cruzi* IV; LL014- T.cruzi V; CL. Brener- *T. cruzi* VI; CL-14- *T. cruzi* VI) and distinct evolutive forms. For this purpose, epimastigote forms of the different strains were cultivated at 28°C in 10% FCS supplemented BHI medium. RNA was extracted using TRIzol® reagent, followed by the quantification and treatment with DNase before the cDNA synthesis, by using the Superscript III kit (Invitrogen). The Real Time PCR assays were performed with primers designed to amplify a 111bp sequence in the ecto-NTPDase I *T. cruzi* gene and, as endogenous controls, a 268bp sequence in the calmodulin *T. cruzi* gene and a 100bp sequence in the GAPDH T.cruzi gene were used. We observed that the strains Cl-Brener, Dm28c, 4167, LL014, 3663 and Y presented mRNA expression levels, respectively, 8,21±1,62; 7,17±1,51; 1,26±0,18; 1,20±0; 1,06±0,04; 0,94±0,08, times higher than the CL-14 strain. Surprisingly, we observed that the epimastigote and amastigote presented expression levels, respectively, 14,35±2,04 and 10,23±1,16 times higher than the trypomastigote form. Moreover, following the parasite growth curve, we also observed that the expression of ecto-NTPDase I raises in a time-dependent manner, which could be related to a parasite response to a purine starvation in the cultivation medium. So far, our results suggest that the ecto-NTPDase I expression is regulated at mRNA level, which is distinct between *T. cruzi* strains and evolutive forms. **Supported by:**:FAPERJ

BM083 - POLYMORPHISMS IN IL-17 AND IL-10 ARE ASSOCIATED WITH THE INDETERMINATE FORM OF CHAGAS DISEASE

SOUZA, N.S.H.[*1]; ROCHA, M.O.C.[1]; OLIVEIRA FILHO, J.[2]; KOH, C.C.[1]; OLIVEIRA, L.M.B.[2]; PIMENTEL, L.[2]; ORNELLAS, A.C.[2]; ALVES, J.F.C.S.[1]; COSTA, G.C.[3]; GOLLOB, K.J.[4]; DUTRA, W.O.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UFBA, SALVADOR, BA, BRASIL; 3.UFV, FLORESTAL, MG, BRASIL; 4.SANTA CASA BH, BELO HORIZONTE, MG, BRASIL.
e-mail:nataliasatchiko@gmail.com

In the chronic phase of Chagas disease (CD), caused by *Trypanosoma cruzi*, about 30% of patients developed the chronic chagasic cardiomyopathy (CCC). This clinical form is characterized by altered cardiac functions and is considered the most severe clinical manifestation. The heterogeneous clinical course of CD suggests that genetic background of human host may contribute to high predisposition/resistance to CCC. Studies of genetic polymorphisms have consolidated a new approach in the analysis of the susceptibility of the human host to CD. IL-17 is considered a pro-inflammatory cytokine and IL-10 is an important immunorregulatory cytokine that plays an important role in balancing the immune response. The aim of this study was to investigate single nucleotide polymorphisms in genes encoding IL-17A, IL-17F and IL-10 and their possible associations with the development of the CCC in Brazilian subjects from Bahia and Minas Gerais. The genotyping of *IL17A*(-197A>G), *IL17F*(+7488T>C) and *IL10*(-1082G>A) genes were performed in a sample of 135 CCC and 59 indeterminate (IND) patients by real time PCR. Patients carrying the ancestral allele (A) for *IL17A* have two times more chance of developing the IND as compared to cardiac form (OR=2.15, p=0.017). A similar result was observed for the allelic frequency (OR=1.81, p=0.023). For *IL17F*, no significant association was found. Patients carrying the variant G for *IL10* have two times more chance of developing the IND form (OR=2.35, p=0.011). A similar result was found for the allelic frequency (OR=1.84, p=0.007). In addition, a combining analysis was made between *IL17A* and *IL10* gene polymorphism. Patients carrying the genotype A+G+ (versus A-G-) have 5 times more chance of developing de IND form (OR=5.24, p=0.0005). Our results showed that polymorphisms in IL-17A and IL-10 are associated with the differential clinical outcome of CD. This study is first one to analyze IL-17 and association of IL-17 and IL-10 polymorphisms. **Supported by:**::CNPq; Ministério da Saúde–Doenças Negligenciadas; INCT-DT; FAPEMIG

BM084 - Characterization of the enzyme iron superoxide dismutase-A in lines of *Leishmania* spp. susceptible and resistant to antimony

TESSAROLLO, N.G.[*]; MURTA, S.F.
CPQRR, BELO HORIZONTE, MG, BRASIL.
e-mail:nayaratessarollo@cpqrr.fiocruz.br

Superoxide dismutase (SOD) is a metalloenzyme that is a central component in antioxidant defence in most organisms. It removes excess superoxide radicals by converting them to oxygen and hydrogen peroxide. In this work the iron superoxide dismutase (FeSODA) gene has been characterized in lines of *Leishmania* spp. (L. guyanensis, *L. amazonensis*, *L. braziliensis* and L. infantum chagasi) 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. Southern blotting analysis using two restriction endonucleases showed the presence of polymorphism in the FeSOD sequence among the different *Leishmania* lines evaluated. Northern blot analysis using a specific probe for FeSOD-A gene showed the presence of a transcript of 4 Kb in all *Leishmania* lines analyzed. The levels of TcFeSOD-A mRNA were similar among all *Leishmania* lines regardless of the drug-resistance phenotype. Functional analysis was conducted to determine whether the overexpression of LbFeSODA gene in the susceptible and Sb-resistant *L. braziliensis* lines would change the Sb-resistance phenotype of transfected parasites. Western blotting analysis showed that the level of FeSODA protein expression was approximately 2 to 3-fold higher in transfected parasites compared to non-transfected ones. The SbIII IC50 analysis showed that the overexpression of this gene in the susceptible *L. braziliensis* line increased the SbIII-resistance phenotype compared to non-transfected line. In contrast, the overexpression of FeSODA in the resistant *L. braziliensis* line reversed the Sb-resistance phenotype. Assays of enzymatic activity of FeSODA these transfected parasites will be conducted to confirm the higher expression this enzyme in these parasites. **Supported by:**:FAPEMIG, CAPES, CNPq, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR.

BM085 - Development of Neospora caninum with resistance to chloramphenicol and expressing beta-galactosidase

PEREIRA, L.M.*; VERRI, M.P.; YATSUDA, A.P.
FCFRP-USP, RIBEIRAO PRETO, SP, BRASIL.
e-mail:miguepereira@yahoo.com.br

Neospora caninum is an obligate intracellular Apicomplexa which has cattle as the intermediate host with most economic impact. One of the current methods for the study and manipulation of Apicomplexa relies on molecular genetic techniques of tachyzoites. For *Toxoplasma gondii* these tools have been applied since 1993, where the first method described was based on resistance against chloramphenicol. As in *T. gondii*, we developed a cassette constituted of the chloramphenicol acetyltransferase gene (CAT) flanked by *N. caninum* Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) promoter (Ncdhfr-CAT). Between the stop codon of CAT and the 3' UTR of DHFR a Lac-Z gene controlled by the *N. caninum* Tubulin was ligated, resulting in a cassette with a reporter gene (Ncdhfr-CAT-NcTub/Lac-Z). The tachyzoites were transfected and chloramphenicol selected (20mM) until the rising of stable resistant forms (6 weeks of selection). To evaluate the beta-galactosidase activity and the application of expressing tachyzoites, an invasion assay was performed. Purified tachyzoites were diluted (1×10^6; 5×10^5; 1×10^5, 5×10^4 and 1×10^4) and applied on a vero cell monolayer in a 24 well plate. The tachyzoites invaded/adhered the cells for 2 hours and the beta-galactosidase activity was evaluated with CPRG reaction for 18 hrs and read at 570 nm. The number of invaded tachyzoites and the absorbance were sufficiently proportional to generate a linear regression curve with R^2 of 0,955. The tachyzoites were also detected by precipitation of X-gal with the beta-gal Staining Set (Roche), both for isolated and adhered/invaded on vero cell culture. The stable expression of a reporter gene in *N. caninum* is an unprecedented event and will allow the development of tools for detection and localization of parasites *in-vivo* and *in-vitro*, in addition to assays for evaluation of drug/sera effects on the invasion process, complementing methods as real time PCR. **Supported by:**:FAPESP

BM086 - Quantitative Analysis of *Trypanosoma brucei* Proteome and Phosphoproteome after Perturbation of MAP2K proteins

KUGERATSKI, F.G.*; BATISTA, M.; DE GODOY, L.M.F.; NOLL, M.A.; KRIEGER, M.A.; MARCHINI, F.K.
ICC FIOCRUZ-PR, CURITIBA, PR, BRASIL.
e-mail:fernanda.kugeratski@gmail.com

In Trypanosomatids the control of gene expression occurs mostly in a post-transcriptional level. The protein phosphorylation, performed by antagonistic action of protein kinases and phosphatases, is a dynamic and reversible post-translational modification essential to eukaryotes biological processes. In the *Trypanosoma brucei* kinome, there is an overrepresentation of mitogen-activated protein kinases (MAPK) pathways members. The MAPK cascades transmit environmental stimuli to the nucleus regulating a range of biological functions, such as proliferation, stress response and turmogenesis. To date, in *T. brucei* no studies have evaluated the function of the components of the MAPK pathways in a global context, comparing the system response at transcipts, proteins and phosphorylation sites levels after perturbation of these kinases. Thus, through the RNAi approach combined with RNAseq and SILAC-based quantitative proteomics and phosphoproteomics, this study aim to identify targets and pathways regulated by the entire MAP2K family - upstream activators of MAPK - and the impacts caused in the transcriptome, proteome and phosphoproteome after RNAi of these signaling components. The results obtained so far showed that the knockdown of four MAP2K proteins decreased the proliferation levels of procyclic cells. In the preliminary proteomics experiments, performed by high-resolution mass spectrometry, the differentially expressed proteins demonstrated involvement with spliceosome, metabolism, biosynthesis of secondary metabolites and biosynthesis of amino acids KEGG pathways. A systems-wide approach to study these signaling proteins is extremely promising, not only to understand how these pathways regulate the gene expression in a three level scale (mRNAs, proteins and phosphorylation sites) but also to test how the biological system respond to the perturbation of these kinases, since several MAP2K inhibitors are available and used in the treatment of different diseases.

BM087 - Expression and genomic organization of amastin family from distinct *Trypanosoma cruzi* strains

MARCOLINO, M.M.K.[*1]; PAIVA, R.M.C.[2]; ARAUJO, P.R.[2]; MENDONÇA-NETO, R.P.[2]; LEMOS, L.[1]; BARTHOLOMEU, D.C.[2]; MORTARA, R.A.[3]; DA ROCHA, W.D.[1]; TEIXEIRA, S.M.R.[2]
1.UFPR - LAB GFP, CURITIBA, PR, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL; 3.UNIFESP, SÃO PAULO, SP, BRASIL.
e-mail:monicamk@gmail.com

Amastins are surface glycoproteins initially described in *Trypanosoma cruzi* and subsequently found to be encoded by large gene families also present in the genomes of several species of the genus *Leishmania* and in other trypanosomatids. Although most amastin genes are organized in large clusters associated with tuzin genes and are up-regulated in the intracellular stage of these parasites, distinct genomic organizations and mRNA expression patterns have been reported. Based on the complete genome sequence datasets of three strains, CL Brener, Esmeraldo and Sylvio X-10, here we showed that T.cruzi presents amastin genes belonging to two of the four previously described amastin subfamilies: 8 copies of β- and about 12 copies of δ-amastins. We also analyzed the genomic organization, gene expression and cellular localization of members of these two amastin sub-families in different *T. cruzi* strains. Whereas δ-amastin genes are organized in two or more clusters with alternating copies of tuzin genes, β- amastins are present as two tandemly arrayed copies located in a distinct chromosome. Similar surface localization of all *T. cruzi* amastins expressed as fusion with green fluorescent protein was determined by confocal microscopy and immnuoblots, although a more peculiar, punctuated localization was observed for a sub-set of δ-amastins. Transcript levels for all δ-amastins were found to be up-regulated in amastigotes of CL Brener, Tulahuen and Y strains, all of them belonging to *T. cruzi* group II, but are significantly reduced in G and Sylvio X-10 strains, both of which are *T. cruzi* I strains known to have lower infection capacity. In contrast to δ-amastins, in all strains analyzed, β-amastins transcripts are more abundant in epimastigotes than in amastigotes or trypomastigotes, suggesting that, in addition of their role in the intracellular amastigotes, *T. cruzi* amastins may also serve important functions in the insect stage of this parasite.**Supported by:**:CAPES/REUNI, CNPq, Fundação Araucária.

BM088 - POLYMORPHISMS IN THE IL-1 CLUSTER ARE ASSOCIATED WITH DIFFERENT CLINICAL OUTCOME OF CHAGAS DISEASE IN PATIENTS FROM RIO GRANDE DO NORTE, BRAZIL

SOUZA, N.S.H.[*1]; KOH, C.C.[1]; ALVES, J.F.C.S.[1]; GOLLOB, K.J.[2]; CÂMARA, A.C.J.[3]; NASCIMENTO, G.B.D.[4]; ANDRADE, C.M.[3]; GALVÃO, L.M.[3]; DUTRA, W.O.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.SANTA CASA BH, BELO HORIZONTE, MG, BRASIL; 3.UFRN, NATAL, RN, BRASIL; 4.SESPRN, NATAL, RN, BRASIL.
e-mail:nataliasatchiko@gmail.com

Trypanosoma cruzi is the etiologic agent of Chagas disease (CD). The chronic chagasic cardiomyopathy (CCC) is considered the most severe manifestation and leads to altered cardiac functions, ventricular arrhythmias, hypertrophy, heart failure and death. The IL-1 cytokine family is mainly produced by monocytes and macrophages and plays an important role in pro-inflammatory reactions caused by parasite-associated molecules. Genetic polymorphisms may affect the rate of gene transcription, the stability of the mRNA and activity of the produced protein. In CD, studies of gene polymorphism associations have received special attention due to the heterogeneous clinical course followed by the disease. Our hypothesis is that IL1 gene polymorphisms are associated with different clinical outcome of CD. The aim of this study was to investigate single nucleotide polymorphisms in genes encoding IL-1α, IL-1β and IL-1ra (IL-1 receptor antagonist) and their possible associations with the development of the different clinical manifestations in Brazilian subjects from Rio Grande do Norte. Genotyping of *IL1A* (-889C>T), *IL1B* (+3954C>T) and *IL1RN* (+2018T>C) genes were performed in a sample of 46 CCC, 60 indeterminate (IND) and 182 control (CT) subjects by real time PCR. The presence and the frequency of the variant T for *IL1A* was approximately two times more frequent in CCC as compared to CT group (T+: OR=1.79, p=0.019; T: OR=1.66, p=0.006). For *IL1B*, patients carrying the variant T have three times more chance of developing the IND as compared to CCC form (OR=3.03, p=0.008). Similar result was found for the allelic frequency (OR=2.86, p=0.004). On the other hand, the C allele of IL-1ra polymorphism is almost two times more frequent in the non-dilated cardiac group (OR=1.97, p=0.042). Our results showed that polymorphisms in IL-1 family are associated with the differential clinical outcomes of CD. This study is first one to analyze IL-1 polymorphisms in the Brazilian population. **Supported by:**:CNPq; MCT/CNPq/CT-Saúde/MS/SCTIE/DECIT; INCT-DT; FAPEMIG

BM089 - Expression of the *Trypanosoma cruzi* trans-sialidase (TcTS) by *Trypanosoma rangeli* alters the parasite-Rhodnius prolixus interaction

GRANUCCI, N.[*1]; STOCO, P.H.[1]; LÜCKEMEYER, D.D.[1]; SCHENKMAN, S.[2]; GRISARD, E.C.[1]
1.UFSC, FLORIANOPOLIS, SC, BRASIL; 2.UNIFESP, SÃO PAULO, SP, BRASIL.
e-mail:edmundo.grisard@ufsc.br

Trypanosoma cruzi trans-sialidases (*Tc*TS) are crucial molecules in the interaction of the parasite with the host cell. Although lacking enzymatic activity, *T. rangeli* genome has genes similar to *Tc*TS family members. The aim of this study was to investigate the interaction of *T. rangeli* expressing an active *Tc*TS (*T. rangeli*-*Tc*TS) with *Rhodnius prolixus*. For that, 4^{th}-5^{th} instar *R. prolixus* nymphs were infected by intracelomic inoculation of *T. rangeli* wild type (WT), *T. rangeli*-GFP and *T. rangeli*-*Tc*TS epimastigotes. As expected, *T. rangeli*-WT and *T. rangeli*-GFP trypomastigotes were observed within the salivary glands three and 10 weeks p.i., respectively. On the other hand, *T. rangeli*-TcTS parasites were not observed in the salivary glands even after 16 weeks p.i. Despite the absence of invasion of the salivary glands, the *Tc*TS gene was detected by PCR in live *T. rangeli*-*Tc*TS parasites obtained from the triatomine hemolymph 50 days p.i. Also, Western blot assays carried out after 70 days p.i. using a polyclonal serum directed to the *Tc*TS catalytic site resulted positive, but still no glands were positive for the parasite. These results demonstrate that expression of *Tc*TS by *T. rangeli* alters the parasite development in *R. prolixus*, not allowing the parasite to invade the salivary glands to perform metacyclogenesis. **Supported by:**:CNPq, CAPES and FINEP

BM090 - A NEW APPROACH TO PREDICT GENE CLUSTERING AND DETERMINE THEIR ESSENTIAL TAG SEQUENCES APPLIED TO *T. CRUZI* POPULATIONS

BAPTISTA, R.P.[*1]; KATO, R.B.[1]; COUTO, B.R.G.M.[2]; SANTOS, M.A.[1]; PAPPA, G.L.[1]; BELCHIOR, J.C.[1]; MACEDO, A.M.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UNIBH, BELO HORIZONTE, MG, BRASIL.
e-mail:rodrigopbaptista@gmail.com

Chagas Disease is a tropical disease caused by the protozoan *Trypanosoma cruzi*, which still being a serious public health problem, affecting approximately 10 million individuals, causing 14.000 deaths per year in Latin America. *T. cruzi* populations were recently classified into six different DTUs named TcI-TcVI, but the epidemiological and clinical relevance of these lineages is not determined yet. Moreover, despite the considerable progress in cellular and molecular biology and in evolutionary genetics, a simple taxonomic strategy for grouping unknown *T. cruzi* populations is still missing. Herein we describe a new computational approach to predict the correct *T. cruzi* population clustering basing on gene sequences and determine the essential DNA tag sequences responsible for this grouping. For that, we initially used 158 amastin gene sequences available in GenBank, derived from six strains belonging to TcI, TcII or TcVI major lineages and through Singular Value Decomposition (SVD), Euclidean distance and K-means algorithms we could classify these sequences into three different groups. To validate our results we also compared our strategy with other current phylogeny reconstruction methods, Kimura-2-parameters and Neighbor Joining. Excellent concordance observed among the three clustering approaches validates this new clustering strategy. To determine the DNA tags regions responsible for each cluster we treated the analyzed data with multivariate logistic regression. For each observed cluster, at least one significant and specific tetranucleotide tag region was identified, demonstrating that our methodology not only allowed the correct clustering of the amastin sequences, but also it is a good strategy to determine polymorphic sites suitable for developing new molecular markers for *T. cruzi* major groups identification. **Supported by:**:FAPEMIG, CNPq, CAPES

BM091 - *Trypanosoma cruzi* DNA detection in different organs from orally infected mice

SILVA, R.T.E.*; BRÍGIDO, P.C.C.N.; NOTÁRIO, A.F.O.; SILVA, F.; RODRIGUES, A.A.; FERNANDES, P.C.C.; SILVA, C.V.
UFU, UBERLÂNDIA, MG, BRASIL.
e-mail:rebeccatavaress@gmail.com

Trypanosoma cruzi, an obligate intracellular parasite that causes Chagas disease, shows tropism for certain organs of the host and can cause serious damage to them. Our study aimed to identify the presence of the parasite in acute and chronic phases of disease in different organs of orally infected mice. For this purpose C57BL / 6 mice were infected with 10^6 metacyclic trypomastigotes from CL strain . After 5, 15, 30 and 180 days of infection, animals were euthanized and organs: brain, heart,
stomach, spleen, liver, small and large intestine were removed for DNA extraction. The organ infection was confirmed by PCR using primers for D7 gene 24Sα rDNA. Our results demonstrated that both in the acute and chronic phases of the disease
the presence of *T. cruzi* was observed in different organs from orally infected mice. The parasite was detected in almost all times of infection in the organs studied, except for the stomach and intestine. The presence of the parasite was more intense in the acute compared to chronic phase. A group of animals were immunosuppressed with dexamethasone 10 days before reaching 180 days of infection and were sacrificed. In this group we found that immunosuppression favors the presence of parasites in the organs studied, comparing them to the acute phase of infection. Our next step is to evaluate the presence of *T. cruzi* in the first hours of infection in different organs, as well as performing the same study with *T. cruzi* strain G and compare them. **Supported by:**: FAPEMIG, CAPES and CNPq

BM092 - Identification and subcellular localization of a UDP- N acetylglucosamine transporter in *Trypanosoma cruzi*

BAPTISTA, C.G.*; MORKING, P.; RODRIGUES, E.C.; AGUIAR, A.M.; RAMOS, A.S.P.
ICC, CURITIBA, PR, BRASIL.
e-mail:li_zahh@hotmail.com

Glycoconjugates play important roles in many different biological processes. A variety of human diseases are caused by defects in glycosylation and key processes for parasite infection such as cell invasion and modulation of the host immune system depend on glycoconjugates. The synthesis of these molecules occurs in the lumen of the endoplasmic reticulum (ER) and Golgi apparatus using nucleotide sugars as substrates. However nucleotide sugars are mostly synthesized in the cytosol and must be transported across the ER and Golgi membranes. The intracellular transport of nucleotide sugars is essential for glycoconjugate biosynthesis and it is carried out by nucleotide-sugar transporters (NSTs). These are hydrophobic proteins with 6-10 transmembrane domains. *Trypanosoma cruzi*, the etiologic agent of Chagas` disease, has a dense coat composed of glycoconjugates whose expression is stage-specific and essential for infectivity. The major glycoconjugates are mucin-like proteins, trans-sialidases and glycoinositol-phospholipids (GIPLs). We have identified a UDP-N acetylglucosamine (UDP-GlcNAc) transporter of *T. cruzi* by heterologous expression in a Kluyveromyces lactis mutant strain defective in UDP-GlcNAc transport. Eleven putative NSTs from *T. cruzi* were analyzed but only one, named TcNST1, was able to rescue the wild-type phenotype as evidenced by flow cytometric analysis. The subcellular localization was analyzed by an amino-terminal fusion with GFP. Our results showed a specific localization at the Golgi apparatus by confocal immunofluorescence and electronic microscopy. We are also checking the expression along the metacyclogenesis – in vitro differentiation of epimastigote forms to infective metacyclics -and life cycle by q-PCR. Preliminary results indicate a constitutive pattern of expression. The study of NSTs in *T. cruzi* is relevant for a better understanding of glyconconjugates` biosynthesis and their importance in the life cycle and infectivity of the parasite. **Supported by:**:CNPQ E FIOCRUZ

BM093 - Studies on the *Trypanosoma brucei* Pseudouridine synthase 7: In vitro silencing effects

LOPES, R.R.S.[*]; POLYCARPO, C.R.
IBQM, RIO DE JANEIRO, RJ, BRASIL.
e-mail:rsoares@bioqmed.ufrj.br

Transfer RNAs (tRNA) play a central role in protein synthesis, being the translators of the genetic code. tRNAs need to undergo several modifications to become mature and functional. One of the most frequent modifications is pseudouridine, a structural isomer of uridine, which is formed by enzymes called Pseudouridine synthases (Pus). The yeast Pseudouridine Synthase 7 (Pus7) is a multisite and multisubstrate enzyme that is able to pseudouridylate rRNAs, snRNAs, mature tRNAs and pre-tRNAs containing introns. In yeast pre-tRNA Tyrosine (pre-tRNATyr), Pus7 acts at U35. In trypanosomatids, the sole tRNA containing intron is tRNATyr, what makes Pus7 an interesting object of study for understanding the role of tRNA processing steps to the biology of these parasites. In this work, the yeast Pus7 homologue of *T. brucei* was identified, allowing the construction of stable *T. brucei* RNAi strain in which we can knockdown the *pus7* gene. The RNAi induction was very successful, with a maximum gene silencing value being achieved on the fourth day after induction (85%). The *T. brucei* Pus7-*like* gene silencing didn't affect the viability of the parasites over 14 days of monitoring. By transmission electron microscopy we observed that the knockdown of the gene induced an autophagic process directed to the mitochondria. Since all the mitochondrial tRNAs of these parasites are imported from the cytoplasm, and the import could be affected by the knockdown, the mitochondrial function was assessed by measurements of oxygen consumption. There were no significant differences in oxygen consumption in the silenced parasites. However, an increase of 20 % in the amount of reactive species (RS) generated was found, using the fluorescent probe DHE. We are now evaluating other effects of the *pus7* gene silencing *in vitro* to understand de role of this enzyme and its substrates to tRNA maturation of these parasites. **Supported by:**::CNPq, FAPERJ, OMS

BM094 - Preliminary studies of *Trypanosoma cruzi* Dipeptidyl aminopeptidase (DPPTc) Single-Allele knockout

MOTTA, F.N.[*1]; FIGUEIREDO, D.T.A.[2]; FEITOSA, G.[2]; SANTANA, J.M.[2]; BASTOS, I.M.D.[2]
1.UNB - FCE, BRASÍLIA, DF, BRASIL; 2.UNB, BRASÍLIA, DF, BRASIL.
e-mail:flavianader@gmail.com

Previous studies by our group had demonstrated that a member of Prolyl oligopeptidase (POP) family (S9) – POP Tc80 (S9A subfamily) - could be involved in the infection process by facilitating *T. cruzi* migration through the extracellular matrix. In order to elucidate if DPPTc (S9B subfamily) also has a role in the pathogenesis of Chagas disease, its knockout was outlined knowing that dpptc is a single copy gene per haploid genome. G418 (neomycin)-resistant *T. cruzi* epimastigotes (CL-Brener strain) were obtained after transfection and recombination of a fragment containing ~400 pb of the 5'UTR and 3'UTR of dpptc interconnected by neomycin phosphotransferase (neo) gene. The *dpptc* was amplified in all G418-resistant parasites. The PCRs using primers of *dpptc* flanking genes corroborate the correct insertion of neo gene in the parasite's genome. Preliminary assays show a significant decrease of DPP activity, using Gly-Pro-AMC, in protein extract of *dpptc*-/+ epimastigotes. Other analysis of this single-allele knockout parasites are under investigation such as their *in vivo* and *in vitro* infectivity. **Supported by:**::CNPq, FAP-DF, Finep, CAPES/COFECUB

BM095 - Molecular characterization of *Trypanosoma cruzi* strains using specific SNPs (Single Nucleotide Polymorphisms) in NADH Dehydrogenase Subunit I mitochondrial gene

BRITO, A.M.[*1]; VALADARES, H.M.S.[1]; LOPES, D.O.[1]; MACEDO, A.M.[2]; SEGATTO, M.[2]; BAPTISTA, R.P.[2]
1.UFSJ, DIVINÓPOLIS, MG, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:amandabrito.bqi@gmail.com

Trypanosoma cruzi, etiologic agent of Chagas disease, displays an extensive genetic polymorphism reflected in the existence of six phylogenetic lineages, called TcI to TcVI. For molecular characterization of *T. cruzi* strains in the major lineages, techniques as PCR-RFLP directed to COII (Cytochrome Oxidase subunit 2) gene are widely used. However, this technique requires the use of restriction enzymes, making it expensive and time consuming. Therefore, our main goal in this work was to develop a faster and economical method based only on PCR assays and agarose gels. For this, we used the BiPASA (Bidirectional PCR Amplification of a Specific Allele) technique, an efficient procedure for SNPs genotyping, because it is based in PCR amplification failure due the presence of mismatches between the primer's 3'-end and DNA template. Initially, DNA sequences of the COII and ND1 mitochondrial genes were submitted to MultAlin program and were found in the ND1 gene SNPs able to classify the *T. cruzi* strains in three groups: TcI, TcII and TcIII to TcVI. To validate the BiPASA assays we used a multiplex PCR system containing primers with specific SNPS corresponding to each cluster and DNA obtained from *T. cruzi* strains belonging to six phylogenetic lineages. The strains belonging to TcI and TcII lineages showed the expected amplification patterns. Surprisingly, the TcIII to TcVI strains presented a double amplification pattern: one characteristic from this cluster and other corresponding to TcI pattern suggesting the occurrence of mitochondrial heteroplasmy, which will be confirmed by DNA sequencing. In addition, we are evaluating the sensitivity of BiPASA technique in Full Nested PCR protocols using serial DNA dilutions and DNA obtained from tissues of chronic chagasic patients. Up to now our results demonstrated that ND1 BiPASA technique presents an innovative potential in *T. cruzi* molecular characterization studies and Chagas disease diagnosis. **Supported by:**:CNPq/ UFSJ

BM096 - SELECTED *Leishmania* eIF4E AND eIF4G HOMOLOGUES ARE DIFFERENTIALLY AFFECTED BY PHOSPHORYLATION EVENTS DURING GROWTH IN CULTURE

PEREIRA, M.M.C.[*1]; NASCIMENTO, L.M.D.[1]; REIS, C.R.S.[2]; DE MELO NETO, O.P.[2]
1.UFPE, RECIFE, PE, BRASIL; 2.CPQAM-FIOCRUZ, RECIFE, PE, BRASIL.
e-mail:larissa@cpqam.fiocruz.br

The translation initiation complex eIF4F functions in the early stages of protein synthesis through interactions performed by its three subunits: eIF4E, the cap binding protein; eIF4G, the scaffolding subunit; and eIF4A, an RNA helicase. Multiple and conserved eIF4E and eIF4G homologues have been identified in trypanosomatids with two likely eIF4F complexes being characterized so far (EIF4E4/EIF4G3 and EIF4E3/EIF4G4). Here we have compared the expression pattern of selected homologues (EIF4E3 and 4, EIF4G3 and 4 and EIF4AI) during different phases of *Leishmania amazonensis* growth. In this organism these proteins are simultaneously expressed in promastigote forms and most can be represented by phosphorylated isoforms. The expression of many of these isoforms vary during growth in culture, with phosphorylated EIF4E4 isoforms being typical of the exponential growth phase, whilst phosphorylated isoforms of EIF4E3 are associated with stationary cells. Despite being phosphorylated, no clear differences were observed in the EIF4G3 and 4 expression profiles during growth and no clear phosphorylation was observed for EIF4AI. Two dimensional gel profiles of EIF4E3, EIF4E4 and EIF4G4 confirmed the presence of many isoforms compatible with multiple modifications by phosphorylation, while only one or two isoforms were observed for EIF4G3. The expression of different isoforms was also investigated using inhibitors of different cellular processes including transcription, translation and mRNA processing. No changes were observed for the expression pattern of EIF4G3 and 4, which suggests that their phosphorylation status is quite stable. However, changes were found for EIF4E3 and 4 in the presence of transcription and translation inhibitors, with modifications similar to those observed in cells at stationary phase of growth. Our results indicate that phosphorylation differentially regulate the activity of different translation initiation factors in trypanosomatids. **Supported by:**:CAPES, UFPE, FIOCRUZ, FACEPE.

BM097 - The sialoglycopeptidase-like protein of *Leishmania* major plays a role in parasite growth

ROMÃO, A.B.D.[*]; DOS REIS, F.C.G.; LIMA, A.P.C.A.
IBCCF - UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:anabeatriz@biof.ufrj.br

Leishmania major was predicted to contain about 154 peptidase genes representing 2% of the genome. We identified in *L. major* a single copy gene with similarity to the sialoglyco metallopeptidase (OSGEP) of *Pasteurella haemolytica*. Bacterial OSGEP specifically hydrolyses peptide bonds between O-glycosylated and sialylated aminoacids and processes surface antigens of leukocytes. Other OSGEP-like proteins are found in bacteria and eukaryotes. The bacterial Kae-1 putative peptidase does not have proteolytic activity and is linked to DNA maintenance and transcription regulation. Yeast Kae1 is part of the chromatin associated multi-proteic complex KEOPS/EKC that is required for telomere maintenance and efficient gene transcription of essential genes. Kae1 was recently associated with the biosynthesis of N6-threonylcarbamoyl adenosine, a universal modification found at position 37 of tRNAs decoding ANN codons. We set out to characterize *L. major* OSGEP. The *L. major* OSGEP gene was cloned and shares 27% sequence identity to *P. haemolytica* OSEGP and 60% identity to bacterial Kae-1. Importantly, the conserved Glu residue, proposed as the catalytic residue of bacterial OSGEP, is replaced by non-conservative Val in *L. major* OSGEP, suggesting that it does not encode a functional peptidase. Recombinant His-tagged OSGEP was expressed in *E. coli* as inclusion bodies, purified and used to raise anti-serum. We generated *L. major* lines overexpressing OSGEP from an episomal vector, and confirmed increased OSGEP protein levels in parasite lysates by Western blot. Overexpressing lines have accelerated growth as promastigotes and reduced parasite infectivity to macrophages. The sub-cellular localization of the protein was assessed by the generation of a *L. major* line expressing OSGEP fused to GFP. Fluorescence microscopy revealed a distribution pattern consistent with perinuclear location. The mechanisms underlying the growth alterations of the mutants are under investigation. **Supported by:**:CNPq

BM098 - Generation and characterization of tunicamycin-resistant *Leishmania braziliensis*

BONFITTO, P.T.[*1]; PINZAN, C.F.[1]; CATTA-PRETA, C.M.C.[1]; DE CARVALHO, F.C.[1]; ALVES, E.V.C.[1]; ALMEIDA, F.B.R.[1]; ROQUE-BARREIRA, M.C.[1]; MOTTA, M.C.M.[2]; CRUZ, A.K.[1]; DE TOLEDO, J.S.[1]
1.FMRP-USP, RIBEIRÃO PRETO, SP, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:pedrobonfitto@gmail.com

Although available, therapeutics against *Leishmania*sis may be hampered by resistance emergence. Therefore, it is relevant to understand drug resistance mechanisms. Tunicamycin (TM), an antibiotic isolated from *Streptomyces lysosuperficus* that blocks the N-acetylglucosilation, has been used to study the mechanisms of virulence and drug resistance in *Leishmania*. Reports on the virulence pattern changes of TM resistant lines are controversial. Our proposal was to extend previous studies and to generate a strain of *L. braziliensis* resistant to high doses of tunicamycin (40 µg/mL) and investigate the host-parasite relationship and the molecular and cellular changes to explain the alterations on the virulence pattern. Herein we showed an increased virulence pattern of TM-resistant line in vivo and in vitro. The cytokines profile was evaluated and indicated a virulence increment. The analysis by scanning electron microscopy revealed a morphological change with longitudinal shortening and transversal thickening. Transmission electron microscopy showed an enlarged contractile vacuole, suggesting an water imbalance and the disorder of lamelles of Golgi apparatus, possibly due to changes on the proteoglycan production. We have also shown, by Pulsed Field Gel Electrophoresis, alterations on molecular karyotype of the resistant strain suggestive of gene amplification. On the amplified DNA was sequenced revealing several genes related to glycosylation pathway. **Supported by:**:CNPq and FAPESP

BM099 - Nitric oxide synthase (NOS) characterization from *Leishmania* brasiliensis

CHARRET, K.S.*; SILVA, F.S.; GUIMARAES, M.L.R.; CANTO-CAVALHEIRO, M.M.; LEON, L.L.; ALVES, C.R.
IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:karenbiologa@gmail.com

*Leishmania*sis is an infectious disease caused by protozoa of the genus *Leishmania*. There is no vaccine in clinical use, and the control of *Leishmania*sis relies mainly on chemotherapy. The Nitric oxide (NO) acts regulating various physiological processes in mammals, it is synthesized by nitric oxide synthase, and one of the isoforms is present in trypanosomatids. Some studies have suggested an association between the NOS and the infectivity and/or an escaping mechanism of the parasite, providing research interest in this molecule. An *in silico* BLAST search of the *Leishmania brasiliensis MHOM/BR/75/M2904* genome databases Gene BD with the amino acid sequence of human brain NOS identified the proteins encoded by LbrM28_V2.1340 p450 reductase as being putative NOS homologues. Subsequently, it was consider suitable mold having PDB ID 3QE2. NADPH-cytochrome protein reductase was obtained from BLAST. The Modeler9v10 program was used to generate the 3D structure. To verify the predicted structures, the DOPE score for the model was obtained from Modeller output. Also the validation of the model was carried using both Ramachandran plot calculations computed with the PROCHECK program and ERRAT. The *LbNOS* gene was amplified by PCR and express by free cell protein expression system. Western blott analyses, Griess reaction and NADPH consumption assays has been realized to identified and confirm the enzyme activity. Here it was provided several lines of experimental evidence that *Leishmania* encodes a homologue of the NOS from its eukaryotic counterparts. The relationship between the NO-generating systems in the parasite and in their host cell warrants further investigation. **Supported by:**:CNPq, CAPES

BM100 - Dissecting the role of XRNA and XRND complexes in the RNA metabolism of *Trypanosoma cruzi*.

KONDRAT, L.*; FERRARINI, M.G.; AVILA, A.R.; MARCHINI, F.K.; GOLDENBERG, S.; HOLETZ, F.B.
ICC-FIOCRUZ/PR, CURITIBA, PR, BRASIL.
e-mail:liandra_kondrat@hotmail.com

Trypanosoma cruzi is the etiological agent of Chagas' disease, a major endemic disease in Latin America. Control of gene-specific expression by RNA polymerase II is not significative and it is mainly regulated post-transcriptionally by mechanisms involving changes in mRNA stability or translation (access to polysomes). Accurate regulation of gene expression requires degradation of mRNA and other RNAs in a well-controlled way. In *Saccharomyces cerevisiae*, two major 5'–3' exonucleases, Xrn1p and Xrn2p, have major role in 5' processing of RNAs: Xrn1p is a cytosolic enzyme involved in degradation of mRNA, whereas Xrn2p is involved in RNA processing in the nucleus. The genome of the kinetoplastids parasites *T. cruzi*, *T. brucei* and *Leishmania* encodes four homologs of the S. cerevisiae Xrn1 and Xrn2, named XRNA, XRNB, XRNC, and XRND. Previous studies revealed that in *T. brucei*, XRND was found in the nucleus, XRNB and XRNC were found in the cytoplasm, and XRNA appeared to be in both compartments. Both proteins XRNA and XRND are essential for parasite growth, and depletion of XRNA increased the abundances of highly unstable developmentally regulated mRNAs. We have been investigating the role of 5'-3' exonucleases proteins XRNA and XRND in *T. cruzi* and we have showed that the TcXRNA protein is regulated throughout the life cycle and partially colocalize with TcDHH1, a marker of RNA granules (similar to P-bodies), indicating a role on mRNA degradation. On the other hand, TcXRND is present in the nucleus, more precisely in the nucleoli. Furthermore, we have been performing immunoprecipitation followed by mass sepctrometry assay in order to identify the partners of TcXRNA and TcXRND complex, while the RNA targets of these exonucleases will be identified by deep sequencing. From this approach, we intend to contribute to get further insight into the RNA metabolism in *T. cruzi*. **Supported by:**:Fundação Araucária, CNPq, Fiocruz

BM101 - Development of a yeast two-hybrid system to investigate protein interactions to the protozoan *Trypanosoma cruzi*, using bait proteins of the Rho signaling pathway

DE CARVALHO, S.S.[*1]; HENRIQUES, D.G.[1]; AMARAL, M.J.D.[1]; ATELLA, G.C.[2]; SILVA-NETO, M.A.C.[2]; DE CARVALHO, M.A.[1]; MELO, L.D.B.[1]
1.IFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:stephanie_serafim@hotmail.com

Introduction: The causative agent of Chagas disease, the *Trypanosoma cruzi*, undergoes profound morphological and metabolic changes along life cycle, indicating the presence of several transduction pathways, in which it is supposed that actin cytoskeleton proteins and monomeric GTPases are involved. However, molecular tools to investigate protein interactions still require development. Objective: Considering this difficulty, we develop a two-hybrid system in Saccharomyces cerevisiae to investigate molecular partners of the orthologs of rho and actin with proteins of T. cruzi. Methods: For production of hybrids in yeast, were used the pGBKT7 to clone our baits in fusion with the DNA Binding Domain of GAL4 (bait-Gal4BD), or the pGAD424 vector to subclone our cDNA library in fusion with the Activation Domain of Gal4 (cDNA-Gal4AD). The pGAD424 had its multiple cloning site modified with inclusion of adapters to allow the subcloning of fragments from normalized cDNA library for the three reading frames with Gal4AD. Occurring in vivo interaction, the two-hybrid formed will be able to activate transcription of genomic markers of S. cerevisiae MAV203 as HIS3, URA3, and LacZ. After clone's selection, they will be sequenced and compared to a database. Results and Conclusions: After cloning Rho and actin coding regions in pGBKT7 we carry out experiments to confirm the expression of baits in yeasts, discarding a possible toxicity. Subcloning efficiency of the original cDNA library with 106 clones, estimated to the three reading frames of pGAD424 vector was approximately 105 clones. The co-transformation of strain MAV203 with actin showed 54 strong interactions, after the selection with the genomic markers. The co-transformation with Rho is in progress to identify positive clones for these interactions. **Supported by:**:PROCIÊNCIA-IFRJ, FAPERJ

BM102 - POSSIBLE ROLES OF ASF1 (ANTI SILENCING FACTOR 1) IN *L. major*

FERREIRA GARCIA, J.B.[*1]; DA ROCHA, J.P.V.[2]; RUY, P.C.[1]; MACHADO, C.R.[2]; CRUZ, A.K.[1]
1.FMRP-USP, RIBEIRÃO PRETO, SP, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:julianaborio@yahoo.com.br

Anti Silencing Factor 1 (ASF1) was identified in different organisms as a histone chaperone that contributes to the histone deposition during nucleosome assembly in newly replicated DNA. Our recent studies of a putative ASF1 from *L.major* showed that increased levels of this protein do not have any influence in the expression of telomeric genes and the overexpression of ASF1 revealed increased levels of proteins involved in chromatin assembly. In an attempt to evaluate the involvement of ASF1 in the cellular response to DNA damage we analysed *L.major* mutants that overexpresses ASF1 by comparison with control lines. The parasites were submitted to hydrogen peroxide (H_2O_2) and the overexpression of ASF1 in *L. major* contributes significantly to the resistance of the cells to the oxidative stress. We investigated the protein profile differences of mutants that overexpresses ASF1 by 2-D gel electrophoresis treated with 400 µM of H_2O_2 for six hours and we detected 40 differentially expressed proteins that were identified by mass spectrometry. The functional annotation was made to improve the protein characterization and to get some information about the twelve hypothetical proteins identified. Two strategies were used, the first one was based on sequence similarity, using BLAST program versus Swissprot, the manually annotated and reviewed database. The second analysis includes the all in one tool for functional annotation of sequences, Blast2GO, which uses the Gene Ontology, KEGG maps, InterPro and Enzyme Codes databases. These methodologies provided the characterization of four hypothetical proteins and obtain further information for the 26 annotated proteins. We also analyzed the cell cycle of *L. major* when they were treated with 200 and 400 µM of H_2O_2 and with gamma irradiation. The in vitro infection didn't show any difference between *L. major* LmASF1 overexpressor and the control line. This set of complementary approaches allows understanding ASF1 roles in *L.major.* **Supported by:**:FAPESP

BM103 - Isolation of *Toxoplasma gondii* in free-range chickens from Vitória, Espirito Santo

FERREIRA, T.C.R.[*1]; COVRE, K.C.[1]; GIOVANINNI, N.P.B.[2]; BELTRAME, M.A.V.[2]; MOREIRA, N.I.B.[1]; PEREIRA, F.E.L.[1]; FUX, B.[1]
1.UFES, VITÓRIA, ES, BRASIL; 2.UVV, VILA VELHA, ES, BRASIL.
e-mail:kamilacovre@gmail.com

Toxoplasma gondii, causes toxoplasmosis, is capable of infecting a wide variety of animals and is highly prevalent in the world. Recent studies have demonstrated that strains of *T. gondii* in Brazil are different from isolates from North America and Europe. Brazil has a diversity of strains, however, little seroepidemiological surveys and no survey genetic and molecular strains of *T. gondii* are reported in Espirito Santo.Thus it becomes extremely important to understand the biological and molecular aspects of the parasite, allowing further integration with the epidemiology of toxoplasmosis. Therefore, serologic was performed by indirect hemaglutination in 35 chickens from Vitória, seven of these chickens had positive serology and the heart was harvested, and the material inoculated in female swiss mice. Tachyzoites were observed in the peritoneum of three animals, seven days after inoculation, and cysts were found in brain's mice, after thirty days. The cysts were reinoculated in two mice and after seven days tachyzoites were collected. All tachyzoites obtained from these animals have been maintained in liquid nitrogen for subsequent biological and molecular characterization. It is believed that this study will result in benefits to knowledge of the distribution of the strains in Espírito Santo state and the biological behavior. These aspects will contribute to future research related to different clinical manifestations, prevention and development of new drugs emphasizing the treatment of toxoplasmosis in Brazil.

BM104 - Isoform analysis and mapping of the phosphorylation target region for two homologues of the translation initiation factor eIF4E from *Trypanosoma brucei* (TbEIF4E3 and TbEIF4E4)

MALVEZZI, A.M.[*1]; MOURA, D.M.N.[1]; DE MELO NETO, O.P.[2]
1.UFPE/CPQAM, RECIFE, PE, BRASIL; 2.CPQAM/UFPE, RECIFE, PE, BRASIL.
e-mail:maramalvezzi@cpqam.fiocruz.br

In trypanosomatids different lines of evidence indicate that protein synthesis is a critical point for regulation of gene expression, possibly involving post-translational modification of key initiation factors (eIFs). In higher eukaryotes the eIF4F complex has a major role during translation initiation, acting through mRNA recognition and ribosome recruitment. Crucial for its activity is its eIF4E subunit, the cap binding protein, known to be regulated by modifications such as phosphorylation. Out of six eIF4E homologues in *Trypanosoma brucei*, two have been implicated in protein synthesis and are characterized by an unique N-terminal segment, *Tb*EIF4E3/*Tb*EIF4E4. In previous studies their expression was analyzed during the parasite procyclic life stage and both were observed to be represented by more than one isoform. Moreover, phosphoprotein purification assays confirmed that they were indeed phosphorylated. The present study sought to study and map within the two proteins the regions targeted for phosphorylation. To accomplish this, full-length and truncated (containing the deletion of its N-terminus) proteins were overexpressed linked to an HA tag in transfected procyclic cells and then analyzed by two dimensional electrophoresis. Both full-length proteins were represented by various spots that decreased in pI with a gradual and small increase in molecular weight, compatible with multiple phosphorylation events. In contrast, for *Tb*EIF4E4, the truncated mutant displayed a significant reduction in the number of spots whilst for TbEIF4E3 the mutant showed a similar profile to the full-length protein. In silico analysis identified potential phosphorylation sites for map/cdk quinases, shown to be implicated in *Leishmania* EIF4E4 phosphorylation, in the N-terminus of both proteins and these could be correlated with the *Tb*EIF4E4 results. Nevertheless our results suggest that different phosphorylation events are involved in regulating the activity of the two proteins. **Supported by:**:CNPq

BM105 - In vivo analysis of translation in *Trypanosoma cruzi* using Ribosome Profiling

HOLETZ, F.B.[*1]; POUBEL, S.B.[1]; SMIRCICH, P.[2]; MUNROE, D.[3]; GOLDENBERG, S.[1]; SOTELO-SILVEIRA, J.[4]; DALLAGIOVANNA, B.[1]
1.ICC-FIOCRUZ/PR, CURITIBA, PR, BRASIL; 2.FACULTAD DE CIENCIAS, MONTEVIDEO, URUGUAI; 3.NIH, FREDERICK, ESTADOS UNIDOS; 4.IIBCE, MONTEVIDEO, URUGUAI.
e-mail:fholetz@tecpar.br

Gene expression in trypanosomatids is mainly regulated post-transcriptionally since all of them show no sign of gene-specific control of RNA polymerase II transcription. The levels of the encoded proteins are determined primarily through regulation of mRNA turnover, translation and protein degradation. So far, little is known about translational regulation in trypanosomatids. Further study on this process is necessary, given that the determination of the amount and identity of the proteins produced by the different stages of the parasites life cycle would inform about all aspects of its biology. Techniques for systematically monitoring protein translation have lagged far behind methods for measuring mRNA levels. Although microarray-based measurements of mRNA abundance have revolutionized the study of gene expression, there is a critical need for techniques that directly monitor protein synthesis. mRNA levels are an imperfect proxy for protein production because mRNA translation is subject to extensive regulation. A recent study from Jonathan Weissman's laboratory (Ingolia *et al* 2009) described a ribosome-profiling strategy that is based on the deep sequencing of ribosome-protected mRNA fragments. They defined the protein sequences being translated and found extensive translational control. Based on this study, we have used this innovative technique to monitor the rate of protein production and to explore the molecular mechanisms used to control the translation process throughout the *Trypanosoma cruzi* life cycle. Preliminary analyzes in *T. cruzi* epimastigotes reveals strong pause sites that could act as key regulatory sites and defined groups of genes with different translational rates. Altogether our data will contribute to get further insight into the mechanisms of gene expression, particularly on translation regulation process in *T. cruzi.* **Supported by:**:CNPq, Fiocruz

BM106 - A multistep computational approach for the identification and classification of ncRNAs: *Trypanosoma cruzi* as a case study

BITAR, M.[*]; GRYNBERG, P.; MACEDO, A.M.; MACHADO, C.R.; FRANCO, G.R.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:mbitarufmg@gmail.com

Non-coding RNAs (ncRNAs) prediction has become a vast field of research and several classes of ncRNAs with different regulatory, catalytic and structural functions have been discovered. Few years ago, some kinetoplastid genomes have been finalized, and a recent study to predict ncRNAs in *Leishmania braziliensis* and *Trypanosoma brucei* has been published. Similarly, we propose to predict and classify ncRNAs for the complete genome of *Trypanosoma cruzi*. For this purpose, we used eQRNA, an algorithm for comparative analysis of biological sequences that performs probabilistic inference on genomic alignments. The entire genomes of *T. brucei* and *T. cruzi* were used to generate the initial alignments submitted to eQRNA, and 4195 ncRNA candidate sequences equal to or longer than 30 nucleotides were found. The candidate sequences were used for blastx search (e-value = 10e-05) against *T. cruzi* annotated proteins. 2813 candidates matched protein-coding sequences and the remaining 1382 candidates were submitted to a pipeline that included search against 25 different ncRNA databases, *ab initio* RNA tools and structural analysis. 1301 candidates had no evidence to be classified as ncRNAs and 49 candidates are tRNAs or rRNAs. Twenty-nine candidates presented similarity with ncRNAs from several databases. Three were considered false-positives. Our next goal is to identify putative regulatory ncRNAs that may be directed to UTR elements by matching the 29 ncRNAs to a catalog of 5' and 3' UTR sequences of *T. cruzi* transcripts retrieved from dbEST. *In silico* approaches concerning energy parameters will be employed to test the validity of these findings. **Supported by:**:Capes, CNPq, Fapemig

BM107 - Evaluation of *Trypanosoma cruzi* mitochondrial activity under ionizing radiation stress

VIEIRA, H.G.S.[*1]; GRYNBERG, P.[1]; MACEDO, A.M.[1]; PELOSO, E.F.[2]; GADELHA, F.R.[2]; MACHADO, C.R.[1]; FRANCO, G.R.[1]
1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.UNICAMP, CAMPINAS, SP, BRASIL.
e-mail:gfrancoufmg@gmail.com

Trypanosoma cruzi is extremely resistant to ionizing radiation withstanding doses of 1000Gy gamma rays. Ionizing radiation can cause DNA double-strand breaks, however, after a dose of 500Gy, *T. cruzi* growth arrests up to 240h returning to normal cell growth rate. The pattern of chromosomal bands is completely restored 48 hours after irradiation. Gamma rays can also induce oxidative stress via reactive oxygen species (ROS) production. Trypanosomatids are characterized by unique mitochondrion containing, the kinetoplast, which concentrates the mitochondrial DNA (kDNA), representing 20-30% of total cellular DNA. The kDNA is composed of a dense 20-50 maxicircles and thousands of minicircles. Mitochondrion is involved not only in the synthesis of ATP through the aerobic oxidative phosphorylation but also in the cell redox control, being the main source of intracellular ROS. In an attempt to understand how *T. cruzi* deal with all the damaged caused by irradiation, we evaluated the mitochondrial activity of irradiated and non irradiated parasites. For this, we measured the oxygen consumption, ATP synthesis and H_2O_2 release of irradiated and non-irradiated parasites during different time points (8h, 24h, 48h 72h and 96h). We also evaluated the expression of maxicircle genes using real time PCR. Irradiated parasites showed higher oxygen consumption rate, slightly higher ATP levels and release less H_2O_2 than non-irradiated parasites. The transcription of the maxicircle genes analyzed by RT-PCR was induced after irradiation and this data corroborated with previous microarray analysis of non-irradiated and irradiated parasites. Therefore, irradiated *T. cruzi* seems to have higher mitochondrial activity, giving support to an increased energy demand to overcome radiation damage and to resist oxidative stress. **Supported by:**:CNPq, FAPEMIG

BM108 - CHARACTERIZATION OF TOR PATHWAY COMPONENTS IN *TRYPANOSOMA CRUZI*

MIOT, H.T.[*]; HOLETZ, F.B.; GOLDENBERG, S.
ICC, CURITIBA, PR, BRASIL.
e-mail:halissontm@gmail.com

Cell signaling mechanisms responsible for detecting nutrients and growth factors in the cell environment , as well as how cells respond to such stimuli, have been studied in various organisms. One of the most signaling pathways studied in recent years, is TOR pathway (Target Of Rapamycin), an important nutritional sensor present in eukaryotes. The main protein of this pathway was discovered in yeast mutants resistant to the drug rapamycin, being so called TOR. At the same time, it was shown that the effect of rapamycin is dependent on its interaction with FKBP12 proteinwhich in turn, interacts with the TOR protein, preventing the access to its substrates. Through the cellular responses induced by drugs, various targets of TOR were identified in eukaryotes, providing an overview of the different processes in which this pathway is involved. We have used an in silico analysis to identify orthologue genes from the TOR pathway in *Trypanosoma cruzi*. These genes were cloned, the proteins were expressed, purified, and inoculated in mice to produce antibodies. Preliminary data showed that the treatment of epimastigotas forms with rapamycin induced morphological changes and reduction of cell proliferation, reinforcing the presence of this pathway in the parasite. Through immunoprecipitation and pull down assays using parasites treated with rapamycin and also parasites subjected to nutritional stress and followed by mass spectrometry, we aim to identify protein complexes involved in TOR pathway . Additionally, we intend to generated knock-out strains for some essential genes involved in the TORC1 and TORC2 complex formation and thus verify their role in the process of cellular differentiation. This approach will allow a major advance in the understanding of how the parasite responds to nutritional stress, as well as about the cellular processes involved in the triggering of differentiation. **Supported by:**:CNPq, Fiocruz

BM109 - Development of an in vivo system to detect protein-protein interaction in *Trypanosoma cruzi*.

LUDWIG, A.[*]; PRETI, H.; BATISTA, M.; KUGERATSKI, F.G.; PAVONI, D.P.; MARCHINI, F.K.; KRIEGER, M.A.; PROBST, C.M.
ICC- FIOCRUZ - PR, CURITIBA, PR, BRASIL. - **e-mail**:adriludwig@gmail.com

A comprehensive study of protein-protein interaction (PPI) networks is very useful for protein functional characterization and for understanding the biology of organisms as an integrated system. We are using yeast two-hybrid (Y2H) screen to study protein interactions in *Trypanosoma cruzi*, but it detects PPIs under heterologous conditions. Thus, we intend to create two parallel homologous in vivo systems for PPI detection, BiFC (bimolecular fluorescence complementation) and BiLC (bimolecular luminescence complementation). Both methods are based on the complementation of two fragments of fluorescent or luminescent proteins when they are fused to a pair of interacting proteins. BiFC and BiLC vectors for *T. cruzi* were created based on the pTcGW Gateway® cloning platform, developed by our group (Batista et al. 2010), with a flexible structure enabling the exchange of its elements. Vectors containing different antibiotic resistance genes were modified to express the N- or C- terminus fragments of proteins (YFP, CFP or luciferase). Vectors were also potentially improved in its efficiency through the exchange of intergenic regions. We are currently validating these vectors by testing interacting candidates. Due to particular characteristics of these approaches we believe BiFC will be very useful to investigate subcellular localization of PPIs and BiLC will be more sensitive and suitable to high-throughput analyses. Our ultimate goal is to combine the Y2H system, BiFC and BiLC results to obtain a comprehensive *T. cruzi* PPI network. In addition to incorporating a system to detect PPI, we are including new fluorescent genes in the pTcGW platform, comprising the entire visible colors spectra and different options within each color range. This set of vectors that we are creating will be a powerful basis to study the biology of this parasite by mapping of PPIs in their endogenous cellular environment and by expanding the options for fluorescence-based protein characterization. **Supported by:**:CNPq and Fiocruz

BM110 - TWO RELATED eIF4G HOMOLOGUES, CONSERVED IN ALL TRYPANOSOMATIDS, PARTICIPATE IN TWO DISTINCT eIF4F COMPLEXES WITH DIVERGED ROLES IN CELL VIABILITY AND PROTEIN SYNTHESIS

MOURA, D.M.N.[*1]; REIS, C.R.S.[2]; DA COSTA LIMA, T.C.[1]; CARRINGTON, M.[3]; DE MELO NETO, O.P.[2]
1.CPQAM/UFPE, RECIFE, PE, BRASIL; 2.CPQAM, RECIFE, PE, BRASIL; 3.UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, REINO UNIDO. **e-mail**:d.moura@cpqam.fiocruz.br

Trypanosomatids are characterized by unique molecular mechanisms acting at various stages of gene expression, with its regulation being mediated mainly by post-transcriptional processes. In protein synthesis they are characterized by a remarkably large number of homologues for two of the subunits of the heterotrimeric translation initiation complex eIF4F. In eukaryotes eIF4F recognizes the mRNAs and facilitates ribosome binding through its eIF4E and eIF4G subunits, respectively. In trypanosomatids two distinct eIF4F complexes have been described by us, formed by the EIF4G3/EIF4E4 and EIF4G4/EIF4E3 subunits. Here we continue the characterization of these complexes investigating different functional properties of EIF4G3 and 4. First, the eIF4E binding sequences in the *Leishmania* orthologues, located to their short N-terminal regions, were targeted by site-directed mutagenesis and binding to eIF4E evaluated in vitro. These uncovered binding motifs which differ for each protein and which are also distinct from the consensus described from other eukaryotes. For studies in vivo, we chose *T. brucei* as model and found that both *Tb*EIF4G3 and 4 are cytoplasmic and essential for cellular viability after RNAi. Knock down of *Tb*EIF4G3 led to a quick reduction in translation with subsequent cellular death. Depletion of *Tb*EIF4G4 produced a growth impairment prior to cell death, but no substantial inhibition in protein synthesis was seen. For both homologues, procyclic cells expressing proteins with mutations in putative motifs for the eIF4G/eIF4E interactions showed minor growth impairment. In contrast, modifications in the eIF4A interacting motif led to an important decrease in cell growth of cultures expressing the in *Tb*EIF4G3 mutant, but no effect was observed upon expression of an equivalent mutation in *Tb*EIF4G4. In all our results are consistent with the existence of at least two distinct eIF4F complexes, with the one containing EIF4G3 having a major role in translation. **Supported by:**:FACEPE, CAPES and CNPq

BM111 - Phylogenomic evidence of ancient horizontal gene transfer from bacteria to *Trypanosoma cruzi* of genes encoding invasion proteins

SILVA, D.C.*; SILVA, R.C.; FERREIRA, R.C.; BRIONES, M.R.S.
UNIFESP, SAO PAULO, SP, BRASIL.
e-mail:marcelo.briones@unifesp.br

The cell invasion mechanism of *Trypanosoma cruzi* has similarities with some intracellular bacterial taxa especially regarding calcium mobilization. This mechanism is not observed in other trypanosomatids, suggesting that the molecules involved in this type of cell invasion were (1) acquired by horizontal gene transfer or (2) the other trypanosomatids have lost the mechanism inherited since the bifurcation Bacteria-Neomura (1.9-0.9x10^9 years ago). Among intracellular bacteria, the mechanism of host cell invasion of genus *Salmonella* is the one that shares the highest similarities with *T. cruzi*. In *Salmonella* inavsion occurs by contact with the host's cell surface and is mediated by the type III secretion system (T3SS) that promotes the contact-dependent translocation of effector proteins directly into host's cell cytoplasm. Here we provide evidence of ancient horizontal gene transfer from intracellular bacteria to *T. cruzi*, in particular T3SS proteins from *Salmonella* spp, by performing exhaustive database searches directed to a wide range of intracellular bacteria and trypanosomatids. Specifically, BLASTP analysis using T3SS aminoacid sequences from *Salmonella* spp as queries revealed significant similarities with MASPs (surface proteins associated mucins) and mucins, which are possibly involved in calcium mobilization during *T. cruzi* invasion. Also, whole genome searches, using the same query, against *Leishmania major* and *T. brucei* shows significantly less similar sequences. Local alignment of the amino acid sequences (SipD, 420, 150, 90) against *T. cruzi* resulted in an alignment with good quality, clearly showing a conserved protein block. Bayesian phylogenetic trees showed the formation of a cluster of *S. typhi* SipD with several *T. cruzi* MASPs with posterior probabilities between 0.79 and 1.00. The secondary structure similarity of SipD with *T. cruzi* proteins ranges from 30 to 45%, indicating that secondary structure is more conserved than the primary structure. **Supported by:**:FAPESP, CNPq, CAPES

BM112 - Diagnosis of congenital toxoplasmosis by real time PCR on neonatal peripheral blood

COSTA, J.G.L.*; CARNEIRO, A.C.A.V.; TAVARES, A.T.; ANDRADE, G.M.Q.; MENEZES-SOUZA, D.; FUJIWARA, R.T.; VITOR, R.W.A.
UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:juju_gatti@yahoo.com.br

Aims: In an attempt to assess the use of an alternative diagnostic method of congenital toxoplasmosis (CT) based on molecular testing, neonatal peripheral blood was used to quantification of parasite load. Methodology/Principal findings: We evaluated a quantitative real-time PCR (qPCR) targeting a non-coding sequence (rep529) with 200-300 folds in genome of *T. gondii* for CT diagnosis and assessment of parasite load. DNA was extracted from 300µL of peripheral blood obtained from 183 newborn suspected of CT that presented specific IgM anti *T. gondii* in neonatal screening performed in the state of Minas Gerais, Brazil. These children were evaluated again after the first year of life to identify the persistence of specific IgG anti-*T. gondii*, considered a standard method for CT diagnosis. CT was confirmed in 150 neonates. The average age of children at the time of blood collection was 58 days. We used human β-globin as internal control for qPCR. Parasites were showed in 84/183 (46%) blood samples. Twelve (6,5%) positive children by qPCR had no detectable IgG anti-*T. gondii* after one year of life. The parasite load was low, ranging from 0,005 parasites/mL to 6,41 parasites/mL of blood. One outlier value was 20,5 parasites/mL. qPCR was positive even in child with 143 days old, but parasite load wasn't correlated to days of life (p =0,6772). Discussion: In countries which pregnant women are not routinely screened for toxoplasmosis, the diagnosis of CT depends only on serologic follow-up after birth, but early diagnosis is essential for rapid initiation of treatment reducing the occurrence of long-term clinical signs. About half of the newborn with congenital toxoplasmosis in Minas Gerais state had parasitemia detected by qPCR, even in children two months of age. Conclusion: qPCR in neonatal peripheral blood appears to be a useful alternative diagnosis approach mainly if performed soon after birth. **Supported by:**:CAPES

BM113 - Ubiquitin-binding complex of *Trypanosoma cruzi*

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

Trypanosoma cruzi, the ethiologic agent of Chagas disease, alternate between quite distinct morphological and functional forms during its life cycle. These changes are driven mainly by post-transcriptional changes in gene expression, which may be controlled at protein level by modulation of the activity, location or amount of stage-specific proteins. This modulation involves a complex combination of signaling systems, in which ubiquitination – modification of target-proteins by ubiquitin (Ub) - plays an important role. However, this system is still poorly characterized in *T. cruzi*, and the identification of proteins that interact with ubiquitination system and its targets may elucidate how this system controls cellular mechanisms. Aiming the identification of these proteins, they were enriched from total cell extract of *T. cruzi* epimastigotes using as bait a recombinant ubiquitin-histidin tagged immobilized in a nickel-agarose matrix. Proteins obtained were than submitted to hydrophobic interaction chromatography, aiming to separate complexes of proteins formed with ubiquitin. With this methodology we could purify 4 major complexes, and, with high resolution, proteins were identified by Orbitrap Mass Spectrometer. The present work has initiated investigations on the molecular and cellular mechanisms regulation by ubiquitination in this primitive organism. Identification of ubiquitin-interacting proteins in *T. cruzi* will help to understand the differentiation mechanism in this pathogenic protozoan.

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BM114 - 5'UTR or protein-coding? The dual role of intergenic segments determined by the alternative trans-splicing sites in *Trypanosoma cruzi* genes

SOUZA, N.P.*; BRANDÃO, A.A.
IOC, FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:nathps@ioc.fiocruz.br

Trypanosoma cruzi, a hemoflagellate protozoan that causes Chagas' disease, show peculiarities in genomic organization and control of gene expression, involving polycistronic transcription, RNA editing and trans-splicing. The availability of genome sequence for the reference strain CL-Brener affords now a detailed look at segments beyond the annotated open reading frames (ORFs). These segments are of special interest because they contain the untranslated regions, which are recognized to play functional roles in mRNA processing and translation in eukaryotes. The 5' untranslated region (5'UTR) is of the utmost importance in *T. cruzi* genome analysis because at the vicinities of this segment occurs the phenomena of trans-splicing, whose site defines the length of 5' end of the mRNA. Here we analyzed *T. cruzi* CL-Brener 5' UTRs under stress conditions using the method of RT-PCR with a primer to the leader sequence combined with an arbitrary primer. After mapping transcripts to *T. cruzi* reference RNA in Genbank, we identified 36 cDNAs from 32 different genes such as mucins, gp63, trans-sialidases, serine carboxypeptidase, hypothetical proteins that displayed a trans-splicing site located inside the annotated ORF. Genes that displayed this alternative trans-splicing were members of multicopy families which share N-terminal segments. Our findings of alternative trans-splicing reinforced by previous reports about similar phenomena in few *T. cruzi* genes, indicate the dual role of these segments: in one moment is 5´UTR and in another is translated in peptides, depending on the environmental, physiological or metabolic signals. Changing the role of a segment from an untranslated region to a protein-coding one should have implications to *T. cruzi* life style and interactions to its hosts. This observation also suggests that trypanosomes may modulate the coding content capacity of their genomes. **Supported by:**:CNPq and IOC/FIOCRUZ

BM115 - Detection and quantification of *Leishmania* (Viannia) sp in DNA obtained from fresh biopsies versus paraffin embedded biopsies by real-time PCR using the gene for the enzyme Glucose 6 Phosphate Dehydrogenase (G6PD).

DE LIMA, A.C.S.[*1]; TOMOKANE, T.Y.[1]; LAURENTI, M.D.[1]; SILVEIRA, F.T.[2]; CORBETT, C.E.P.[1]; GOMES, C.M.C.[1]
1.FMUSP, SÃO PAULO, SP, BRASIL; 2.IEC, BELÉM, PA, BRASIL.
e-mail:thaisey@yahoo.com.br

The use of real-time PCR (qPCR) for detection and quantification of parasites in biopsies is very important for the diagnosis and understanding of some aspects of *Leishmania*sis. This tool is able to be applied in fresh biopsies, but also in material collection, basically consisting of tissue fixed in formalin and embedded in paraffin, which is known to present a high DNA degradation. However, it is known that, although degraded, this material can be used in molecular techniques. This study aimed to compare the DNA detection using fresh and paraffin tissues from five cases of ACL in the G6PD qPCR reaction. Were also analyzed data such as time of lesion versus parasitism of detection by qPCR and compared with conventional methods, like as isolation in culture and parasite detection in smear.
DNAs were extracted by phenol-chloroform method and subjected to qPCR G6PD reaction for absolute quantification of the reaction using a standard curve. The nucleotides used were LVF-LVR (Castilho et.al, 2008). The DNA obtained from fresh tissues showed more suitable for PCR amplification. The qPCR presented higher sensitivity than conventional methods for a parasite detection. A negative correlation was observed between time of lesion and parasitism in the biopsies. In conclusion, the results showed that qPCR is more sensitive than conventional methods, the best sample for DNA application using qPCR was fresh tissue. The negative correlation between time of lesion and parasitism is compatible with lesion caused by parasite belongs to Viannia subgenus. **Supported by:**:LIM-50/HCFMUSP, FAPESP (2006/56319-1)

BM116 - A model for analysis of RNA-binding proteins divergence and evolution in trypanosomatids

SLOMPO, E.P.G.[*1]; HOFFMANN, F.G.[2]; DALLAGIOVANNA, B.[1]
1.ICC, CURITIBA, PR, BRASIL; 2.MSU, MISSISSIPI, ESTADOS UNIDOS.
e-mail:eloguerra@yahoo.com.br

Life is adaptive. There still remain outstanding questions in biology concerning regulatory networks evolution. It is known that genes can duplicate, but how they evolve to new functions, especially considering genes that code for proteins with regulatory functions, is still an open discussion. *Trypanosoma cruzi*, beyond its medical importance, is a well-established model organism for studying post-transcriptional regulation. Due to its early branching from eukaryotic phylogeny, is also a model in evolutionary biology. Several studies have being conducted on RNA-binding proteins (RBPs), with the aim to understand gene regulatory networks in an organism that relies on post-transcriptional mechanisms to control gene expression. TcRBP40 is an RBP that selectively bind to mRNAs and is mainly localized on reservosomes. Phylogenetic analysis revealed that its coding gene arose from the duplication of an ancestral gene, such as its ortholog (TbRBP7) on *T. brucei*. According to the phylogenetic tree, these duplication events occurred after the divergence of both species and the genes are asymmetrically accumulating mutations. Thus, they can be considered as a model study on gene network evolution. We are performing the functional characterization of these 4 proteins. They were expressed and purified in their soluble form, for RNA pull-down assay. Genes were also cloned on tag vectors for affinity purification of the RNP complexes, to obtain the bound RNA. These will be sequenced on SOLiD™ platform, so the targets of all 4 proteins will be identified and compared. Their regulatory regions will be analyzed looking for a common recognition motif, which will be tested against each protein. The same transfectants will allow cellular localization and overexpression analysis. Altogether, these data will provide information about functional divergence of the duplicates, and possibly bring new insights into how genes functionally evolve so the proteins can acquire new features. **Supported by:**:CAPES

BM117 - Characterization of native kinesins in *Trypanosoma brucei* using RNAi approach

IRAZAZABAL, L.N.[*1]; LUIGGI, S.[2]; MARANDE, W.[2]; KOHL, L.[2]
1.UNB, BRASILIA, DF, BRASIL; 2.MNHN (MUSÉUM NATIONAL D´HISTOIRE NATURELLE), PARIS, FRANÇA.
e-mail:luzirazazabal@gmail.com

Kinesins are motor proteins able to move towards the positive end of microtubules, using the energy provided by the hydrolysis of ATP. They perform many roles in cells and they carry a wide variety of cargo such as vesicles, chromosomes or, as in the case of intraflagellar transport, protein complexes - a mechanism essential for the formation of flagella in most eukaryotes. We recently characterized a kinesin involved in the assembly of a flagellar extra-axonemal structure (TbKIF9B).
In *Trypanosoma brucei* genome, we identified 40 genes encoding putative kinesins. However, the functions and locations of the corresponding proteins are often unknown. We have now undertaken a functional analysis of multiple kinesins targeting three groups of kinesins:
1) kinesins specific of trypanosomatids that can play a role in the life cycle of the parasite;
2) kinesins conserved in eukaryotes with flagella that can provide general information about the roles of these motor proteins;
3) kinesin genes whose expression can be regulated by epigenetic modifications such as cytosine methylation.
We first investigated the signatures of proteins by comparing the coding sequences. To determine their function in the trypanosome, we decided to turn off their expression by inducible RNA interference (RNAi). For accomplished that, specific fragments of each of kinesins were cloned into the expression vector for the extinction of the expression of the proteins targeted by RNAi. After verification by sequencing, these vectors were electroporated into procyclic forms of T. brucei. In parallel, we wanted to investigate the protein localization (flagellum, cytoplasm, membrane, nucleus). We cloned the genes targeted in frame with YFP (yellow fluorescent protein) in an appropriate expression vector. The constructs were transfected into wild-type procyclic cells. Thus we could observe the protein localization in living cells or fixed.
We present here the preliminary results of this functional analysis. **Supported by:**:CAPES-COFECUB, CNRS, INSTITUT PASTEUR, ANR

BM118 - Global analysis of trans-splicing sites in *Trypanosoma cruzi*

PROBST, C.M.[*]; PAVONI, D.P.; MARCHINI, F.K.; KRIEGER, M.A.
FUNDAÇÃO OSWALDO CRUZ, INSTITUTO CARLOS CHAGAS, CURITIBA, PARANÁ, BRASIL, CURITIBA, PR, BRASIL.
e-mail:cprobst@tecpar.br

Trypanosoma cruzi is a human parasite which causes Chagas disease, with a significant burden regarding morbidity and mortality mainly in Latin America. It is a member of the class Kinetoplastea, composed of free-living and parasitic protozoan. As an early branch of the eukaryote lineage, this class presents several peculiar biological characteristics, among them the mRNA processing through trans-splicing, where a specific sequence (mini exon) as well as a poly-A tail is added to the 5' and 3' ends, respectively. Post-transcription mechanisms play an essential role in gene expression regulation in these organisms and the identification of the trans-splicing sites is of utmost importance for delimiting the mRNA boundaries, specially the untranslated regions (UTR), enabling a more specific analysis of cis elements involved in gene expression regulation. A total of 2.4 billion SOLiD RNA-Seq reads generated by us in other transcriptomics projects, mainly from the epimastigote stage, were screened for poly-A and mini exon sequences. Using diverse similarity criteria, the mini exon sequence was identified in 1.2 to 15.3 million reads, and the poly-A tail in 3.4 to 25.1 million reads. Mapping these reads to the reference *T. cruzi* genome identified ~15,000 distinct trans-splicing sites, comprising nearly all *T. cruzi* genes. Due to its fragmented assembly stage, we had to analyse these reads with special care, creating several quality metrics. These results were compared with similar studies with T. brucei, a related species. The results presented here represents the first initiative to produce a global view of trans-splicing in T. cruzi, providing an essential information for regulatory gene expression network analysis. Further perspectives are directed sequencing of mRNA ends and the comprehensive analyses of trans-splicing sites in other life cycle stages of *T. cruzi*. **Supported by:**:CNPq, Fundação Araucária, FIOCRUZ

BM119 - COMPARATIVE TRANSCRIPTOMICS OF *TRYPANOSOMA CRUZI* PRIMARY AND SECONDARY AMASTIGOGENESIS

KESSLER, R.L.*; KRIEGER, M.A.; PROBST, C.M.
INSTITUTO CARLOS CHAGAS-ICC, CURITIBA, PR, BRASIL.
e-mail:polacokessler@hotmail.com

Trypanosoma cruzi is the protozoan that causes Chagas Disease, affecting ~15 million people in the Americas. This parasite has a biphasic life cycle where at least four cellular forms alternate between the insect vector (epimastigotes and metacyclic trypomastigotes) and the mammalian host (amastigotes and bloodstream trypomastigotes), with several differentiation processes. One of them, amastigogenesis naturally occurs when trypomastigotes penetrate nonphagocytic and phagocytic cells and differentiates to amastigotes in the mammal cell cytoplasm. The amastigogenesis can be induced in vitro by exposing trypomastigotes to specific media with acidic pH, and can be classified as primary or secondary if started from metacyclic or blood trypomastigotes, respectively. The present work aims to analyze the transcriptome of this parasite during in vitro primary and secondary amastigogenesis using RNA-Seq technology. Metacyclic trypomastigotes were obtained by in vitro metacyclogenesis and cell derived trypomastigotes were recovered from the supernatant of infected Vero cells. Both trypomastigotes were purified by ion exchange chromatography in DEAE-cellulose and the amastigogenesis were induced by exposing them to high glucose DMEM medium, pH 5. As both processes have different kinetics, total RNA samples were obtained at 0, 12, 24, 48, 36 and 72 hours for primary amastigogenesis and 0, 2, 6, 12, 24 and 48 hours for secondary amastigogenesis. mRNA were amplified and analyzed by RNA-Seq with the SOLiD4 platform. Currently we are analyzing the 36 libraries produced (triplicate experiments) in search for differentially expressed genes and patterns of gene expression in primary and secondary amastigogenesis. The data generated constitute the first global assessment of the transcriptional program of *T. cruzi* during amastigogenesis, and their conjunction analysis with other datasets enriches our understanding of *T. cruzi* regulome. **Supported by:**:CNPq, CAPES, NIH, Fundação Araucária and FIOCRUZ

BM120 - Study of Otubain role in *Trypanosoma cruzi* infection by knockout

FIGUEIREDO, D.T.A.*; MOTTA, F.N.; AZEVEDO, C.S.; SANTANA, J.M.; BASTOS, I.M.D.
UNB, BRASÍLIA, DF, BRASIL.
e-mail:dtafigueiredo@gmail.com

An estimated 10 million people are infected with *Trypanosoma cruzi* (the parasite that causes Chagas disease) worldwide, mostly in Latin America. Chagas` disease is a public health burden since available treatments are often ineffective in chronic stage and cause severe side effects. The aim of our research team is to study parasite proteases as potential new therapeutic targets. In this work, we present a preliminary functional study of otubain (OtuTc), a cysteine protease of T. cruzi. Otubain belong to the deubiquitylating enzymes (DUBs) family important in gene regulation and others several biological processes such as immune response activation. otutc presents a single copy gene in genome, which allows us to study, by knockout (KO), the role of the protein in parasite viability and infection process. The KO cassette was constructed with neomycin phosphotransferase gene (neo) flanked by 5'UTR and 3'UTR of otutc. They were transfected into epimastigotes (CL-Brener strain) producing G418-resistant parasites. Several PCRs were carried out to confirm the presence of otutc and neo in the KO parasite genome. The effect of otutc KO was analyzed by in vitro parasite growth and L6 host cells infection. Furthermore, to provide a deeper understanding of this protease function in infection process, parasites double-allele KO, as well as, in vivo mice infection are under analysis. **Supported by:**:CNPq, DPP/UNB, FAP-DF, Finep and CAPES/COFECUB

BM121 - Descriptive and comparative analysis of the global transcriptome response of *Trypanosoma cruzi* to different medium conditions

PROBST, C.M.*; LEPREVOST, F.V.; MARCHINI, F.K.; PAVONI, D.P.; KRIEGER, M.A.
FUNDAÇÃO OSWALDO CRUZ, INSTITUTO CARLOS CHAGAS, CURITIBA, PARANÁ, BRASIL, CURITIBA, PR, BRASIL.
e-mail:cprobst@tecpar.br

During its life cycle, *Trypanosoma cruzi* has to face several distinct environments, which are characterized by a dramatic shift in ambient conditions, as temperature, pH, nutrient disponibility and oxidative bursts, among others. These changes have to be coupled by the parasite, so a specific response probably evolved as a response to these challenges. Understanding them is very important to increase our knowledge about the molecular biology mechanisms underlying these processes, reinforcing its potential use as a guide to target important processes in the pursuit of new therapeutic approaches; besides, as its transcriptome profiles have to change to deal with the new conditions, specific modules of co-expression and co-regulation must have evolved, so studying these processes is very powerful for the regulome initiative, where these modules are being identified. We have transferred *T. cruzi* Dm28c epimastigotes in exponential growth to distinct media, evaluating nutrient disponibility (TAU, TAU3AAG, PBS), temperature (10^{o}C, 16^{o}C, 37^{o}C, 41^{o}C), pH (4.0, 5.8, 8.5) and oxidative (20mM H_2O_2, 200mM H_2O_2), and samples in triplicate were taken from 0, 1, 2, 4, 6 and 24 hours. Total mRNA was extracted and sequenced in a SOLiD4 equipment. We have generated ~3 billion reads, comprising 216 samples, and the least sequenced sample had >10 million reads. We have mapped these reads to the CL Brener reference genome and the differential expression was analyzed using the edgeR software, from the Bioconductor project, in the R environment. The amount of differentially expressed genes (DEG) at 10% FDR ranged from ~300 to ~1,500 genes, depending on the evaluated medium. The distinct classes of DEG were mainly constituents of metabolic pathways; also, we have observed different response of protein classes usually considered as regulated by stress (heat shock proteins, for instance), i. e., genes included in these classes showed distinct behaviors. More interestingly, comparing the different transcriptome responses against each other, we were able to identify a general modulated core, but most of the genes were modulated in specific responses, what was not seen in classical model organisms, but in an intracellular parasite, *Candida albicans*, reinforcing the different evolutionary pressures. Taken together, these results represent the first global transcriptomics analysis of *T. cruzi* response to several environment changes; these data is integrated in our database of gene expression regulation and being used for identification of co-regulation modules. **Supported by:**:CNPq, Fundação Araucária, FIOCRUZ

BM122 - Transformation of *Leishmania* using cross-species whole genomic DNA

COELHO, A.C.*; LEPROHON, P.; OUELLETTE, M.
CENTRE DE RECHERCHE EN INFECTIOLOGIE, LAVAL UNIVERSITY, QUEBEC CITY, CANADÁ.
e-mail:ccadriano@gmail.com

Leishmania spp. are pathogenic protozoa characterized by a substantial diversity in pathogenesis and virulence despite their considerable synteny at the genome level. Although the occurrence of hybrid strains in the field had already been reported previously by several authors, the existence of genetic exchange in the sand fly vector was only recently proven when hybrid parasites were isolated and generated (Akopyants *et al.*, 2009). Given the frequency of hybrid parasites in the field and their importance in shaping *Leishmania* population's heterogeneity, we assessed the possibility of generating cross-species recombinants in vitro by heterologous genomic DNA transfection. We describe a knock-in protocol based on whole genome transformation (WGT) by introducing a drug resistance marker in the donor *Leishmania* cells that could be used for selecting recombinant recipient parasites. These parasites were shown to acquire the phenotype derived from the donor cells, as demonstrated for the transfer of drug resistance genes from *L. major* into *L. infantum,* with integrations of exogenous DNA fragments as large as 40kb for several chromosomal regions and that took place at homologous loci in recipient strains. We also found that inactivating the mismatch repair gene *MSH2* can further facilitate the recovery of cross-species hybrids. Our observations are the first step for the generation of in vitro hybrids for assessing gene function under natural genomic contexts and this technology may be applicable to other pathogens.

BM123 - Comparison of PCR and kDNA addressed to hsp70 for the detection of *Leishmania* in small mammals of the Indigenous Land Xakriabá, MG, Brazil

PEREIRA, A.S.*; FERREIRA, E.C.; REPOLES, L.C.; REGO, F.D.; GONTIJO, C.M.F.
CPQRR, BELO HORIZONTE, MG, BRASIL.
e-mail:agnes.sampaio@hotmail.com

Since 2001 have been reported autochthonous cases of Cutaneous *Leishmania*sis (ATL) in Xakriabá Indian reservation. In the period 2001 to 2009 were more than 200 cases diagnosed among residents of reservation (MS/FUNASA, 2010). The aim of this study is the comparison of techniques for detection of *Leishmania* DNA in tissues of small mammals trapped in the Indigenous Land Xakriabá. Recently PCR directed at different targets have been used for the detection of *Leishmania* in different hosts. In this study we used skin samples from tail and ear of 94 small mammals, captured in Indigenous Xakriabá, including marsupials, like Didelphis albiventris, Marmosops incanus and Gracilinanus sp and rodents, as Trychomys apereoides, Rhipidomys sp. and Rattus rattus. DNA extraction was performed on tissue samples using the kit & Tissue Cells genomicPrep Mini Spin - GE Healthcare, following the manufacturer's instructions. For detection of DNA from *Leishmania* PCRs were performed with kDNA directed to the primers: 5 '(C/G) (C/G) (G/C) CC (A/C) T CTA (T/A) T TAC CCC AAC ACC 3 'and B: 5' GGG CGT GAG GGG TCT GCG AA 3 ', generating a fragment of 120pb, and to hsp70 gene with primers a: 5' GACGGTGCCTGCCTACTTCAA 3 'and B: 5' CCGCCCATGCTCTGGTACATC 3 ', generating a fragment of 1300 bp PCR directed to kDNA *Leishmania* showed 36 positive when the fabric used was 14, and tail skin positive ear skin when used. hsp70 showed 15 positive when using the skin of the tail and seven positive skin when used in the ear. When analyzed skin samples from the tail (n = 89), we obtained an agreement of 0.408, which is considered tolerable. With the ear skin samples (n = 91), the correlation found was of 0.097, which is considered weak. The PCR analysis using the target kDNA shown more sensitive when the hsp 70 is more effective characterization of the parasite, which indicates the importance of using two associated techniques for achieving a more reliable in determining hosts possible reservoirs *Leishmania*. **Supported by:**:FAPEMIG