BM001 - **STABILITY OF THE EPISOMAL PX63-GFP PLASMID IN LEISHMANIA CHAGASI**

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Objective: Evaluate the fluorescence stability of the pX GFP plasmid in the absence or presence of drug pressure Methods: *Leishmania chagasi* (MHOM/BR/BA262) strain was transfected with 20µg of supercoiled pX GFP plasmid and we used 30 µg/ml of geneticin as a drug selection. After growth to saturation, parasites were harvested and plated in a solid medium. We analyzed the fluorescence of six clones by FACS and picked the ones with highest and lowest Fluorescence Intensity (FI). For each of these clones were made 14 successive passages with the following conditions: Total absence of drug pressure, keeping the concentration of 30µg/ml of G418 or with a slow increase in concentration until it reached 750µg/ml, followed by total withdraw of the drug. The most fluorescent clone was also inoculated in Siberian Hamster. After 75 days the animal was euthanized and 5 mg of its liver was passed to the Schneider to obtain promastigotes. After saturation, the IF was analysed by FACS. Results: When we cultivated with the absence of drug selection, after 14 passages the Leishmania had only 3% of its initial IF. In the case of maintaining the initial concentration of G418 (30µg/ml), after 14 passages still was present an Intensity of fluorescence equal to 46% of its original. In the case of the gradual increase of drug selection, we observed an increase in IF, obtaining the maximum value for the concentration of 500µg/ml of G418, which corresponded to 138% of its initial IF. In the case of the passage thought hamster, the leishmania had only 5% of its initial IF. Conclusions: The compared clones with lower and higher initial IF showed maintenance of IF in a similar way. These results suggests that the plasmid pX GFP can be routinely used to express fluorescent proteins in *L. chagasi*, since the acquisition time without drug pressure doesn't exceed a few months.
Supported by:CNPq 141032/2009-0

BM002 - **CHARACTERIZATION OF A PROTEIN TYROSINE PHOSPHATASE IN *TRYPANOSOMA RANGELI* (*TR*PTP2) USING MONOCLONAL ANTIBODIES**

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Protein tyrosine phosphatases (PTPs) are enzymes involved on regulation of processes such as cell division and differentiation. Kinetoplastid PTPs, such as *Tb*PTP1 in *Trypanosoma brucei*, have already been reported to be essential for these parasites' biological cycle and, due to their significant differences from human PTPs, are considered as promising therapeutic drug targets. In the present work we have evaluated the cellular location and expression levels of a *Trypanosoma rangeli* PTP (*Tr*PTP2), which is 58% identical to *Tb*PTP1. Recombinant PTPs from *T. rangeli* (*Tr*PTP2) and from *T. cruzi* (*Tc*PTP2) were expressed in *Escherichia coli* BL21 (DE3), purified and then used to produce monoclonal antibodies (MAb) in Balb/C mice. Polyclonal serum showed cross-recognition of both *Tr*PTP2 and *Tc*PTP2. So far, Western blots using anti-*Tr*PTP2 revealed a single ~65 kDa protein in total extracts of *T. rangeli* epimastigote and trypomastigote forms. Since this molecular mass is higher than the predicted 36 kDa for *Tr*PTP2, analysis of the deduced amino acidic sequence revealed glycosylation sites, which were corroborated by gel staining with Schiff's reagent. Immunofluorescence essays using anti-*Tr*PTP2 MAb suggests that there is a concentration of *Tr*PTP2 in the flagellum of both epimastigote and trypomastigote forms of *T. rangeli*. As tyrosine-phosphorylated proteins have already been recognized in the flagellum of *T. brucei*, the detection of a protein responsible for tyrosine dephosphorylation in the *T. rangeli* flagellum could be involved on regulation in this structure; particularly because the flagellum is an important structure for cell differentiation in trypanosomatids and an interface of interaction with their hosts. Supported by CNPq, FINEP, CAPES and UFSC.
Supported by:CNPQ, FINEP, CAPES, UFSC

BM003 - **COMPARATIVE ANALYSIS OF GENE EXPRESSION PROFILE IN ANTIMONY-RESISTANT AND SUSCEPTIBLE NEW WORLD LEISHMANIA SPECIES**

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Drug resistance is a major problem in leishmaniasis chemotherapy. RNA expression profiling using DNA microarrays is a suitable approach to study simultaneous events leading to a drug-resistance phenotype. Gene expression analysis has been performed primarily with Old World Leishmania species and here we investigate molecular and functional alterations in antimony (SbIII) resistance in the New World Leishmania species. Promastigote forms of L. (L.) amazonensis, L. (V.) guyanensis and L. (V.) braziliensis were selected for resistance to antimony by step-wise drug pressure. Gene expression of highly resistant mutants was studied using DNA microarrays. RNA expression profiling of antimony-resistant L. amazonensis revealed the overexpression of genes involved in drug resistance including the ABC transporter MRPA and several genes related to thiol metabolism as trypanothione synthase. A comparative analysis of commonly modulated genes in the three species studied revealed 168 upregulated and 71 downregulated genes. Although it is noteworthy the presence of several genes encoding hypothetical proteins, we highlight the overexpression of genes that encode proteins as: dihydrofolate reductase-thymidylate synthase, S-adenosylhomocysteine hydrolase, tryparedoxin peroxidase, trypanothione reductase, cyclophilins, GP63, nucleobase transporter (NT3) and some ABC proteins. The functional annotation analyses pointed shared overexpressed genes involved in the glutathione metabolism, redox homeostasis, protein and RNA metabolism. Glucose and folate transporters as well as MDR2 (multidrug resistance-associated protein 2) were found to be downregulated. Several of these gene-encoding proteins were not previously linked to antimony resistance. An integrated overview of the alterations suggests the presence of shared mechanisms that leads to compensate the metabolic imbalance generated by the drug. Additionally, the intracellular thiol levels were found enhanced in most of the antimony-resistant Leishmania spp. mutants. Mechanisms classically found in Old World antimony resistant Leishmania were also highlighted in New World antimony-resistant Leishmania. These studies were useful to the identification of resistance molecular markers.
Supported by:CNPq, CAPES, FAPEMIG, CIHR

BM004 - **TCOLA, AN OBG ATPASE THAT PLAYS A ROLE IN THE OXIDATIVE STRESS IN TRYPANOSOMA CRUZI**

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We have identified a putative Obg-like ATPase in Trypanosoma cruzi (TcOLA), which is highly conserved in bacteria and eukaryotes, suggesting that it may be involved in a ubiquitous biological pathway. Accordingly, TcOLA was identified associated to polysomes, suggesting that it plays a role in translation process. To gain insight into its cellular function we performed immunoprecipitation assays combined with mass spectrometry to determine the composition of biological complexes associated with TcOLA. Our analysis showed that TcOLA was associated with several ribosomal proteins, translation factors and the protein RPN7 from the regulatory subunit of the T. cruzi proteasome. A TcOLA null mutant was obtained by targeted gene deletion. The epimastigote from TcOLA mutant showed no abnormal morphology by light microscopy. In addition there is no impairment in its proliferation and differentiation. In yeast, the TcOLA ortholog (YBR025c) is involved in the degradation of oxidized proteins in cells subjected to oxidative stress. In view of that, we performed an assay for detection of protein carbonyls formed on oxidized proteins in T. cruzi (wild type and TcOLA mutant). Indeed, we observed an increase of protein carbonyls in the knockout parasites. Moreover, TcOLA mutant when submitted to oxidative stress showed a significant reduction in the proliferation when compared to wild type parasite. Taken together, our data suggest that TcOLA might play an important role as a molecular link between the translation apparatus and the proteasome for degradation of proteins damaged during translation.
Supported by:CNPq

BM005 - **TOWARDS THE ESTABLISHMENT OF A CONSENSUS REAL-TIME QPCR TO MONITOR TRYPANOSOMA CRUZI PARASITAEMIA IN CHRONIC CHAGASIC CARDIOMYOPATHY PATIENTS**

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Real-time PCR is an accurate method to quantify *Trypanosoma cruzi* DNA in clinical samples and can be used to follow-up specific chemotherapy in Chagas disease. Recently a qPCR workshop was held at FIOCRUZ/RJ in order to generate a consensus protocol to be applied to the *Benznidazole Evaluation for Interrupting Trypanosomiasis* (BENEFIT) study, with the participation of 3 PCR Core Laboratories (Argentina, Brazil and Colombia). This report describes the evaluation of distinct DNA extraction procedures and qPCR protocols for the absolute quantification of *T. cruzi* DNA in Guanidine-EDTA blood lysates (GEB). Four primer sets directed to the conserved motifs within the repetitive *T. cruzi* satellite DNA sequence, and one targeted to the kDNA minicircles were compared in SYBR Green and TaqMan systems. Standard curve parameters, such as amplification efficiency, coefficient of determination and intercept were evaluated accordingly to the procedure used for obtaining DNA samples with known parasite concentrations. SYBR Green performance was assessed for each primer set to estimate parasitic load in six chronic chagasic cardiomyopathy (CCC) BENEFIT patients prior to drug/placebo administration. The results suggested a more accurate quantification using the commercial kit based on silica-membrane technology, to enable efficient processing of GEB samples and to minimize sample contamination, comparing with the in-house phenol-chloroform method. Considering the complex profile revealed by kDNA melting curve analysis, the quantification data obtained with this primer set could not be precisely supported by SYBR Green. Regardless kDNA, all primers tested revealed good performance to quantify the parasitic load in CCC patients. The average *T. cruzi* DNA concentration from CCC patient triplicate samples varied from 0.02±0.01 to 56.54±6.00 parasites/mL, with 5 out of 6 patients presenting very scarce parasitaemia with less than 10 parasites/mL of blood.
Supported by:CNPq

BM006 - **COMPARATIVE PROTEOMICS OF WILD TYPE AND TOR KNOCK DOWN IN TRYPANOSOMA BRUCEI**

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Target of rapamycin (TOR) proteins are eukaryotic serine/threonine kinases that act in complexes and control cell growth in response to external stimuli. Four TOR were described in T. brucei: the orthologs TbTOR-1 and TbTOR-2 and two other homologs TbTOR-like 1 and TbTOR-like 2. TOR signaling pathways are unknown in trypanosomes and might be relevant to control cell growth in the different hosts. By using RNAi based approach, we have previously found that TOR-like 1 and TOR-2 knockdown parasites have growth defects. Here we compared procyclic T. brucei proteome of these knockdowns with control cells. Comparative data was generated by 2D gel electrophoresis of protein stained by Coomassie blue. Protein expression was detected in biological triplicates using PD Quest 7.3.1 (Bio Rad) analysis software and spots showing differences ($p<0.05$) were cut, digested with trypsin and subjected to LC MS/MS analysis. We identified 31 distinct proteins affected by TbTOR-like 1 and 15 affected by TbTOR-2 knockdown. These data were applied to the String database and used to generate an interatoma profile. The results show that while TbTOR-like 1 affects proteins related to stress, glycolytic pathways and aminoacid transport, TbTOR-2 seems to affect mainly proteins related to cell cycle control. These findings delineate TbTOR-2 containing complexes in cellular growth and TbTOR-like 1 in response to environmental changes in T. brucei.
Supported by:FAPESP E CNPQ

BM007 - **HORIZONTAL GENE TRANSFER CONFERS FERMENTATIVE METABOLISM IN THE RESPIRATORY-DEFICIENT PLANT TRYPANOSOMATID PHYTOMONAS SERPENS**

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A survey of expressed sequence tags in the plant trypanosomatid *Phytomonas serpens* revealed a transcript encoding for a putative pyruvate decarboxylase (PDC), a hallmark of fermentative energy metabolism occurring in yeasts and some bacteria. Sequence analysis showed that this gene shares high similarity with genes from phytobacteria and has an ortholog in *Leishmania* spp. The goals of this study were: (i) to obtain phylogenetic evidence supporting that a horizontal gene transfer (HGT) event may have acted in the acquisition of the PDC by *Phytomonas* and *Leishmania*; (ii) to investigate the gene copy number and transcript abundance in the two trypanosomatids. Phylogenetic analysis of indolepyruvate decarboxylases (IPDCs) and PDC sequences of bacteria and fungi indicates that *Phytomonas* PDC is robustly monophyletic with the *Leishmania* enzyme. In the trees obtained by different methods this clade appears as a sister group of gamma-proteobacteria IPDCs, with 98% bootstrap support in the Maximum Parsimony tree. *P. serpens PDC* gene copy number was investigated by Southern blot. While *Leishmania* has a single copy gene, at least six, tandemly-repetead gene copies with an intergenic region of 130bp were estimated in *P. serpens*. Real time RT-PCR indicates a 5.7-fold increase in the abundance of gene transcripts in *P. serpens* as compared with *L. major*. Finally, southern hybridization using a pair of degenerated primers designed based on the *P. serpens* and *Leishmania* spp. sequences suggests the presence of *PDC* orthologs in other trypanosomatids like *Herpetomonas*, *Crithidia* and *Leptomonas*. *Phytomonas*, but not *Leishmania*, produce ethanol when cultured in glucose-rich media. This observation is suggestive that the *Phytomonas* enzyme is involved in alcoholic fermentation, converting pyruvate to acetaldehyde. Taken together the data suggest that the acquisition of a PDC activity represents a well suited adaptation for *Phytomonas* endurance in the plant host carbohydrate-rich medium.
Supported by:FAPESP

BM008 - **SIRTUIN-MEDIATED DOWNREGULATION OF GENES CO-RELATES TO H3AC MODIFIED CHROMATIN REGIONS IN LEISHMANIA**

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The acetylation/deacetylation of histone lysines is an important mechanism to control chromatin structure and transcription initiation in different eukaryotes. In yeast, the implication of Sir2p, a member of the NAD^+-dependent histone deacetylase (HDAC) family, in histone modification and chromatin remodeling has been widely documented. Recent studies have demonstrated histone modifications within putative sites of transcription initiation along trypanosomatid chromosomes (Respuela et al., 2008. JBC. 238:50 and Thomas *et al.,* 2009. BMC Genomics. 10:152). *Trypanosoma brucei* Sir2 homolog has been characterized and was shown to participate in gene silencing. We have investigated the Sir2 homologs of *Leishmania major* and hypothesized that these proteins would be involved in epigenetic mechanisms in this parasite. Among the putative Sir2 genes of *Leishmania*, LmSir2rp1 has been characterized as a cytosolic protein and LmSir2rp2 has a mitochondrial localization peptide signal. *In silico* analyses of LmSir2rp3, revealed the conservation of residues necessary for the deacetylase activity. To determine the subcellular localization of LmSir2rp3, we performed protein fractionation followed by western blotting analysis and confirmed that LmSir2rp3 is a nuclear protein. To characterize LmSir2rp3 function, we performed a yeast complementation assay, using a ScSir2 knockout strain, which showed that LmSir2rp3 was able to restore the silencing effect of a telomere-located *URA3* auxothophy marker. To further investigate the silencing effect of LmSir2rp3, we generated a cell line overexpressing LmSir2rp3. The expression profile of the LmSir2rp3 cell line was investigated in microarray hybridization analyses. These experiments revealed that LmSir2rp3 overexpression led to changes in mRNA levels of genes involved in transcription regulation, translational control, chromatin modification and processing of small non-coding RNAs. Interestingly, the location of 16% of the down-regulated genes was coincident with the acetylated histone H3-rich regions (H3ac) across the parasite chromosomes. As previously described, H3ac regions are typical of strand-switch regions (SSR), which are considered to be sites for transcription initiation in trypanosomatids. Our data suggest the participation of LmSir2 in a possible epigenetic control of gene expression at the level of transcription initiation. Ongoing work is focused in the demonsration of the histone deacetylase activity of LmSir2 *in vitro* and in the investigation of LmSir2 influence in chromatin remodeling processes.
Supported by:FAPESP and CNPq

BM009 - **STRUCTURE AND ORGANIZATION OF TELOMERIC AND SUBTELOMERIC REGIONS FROM TRYPANOSOMA CRUZI**

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T. cruzi trypomastigotes express surface glycoproteins of the trans-sialidases (TS) superfamily which are implicated in the host cell invasion. Single populations of *T. cruzi* may express different antigenic forms of TS. Analysis of TS genes located at the telomeres suggested that chromosomal ends could have been the site for generation of new TS variants. Preliminary work of our laboratory showed that *T. cruzi* telomeres are formed by an array of tandemly arranged hexamerrepeats (5' TTAGGG 3'), followed by a 189-bp junction sequence and the subtelomeric region. The aim of our work is to characterize the structure and organization of the telomeric and subtelomeric regions of *T. cruzi.* We identified 49 contigs carrying the telomeric repeat (TTAGGG) in the TriTryp data base, all contigs display adjacent to the hexamer repeat a conserved 189 bp junction, which represents a signature of *T. cruzi* chromosomal ends. We found that 40 telomeric contigs could be assigned to T. cruzi chromosome-sized scaffolds. The subtelomeric region was defined as the sequence between the 189-bp junction until the beginning of the interstitial chromosome region; the size of subtelomeric regions varies from 5 kb-182 kb. T. cruzi subtelomeric sequence structure varies widely, mainly as a result of large differences in the abundance and organization of genes encoding surface proteins (TS and DGF-1), retrotransposon hot spot genes (RHS), retroposons elements, RNA-helicase and N-acetyltransferase genes at individual telomeres. The subtelomeric regions are enriched in pseudogenes, but also contain complete gene sequences, indicating that these regions may be functional parts of the expressed genome. We were able to connect some telomeric ends to the chromosomal bands separated by pulsed field gel electrophoresis. The lack of synteny in the subtelomeric regions suggests that genes located in these regions are subject to recombination, which increases its variability, even in homologous chromosomes.
Supported by:FAPESP, Cnpq, Capes

BM010 - **SEARCHING REGULONS IN LEISHMANIA USING CONSERVED INTERCODING SEQUENCES AS BAIT**

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The mode of *Leishmania* transcription transfers to the post-transcriptional level most of the control of gene expression and understanding the machinery involved in the regulatory processes must be pursued to better understand the parasite's biology. Aiming to identify putative regulatory cis-elements present in the non-coding DNA, we established a computational method for extracting conserved intercoding sequences (CICS) from *Leishmania spp*. genomes. These conserved elements were mapped within predicted mRNA UTRs and genes bearing common CICS were clustered. We generated a database with 9,225 groups of CICS common to *L. major, L. braziliensis* and *L. infantum* genomes, which we called LeishCICS-clusters. We then investigated the transcript levels of *L. infantum* genes, present in the LeishCICS-clusters, making use of an available expression data for eight timepoints during *L. infantum* differentiation between promastigote and amastigote stages (microarray analysis, Lahav et al., 2011). We obtained 730 LeishCICS-clusters, containing from 2 to 17 genes analyzed in the microarray experiment, whose presented a Pearson's correlation coefficient greater than 0.5. Our results indicate that the developed in silico approach associated with this high-throughput functional assay may contribute to the identification of *Leishmania* post-transcriptional regulons.
Supported by:Fapesp

BM011 - **THE ROLE OF LEISHMANIA BRAZILIENSIS STRAIN POLYMORPHISM ON DISEASE OUTCOME AND DISTRIBUTION**

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American tegumentary leishmaniasis is caused by parasites of the Leishmania mexicana and Leishmania braziliensis complexes in the New World. Three forms of ATL can result from human infection with Leishmania braziliensis: localized cutaneous (CL), mucosal (ML) and disseminated (DL) leishmaniasis. All these ATL outcomes can be found in Corte de Pedra were a complex population of L. braziliensis is endemic. The present study aims to characterize in finer detail the population structure of these parasites, how the endemicity is maintained over time and identify genotypes more prevalent in subpopulations of L. braziliensis in Corte de Pedra and their association to disease form. For this propose, we first cloned and sequencied six amplicons generated by RAPD performed in nine L. braziliensis isolates. None of the six loci presented evidence of more than two alleles for each of the nine isolates initially tested. After screening for discriminatory loci, we designed specific primers and applied them to clone and sequence three loci out of 35 L. braziliensis isolated from patients of CL (17), ML (9) and DL (9). We are using the data generated with these loci to validate a sequence based typing method against the RAPD data previously used. These three loci (ABI 04-B1, ABI 04-B2 and A8B2) reinforced the previous data that the majority of human infections may be clonal or oligo-clonal in Corte de Pedra. When the three loci, from 35 isolates tested, were concatenated, 13 possible genotypes were found and only two were shared by 9 DL patients, suggesting some genotypes may be more frequently recovered from specific forms of ATL. We are now expanding these analyses for a sample of 289 specimens obtained from prospectively enrolled patients: 186 CL, 31 ML and 72 DL. Although the genotipic data is still lacking, the ongoing analysis of geographic distributions with 209 such patients suggests that disease forms present different frequencies among the four sectors of Corte de Pedra.
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BM012 - **ADAPTIVE PATTERNS IN EXTRA AND INTRACELLULAR LIFESTYLE OF PROTOZOAN PARASITES REGARDING THE REPETITIVE CONTENT OF THEIR PROTEOMES**

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Adaptive sequence patterns have been fingerprinted into the parasite's genomes over time, leaving clues that might help understanding selective forces and adaptive mechanisms involved in host-parasite interactions. In parasites, repetitive proteins play several important roles, such as host cell adhesion/invasion and immune evasion. Here, we identified repetitive amino acid sequences from proteins of distinct subcellular compartments in the proteome of 12 protozoan parasites and compare such findings with their lifestyle. We found that intracellular parasites have higher repetitive content than those exclusively extracellular. Surface proteins and potentially N- and O-glycosylated amino acids are enriched with repeats. Ortholog proteins from intracellular parasites *T. cruzi*, *L. major*, *L. infantum* and *L. braziliensis* have higher expansion of repetitive amino acids than those from the extracellular T. brucei. Also, intracellular parasites have the highest proportion of repeats in the developmental stages with the ability to invade host cells. We have also analyzed the repetitive content of parasite proteins known to be involved in host cell adhesion and related to immune evasion mechanisms. In this case, proteins from extracellular parasites have higher ratio of degenerate/perfect repeats than those from intracellular parasites. We speculate that this may be related to a pressure for diversification of surface antigens involved in immune evasion mechanisms in extracellular parasites. The lower ratio in intracellular parasites may due to a balance between the pressure for expansion of degenerate repeats, since these parasites must also evade the host immune response, and pressure to maintain and expand perfect repeats. As adhesion depends on the interaction between specific motifs of parasite's proteins and host cell receptors, conservation and/or expansion of perfect repeats involved in adhesion may represent an evolutionary advantage favoring host cell invasion.
Supported by:WHO, FAPEMIG, CNPq, CAPES

BM013 - **SEX OR NOT: THAT'S THE QUESTION FOR TRYPANOSOMA CRUZI**

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Trypanosoma cruzi strains were recently classified into 6 DTUs (TcI-TcVI), from which TcII is related to severe cases of Chagas disease in the Southern Cone countries. Despite the considerable progress in the biology of the parasite, population structure and reproductive mode of *T.cruzi* is under debate. Two major hypotheses of clonality versus sexuality coexist, but due to scarcity of evidences of sex among *T.cruzi* parasites it has been accepted that parasite reproduction is mainly clonal with infrequent genetic recombination. This assumption has being based on Hardy-Weinberg (HW) imbalances observed among different genetic markers in *T.cruzi* populations. However, since the analyzed populations were isolated from different geographic areas the possibility of Wahlund effect as generating these HW imbalances cannot be ruled out. In order to investigate this possibility, we compared allele's frequency of 12 different polymorphic loci (9 nuclear and three mitochondrial) using 2 groups of TcII strains: one including 86 isolates from Latin America patients and another of 74 isolates from patients from Minas Gerais State (MG) only. We assumed as null hypothesis that population structure of *T.cruzi* is essentially clonal, and therefore HW disequilibrium and sharp association between the clusters obtained by analyzing both genomes in both groups of strains should be observed regardless of geographic origin. Analyses of 4 microsatellite loci showed that the number of loci in linkage disequilibrium decreased from 4 to 1 when only MG strains were analyzed. Moreover, we did not observe in this last group any correlation between the clusters by analyzing nuclear and mitochondrial loci suggesting independent inheritance of these genome markers. Our results do not support a mainly clonal population structure for *T.cruzi* and suggest that, at least for TcII populations coexisting in a same area, the genetic exchanges among the strains are much more frequently than currently expected.
Supported by:FAPEMIG, CNPq, CAPES

BM014 - **LEISHMANIA CHAGASI ANTIGENS ASSOCIATED WITH RESISTANCE TO VISCERAL LEISHMANIASIS**

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Background: Resistance and recovery from Visceral Leishmaniasis (VL) have been associated with cellular immune response, characterized by a positive Delayed Type Hypersensitivity (DTH) test but a poor humoral response against specific parasitic antigens. In this study, we characterized the IgG recognition during resistance conversion in post-treatment patients and asymptomatic infected people.
Methodology: We used sera from VL patients (DTH-), VL recovery patients (DTH+) and asymptomatic infected people (DTH+) to recognize antigens from Leishmania chagasi by conventional and 2D western blot assay. Next, proteomics methods (MALDI ToF/ToF-MS) were used to identify antigens recognized by each group. Genes coding for the identified antigens were cloned, expressed and tested to serology by ELISA using sera from same groups.
Results: We observed a specific recognizing pattern of Leishmania antigens associated to DTH status and indentified several antigens with a specific recognizing. In addition, a mixture the identified recombinant proteins against asymptomatic infected people (DTH+) sera showed not only a higher recognizing than total soluble Leishmania antigens (SLA), but also less cross-reactivity against Chagas disease patients sera.
Conclusion: Our results are the first evidence of a recognizing pattern associated with VL resistance. These data bring new perspectives in the development of a differential serological test for asymptomatic infected individuals using a specific set of proteins that also could be new vaccine candidates.
Supported by:fapesb

BM015 - **GLOBAL TRANSCRIPTOME ANALYSIS OF HOST-PARASITE INTERACTION BY RNA-SEQ IN A MURINE CARDIOMYOCYTE-TRYPANOSOMA CRUZI MODEL**

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Chagas' disease affects millions of people in South and Central America and is caused by *Trypanosoma cruzi*. Chronic chagasic cardiomyopathy occurs in approximately one-third of infected individuals, having a high morbidity and mortality impact. One study model is the response of murine embrionic cardiomyocyte to *T. cruzi* infection, which has been recently investigated in greater detail using high-throughput techniques. To deepen our molecular understanding of this host-parasite interaction model, we have conducted a broader analysis using next generation sequencing (RNA-Seq). Briefly, we have extracted mRNA from control and infected cardiomycites in 1, 2, 3, 4, 5, 6 and 24h, in triplicate experiments, and submitted to SOLiD sequencing. We have obtained 662,468,476 reads (~15.8 million per sample), consisting in the largest evaluation of transcriptome modulation in *T. cruzi*-host interaction studies. Besides the intrisic qualities of RNA-Seq (higher reproducibility, sensitivity, dynamic range and accuracy), it allows us for the first time to quantitate the parasite response in the same sample, which represents a major breakthrough. We were able to identify more than 500 murine genes modulated, which are associated with the immune response, inflammation, cytoskeleton organization, cell-cell and cell-matrix interactions, apoptosis, cell cycle, and oxidative stress, as previously described. However, there are new candidates identified in this study, and most important, the higher accuracy of the modulation patterns allowed us for the first time to temporarily characterize the order of events. Regarding *T. cruzi* response to the interaction and invasion process, we have identified genes usually known to be involved in the process, trypomastigote to amastigote differentiation markers, and new candidates. So, this dataset represents an extremely rich resource to study *T. cruzi*-cardiomyocyte interaction.
Supported by:CNPQ, FIOCRUZ

BM016 - **THE EXPRESSION OF ECTO-NTPDASE I IN DISTINCT STRAINS OF TRYPANOSOMA CRUZI**

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Chagas Disease affects millions of people in Central and South America and still represents a serious threat to public health, causing major economic and social impacts. The different *Trypanosoma cruzi* strains involved in the infection have been suggested to cause different clinical manifestations, diagnostic responses and therapeutic efficiency. The ecto-NTPDase I is an apyrase whose active site is located on the external face of the *T. cruzi* plasma membrane, and previous studies have proposed a contribution of 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 (CL. Brener - *T. cruzi* VI; Dm28c – *T. cruzi* I; Y - *T. cruzi* II; 3663 - *T. cruzi* III; 4167 – *T. cruzi* IV; CL-14 – *T. cruzi* VI) and distinct evolutive forms (Epimastigote, Amastigote and Tripomastigote). 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 111 bp sequence in the ecto-NTPDase I *T. cruzi* gene (AY540630) and, as endogenous control, a 268 bp sequence in the calmodulin *T. cruzi* gene (X 52096.1). We observed that the strains Cl-Brener, Y, Dm28c, CL-14 and 4167 showed expression levels, respectively, 3.62±0.23, 3.47±0.77, 2.26±0,15, 2.21±0.28, 1.49±0.08 times higher than the 3663 strain. Regarding the evolutive forms, 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. So far, our results suggest that the distinct infectivity patterns, observed among the different strains and evolutive forms could be related to the expression of the ecto-NTPDase I gene in *T. cruzi*.
Supported by:FAPERJ

BM017 - **PRELIMINARY CHARACTERIZATION OF THE TRIATOMA BRASILIENSIS ANTERIOR MIDGUT TRANSCRIPTOME BY PARTIAL CDNA SEQUENCING**

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Triatoma brasiliensis is one of the arthropod vectors responsible for *Trypanosoma cruzi* transmission in Brazil. Since *T. cruzi* host infection is modulated by the amount of blood ingested, understanding what influence the insect feeding performance is of utmost importance. Very little is known about regulation of gene expression in *T. brasiliensis* and what genes are induced in the insect gut after feeding. In this work we propose the characterization of the anterior midgut transcriptome of *T. brasiliensis* by partial sequencing of cDNAs and generation of Expressed Sequence Tags (ESTs). Messenger RNA was isolated from the midgut of fifth-instar nymphs and cDNA library was constructed using the Creator™ Smart™ cDNA Library Construction Kit (Clontech). cDNA molecules were cloned into the pDNR-LIB vector and clones were randomly selected and sequenced on the MegaBace™ 1000 DNA sequencer (GE Healthcare). ESTs were edited to remove sequences of vector, adapters and poli(A) tail using the softwares DNA Baser and SeqClean. Similarity searches were done using BLASTx and BLASTn programs. Sequences were then clustered to produce contigs using the CAP3 assembler. We obtained 146 unique sequences after clustering, 13 of them corresponding to contigs composed of two to ten sequences and 133 singlets. Five singlets represented contaminant transcripts from rat and chicken that were used for insect feeding. A total of 58 uniques did not match to any sequences in the NCBI databases. Annotated transcripts matched genes coding proteins belonging to energy production and conversion, but the most abundant transcripts were similar to defensin, a conserved secreted protein from *Oncopeltus fasciatus* and a polyprotein from slow bee paralysis virus. The characterization of the *T. brasiliensis* midgut transcriptome will help us to understand the feeding processes of arthropod vectors and to provide useful information for the discovery of novel pharmacologically active compounds.
Supported by:FAPEMIG, CNPq

BM018 - **CATHEPSIN B-LIKE OF EIMERIA SPP.**

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Coccidiosis is one of the most economically important diseases of domestic poultry. The disease is caused by intracellular protozoan parasites from genus *Eimeria* belonging to the subclass *Coccidia* (with other parasites such as *Toxoplasma*). Coccidiosis seriously impairs the growth of infected chicken resulting in high economic losses of more than 3.5 billion dollars per year and there is a rising problem of parasites becoming resistant to the usual coccidiostats. Many efforts have been done to understand the biology of these parasites, including genome and transcriptome studies. In parasitic protozoa, proteases such as cathepsin B, papain like cysteine protease, play key roles in host-parasite interactions and pathogenicity, and may constitute promising targets for new anti-parasite drugs. The aim of this work is to identify the catalytic domain of this cathepsin in *Eimeria* of domestic poultry. Sequences encoding a cathepsin B-like protein were identified in the genome (Sanger Intitute) and from ORESTEs contigs (http://www.coccidia.icb.usp.br/) of *E. tenella*. The catalytic domains of cathepsins B were amplified, the products were purified, cloned and sequenced. All nucleotide sequences were submitted to an automated sequence processing pipeline (EGene system - Durham et al. – Bioinformatics 21: 2812-3, 2005). Sequences encoding catalytic domain of cathepsins B were successfully obtained in four species (*E. tenella* H, *E. maxima* H, *E. necatrix* C and *E. praecox* H), showing all catalytic residues and the characteristic His-His of the occluding loop. The sequences were submitted to phylogenetic analysis and trees were inferred with nucleotide and amino acid sequences by parsimony, neighbor joining and maximum likelihood methods using PAUP* 4.0b10 software package. The tree generated by maximum likelihood method showed better biological relation between the taxa than others methods. This is the first data describing cathepsin B-like in four protozoan of genus *Eimeria*.
Supported by:CNPq

BM019 - **EVIDENCE FOR TRYPANOSOMA CRUZI IN ADIPOSE TISSUE IN HUMAN CHRONIC CHAGAS DISEASE**

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The adipocyte, once considered as a static storage compartment for triglycerides is now appreciated to be an active endocrine cell playing critical roles in different metabolic and immune responses and in the development of chronic diseases such as diabetes, obesity, steatohepatitis, cancer and others. However, the involvement of adipose tissue in infectious diseases has been poorly investigated. Our group has pointed the adipose tissue as an important target for Trypanosoma cruzi infection which resulted in an inflammatory phenotype of cultured adipocytes. The question arose whether the parasite directly invades human adipose tissue and mouse primary adipocyte cells. To verify this ability of T. cruzi, we isolated primary adipocytes from epididymal adipose tissue of BALB/c mice, exposed them to trypomastigotes of T. cruzi Y strain and evaluated the presence of amastigotes in these cells by immunofluorescence. Our results demonstrated amastigotes forms close to the nucleus of adipocytes indicating that the parasite is able to infect this cell type. Despite significant evidence of adipose tissue involvement in the systemic interaction with infectious agents, no work, so far, examined if patients with chronic chagasic patients presented adipose tissue infected with T. cruzi. In order to address this question, biopsies of subcutaneous fat tissues were recovered from 10 patients presenting chronic Chagas heart disease and 10 control patients who underwent pacemaker exchange at the Clinical Hospital of the UFMG, Brazil. T. cruzi kDNA were detected in 3 of the 10 Chagas patient's pericardial fat tissues (30%) and two of these samples were genotyped. In one patient, it was detected the presence of TcII. In the other, we detected the occurrence of a mixed infection consisting of TcII and TcVI. Taking these findings together, we postulate that in chronic Chagas disease the adipose tissue may be an important parasite reservoir from which recrudescent of infection can occur.
Supported by:Albert Einstein College of Medicine, CAPES, CNPq, Diabetes Research Center, FAPEMIG and NIH

BM020 - **RNA-BINDING PROTEINS INTERACTOME IN TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is a protozoan parasite, which causes Chagas' disease, and is a interesting model of study for post-transcriptional control of gene expression. RNA-binding proteins (RBPs) are extremely important in regulating processing, storage, translation and degradation of mRNA, and through their interaction with specific mRNAs elements, they can define mRNA fate. We are conducting a large project to build a complete map of protein-protein interaction (PPI), which will be extremely valuable for functional gene annotation and data integration. Using the ORFeome developed in our laboratory, we have selected ~700 proteins, consisting of all putative RNA-binding or those that are part of potential interacting functional complexes, as ribosome, splicing and decay machinery. These genes were transferred to appropriate yeast two-hybrid vectors, and interactions were tested using a Tecan 200 robot in HIS- and URA-selective media. Interactions and self activators rates were similar to the literature. Currently, the screening results are being scrutinized using the Cytoscape software and the interaction modules are being used as scaffolds for plotting transcriptomics, proteomics and ribonomics datasets that are available in our Institute. A small fraction of these interactions were present in other interactome datasets. Although it raises concerns about false positives, we hypothesize that, due to large evolutionary distance from other model organisms whose interactome is well studied, and to the specificity of its post-transcriptional gene expression regulation, comparison of interactome datasets based on orthologs is of low power to detect true interactions. We are also developing a system for studying PPI in vivo in *T. cruzi*, based in fluorescent and luminescent complementation assays. These vectors will be helpful to increment and validate Y2H results.
Supported by:CNPq; FIOCRUZ

BM021 - **STUDIES ON THE TRYPANOSOMA CRUZI AND TRYPANOSOMA BRUCEI PSEUDOURIDINE SYNTHASE 7-LIKE**

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The transfer RNAs (tRNA) play a central role in protein synthesis, being the translators of the genetic code. As many other molecules, they need to undergo several modifications to become mature and functional. The yeast Pseudouridine Synthase 7 (Pus7) is a multisite and multisubstrate enzyme that is able to modify uridines in several tRNAs and others RNAs. In yeast pre-tRNA Tyrosine (pre-tRNA^{Tyr}), Pus7 acts at U35 catalyzing the formation of pseudouridine (ψ), a highly conserved phylogenetically modification. Most of the studies involving Pus7 are performed in yeast. We identified in the tritryps genomes, homologues of this gene which haven't been studied yet. Here we show the successful amplification of the *Trypanosoma cruzi pus7* gene (1977 base pairs). The fragment was subcloned into pTZ57R/T (Fermentas®), the sequence confirmed and cloned into pET15b for overexpression of the recombinant enzyme in *Escherichia coli* and its characterization *in vitro*. Furthermore, we have cloned a 500 base pairs internal region of the *pus7* gene from *Trypanosoma brucei* and constructed a plasmid for RNAi induction. The transfected parasites were selected with phleomycin and the RNAi induced with tetracycline, the knockdown was confirmed by qPCR. We show the *pus7* gene silencing does not affect the growth rate of the *T. brucei* procyclic form. We are now evaluating other effects of the pus7 gene silencing.
Supported by:FAPERJ, OMS

BM022 - **YEAST MUTANTS COMPLEMENTED WITH TRYPANOSOMA CRUZI GENES ENCODING ENZYMES OF THE GPI BIOSYNTHETIC PATHWAY AS NEW TOOLS FOR DRUG DEVELOPMENT**

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Glycosylphosphatidylinositol (GPI) is an important anchoring molecule for cell surface proteins involved in many aspects of host-parasite interactions, such as adhesion and invasion of host cells as well as in host immune response. Protozoan-derived GPI anchors exert various immunostimulatory and regulatory activities, including their ability to elicit the synthesis of pro-inflammatory cytokines by host macrophages. Therefore, the biosynthesis of *T. cruzi* GPI anchors offers potential new targets for drug development. Here we show the characterization of *T. cruzi* genes encoding homologs of 10 proteins involved in GPI biosynthesis: DPM1, GPI1, GPI2, GPI3, GPI8, GPI10, GPI12, GPI14, GPI18, and GAA-1. Sequences corresponding to GPI3 and GPI12 genes of *T. cruzi*, cloned in fusion with GFP and transfected into epimastigotes, showed a cellular localization compatible with endoplasmic reticulum. Similar localization was observed after transfecting HT1080 human fibrosarcoma cells with *T. cruzi* DPM1, GPI3, GPI12, and GPI8 genes in fusion with GFP. To verify whether these genes encode homologous enzymes in *S. cerevisiae*, we transformed yeast mutants defective in each GPI gene. We showed that DPM1, GPI10, and GPI12 from *T. cruzi* restore the growth of yeast mutants in non-permissive conditions. The presence of complete GPI anchors synthesized by yeast mutants complemented with *T. cruzi* genes was confirmed by thin layer chromatography (TLC) analyses of yeast cell extracts that were cultivated in medium containing [2-^{3}H]*myo*-inositol. These transformed yeast mutants are now being used for drug screening assays. 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. PCR analyses of hygromycin resistant parasites, confirmed the integration of the HYG gene and disruption of one allele of the GPI8 gene. Further characterization of this single allele mutant, as well as deletion of the second allele, are also underway.
Supported by:CNPq, HHMI

BM023 - **CHARACTERIZATION OF A MIF4G HOMOLOG PROTEIN IN TRYPANOSOMATIDS**

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NOM1 (nucleolar MIF4G-containing protein 1) is a nucleolar protein in humans, codified by a gene located at the chromosome 7q36 breakpoint involved in 7;12 translocations and found in patients with the aggressive form of acute myeloid leukemia. This protein contains the MIF4G and MA3 conserved domains, found in several proteins that impact translation (like eIF4G), cell growth, transformation and apoptosis. In humans and in yeast NOM1 was found to interact with proteins of the eIF4A family. Several homologues were found throughout different organisms and, through genome analysis, sequences encoding NOM1 homologues were also identified in Trypanosomatids. We recently started its characterization in *T. brucei* and *L. major* and for that its genes were amplified by PCR followed by cloning into pET21a and pGEX4T3 vectors for expression in *E. coli* cells. The his-tagged *T. brucei* recombinant protein was then used for production of polyclonal antiserum in rabbit. The resulting immunopurified antibody was used to analyze the protein expression through Western blots. For subcellular localization we used immunofluorescence microscopy in cells of procyclic and bloodstream stages. The results indicated that NOM1 is expressed in a constitutive way in both stages and surprisingly it is located in the cytoplasm, with a higher expression around the kinetoplast. We also performed pull-down assays *in vitro* to verify the ability of *Tb*NOM1 to interact with the eIF4A homologues of *T. brucei* (*Tb*EIF4AI and *Tb*EIF4AIII). It bound weakly to *Tb*EIF4AI and *Tb*EIF4AIII but strinkingly it appears to interact to other *Tb*NOM1 molecules in a stronger manner. Searching for new evidences regarding the role of this protein, we are performing transfections in procyclic cells for generating EYFP (enhanced yellow fluorescent protein) fusions and RNAi and performing immunoprecipitations assays to identify in which processes the *Tb*NOM1 is functionally involved.

Supported by:FACEPE/CNPQ

BM024 - **EVALUATION OF CHEMICAL STRUCTURES OF FERROCENE DIAMINES ON THE OXIDATIVE METABOLISM IN TRYPANOSOMA CRUZI STRAINS**

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Studies have searched potential targets for trypanocidal substances, some reporting an increase on the production of enzymes involved in the oxidative stress which probably could be responsible for the benznidazole (only drug available in Brazil for Chagas disease) resistance of certain strains. Such enzymes have important roles in the survival and growth of the parasites as well as: peroxiredoxin (PRX), which catalyzes the reduction of peroxides; superoxide dismutase (SOD), a metaloenzyme which eliminates superoxide radicals converting them into hydrogen peroxide and molecular oxygen and old yellow enzyme (OYE), an NADPH-flavin oxidoreductase which may be involved in the reduction of some trypanocidal substances. This work aims to evaluate the susceptibility of some different *T. cruzi* strains to diamine derivatives of ferrocene and analyze the difference in their expression level which would be related with the resistance of parasites. By now the results showed that some structures derived from diamines of ferrocene demonstrated trypanocidal activity higher than the benznidazole. The susceptibility of epimastigote forms to diamines of ferrocene was performed using colorimetric MTT and IC50 values of each strain were, respectively: Y = 12,84 µM, Bolívia = 11,40 µM, SI1 = 9,20 µM, SIGR3 = 21,40 µM, SI8 = 19,00 µM and QMII = 15,60 µM. The ORFs (Open Reading Frame) of the three enzymes were cloned and all recombinant proteins were purified. These were used in the production of polyclonal antibody in rabbit. Western blot will help to characterize the difference in the expression level of proteins of *T. cruzi* strains after treatment to diamine derivatives of ferrocene. Next step will be tested the cytotoxicity of these diamines of ferrocene in HepG2 as a model for human liver cell.

Supported by:CNPq

BM025 - **ANALYSIS OF GENIC TRANSCRIPTS FROM TRYPANOSOMA CRUZI CL-BRENER AND BLASTOCRITHIDIA CULICIS UNDER STRESS**

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The members of the family Trypanosomatidae are flagellated protozoa parasites which can infect vertebrates and invertebrates. According to the number of hosts they need to acomplish their life cycle, they are classified either as monoxenics or heteroxenics parasites. The goal in this work is to perform a comparative analysis of genic transcripts that have been amplified through RT-PCR in both *Trypanosoma cruzi* clone CL-Brener (heteroxenic species), and *Blastocrihthida culicis* (monoxenic species). Both species were grown under to physical, chemical and nutrition stress. We ask whether two species of trypanosomatids with distinct life styles respond to the environmental pressure (tension in culture medium) expressing the same genes. To this end, we carried out the growth of both species in different stress conditions, such as changes in temperature and in pH, nutrient content of the culture medium, addition of chemicals at maximal concentration, among others. Transcripts were generated after RT-PCR with selected arbitrary primer (RNA fingerprinting) and compared both to the standard growth condition (control) and the altered ones between the two species. Different responses were observed in the trypanosomatid physical demands, such as the extreme of temperatures. Through this methodological approach, which tends to select the most abundant genes under stress conditions, we obtained a sample of the transcription activity of protozoan. After fragment sequencing 722 ESTs were obtained under tension for *T. cruzi* and *B. culicis*. In addition ESTs in standard condition (control) for *B. culicis* were also generated. Analysis of these sequences reveal a high frequency of gene related to surface proteins (mucins, trans-sialidades, gp63, etc.) under tension in both *T. cruzi* and *B. culicis*. Transcripts of regulatory proteins were observed in small number. In both species the additional trans-splicing through the use of internal acceptor sites (AG) before the ORF, or the alternative trans-splicing using sites internal to the predicted ORF have been detected. In conclusion, trypanosomatid species with different life styles share only a few genes when they are exposed to stress and many transcripts appears to be exclusive for each species.
Supported by:CNPq,FIOCRUZ

BM026 - **SEARCHING FOR NEW ANTIGENS FOR CANINE VISCERAL LEISHMANIASIS SERODIAGNOSTIC: STUDY OF RLC36 GENE**

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Visceral leishmaniasis (VL) is the most severe clinical form of the disease and can be fatal if untreated. A specific and accurate diagnosis is required for the identification of infected dogs, which are important Leishmania sp reservoirs, to provide better epidemiological control in order to do the interruption of disease transmission to humans. Nowadays, parasite demonstration and serological tests present serious limitations due to low parasitemia and false positive (use of entire parasite as antigen causing cross reactions), respectively. In such a way there is an urgent need of development of more trustworthy diagnosis tests based on recombinant proteins. This work reports the isolation and characterization of one specific *Leishmania chagasi* gene, named rLc36, and its product as a hypothetical antigen for a new diagnosis method.
On-line tools were used to obtain the L. chagasi sequences (http://www.genedb.org). Partial rLc36 gene was amplified with specific primers, cloned in pET28a, and expressed in different *Escherichia coli* strains (BL21 DE3, BL21 DE3 pLysS, BL21 DE3 CodonPlus, BL21 Rosetta KM71H). The rLc36 recombinant protein was purified by nickel column. Different concentrations of the protein were tested by ELISA using serum from dogs with LV and serum from health dogs.
The protein concentrations of 0.1 and 0.01µg/mL were detected in the ELISA tests. The recombinant protein rLc36 strongly reacted with serum of infected dogs in preliminary results.
In such a way, the recombinant protein has shown potential to be used in new diagnostic tests.
Supported by:CNPq

BM027 - **MRNA DECAY PATTERNS IN TRYPANOSOMA CRUZI EPIMASTIGOTES**

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The protozoan *Trypanosoma cruzi* controls its gene expression mainly by post-transcriptional mechanisms. In a previous work, microarray experiments were made with 10,816 probes to evaluate the mRNA decay in *T. cruzi* Dm28c epimastigotes treated from 15 to 240 minutes with actinomycin D. Each experimental unity was made in replicate comprising 40 samples. Between 2 and 3 thousand differentially expressed genes were identified comparing the extreme time points (0 and 240 minutes). This data are a rich repository for mRNA decay analysis, however, the need to hybridize nucleic acid targets and probes to yield a relative measure and the dependency of signal/background noise ratio produce less conclusive results. In this way, the present project covers earlier (0, 5, 10, 15, 20 and 25 minutes) mRNA decay time points and utilizes the RNA-Seq WT sequencing technique. Among the advantages of RNA-Seq, the measure is quantitative (absolute), with better resolution and probe-independent, which confers wider genomic coverage, no cross-hybridization and smaller background noise. We used epimastigote cultures treated with the inhibitors sinefungin and actinomycin D to block trans-splicing and transcription, respectively. After mRNA purification of the 12 samples (with biological replicates), sequencing and genome mapping, read counts per gene were normalized using TMM method of the *edgeR* software package in the *R* statistical platform. By Digital Gene Expression analysis our experiments show that during parasite's life cycle in epimastigote forms can be partially explained by mRNA decay rates. Moreover, it is possible to correlate transcript decay rates with some gene functions resembling cytoskeleton and organelle organization. Mainly genes related with macromolecular complexes such as ribosomes and ribonucleoproteins are enriched in Gene Ontology annotation terms. Our results emphasize the complexity of how *T. cruzi* regulates its gene expression by post-transcriptional mechanisms.
Supported by:CNPq, Fiocruz and Fundação Araucária

BM028 - **IDENTIFICATION OF MODULATORS OF LEISHMANIA (LEISHMANIA) AMAZONENSIS METACASPASE USING PHAGE DISPLAY**

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Leishmaniasis is considered an important public health problem. Among the many species described as causing human disease in Brazil, Leishmania L. amazonensis is an important etiologic agent of human cutaneous Leishmaniasis, and is responsible for a wide range of clinical forms. During its life cycle, Leishmania amastigotes live inside phagolysosomes of phagocytic cells of vertebrate hosts, while promastigotes live inside the invertebrate vector. The two forms explored apoptotic mimicry or apoptosis as ways to reduce macrophage inflammatory response and allow parasite proliferation in the vertebrate host. Intracellular proteases such as caspases are key effectors in the apoptotic process. Metacaspases (MCAs) are distant evolutionary forms of metazoan caspases and are involved in apoptosis and life-cycle of Leishmania. They may be potential targets to damage the parasites without affecting the host, but little is known about their modulators. Phage Display is based on the expression of synthetic proteins/peptides in the phage capsid, and is used for selecting ligands for proteins, cells or tissues. We employed Phage Display on L. L. amazonensis recombinant MCA to find peptide ligands that may provide information about MCA regulation and possibly control of apoptosis. We obtained the recombinant MCA by expression on pET28a bacterial vector, validating by Western blot, ELISA and measurement of tripsin-like activity. Phage Display using three different peptide libraries led to some enrichment of bound phages over four rounds of selection over MCA. Some of the selected phages will be sequenced and analyzed in search of MCA modulators. Treatment of Leishmania L. amazonensis promastigotes with reactive oxygen species (H_2O_2) or heat shock induced tripsin-like (possibly MCA) activity may be used, along with cell viability, to evaluate the effect of the selected MCA binding peptides.
Supported by:CAPES

BM029 - **TRANS-SPLICING IN VITRO USING CELL-FREE NUCLEAR EXTRACT AND PARTIAL SEQUENCE OF ALPHA-TUBULIN PRE-MRNA FROM TRYPANOSOMA CRUZI**

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Trypanosomatidae family comprises a large number of protozoan parasites, including important etiological agents of neglected human diseases. Among the diseases causative agents, *Trypanosoma brucei* (responsible for sleeping sickness) and *Trypanosoma cruzi* (ethiological agent of Chagas' disease) are the most important parasites. Both have trans-splicing as a mechanism for the processing of its mRNA, which involves the excision of introns and union of exons to form two independent transcripts, the splice-leader (SLRNA), and the acceptor pre-mRNA. The reaction of cis-and trans-splicing *in vitro* with nuclear extract of HeLa cells has already been standardized and used as a model in several experiments. However, in trypanosomes, the only references on the reaction of trans-splicing *in vitro* are from Vianna et. al. (2001) and Skaked et. al. (2010), using parasite-free extracts prepared on different ways. For trypanocidal drugs analysis, trans-splicing reaction using permeable cells of *T. cruzi* were used as a model (Barbosa et. al. 2007), but this is not the same as free cell-nuclear extract as mentioned above. Thus, this work shows *in vitro* trans-splicing reaction using nuclear extracts either from *T. cruzi* epimastigote forms and/or T. brucei procyclic forms reacting with the *T. cruzi* alpha-tubulin cloned sequence as pre-mRNA acceptor. The standardization of this *in vitro* reaction will be able to promote advances to understanding the trans-spliceosome machinery, as well as occurred in mammalian cis- and/or trans-splicing, becoming also an interesting model for evaluating interference of trypanocidal drugs.
Supported by:CAPES

BM030 - **IDENTIFICATION OF MOTIFS INVOLVED IN THE INTERACTION BETWEEN HOMOLOGUES OF THE TRANSLATION INITIATION FACTORS EIF4G AND EIF4E IN TRYPANOSOMATIDS**

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Trypanosomatids rely almost entirely post-transcriptional mechanisms for control of gene expression, mediated through mRNA maturation and degradation along with control of the frequency of translation, presumably at its initiation. In the majority of the eukaryotes, translation initiation starts with the binding of the complex eIF4F (eIF4A, eIF4E and eIF4G) to the cap at the 5’ end of the mRNA, facilitating its recognition by the 40S subunit. Several homologues to the eIF4F subunits have been previously identified in trypanosomatids, including five eIF4Gs (EIF4G1-5), four eIF4Es (EIF4E1-4) and one eIF4A (EIF4AI). Their functions have been investigated in *L. major* and *T. brucei* and it has been shown *in vitro* that both EIF4G3 and 4 interact with EIF4AI, however mutations in a motif within these proteins containing the residues LNK abolish this interaction. EIF4G3 and 4 also bind to EIF4E3 and 4, and different residues were associated to these interactions. Substitution of residues FSL in EIF4G3 abrogates its interaction with EIF4E4 and mutagenesis of residues IL in EIF4G4 abolishes its interaction to EIF4E3. Here we evaluated *in vivo* the role of the same motifs in the eIF4G/eIF4E and eIF4G/eIF4A interactions in *T. brucei*. Procyclic cells expressing TY-tagged proteins with substitutions in the corresponding amino acids were analyzed through growth curves immunoprecipitation assays (IPs). Minor growth impairment was noticed in cells expressing mutated proteins for the motifs FSL and LL in EIF4G3 and 4, respectively. Strikingly, the mutation in the LNK residues in EIF4G3, but not in EIF4G4, was responsible for a significant decrease in cell growth. The IPs confirmed the involvement of the motifs in the cited interactions. Our findings corroborate the existence of at least two different eIF4F-like complexes, with distinct binding sites responsible for the eIF4G/eIF4E interactions, with the one containing EIF4G3 appearing to have a more effective role for cell growth.
Supported by:FACEPE, CAPES and CNPq

BM031 - **STRUCTURAL AND FUNCTIONAL ANALYSIS OF SUBTELOMERIC GENE FAMILIES IN TRYPANOSOMA CRUZI**

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Subtelomeric regions show characteristics unique to eukaryotic genomes. Genes located in the subtelomeric regions are subject to elevated rates of mitotic recombination and epigenetic regulation. The subtelomeric regions of *Trypanosoma cruzi* harbour many genes encoding surface proteins [trans-sialidase-like proteins (TS); Dispersed Gene Family (DGF-1)], retrotransposon hot spot genes (RHS) and retroelements that are involved in host-parasite interactions and immune evasion. We studied the structural and functional organization of subtelomeric gene families in clone CL Brener. Approximately 9.5% of TS, 12% of DGF-1 and 19% of RHS genes annotated in the genome were located at the chromosomal ends. Hybridization of probes to pulsed field gels of chromosomal DNA confirmed that TS, DGF-1 and RHS genes are distributed in subtelomeric regions of multiple *normal">Trypanosoma cruzi* chromosomes. Of the 127 TS subtelomeric sequences analyzed, 35 are complete genes, while 93 copies of sequences are truncated by stop codons. All groups of TS superfamily are represented in the subtelomeric regions, most of them (83) are variants of group II (GP82, GP85, TC85). We found 70 subtelomeric copies of DGF-1, 37 intact and 33 truncated. The complete copies show the transmembranic and signal peptide domains, suggesting a structural role for this protein. DGF-1 subtelomeric copies are always accompanied by RHS genes. There are 141 subtelomeric RHS sequences, most of which are pseudogenes (105). The RHS complete copies showed the characteristic features of the family, such as the ATP/GTP binding motif and site of insertion of retrotransposons. Taken together, our computational and experimental analyses show that the variability of *Trypanosoma cruzi* subtelomeres supports the generation of new antigenic variants by promoting gene recombination. The presence of complete genes copies suggest that subtelomeres may be sites for gene expression.
Supported by:FAPESP, CNPq, CAPES

BM032 - **POPULATION DYNAMICS OF LEISHMANIA CHAGASI KINETOPLAST MINICIRCLES**

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The mitochondrial DNA of the kinetoplastid protozoa is known as kinetoplast DNA (kDNA). This DNA is organized in a network containing maxicircles responsible for respiratory functions and minicircles involved in the production of guide RNAs, which play a role in the RNA editing of maxicircles. The minicircles are divided into an approximately 120 pb conserved region and an approximately 600 pb variable region. The focus of this study is on the sequence analysis of the minicircles variable region in order to understand the minicircle distribution on different life stages of L. chagasi. The samples were collected from dogs and symptomatic patients. DNA extraction was carried out with the commercial kit Núcleo Spin Blood Kit (Macherey - Nagel) following its protocol. kDNA was amplified by PCR, using a pair of oligonucleotides LIN R4 - forward (5'-GGT TGG TGT AAA ATA GGG-3) e LIN 19 - reverse (5'-GAA CGC CCC TAC CCG-3'), producing a fragment of 720 b.p. PCR products were cloned in pTZ57R/T vector according to the InsTAclone PCR cloning kit protocol. The resulting clones (approximately 140 sequences) were sequencing. The sequences were individually compared with the ones deposited in the GENBANK, aligned with Clustal X software and constructed a phylogenetic tree utilizing MEGA 4.0 software adopting UPGMA algorithm and choosing bootstrap with 1000 replicates. Sequences distribution between different hosts is homogeneous. In a first analysis high polymorphism was observed, conversely, when analyzed in more detail, i.e. by branch, sequences proved to be conserved and minimal SNP (Single Nucleotide Polymorphism) was found. Based on the data different sequences were categorized in 57 classes. There was an interesting discovery while comparing sequences with the ones available in the GenBanK. Despite limited coverage similarity of chromosome 27 of the *Leishmania donovani* was observed in all sequences, which leads us to question whether the gRNA could have a post transcriptional editing role not only in maxicircles but also in nuclear DNA. These are preliminary findings and more sequences will be analyzed prior to the conclusion of this research allowing for better understanding of the minicircles distribution validating or not this theory. Due to the complexity of this matter further researches should be developed.
Supported by:CAPES

BM033 - **CLONING, EXPRESSION AND PURIFICATION OF THREE TRUNCATED MUTANTS OF RPA-1 FROM LEISHMANIA AMAZONENSIS.**

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Leishmaniasis is an emerging human disease, caused by protozoa belonging to the Leishmania genera. The disease is endemic in many countries including Brazil. WHO estimates that ~350 million people are at risk and 12 million are infected. There are no efficient methods for controlling or eradicating leishmaniasis and therefore, control efforts and the establishment of intensive research to better understanding of the molecular biology of these protozoa may facilitate the discovery of new anti parasitic therapies. Actually, telomeres have been considered good targets for the development of new therapies. The telomeric complex is formed by repetitive DNA and protein-forming complexes. In most eukaryotes, proteins such as replication protein A, play many roles in DNA metabolism, including telomere maintenance. In Leishmania spp., unlike other eukaryotes, only RPA subunit 1 interacts in vitro and in vivo with the G-rich telomeric strand using an N-terminal OB fold domain. The present project has the aim to characterize three truncated mutants of LaRPA-1 that will be useful tools to capture by pull-down, LaRPA-1 protein-interacting partners. To reach our goal, we used PCR-based cloning strategies to obtain three LaRPA-1 mutants, here named LaRPA-1ΔObfold1, LaRPA-1ΔCterm and LaRPA-1ΔObfold-1-2. All mutants were expressed with an N-terminal 6xHis tag using pET 28a+ expression vector. Protein expression was induced by 1mM IPTG and bacterial extracts were fractionated onto 12% SDS-PAGE to verify protein´s solubility. Recombinant proteins were obtained in non soluble form and were affinity purified on Hi-trap Chelating columns in the presence of 8M urea. Subsequently, they were renatured by dialysis and analysed by SDS-PAGE and by Western blot probed with anti-LaRPA-1serum. Circular dichroism spectroscopy analyses showed that all three purified recombinant proteins were correctly folded. Pull-down experiments using these proteins as baits are under standardization.
Supported by:FAPESP, CNPQ

BM034 - **HETEROLOGOUS EXPRESSION OF A *TRYPANOSOMA CRUZI* TRANS-SIALIDASE (TCTS) BY *TRYPANOSOMA RANGELI***

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Trypanosoma rangeli is a hemoflagellate parasite capable of infecting a variety of mammals, including humans, in Central and South America. Geographical distribution, reservoirs and vectors are shared with *Trypanosoma cruzi*, etiological agent of Chagas disease. The *T. cruzi* trans-sialidases (TcTS) are crucial molecules on the interaction, penetration of the parasite in the host cell and its evasion from parasitophorous vacuole. *T. rangeli* has genes similar to TS family members but with no catalytic activity has been proved so far. The goal of this study was to perform the heterologous expression of TcTS in *T. rangeli* aiming the study of host-pathogen interaction. Expression of TcTS by *T. rangeli* was achieved by transfection with a modified version of the pTEX-eGFP plasmid containing the TcTS ORF. Expression was confirmed by fluorescence microscopy and Western blot using a polyclonal serum directed to the catalytic site and a monoclonal antibody directed to the terminal SAPA repeats of TcTS (mAb39). Furthermore, our results pointed out that TcTS expressed by *T. rangeli* is active and capable of transferring sialic acid to a 4-Methylumbelliferyl-β-D-galactopyranoside (MuGal) acceptor. Supported by CNPq, CAPES, FINEP and UFSC.

BM035 - **TEMPORAL ANALYSIS OF AEDES AEGYPTI POPULATION DYNAMIC**

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Dengue virus is a major public health problem worldwide. *Aedes (Stegomyia) aegypti* is the main dengue vector. Since there is no specific treatment or effective vaccine, control measure is focused on vector control. It is believed that population density is higher in the warmer/rainy season than in cold/dry. The study aimed to genetically characterize population dynamics of *Ae. aegypti* during climatic variations. Collections were performed at least once in both periods over five years by oviposition traps at Botucatu city. The technique of TaqMan allelic discrimination was used for genetic analysis, in which SNPs from nine genes distributed on three chromosomes of the mosquito were genotyped. Bayesian analysis did not show variance on population structure over the five year period. The percentage of variation among samples in statistical analysis was low (*Fst* = 0.0028, p = 0.7634), furthermore the allele frequencies were constant. The results show that despite wide variation in the density of adults, population size does not vary. Therefore, there is variation in the prevalence of the species life stages: adults in warmer/rainy, and possibly eggs in cold/dry, resulting in different control strategies for each period. Moreover, estimation of population size should not consider only winged adults, but all other found life stages forms.
Supported by:Fapesp

BM036 - **EVALUATION OF HETEROLOGOUS EXPRESSION OF THE *TRYPANOSOMA CRUZI* 82-KDA SURFACE GLYCOPROTEIN (GP82) BY *TRYPANOSOMA RANGELI***

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Trypanosoma rangeli is a hemoflagellate kinetoplastid parasite that shares geographic distribution with *Trypanosoma cruzi*. In addition, these two species also share reservoirs and vectors where single or mixed infections may occur, leading to false-positive diagnosis of the American trypanosomiasis. The life cycle of *T. rangeli* within mammalian hosts is still unknown, being controversial the ability of the parasite to invade mammalian cells. Despite these facts, host cell-parasite interaction undoubtedly occurs and involves different molecules, especially surface antigens that may be targets for the mammalian immune system. This study addressed one of the molecules involved on the parasite-host cell interaction through the heterologous expression of *T. cruzi* 82-kDa glycoprotein (Tcgp82) by *T. rangeli*. Expression of Tcgp82 by *T. rangeli* (Choachí strain) did not affect the growth pattern of the parasites *in vitro*, even when submitted to metacyclogenesis. The cellular localization of gp82 was similar for *T. rangeli* -gp82 and *T. cruzi* (Y strain), being distributed across the surface of non-permeabilized parasites and in the central region of permeabilized parasites as revealed by 3F6 MAb. The number of internalized parasites 16 hours after parasite-host cell (Vero cells) interaction was 46% higher for *T. rangeli* -gp82 compared with non-transfected *T. cruzi* and *T. rangeli*. This result indicates a possible phenotypic change on the host cell-parasite interaction due the expression of gp82 by *T. rangeli*, facilitating the entry of the parasite into the cell. However, at 72 hours of interaction, the number of *T. rangeli* -gp82 and *T. rangeli* wild-type (WT) within cells did not differ significantly. At this same time-point, the number of *T. cruzi* amastigotes was 91% higher when compared to *T. rangeli* WT or *T. rangeli* -gp82, demonstrating the clear involvement of the gp82 on the process of host cell recognition and penetration, but not on the intracellular development.
Supported by:CNPq, CAPES, FINEP and UFSC

BM037 - **A PROTEOMIC SURVEY OF *TRYPANOSOMA RANGELI* EPIMASTIGOTES**

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Trypanosoma rangeli is a hemoflagellate parasite that infects several species, such as triatomines and mammals, including man. Although *T. rangeli* is non pathogenic for humans, the sharing of several antigenic determinants with *Trypanosoma cruzi* leads to inconclusive or mis-diagnosis of human infections. The aim of this study is to characterize the repertoire of proteins expressed by *T. rangeli* replicative and non-infective epimastigote forms. Protein extracts were produced from epimastigotes of the Choachi strain cultured in liver infusion triptose (LIT) medium. Two distinct approaches were used for protein identification, 2DE-MALDI-MS/MS and LC-MS/MS. A total of 57 proteins were identified so far, being 12 identified by both techniques, 33 identified exclusively by 2DE-MALDI-MS/MS, and 12 identified only by LC-MS/MS. Identified proteins were then functionally classified according to the Cluster of Orthologous Groups for Eukaryotes (KOG). The most represented classes were those of post-translational modification, and amino acid transport and metabolism, with 6 and 7 proteins, respectively. Among the identified proteins were some related to virulence, including cystathionine, zinc finger protein 2 and sialidase. This study represents the first effort for large-scale proteomic analysis of *T. rangeli* epimastigotes and, along with the complementary study of the protein repertoire of infective trypomastigotes (see abstract of Lückemeyer et al.), will allow intraspecific and interspecific comparative proteomic analysis, providing original and valuable information on the biology of *T. rangeli* and other trypanosomatids.
Supported by:Supported by FINEP, CNPq, and CAPES.

BM038 - **CARACTERIZATION OF THE GENE PTERIDINE REDUCTASE 1 (PTR1) IN LEISHMANIA BRAZILIENSIS RESISTANT TO POTASSIUM ANTIMONY TARTRATE**

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Pteridine reductase is a NADPH-dependent short-chain reductase that participates in the salvage of pterins in trypanosomatid protozoans, converting biopterin to tetrahydrobiopterin. In Leishmania, PTR1-dependent synthesis of tetrahydrobiopterin may also be involved in the reduction of the oxidative stress generated by antimonial. Recently, we selected in vitro populations of Leishmania braziliensis, which are 20-fold more resistant to potassium antimony tartrate Sb III (LbSbR) than its susceptible counterpart (LbWTS) (Liarte & Murta, 2010). In further studies, we identified transcripts differentially expressed in LbWTS and LbSbR populations using DNA microarray methodology (Liarte et al, in preparation). Interestingly, the LbPTR1 gene encoding pteridine reductase 1 showed higher expression (3-fold) in the LbSbR population compared with its LbWTS counterpart. In order to confirm these findings, here the levels of LbPTR1 mRNA and protein were compared in LbWTS and LbSbR populations. The levels of LbPTR1 mRNA in both parasite populations were determined by quantitative real-time RT-PCR experiments. The amount of LbPTR1 cDNA in the Leishmania populations was determined by linear regression analysis using the PCR threshold cycle (CT) values obtained from the standard curve generated with known amounts of the LbPTR1 plasmid. The levels of transcription of the LbPTR1 gene were 7-fold higher in the resistant population LbSbR compared with the LbWTS population. In Western blot analysis, anti-LmPTR1 polyclonal antisera from L. major recognised a 30 kDa protein in both L. braziliensis populations. The level of expression of this polypeptide was approximately 6-fold higher in the LbSbR population than in the LbWTS population. Studies to determine whether overexpressing LbPTR1 in the susceptible population will confer antimony-resistant to these parasites are being performed to confirm our hypothesis that LbPTR1 may be involved in L. braziliensis resistance to antimony.
Supported by:CNPq, FAPEMIG, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR

BM039 - **MOLECULAR STUDY OF TRYPANOSOMA CRUZI STRAINS ISOLATED FROM THE NORTHEAST AND SOUTH BRAZIL**

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Trypanosoma cruzi is classified as a single species, although there is substantial genetic and phenotypic diversity among isolates. Strains can be divided into six lineages through protein and genetic markers currently designated as discrete typing units (DTUs). This study aims to determine the lineages of the strains SI5, SI8 T. cruzi isolated from Triatoma sordida; QMM3 and QMM5, T. rubrovaria, T m and T lenti, isolated, respectively, from T. melanocephala and T. lenti. The strains were isolated from abdominal compression of these triatomine species collected in the wild environment. Genotyping was performed by PCR-RFLP by amplifying the genes LSU rDNA, HSP60 and GPI. The products of PCR reactions were subjected to electrophoresis on agarose gel 1% (HSP60) and 3% (LSU and GPI), stained with ethidium bromide and visualized with UV light. The products of genes HSP60 and GPI were digested with restriction enzymes EcoRV and HhaI, respectively. According to the methodology, the LSU gene amplification from strains SI5 and SI8 originated fragments of 125 bp. For the isolated T lenti, Tm, QMM3 and QMM5 the fragments were 110 base pairs. Moreover for the HSP60 gene product showed fragments between 432-462 base pairs and GPI, 1264 base pairs. According to these results the studied strains were classified as group I (T. lenti), II (SI5 and SI8) III (Tm) and V (QMM3 and QMM5). Genotyping by PCR-RFLP is used for the characterization of strains isolated from humans, insects and animal reservoirs and indicates the existence of heterogeneity among isolates. Therefore, our work confirms the studies indicating the existence of distinct genotypes. Through the molecular studies six strains (T lenti, Tm, QMM3/QMM5 and SI5/SI8) were identified as belonging to groups I, III, V and II of T. cruzi, respectively.
Supported by:Capes

BM040 - **A PRELIMINARY PROTEOMIC SURVEY OF *TRYPANOSOMA EVANSI* TRYPOMASTIGOTES USING LC/MS/MS**

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The *Trypanosoma evansi* parasite infection, commonly called “surra”, affects many animal species such as horses, cattle and camels, causing significant economic losses to livestock industry around the world. Recently, human cases have been described. The aim of this study is to identify proteins expressed by *T. evansi* trypomastigotes in mouse experimental infections, with emphasis in the identification of potential drug targets and diagnostic antigens. In a preliminary proteomic survey using LC-MS/MS, 33 proteins from *T. evansi* trypomastigotes were identified and assigned to KOG functional groups, with most of them belonging to the group of cellular processes and signaling and metabolism proteins. Among the identified proteins there were cyclophilin and cysteine peptidase, which have been described as virulence factors and potential drug targets in other trypanosomatids, for being involved in parasite growth and survival in mammalian hosts. Furthermore, we identified several glycolytic enzymes, such as ATP-dependent phosphofructokinase, enolase and glycosomal fructose-bisphosphate aldolase. In *T. evansi* these enzymes are especially important for flagellar movement, a process that is dependent of the environmental glucose concentration. Considering that flagellar movement is essential for parasite infection, glycolitic enzymes also become attractive targets for future studies on their drug target potential. The *T. evansi* proteomic survey will also be extended with the analysis of more trypomastigote samples by both LC-MS/MS and 2DE-MALDI-MS/MS, in order to provide a comprehensive coverage of the repertoire of proteins expressed by this stage of the parasite. For the identification of antigenic proteins with potential for use in surra immunodiagnosis, we will also perform 2DE-immunoblots with sera from animals infected with *T. evansi* trypomastigotes.
Supported by:Supported by FINEP,CNPq and FAPESC.

BM041 - **BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE STRAIN QMM 12 OF TRYPANOSOMA CRUZI ISOLATED FROM TRIATOMA RUBROVARIA**

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Chagas disease, also known as American trypanosomiasis, is caused by the protozoa Trypanosoma cruzi. It affects 10 milion people mainly in Latin America, but nowadays it has been found in other countries. It is known that this parasite is classified into two major groups (TcI and TcII) and six subgroups named DTUs (Discrete Typing Unit). The strain QMM12 was isolated from T. rubrovaria by Rosa et al. in 2008, since then it has been maintained by peals in vivo and in vitro. It was biologically and molecularly characterized through parasitemic profile and the sequence of the gene 24Sα from ribossomal RNA, HSP60 e GPI. For the study of parasitemic profile, Swiss mice were intraperitoneally inoculated with trypomastigotes forms with the purpose of studying the parasitemic peak, mortality rate and prepatent period. The counting of the tripomastigotes forms was done in alternate days in 5µL of blood obtained from the tail of the animals and observed in optical microscope. The extraction of a genomic DNA and subsequent gene amplification 24Sα of rRNA, HSP60 and GPI by PCR-RFLP was conducted for the molecularly study. The tripomastigotes forms had been observed in peripheral blood since the fourth day of infection, the parasitemic peak ranged between 20 – 40 days after the initial inoculum. The forms disappeared on the 58th day and the mortality rate was 20%. PCR amplification generated a fragment of 110bp for 24Sa gene, two bands for HSP60 and two bands for GPI genes. The molecular results revelead that the QMM12 strain belongs to group III.

Supported by:Paulista State University - School of Pharmaceutical Sciences

BM042 - **MOLECULAR KARYOTYPE OF SYMBIONT CONTAINING TRYPANOSOMATIDS**

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Many species of the Trypanosomatidae family are monoxenics, thus inhabiting a single invertebrate host, usually an insect, during all its life cycle. Four monoxenic species harbor a symbiotic bacterium in the cytoplasm, which maintains an obligatory association with the host protozoan, constituting excellent model to study the origin of organelles and cellular evolution. In this work, our main goals are to characterize the molecular karyotype and determine the genomic sequencing of two monoxenics species: *Crithidia deanei* (recently renamed as *Angomonas deanei*) and *Blastocrithidia culicis* (recently renamed as *Strigomonas culicis*). For these purposes, DNA molecules were separated by pulsed field gel electrophoresis (PFGE) revealing that *C. deanei* and aposymbiotic strains present 15 chromosomal bands that range from 0.41 to 1.60 Mb and 0.45 to 1.53 Mb, respectively. *B. culicis* has 16 chromosomal bands ranging from 0.49 to 2.14 Mb. Furthermore, *Trypanosoma cruzi* genetic markers, such as the genes GADPH, α-tubulin, 18S rDNA and HSP85 were used to locate genes on chromosomal bands of *C. deanei* and *B. culicis*. The chromosomal organization differs on both strains of *C. deanei* (symbiotic and aposymbiotic) and for *B. culicis*. In the symbiotic strain of *C. deanei*, the GAPDH and HSP85 genes are located on chromosome 10, the α-tubulin gene on the chromosome 6 and the 18S rDNA genes are on chromosomes 1, 3, 6, 7, 8, 11 and 12. In contrast the HSP85 gene is located on chromosomes 8 and 9 in the aposymbiotic strain of *C. deanei* and in chromosome 3 of *B. culicis*. The obtained data will contribute to establish a comparative study using the genomic sequencing of *C. deanei* and *B.culicis*, which were estimated to be 24.2 Mb and 21.5 Mb, respectively. Other genetic markers specific for the symbiont containing trypanosomatids, as the kinetoplast associated-proteins (KAPs), as well as the 16SRNAr and FtsZ genes of the symbiotic bacterium will be also used to define the chromosomal bands of each species in order to elucidate important aspects of their biology such as gene lost and synteny in these microorganisms.

Supported by:CNPq, FAPERJ, LNCC

BM043 - **INVESTIGATION OF THE ROLE OF AN ARGININE N-METHYLTRANSFERASE (LMJPRMT7) IN LEISHMANIA MAJOR**

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Protozoan parasites of the *Leishmania* genus are early-branching unicellular eukaryotes displaying their coding genome organized into polycistronic clusters in the absence of canonical promoter sequences for transcriptional regulation. Gene expression in these parasites requires RNA processing through coupling of *trans*-splicing and polyadenylation. Additionally, control of gene expression in *Leishmania* occurs post transcriptionally, involving regulatory mechanisms such as modulated degradation of transcripts, which primarily requires a large set of RNA binding proteins (RBPs), and protein functional regulation. Several studies conducted on mammals and other trypanosomatids have identified critical roles for protein arginine N-methyltransferases (PRMTs) as modulators of RBP function. These enzymes transfer a methyl group from a methyl donor (usually S-adenosyl methionine) to specific protein arginine residues. Results from a previous study in our lab have identified a putative PRMT7 in *L. major* genome and indicated a correlation between its expression and wide modulation of gene expression in a spliced leader mutant. We have generated several tools to provide a global investigation of its role in *L. major*. We have engineered constructs to knockout or overexpress LmjPRMT7 and to insert a *myc*-tagged version *in vivo*. Generation of Leishmania transfectants are underway and will be important to associate phenotype and gene expression changes to the role of this enzyme in the parasite throughout its life cycle. We have also purified the heterologous protein from *E. coli* and generated polyclonal antibody anti-LmjPRMT7. The antibody will be used to investigate the enzyme subcellular location and to follow its expression under different conditions. Therefore, such tools are now in use to investigate the role of LmjPRMT7 as a regulatory protein in the panorama of gene expression control in *Leishmania*.
Supported by:FAPESP, CNPq

BM044 - **FUNCTIONAL CHARACTERIZATION OF 8-OXOGUANINE DNA GLYCOSYLASE OF TRYPANOSOMA CRUZI**

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One of the biggest threats to genome's integrity is the oxidation of nitrogenous bases, being 8-oxoguanine (8-oxoG) the most frequent oxidative lesion. 8-oxoG can erroneously pair with adenine, leading to mutation. Base excision repair (BER) is the most important cellular process in the excision of 8-oxoG. It begins with the activity of 8-oxoguanine DNA glycosylase (Ogg1/Fpg) that cleaves the lesion, leaving in the DNA an abasic site, which is further processed by other enzymes. Removal of 8-oxoG enables insertion of a normal guanine, preventing mutation. Trypanosoma cruzi, like most living organisms, is susceptible to oxidative stress. Hence, DNA repair is essential for its survival and improvement of infection. Given the importance of DNA repair to this organism, we have characterized the gene OGG1 of T. cruzi. First, we performed a heterologous complementation assay. The experiments were carried out in E. coli, however, expression of TcOGG1 was toxic both to wild type (WT) cell (AB1157) and to fpg -/- cells (BH20). Nevertheless, expression of TcOGG1 was not toxic to Saccharomyces cerevisiae. Yeast mutants ogg1-/- (CD138) have an increased mutation frequency, but when they expressed TcOGG1, this frequency was similar to the one seen for the WT cell (FF18733). We have also constructed a T. cruzi strain that overexpresses OGG1. Although in standard conditions this strain has a similar growth in comparison with the WT cell, the former is more sensitive to hydrogen peroxide than the latter. The overexpressing cells also show reduced levels of 8-oxoG both in the nucleus and in the kDNA, when compared to WT, and when treated with H2O2, this difference increases. Reduction of 8-oxoG must be associated with TcOgg1 enzyme, since localization experiments using TcOgg1-GFP protein revealed that it is directed both to nucleus and to mitochondria. Our data suggests that T. cruzi has a functional Ogg1 enzyme which participates in nuclear and mitochondrial BER.
Supported by:CAPES,Fapemig,CNPq, HHMI

BM045 - **IMMUNOPROTEOMIC ANALYSIS OF SURFACE PROTEINS OF *TRYPANOSOMA RANGELI* TRYPOMASTIGOTES**

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Trypanosoma rangeli is a non-pathogenic protozoan parasite that infects several invertebrate and vertebrate hosts. Due to antigen sharing with *T. cruzi*, misdiagnosis of Chagas disease might occur in a wide geographical area, often leading to improper epidemiological inferences. Secreted and surface proteins have been described as important shared antigens such as the GPI-anchored proteins, which are the most abundant proteins in *T. cruzi* surface, being reported as important in the host-parasite interaction. In this work, a comparative analysis of surface proteins from *T. cruzi* Y and *T. rangeli* Choachí obtained via Triton X-114 fractionation was carried out by gel-LC/MS/MS and imunoblotting. Resolution of proteins by SDS-PAGE revealed similar profiles for *T. rangeli* epimastigotes and trypomastigotes, however when comparing the profiles of these forms of *T. cruzi* they were very distinct. Comparison between the profiles of the trypomastigote forms of these two parasites, pointed out to several *T. rangeli* proteins with Mr of 24kDa and 50-75kDa that were not present in *T. cruzi*. These *T. rangeli* differential bands were analyzed by LC-MS/MS and protein identification by MASCOT, using the *T. cruzi* genome and *T. rangeli* transcriptome databases. The proteins identified in *T. rangeli* were sialidase (75kDa), a GPI-anchored protein, and flagellar calcium binding-protein (24kDa). Aiming to understand if these trypomastigote surface proteins could be used in differential diagnosis, immunoblotting assays were carried out using serum from mice experimentally infected. As expected, pattern recognition of proteins from *T. rangeli* and *T. cruzi* by homologous serum is more specific than by heterologous serum. Among others, a *T. rangeli* ~24kDa protein and a *T. cruzi* ~35kDa protein, whose identifications are under way, were exclusively recognized by homologous serum, indicating that these surface proteins could be used in differential serodiagnosis of infections with these two parasites.
Supported by:CNPq, FINEP, CAPES, Unoesc and UFSC

BM046 - **CHARACTERIZATION OF RNA BINDING PROTEINS WITH RRM DOMAIN IN TRYPANOSOMA CRUZI**

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The regulation of gene expression in trypanosomatids occurs mainly at post-transcriptional level, and few RNA binding proteins have been characterized. The RRM domain (RNA Recognition Motif) is one of the most abundant domains found in RNA binding proteins in higher eukaryotes. Proteins containing RRM domain are involved in most post-transcriptional processes, such as splicing, transport and stability. To characterize protein complexes associated with non-translated or translated mRNAs in Trypanosoma cruzi, mRNPs from epimastigotes and epimastigotes under nutritional stress were isolated using poly-(T) beads and the protein complexes bound to poly-(A+) mRNAs were analyzed by mass spectrometry (LC-MS/MS). Two proteins with RRM domain were chosen for further characterization, DRBD3 and NRBD1. The genes encoding these proteins were cloned and recombinant proteins were used to obtain polyclonal antibodies. In silico analysis showed that both proteins have two RRM domains in their structure. Western blot assay during metacyclogenesis was performed and showed that the proteins are expressed throughout the life cicle. Immunofluorescence was also carried out, showing that the protein NRBD1 presents a perinuclear location, while DRBD3 presents a slightly granular dispersion in all cytoplasm. Northern blot and RT-PCR are being conducted with DRBD3. Polysome profile in sucrose gradient and immunoprecipitation with both proteins will be performed in order to get further insight in their function, mRNA tragets and partner proteins. The characterization of NRBD1 and DRBD3 will help to elucidate the post-transcriptional regulation in T. cruzi.
Supported by CAPES/FIOCRUZ and CNPq.

BM047 - **DEVELOPMENT OF A SEMI-AUTOMATED SEQUENTIAL KNOCKOUT SYSTEM IN TRYPANOSOMA CRUZI, PART I: PRIMER PREDICTION SOFTWARE**

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The Trypanosoma cruzi genome has several unknown function genes, comprising ~46.5% of the possible protein coding genes. The majority of them was characterized by in silico studies; however, few genes have been studied in T. cruzi through reverse genetics techniques, in part due to the absence of a fast and highly effective methodology. Most researches regarding this subject rely on classical cloning techniques, with the construction of DNA cassettes for gene disruption through homologous recombination, which are very time-consuming. In this context, we started to develop a semi-automated system for sequential knockouts in T. cruzi strain Dm28c, which will allow functional characterization of multiple genes through large scale gene disruption. The experimental design consists of three parts: development of a primer prediction software - which is the main subject of this abstract -, construction of knockout cassettes and sequential gene disruption. The cassettes will be made using fusion PCR instead of classical cloning, to speed up the process; and for the sequential knockouts we will use the CRE/lox system, which avoids the use of multiple selection markers. The primer prediction software was made using Perl programming language and Primer3 algorithm. Briefly, a protein coding sequence and flanking intergenic regions are extracted from *T. cruzi* strain CL Brener scaffolds and compared to strain Dm28c contigs using BLASTn algorithm. From the HSPs, the identical sequences between the intergenic regions of the two strains are extracted and delivered to Primer3 as input. The Primer3 output is then parsed and fusion tags are added to the 5' ends of the top quality inner primers, which will have a major role in the fusion PCR. Considering the high sequence divergence between different T. cruzi strains, we hope to achieve a higher effectiveness in the reactions using this new primer design strategy, in comparison with the primers designed directly from strain CL Brener.
Supported by:CNPq, FIOCRUZ, Fundação Araucária

BM048 - **MSH2 INVOLVEMENT IN OXIDATIVE STRESS RESPONSE IN TRYPANOSOMA CRUZI**

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MSH2 is primarily described as a protein involved in DNA Mismatch Repair (MMR). However, recent results from our group suggest that in Trypanosoma cruzi it can also be involved in the oxidative stress response. Gene characterization based on nucleotide polymorphisms of MSH2 identified three different isoforms, named TcMSH2 A, B and C, present in different T. cruzi strains. Functional characterization suggests that strains presenting TcMSH2A have a more efficient MMR when compared to strains presenting TcMSH2 B or C. Also, strains presenting TcMSH2 B or C, as well as MSH2 single knockouts are more susceptible to hydrogen peroxide treatment and accumulate more oxidized guanine in the kinetoplast DNA when compared to TcMSH2A strains and wild type parasites. Analyses of the sequences of the two MSH2 alleles present in the CL Brener genome – corresponding to MSH2 B and C – suggest that only one of the isoforms has a mitochondrial localization signal, in addition to nuclear localization. A mitochondrial localization signal has not been identified in TcMSH2 A isoform. Cellular localization analyses indicated that, in the CL Brener clone, a MSH2 protein with a molecular weight lower than the predicted MW is present in the mitochondria. Since T. cruzi is an intracellular parasite, it must be able to resist to the oxidative stress generated inside the infected cell. Preliminary data indicating that MSH2 single knockouts are less infective than wild type cells are in agreement with the proposed role for this protein in the repair of oxidative damage in parasite DNA.
Supported by:CNPq, FAPEMIG and HHMI

BM049 - **THE ABC TRANSPORTER GENE FAMILY OF TRYPANOSOMA CRUZI**

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The ATP-binding cassette (ABC) protein superfamily is one of the largest evolutionarily conserved families. Most of these proteins are involved in the ATP-dependent transport of molecules across biological membranes, including chemotherapeutic drugs. The goals of this study were: (i) to identify and classify ABC transporters of T. cruzi; (ii) to characterize the structure of one ABC transporter (TcABCG) potentially involved in benznidazole (BZ) resistance in T. cruzi strains. The combined use of systematic BLAST searches against the T. cruzi genome using the ABC signature sequence as a query allowed the identification of 56 ORFs coding for putative ABC proteins. Multiple sequence alignments were performed on the amino acid sequences of the ATP binding domains by using CLUSTAL W with the default settings. The resulting multiple sequence alignments were subjected to analyses using neighbor-joining method. T. cruzi ABC proteins were classified into ABCA to ABCH subfamilies, following the HUGO nomenclature adopted for eukaryotes ABC proteins. Evidence that TcABCG is potentially involved in BZ resistance in T. cruzi was obtained by our group following DNA microarray hybridization, real time RT-PCR assays and gene transfection. TcABCG is a “half-size” transporter, composed of an ATP binding domain (NBD) fused to a transmembrane domain (TMD). CL Brener Esmo and Non-Esmo TcABCG haplotypes display 41 nucleotide variations, 28 of which code for synonymous substitutions. Non-synonymous variations are concentrated in the intracellular NDB. The TcABCG gene of eight T. cruzi strains of four DTUs was amplified with Pfu polymerase, the products cloned in pGEM-T Easy and six clones from each strain were sequenced. Multiple sequence alignments were performed. Data indicate that TcABCG gene structure is characteristic of each DTU and that intra-DTU SNPs are conserved. The data also support the hybrid nature of TcV strains and indicate intragenic recombination.
Supported by:FAPESP, CAPES, CNPq

BM050 - **TCNUP-1: A NUCLEAR PERIPHERY COILED-COIL PROTEIN, ASSOCIATES WITH CHROMATIN IN TRYPANOSOMA CRUZI.**

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The nuclear envelope is a complex structure composed of an outer and inner nuclear membrane, nuclear pore complexes and the nuclear lamina. The nuclear lamina is a dense fibrillar network inside the nucleus of eukaryotic cells, having an important role in anchoring and recruiting elements to the nuclear periphery. Lamins are the principal component of nuclear lamina and are essential in metazoans. To fulfill these important functions, organisms lacking lamin genes have distinct proteins. Some years ago, a coiled-coil protein in *Trypanosoma brucei*, NUP-1, was identified on the inner face of the nuclear envelope, suggesting that it could be the major filamentous component of its nuclear lamina. Recently we have characterized a homologous protein in *Trypanosoma cruzi* (TcNUP-1). Considering that TcNUP-1 should play a role in the nuclear lamina, we investigated its in vivo DNA binding sites using a chromatin immunoprecipitation assay (ChIP). We demonstrate for the first time that TcNUP-1 associates with chromosomal regions containing large non-tandem arrays of genes encoding surface proteins. To confirm the co-localization of these regions with TcNUP-1, we used fluorescent in situ hybridization (FISH) assays. Our data support the hypothesis that TcNUP-1 has a role in chromatin organization by anchoring *T. cruzi* chromosomes to the nuclear envelope.
Supported by:CNPq; Fundação Araucária; Fundect.

BM051 - **TESTING OF LEISHMANIA AMAZONENSIS TELOMERASE ACTIVITY USING A NONISOTOPIC PCR-BASED ASSAY**

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Leishmaniasis comprises a spectrum of diseases that affect millions of people around world, with 350 million individuals at risk. Current treatments rely on drugs that have high toxicity, prolonged therapy, low efficiency and high cost. For these reasons, the World Health Organization (WHO) has encouraged the development of new therapies for the treatment of this disease. Leishmania telomeres are composed by the conserved TTAGGG repeats maintained by telomerase. In our laboratory the gene encoding the protein component of telomerase (TERT) was cloned and telomerase activity was detected in semi-purified extracts using affinity chromatography. Enzyme activity was assayed using modifications of the conventional TRAP (Telomere Repeat Amplification Protocol) assays and to detect enzyme products it was necessary to use radioisotopes. Here we show that it is possible to detect L. amazonensis telomerase activity in both total and semipurified (DEAE chromatography) extracts using the TRAPEZE® XL Telomerase Detection Kit (Millipore). The presence of activity in these extracts was undoubtedly detected when compared with the internal PCR control and the non-telomerase control. The TRAPEZE® XL Kit showed to be highly sensitive and a very efficient non-isotopic in vitro assay for the fluorometric detection of Leishmania telomerase activity. Our preliminary results also showed that the L. amazonensis extracts tested as positive for telomerase activity were also highly sensitive to RNAse A pretreatment. RNAse A treatment is the universal control used to confirm the presence of telomerase activity in vitro assays. The quantitative analysis of telomerase activity in parasite extracts is underway.
Supported by:FAPESP, CNPq

BM052 - **LEISHMANIA (LEISHMANIA) AMAZONENSIS ARGINASE COMPARTMENTALIZATION IN THE GLYCOSOME IS IMPORTANT FOR PROPER ENZYME ACTIVITY AND PARASITE INFECTIVITY**

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Polyamines are ubiquitous molecules that are present in all eukaryotes and participate in many important cellular processes. In *Leishmania*, arginase (E.C. 3.5.3.1) catalyzes L-arginine hydrolysis to urea and L-ornithine, a polyamine precursor. It had been shown that, besides the reported infectivity improvement, arginase is an essential enzyme for the parasite's in vitro proliferation, wthat made the search and study of arginase competitive inhibitors attractive in the pursuit of new treatments for leishmaniasis. However, little attention has been paid to the arginase glycosomal compartmentalization effects to the parasite physiology. In this study, we show that arginase remains in the amastigote glycosome during macrophage infection by immunolabeling electron microscopy. To verify the importance of arginase compartmentalization, we first generated an *L. (L.) amazonensis* arginase knockout mutant (arg^-). The obtained mutant did not present arginase activity and was auxotrophic for polyamines besides presenting an attenuated infectivity behavior. From the knockout mutant, we generated two genetically complemented mutants integrating the native arginase ORF (arg^-/ARG) or the ORF lacking the SKL glycosomal addressing signal (arg^-/argΔSKL) into the SSU rRNA locus. The arg^-/ARG parasite had partially recovered ARG activity that was sufficient to rescue in vitro infectivity. Remarkably, despite a recovery in mRNA expression levels, the arg^-/argΔSKL mutant lacked arginase activity and maintained impaired infectivity. Furthermore, arg^-/argΔSKL infection in mice was even more attenuated than infection with arg^- parasites. The evaluation of the amino acids internal pools in the mutants showed an increase in the internal arginine concentration in both arg^- and arg^-/argΔSKL parasites. Our results indicate that the proper compartmentalization of *L. (L.) amazonensis* arginase in the glycosome is important for enzyme activity and optimal arginase performance during infection. Our conjecture is that parasite arginase participates in a complex equilibrium between parasite/host enzymes that define the fate of L-arginine and its proper sub-cellular location may be essential for this physiological orchestration.
Supported by:FAPESP, CNPq, NIH

BM053 - **MRNA CONTENT OF SPECIFIC MRNPS IN TRYPANOSOMA CRUZI SHOW THE COORDINATE GENE EXPRESSION BY POSTTRANSCRIPTIONAL REGULONS**

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Trypanosomatids gene expression is regulated almost exclusively at the post-transcriptional level, with mRNA metabolism, such as stability, storage and degradation playing a decisive role. In other eukaryotes like budding yeast, fruitfly and mammalian cells, there is evidence that multiple mRNAs are co-regulated by RNA-binding proteins to form ribonucleoprotein complexes (mRNPs) that regulate their export, stability, localization and translation. These observations have led to a model in which mRNAs that encode functionally related proteins are coordinately regulated during cell growth and differentiation as post-transcriptional RNA operons or regulons. CCCH zinc finger proteins (ZC3Hs) are a novel class of RNA binding protein involved in posttranscriptional mechanisms controlling gene expression. Here we characterize the mRNA content of TcZF211.70 from *Trypanosoma cruzi*, an RNA binding protein with a zinc finger domain, in epimastigote forms during exponential growth and under stress conditions. Using the cell under stress it was possible to observe a dynamic change in TcZF211.70 cellular localization, the granules were larger in stressed compared to epimastigotes under normal growth conditions. As well as cellular distribution alteration, the mRNA content of this protein also changed. In epimastigotes most mRNAs target of TcZF211.70 encode for membrane bound proteins such as Mucin II and MASP (Mucin associated protein), which are predominantly expressed in metacyclic forms, indicating that these mRNPs might function as storage granules. However, when the cells are under stress, the targets bound to TcZF211.70 change drastically and are formed by mRNAs that encode for ribosomal proteins, nuclear and nucleolar proteins. This dynamic rearrangement suggests that this protein might have a different function in stress, sequestrating the mRNAs that encode for ribosomal proteins to attenuate translation in response for the stressing condition. Our results suggest that *Trypanosoma cruzi* genes form posttranscriptional regulons and they may play an important role in gene expression regulation in this parasite.
Supported by:CNPq, CAPES, Fiocruz

BM054 - **IDENTIFICATION OF RNA TARGETS ASSOCIATED TO GAP2-CONTAINING RIBONUCLEOPROTEIN COMPLEXES IN TRYPANOSOMA CRUZI**

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In trypanosomatids, regulation of gene expression occurs mainly at the post-transcriptional level. The association between mRNAs and certain proteins determine mRNA fate, directing them to translation, repression or degradation. TcGAP2 was previously identified by mass spectrometry as one of the proteins associated to poly-(A+) mRNAs in epimastigotes and epimastigotes under nutritional stress. The gene encoding GAP2 was cloned and expressed to produce antisera against this protein. GAP2 was shown to localize mainly in the kinetoplast. To identify the mRNAs associated to TcGAP2 mRNP complexes, we carried out immunoprecipitation assays with anti-GAP2 serum using epimastigotes and epimastigotes under nutritional stress lysates. The RNA targets of GAP2 were identified by deep sequencing technology (RNA-seq - SOLiD™). A larger number of mRNAs were identified in epimastigotes under nutritional stress (n=235) as compared to epimastigotes (n=37). GAP2-associated RNAs in epimastigotes were highly enriched for genes enconding cytoplasmic (37%) and nuclear proteins (25%). Conversely, RNAs associated to GAP2 in epimastigotes under nutritional stress were highly enriched for genes enconding mitochondrial (45%) and cytoplasmic proteins (30%). MEME algorithm analysis was used to identify commonly occurring sequence motifs among the identified RNAs. No apparent motifs were found in the 5' upstream region nor in the coding sequence. However, in the 3' downstream region a 21-nucleotide long G-rich motif was found, present in 94 out of 235 transcripts. These data indicate that GAP2 is a component of a ribonucleoprotein complex that might be regulating the processing of specific mRNAs.
Supported by:CNPq, CAPES e Fiocruz

BM055 - **HETEROLOGOUS EXPRESSION AND PURIFICATION OF NAEGLERIA GRUBERI SELENOPHOSPHATE SYNSTHASE**

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Selenium (Se) is an essential trace element in vivo, which is mainly found in selenoproteins as the 21st amino acid (Selenocysteine – Sec – U). The selenoproteins generally participate in the cellular redox state balance, playing an important role on cell growth and proliferation. The publication of Naegleria gruberi (ATCC 30224) genome allowed us to investigate the presence of the Sec-incorporation pathway in this “primitive” eukaryote. Using a thorough bioinformatics approach; we identified various genes involved in Sec-incorporation machinery. Among these genes, it was identified a homolog of Selenophosphase synthase (SelD or SPS). SelD is responsible to produce the biologically active selenium donor compound, monoselenophosphate, from ATP and selenide, for the synthesis of selenocysteine. The N. gruberi SelD is a 737 amino acids enzyme divided into two domains. The C-terminal domain has high sequence identity with bacteria SelD and phylogenetic analysis shows that N. gruberi SelD homologue nested within Clostridium botuluim and Sebaldella termitidis. Functional complementation experiments using an SelD-deficient E. coli strain demonstrated that N. gruberi SelD is active and restored the E. coli selenoproteins synthesis. N. gruberi SelD N-terminal domain showed identity with eukaryotic methyltransferases, enzymes able to bind S-adenosylmethionine. The full length N. gruberi SelD and the C-terminal domain were cloned into pET32a and transformed in BL21(DE3) codon plus for recombinant expression. Recombinant proteins with approximately 100 and 60 kDa respectivelly were obtained for molecular and functional characterization.
Supported by:FAPESP/CNPQ

BM056 - **MOLECULAR EPIDEMIOLOGY OF LEISHMANIA CHAGASI IN BRAZIL: USE OF KDNA AS A MOLECULAR MARKER**

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Leishmaniasis is a parasitary disease caused by Leishmania protozoans and transmitted by female Phebotomidae sandflies. Clinical manifestations are diverse, being visceral leishmaniasis (VL) the most severe form. In Brazil VL is caused by Leishmania chagasi and transmitted by Lutzomyia longipalpis sandfly; the main zoonotic reservoirs are dogs and wild canids. Due to a broad range of disease manifestations and great variety of host species infected, Leishmania parasites are thought to possess great genotypic variability.This is of major significance in epidemiological features and in disease transmission. The aim of this study is to identify different genotypic strains of L. chagasi from distinct sources in three endemic areas: fresh amastigotes from dog bone marrow samples, and cultured promastigotes from human patients in Teresina, Piauí State; fresh amastigotes from wild canids bone marrow samples, and cultured promastigotes from human patients in Campo Grande, Mato Grosso do Sul State; and fresh amastigotes from human bone marrow samples in Bauru, São Paulo State.PCR-RFLP of kinetoplast DNA (kDNA) was used in order to compare genetic profiles in parasites from different geographical origins.Results have shown different genotypes depending on geographic origin and parasite source. Amastigotes showed overall high degree of genetic variability when compared to promastigotes; and Campo Grande and Bauru isolates presented much more similarity when compared to Teresina isolates, this genetic structure could represent two different strains with independent introductions into Brazil. Moreover, parasites isolated from wild canids in Campo Grande showed completely different genotypes compared to human isolates. To our knowledge, this is the first description of sympatric parasite strains within different hosts.There were at least two clearly different parasite lineages in Bauru samples from symptomatic individuals.The significance of this finding remains unclear.
Supported by:FAPESP

BM057 - **AN ESSENTIAL NUCLEAR PROTEIN IN TRYPANOSOMES IS A COMPONENT OF MRNA TRANSCRIPTION/EXPORT PATHWAY**

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In trypanosomes, post-transcriptional events are the major points for regulation of gene expression, however the export pathway of mRNA from the nucleus is poorly understood. For this reason, we investigated the function of trypanosome Sub2 (Tryp-Sub2), a highly conserved protein ortholog to Sub2/UAP56, component of the complex connecting transcription with mRNA export in yeast and human. Similar to its orthologs, TcSub2 is a nuclear protein, localized in dispersed foci all over the nuclei, at the interface between dense and non-dense chromatin areas. The association of Tryp-Sub2 with transcription/processing sites was analyzed by BrUTP incorporation assays and confirmed that it is associated with active RNA polymerase II, but not RNA polymerase I or SL transcription in *T. cruzi*. The TcSub2 double knockout is lethal, suggesting it has an essential function. Alternatively, RNAi assays in *T. brucei* demonstrated that its knockdown causes mRNA accumulation in the nucleus and decrease of translation levels, reinforcing that Tryp-Sub2 is a component of mRNA transcription/export in trypanosomes. To further characterize what mRNAs are exported by Tryp-Sub2 pathway, we intend to sequence by SOLiD nuclear transcripts from parasites befor and after RNAi induction to identify which ones have the export affected by silence of the protein. For investigation of proteins associated with Tryp-Sub2, we will make immunoprecipitation of protein complex and submit it to mass-spectrometry for peptide identification. Besides, two-hybrid assays will be performed using selected targets to identify which factors directly interact with Tryp-Sub2.

Supported by:CAPES, CNPq, Fundação Araucária e FIOCRUZ

BM058 - **EXPRESSION AND ANTIGENIC PROFILES OF MASP FAMILY OF TRYPANOSOMA CRUZI IN ACUTE PHASE OF EXPERIMENTAL INFECTION**

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A major finding of the T. cruzi genome project was the discovery of the large multigene family MASP, which is preferentially expressed in infective forms of the parasite. Although MASP family function is unknown, their extended sequence variability and presence of repetitive motifs associated to the localization at the parasite surface suggest this family participates in parasite-host interactions such as host cell adhesion/invasion or/and immune evasion mechanisms. In the present study, we have analyzed the MASP expression profile in trypomastigotes derived from distinct host cells and from acutely infected mice after sequential passages. Also, in order to investigate the MASP antigenic profile we have performed B cell linear epitope prediction on the MASP proteome and further designed a SPOT peptide array with 200 putative epitopes. This peptide array was screened with sera from acutely infected mice. The results from the analysis of 7 expression libraries suggest that, although several MASP genes are co-expressed in the parasite population, the repertoire of expressed genes is distinct in trypomastigotes derived from tissue culture and bloodstream trypomastigotes recovered from sequential passages in mice, which was further confirmed by Real Time RT-PCR. The B cell epitope screening showed that mice IgG and IgM are reactive against several MASP peptides during acute phase. This is the first report on MASP antigenic property and profile. We speculate that variations in the large repertoire of potentially antigenic peptides derived from MASP family may favor the parasite escape of immune response during the acute phase of infection.

Supported by:WHO, FAPEMIG, CNPq, CAPES

BM059 - **THE EXPRESSION OF LARBP38, OGG1 AND TRYPAREDOXIN PEROXIDASE IS ALTERED UPON H2O2-INDUCED STRESS IN LEISHMANMIA AMAZONENSIS PROMASTIGOTES**

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Rbp38 is a protein exclusively expressed in trypanosomatid parasites. The protein is encoded by a nuclear gene and is probably multifunctional and implicated with kinetoplast and nuclear DNA replication. In an attempt to discover if LaRbp38 is also involved with DNA-damage response caused by oxidative stress, we used H2O2 to treat L. amazonensis promastigotes. The IC50 for H2O2 was determined as 2 mM, and cells were treated with the drug for different period of time. Parasite extracts were obtained from each time point and submitted to Western blotting analysis using anti-LaRbp38 serum. The extracts were also probed with anti-LaRPA-1 and anti-OGG1 sera, used respectively as markers for signaling damage and base excision repair. The expression of LaRbp38 increased after 30 min. of drug exposure, whereas the amount of LaRPA-1 showed a slightly increase after 15 min., and the amount of OGG1 slightly increase after 2h of H2O2 treatment. Anti-2Cys-peroxiredoxin tryparedoxin peroxidase (TryP), was used as a positive control of the assay, since TryP catalyses the reduction of H2O2 and organic hydroperoxides to water or alcohols. In our experimental conditions the expression of TryP gradually increased as soon as parasites were exposed to H2O2. Alfa-tubulin was used as the loading control. Parasite samples from different time points were also analysed by immunofluorescence using anti-LaRbp38 and anti-OGG1 sera. After 1 hour of treatment with H2O2, LaRbp38 was visualized in the nucleus and in the kinetoplast whereas, in control parasites, the protein was predominantly found in the kinetoplast. Similarly, the number of cells showing OGG1 in both subcellular compartments after H2O2 treatment, increased compared to the controls where OGG1 is also found predominantly in the kinetoplast. Altogether these results strongly suggest that LaRbp38 may participate in the DNA-damage response triggered by oxidative stress. The implications of these findings will be discussed.
Supported by:Fapesp

BM060 - **PRELIMINARY RESULTS SHOW THAT OXIDATIVE STRESS INDUCES CHANGES IN THE EXPRESSION LEVELS OF LEISHMANIA AMAZONENSIS RPA-1**

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Leishmaniasis is a spectrum of diseases caused by parasites of the genus Leishmania. Infection occurs during the bite of infected sandflies. During infection, parasites use different mechanisms to evade host defenses, which include the exposition to reactive oxygen species (ROS). To overcome these barriers they usually increase the expression of specific peroxidases which avoids parasite´s death. However, it is still unknown how parasites protect their genome and their telomeres from ROS attack. It is well known that telomere integrity plays a crucial role in the capacity for continuous cell proliferation but it is the main target for oxidative damage. Therefore, our goal is to understand how parasite telomeres respond to oxidative stress. Replication Protein A (RPA) has been found to be an indispensable player in almost all DNA metabolic pathways including DNA repair and telomere maintenance. Leishmania RPA-1 was well characterized as a telomeric protein and is also probably involved in damage response, by participating directly in DNA replication and telomere protection during damage. We first estimated the IC50 for H2O2 and then we exposed promastigotes growing in axenic cultures, to sublethal doses of H2O2 for different time points. Parasites treated with H2O2 and the non-treated controls, were analysed by indirect immunofluorescence using anti-LaRPA-1, anti-Ogg1 and anti-TP (Tryparedoxin peroxidase) sera. Nuclear extracts obtained from parasites treated in the same conditions as above, were tested by Western blot probed with the same sera. Preliminary results showed that LaRPA-1 expression increased after the first minutes of H2O2 treatment followed by an increase in nuclear Ogg1, suggesting that LaRPA-1 is rapid recruited in response to oxidative damage. Assays using parasites treated with H2O2 and in the presence or absence of an anti-oxidant, i.e. N-Acetylcystein (NAC) as well as pull down and co-immunoprecipitation experiments are under way.
Supported by:FAPESP

BM061 - **CHARACTERIZATION OF EUKARYOTIC INITIATION FACTOR 5A (EIF-5A) IN TRYPANOSOMA CRUZI POPULATIONS SUSCEPTIBLE AND RESISTANT TO BENZNIDAZOLE.**

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Eukaryotic initiation factor 5A (eIF-5A) is a protein of 18 kDa, acidic (pI 4.5), highly conserved and essential for the eukaryotic cells. Although it was identified as a translation initiator factor, its functional role in eukaryotic cells is not defined. Recent evidence suggests that it is involved in the stress response of several organisms. 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 BZ-resistance in a *T. cruzi* population (BZR) when compared to a BZ-susceptible *T. cruzi* population (BRS). In the present study, we compared the eIF5A protein expression levels in *T. cruzi* populations susceptible (CL Brener) or naturally resistant to BZ (Colombiana), or which were selected *in vivo* (BZR) or *in vitro* (17LER) to BZ with their respective sensitive pairs (BZS and 17WTS). Western blot experiments using anti-TceIF5A polyclonal antibody recognized a 18 kDa protein in all *T. cruzi* strains analyzed. The expression level of this polypeptide was approximately 2-fold lower in BZR than in BZS. In contrast, no differences in TceIF5A protein expression levels were observed between the remaining *T. cruzi* samples. Western blot analysis using two-dimensional gel electrophoresis (2-DE) confirmed this result. The anti-TceIF5A antibody recognized one spot with lower signal intensity in BZR when compared to BZS. Both *T. cruzi* populations were stably transfected with TceIF5A gene in order to overexpress this gene in these parasites. Molecular characterization of transfected and non-tranfected parasites is being performed to determine a possible involvement of TceIF5A in the drug resistance phenotype in *T. cruzi.*

Supported by:CNPq, FAPEMIG, CPqRR and PDTIS/FIOCRUZ

BM062 - **IDENTIFICATION OF THE GENE ENCODING THE MAJOR SURFACE PROTEASE (MSP) IN TRYPANOSOMA RANGELI**

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Trypanosoma rangeli is a flagellate protozoan which is studied in the same epidemiological context of *Trypanosoma cruzi*, although it is non-pathogenic for the vertebrate host. The surface molecules of parasites play a key role in the parasite-host interaction. One of these molecules is the Major Surface Protease (MSP or gp63), a zinc-dependent hydrolase which is anchored to the cell surface via a glycosylphosphatidylinositol (GPI) anchor. MSPs were initially described in the genus Leishmania, and later in other trypanosomatids as an important virulence factor. The objective of this study was to identify an entire copy of the TrMSP gene, as well as perform in silico characterization of the predicted enzyme. The TrMSP ORF (open reading frame) is composed by 1,767bp and encodes a putative protein of 588 amino acids with isoeletric point of 7.13. TrMSP has 58% of identify with the TcMSP (*T. cruzi*) and has all the elements necessary for its processing, such as a signal peptide, a propeptide and a site for addition of GPI-anchor. The preliminary characterization of 5' and 3' UTRs (untranslated region), generated during the identification of the TrMSP ORF revealed the presence of a potential acceptor site of spliced leader, as well as a possible sequence involved in post-transcriptional regulation. The presence of MSPs in *T. rangeli*, a non-pathogenic human parasite, opens new possibilities for understanding the host parasite relationship, especially in the vertebrate host whose biological mechanisms of parasitism are poorly understood.

Supported by:Fundação de Amparo À Pesquisa do Estado de Minas Gerais

BM063 - **A SENSITIVE MULTIPLEX PCR FOR MOLECULAR CHARACTERIZATION OF TRYPANOSOMA CRUZI STRAINS**

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Trypanosoma cruzi, aetiologic agent of Chagas disease, presents a great genetic heterogeneity evidenced by several molecular markers. Recently, a second consensus was reached for T. cruzi nomenclature: T. cruzi strains are now referred to six Discrete Taxonomic Units (DTUs) named T. cruzi I to T. cruzi VI. However, there is no consensus about how many and which markers should be used to have a suitable classification for T. cruzi strains within these six DTUs. In addition, to date there is no methodology able identifying the T. cruzi DTUs and simultaneously characterize the intraspecific variability among the parasite strains. Here, we proposed a sensitive multiplex PCR system based on a Full Nested PCR composed of seven polymorphic markers: RFLP-Cytochrome Oxidase subunit II, Spliced Leader Intergenic Region, 24Sα rDNA and four microsatellite loci (TcTAc15, TcTAT20, TcATT14 and TcAAAT6), to improve the procedure for molecular characterization of T. cruzi strains. Preliminary results revealed that after second round of PCR assays all the markers presented the expected amplification patterns while was used 1ng of parasite DNA, demonstrating that the multiplex strategy is suitable for typing cultured parasite strains. However, when evaluating the sensitivity of this multiplex system employing serial parasite DNA dilutions, positive amplifications were obtained only up to 10 picograms, quantity much higher than the 200 fentograms frequently found in biological samples. These findings demonstrated that further experiments will be necessary to improve the sensitivity of this methodology to detect parasite DNA directly in biological samples such as blood and other tissues from chronic chagasic patients. The optimization of PCR sensitivity will open new possibilities in the molecular characterization T. cruzi procedures allowing to determine with assurance the T. cruzi DTUs as well as individually characterize each parasite strain using only a single sample of DNA.
Supported by:FAPEMIG, CNPq, CAPES

BM064 - **SYSTEMIC ANALYSIS OF GENE EXPRESSION MODULATION AFTER NUTRITIONAL STRESS IN TRYPANOSOMA CRUZI.**

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Due to methodological advances, the study of organisms as complex systems is relatively new, requiring a multidisciplinary approach, including the development of new experimental methodologies and analytical methods. These advances provide new opportunities to study different biological features in a large-scale framework. Among different biological phenomena, the response to changing environments has several interesting characteristics, as being common for different types of organisms, requiring specialized adaptations, and the rigid control of these modulations. The stress resulting from environmental changes demands a fast response from organisms, requiring the modulation of different, and often overlapping, regulatory networks, which confers to the organism the capacity to overcome these changes. The trypanosomatids are eukaryotes whose gene expression regulation occurs predominantly post-transcriptionally and the current knowledge about the organization and regulation of the gene expression regulatory network in these organisms is limited. A comprehensive study of these networks is very important for understanding the systems of post-transcriptional control. Among these, the response to environmental stresses is of utmost relevance for biological characterizations, influencing important life cycle processes such as *T. cruzi* metacyclogenesis. We are now conducting a general characterization of stress response in *T. cruzi*, including morphological, physiological and biochemical analysis, but with a larger effort in creating *omics* maps. We analyzed cell morphology and behavior during long periods of stress as also the capability of growth after up to fourteen days of nutritional stress. For transcriptome analysis we collected total and polissomal RNA samples up to six hours of stress and the data is being generated by SOLiD sequencing. At present other types of stress are being evaluate to characterize as this parasites respond to different environmental stresses.
Supported by:CNPq, , Fundação Araucária, Fiocruz

BM065 - **MOLECULAR DIAGNOSTICS FOR DETECTION AND DIFFERENTIATION OF LEISHMANIA COMPLEXES**

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Leishmaniasis is a vector-borne zoonosis caused by Leishmania protozoa. The epidemiology of the disease is complex, being that 1) is present on four continents, being endemic in Brazil, 2) a lot of animals reservoirs, 3) Million people are infected, 4) there are more than 30 species of potential vectors and 5) has more than 20 pathogenic species to humans. The aim of this study was to develop a kit for molecular diagnosis of leishmaniasis, with can detect and differentiate the complexes of the genus Leishmania, which have different clinical presentations, such as mucocutaneous, cutaneous and visceral and differentiate from areas of co-existence with Chagas disease. We performed DNA extraction with phenol/chloroform (Sambrook, 1989) from 28 human blood samples whose patients were suspected for leishmaniasis at the University Hospital of Uberlândia, MG, Brazil. Components used in the reaction were performed undergo a process of lyophilization and the samples were tested by Multiplex PCR. The primers were previously described by Harris (1998) with amplify three complex bands with very different molecular weights: 146-149, 218-240 and 351-394pb for the complex L. braziliensis, L. donovani and L. mexinaca, respectively. The reaction was observed in 1.5% agarosis gel electrophoresis and visualized at ImageQuant® Biosystems. With the multiplex PCR analysis were possible to identify and differentiate samples with Leishmaniasis. It was also possible to verify the co-infection of Leishmania species and no cross-reaction was observed with Trypanosoma cruzi, suggesting a good specificity of the technique. The lyophilization process appears to allow storage of compounds for long periods of time without loss of quality. The multiplex PCR was able to make the differential diagnosis of Leishmania complexes, which may be applicable in the future for the laboratory diagnosis and clinical improvements. More samples will be tested to optimize de multiplex PCR reaction.
Supported by:Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG

BM066 - **MOLECULAR DETECTION OF TRYPANOSOMA CRUZI FROM THE AMAZON AND OTHER ENDEMIC AREAS OF BRAZIL, IN MICE TREATED WITH BENZNIDAZOLE**

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Currently, Chagas disease is controlled in most endemic classical areas of Brazil including the states of Paraná (PR) and Minas Gerais (MG). However, in the Amazon it emerges as a major public health problem and presents with peculiar clinical and epidemiological characteristics. The PCR has been highlighted as an important tool to detect Trypanosoma cruzi in humans and animals untreated and etiologically treated. The aim of this study was to assess the ability of PCR to detect DNA of T. cruzi from the states of Amazonas (AM), PR and MG, in the blood of mice treated with benznidazole. We analyzed 16 isolates of T. cruzi: 12 AM, 3 PR, and 1 MG. For each isolate, male Swiss mice, from 21 to 28 days were inoculated intraperitoneally with 10,000 blood trypomastigotes. Ten animals were treated orally with benznidazole (BZ-LAFEPE) 100mg/kg/day for 20 consecutive days, comprising the TBZ group. The untreated group (NT) consisted of animals inoculated with four isolates (3 AM and 1 PR, 10 animals per isolate). For PCR, we collected 200 mL of blood into tubes containing 400 mL of guanidine/EDTA 6.0M/0.2M. DNA was amplified with primers 121 and 122, visualized in polyacrylamide gel at 4.5% and revealed by silver. In the NT group, PCR positivity ranged from 28.6 to 80.0% for animals infected with AM isolates and was 83.3% for the PR isolate. In TBZ animals, PCR was negative for four isolates: 2 AM, 1 PR, and 1 MG. PCR positivity of 15% was observed for 1 AM isolate, between 20 and 60% for 6 AM isolates and 1 PR, and between 70 and 73% for 3 AM and 1 PR isolates. The higher capacity of detecting T. cruzi DNA was recorded in the NT group animals inoculated with the PR isolate. For AM isolates the percentages of positive PCR in the NT group varied and were higher than those observed in TBZ animals. The PCR could detect T. cruzi DNA in mice with low parasitemia, as those inoculated with AM isolates, even when treated with BZ.
Supported by:Fundação Araucária e CNPQ.

BM067 - **THE INTRA-SPECIES DIVERSITY OF LEISHMANIA BRAZILIENSIS INFECTION**

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The association between Leishmania species and different forms of disease supports the role of the genetic content of the parasite on the clinical manifestations and prognosis of infections. American Tegumentary Leishmaniasis (ATL) is characterized by a wide spectrum of clinical and immunological manifestations, which involves the skin and mucous membranes. Three different forms of ATL resulting from human infection with Leishmania braziliensis – Localized Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (ML) and Disseminated Leishmaniasis (DL) - can be simultaneously found in Corte de Pedra, an area endemic for L. braziliensis in the state of Bahia. This suggests that clinical spectrum may be caused, in part, by intra-specific variability among strains of the parasite in that area. Our hypothesis is that L. braziliensis from different clades cause distinct behaviors in infected immune cells of the host and the resulting events could be responsible for innate and adaptive responses, which would determine the immunity to the parasite and the immunopathology that leads to the clinical outcomes of ATL. We evaluate whether strains belonging to different clades of L. braziliensis of Corte de Pedra cause distinct gene expression patterns in infected macrophages. In vitro infection of human monocyte-derived macrophages from healthy donors was performed for four hours with one isolate of L. braziliensis from each of different clades - A, B and C, which are associated with DL, CL and ML, respectively – then total RNA was collected for gene expression analyses. Using DNA microarrays, we compared the global gene expression profiles among non-infected and infected macrophages and evaluate the expression levels of approximately 3,000 genes in the human genome. Our results indicate that the large majority of them are repressed at this early stage in the infected cells, and that gene expression may differ among macrophages infected with parasites of the different clades.
Supported by:NIH

BM068 - **TWO MOLECULAR TOOLS FOR DETECTION OF NATURAL AND ARTIFICIAL MIXED INFECTIONS OF TRYPANOSOMA CRUZI**

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Literature data have been shown the simultaneous presence of more than one DTU (Discrete Typing Units) of *Trypanosoma cruzi* in triatomines, wild reservoirs and humans. Concomitant occurrence of *T. cruzi* I (TcI) and *T. cruzi* II (TcII) has been more frequent. The purpose of this study was identify mixed infections in samples isolated from naturally infected triatomines and artificial mixtures constituted from forms of acellular culture. Nine strains isolated from triatomines (*Triatoma sordida* and *Panstrongylus megistus*), and seven of artificial mixtures containing TcI and TcII in different proportions (10, 25, 50, 75 and 90%) were evaluated by analysis of ribosomal RNA gene 24Sα (rRNA) and multiplex PCR that differentiates *T. cruzi* of *T. rangeli*. Band patterns of these samples were compared with reference samples: Sylvio (TcI) and Esmeraldo (TcII). The analysis of rRNA samples of triatomines showed banding patterns similar to TcII, and in artificial mixtures a strong band of TcII in all proportion, and a weak band in TcI in the proportions of TcI/TcII 90/10, 50/50 and 75/25. The analysis of multiplex PCR for the artificial mixtures showed a banding pattern consistent with that of two DTUs together, without differentiating clearly TcI and TcII, but with very different patterns of TcI and TcII individually. Isolates from triatomines showed banding patterns similar to those of the artificial mixtures. We conclude that, mixed infection in artificial mixtures was detected by rRNA analysis, when TcI was in greater proportion than TcII and that it was not effective for natural mixed populations. The multiplex PCR indicated better the presence of mixed infections in natural and artificial populations, but with unsatisfactory performance. Other molecular markers should be tested for more clearly evidence mixed populations in the studied samples and other isolates including those obtained from other hosts.
Supported by:CNPq, PPG/UEM, CAPES, PROAP-CAPES

BM069 - **CHARACTERIZATION OF TRRM2 PROTEIN IN T.CRUZI: INVOLVEMENT IN REGULATION OF GENE EXPRESSION AND STRESS RESPONSE**

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Trypanosoma cruzi, the etiological agent of Chagas' disease, is widely studied due to its medical importance and particular features that make it an alternative model for basic biological studies. Repression of messenger RNAs in cytoplasmic granules composed of mRNA-protein (mRNP) complexes is an important pathway of posttranscriptional regulation in eukaryotes, and it was recently shown that mRNA granules are present in *T. cruzi*. TIA1/TIAR are proteins involved in splicing, apoptosis and post transcriptional regulation, and play an important role in eukaryote´s stress response, since under stress conditions they act in the assembly of cytoplasmic stress granules. TRRM2, a *T. cruzi* protein identified by sequence similarity with human TIA proteins, was chosen for this study. Preliminar results showed that this protein is expressed throughout the parasite's life cycle, and that it is conserved among trypanosomatids. This protein shows a mainly nuclear localization, and sodium arsenite treatment showed that TRRM2 does not have the distribution pattern of a canonical TIA protein, since the formation of TIA like stress granules is not observed. However, it is interesting noticing that, SOLiD™ sequencing results showed that TRRM2 binds to different subsets of mRNAs under stress conditions, when compared with normally grown epimastigotes These results suggest that in *T. cruzi* TIA like proteins may be involved in different stress response pathways, not involving stress granules. The characterization of this protein can help to elucidate the mechanisms of posttranscriptional regulation in T. cruzi.
Supported by:CNPQ / FIOCRUZ

BM070 - **COMPARATIVE PROTEOMIC ANALYSIS OF POPULATIONS OF LEISHMANIA INFANTUM CHAGASI SUSCEPTIBLE AND RESISTANT TO POTASSIUM ANTIMONY TARTRATE.**

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Recently, we selected in vitro populations of *L. infantum chagasi* that are 4-fold more resistant to potassium antimony tartrate Sb III, LcSbR than theirs susceptible counterparts LcWTS (Liarte & Murta, 2010). In the present study, proteomic analysis of *L.infantum chagasi* susceptible (WTS) and SbIII-resistant (SbR) populations was performed using two-dimensional gel electrophoresis (2-DE). For each pair of samples, proteins from three independent experiments were obtained, and gels in triplicate were analyzed for each cultivate. The differential expression analysis was performed comparing the quantity of matched spots in each pair of sample. A protein was considered differentially expressed when the ratio between the intensities of S (susceptible) and R (resistant) spots were ≥ 2-fold. The spots were submitted to mass spectrometry (LC-MS-MS) by using a quadrupole time-of-flight analysis for protein identification. Tandem mass spectra were searched against the NBCI nonredundant database using the MASCOT algorithm (http://www.matrixscience.com) or MS-Fit algorithm against the Swiss Prot database. Analysis of the gel images showed that *L.infantum chagasi* presented 28 and 94 spots more expressed in the susceptible and resistant populations, respectively. Out of the 103 spots analyzed through MS, 55 were identified as being 11 overexpressed in susceptible and 44 in Sb-resistant populations. Among the proteins identified in Sb-resistant population LcSbR, 24% are involved in protein metabolism, synthesis and degradation; 16.4% are proteins related to energy metabolism; 17% are heat shock proteins and antioxidant enzymes; 10% are associated with cytoskeleton and 4% correspond to hypothetical proteins. Functional annotation data show a decreased expression of proteins associated with DNA synthesis and transcription. Further studies will be performed in order to better investigate the role of some these proteins in the drug-resistance phenotype in Leishmania infantum chagasi.
Supported by:CNPq, PDTIS, FAPEMIG, CPqRR, UNIMONTES and UNICEF/UNDP/World Bank/WHO/TDR

BM071 - **DEVELOPMENT OF A DUAL REPORTER SYSTEM TO IDENTIFY REGULATORY CIS-ACTING ELEMENTS IN UNTRANSLATED REGIONS OF TRYPANOSOMA CRUZI MRNAS.**

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In trypanosomatids, transcription is polycistronic and gene expression control occurs mainly at the post-transcriptional level. To investigate the role of sequences present in the 3'UTR of stage-specific mRNAs of *Trypanosoma cruzi*, we generated a new vector, named pTcDUALuc, containing the firefly and Renilla luciferase reporter genes. To test this vector, sequences derived from the 3'UTR plus intergenic regions of the alpha tubulin gene, which is up-regulated in epimastigotes, and amastin, which is up-regulated in amastigotes, were inserted downstream from the firefly reporter gene and luciferase activity was compared in transient and stable transfected parasites. As expected, increased luciferase activity was detected in epimastigotes transiently transfected with pTcDUALuc containing tubulin sequences. Using stable transfected cell lines that were allowed to differentiate into amastigotes, we observed increased luciferase activity and mRNA levels in amastigotes transfected with pTcDUALuc containing amastin sequences. We also showed that the spliced leader sequence and poly-A tail were inserted in the predicted sites of the firefly luciferase mRNA and that deletions in the alpha tubulin 3'UTR resulted in decreased luciferase expression because it affects polyadenylation. In contrast to the constructs containing 3'UTR sequences derived from tubulin and amastin genes, the presence of the 3'UTR from a trans-sialidase gene, whose expression is higher in trypomastigotes, resulted in increased luciferase activity in trypomastigotes without a corresponding increase in luciferase mRNA levels.
Supported by:CNPq/CAPES/FAPEMIG/HHMI

BM072 - **OXIDATIVE STRESS AND DNA LESIONS: ROLE ON TRYPANOSOMA CRUZI CELL VIABILITY**

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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, G-C to T-A transversion mutations can occur, which makes this lesion particularly deleterious. All organisms evolved repair mechanisms to deal with 8-oxoG. In higher eukaryotes, the enzymes OGG1, MutY and MutT constitute the 8-oxoG repair pathway. Trypanosoma cruzi needs to deal with various oxidative stress situations that it is exposed to, such as the mammalian intracellular environment and the triatomine insect gut where it replicates. We focus on the MutT enzyme, which is responsible to remove the 8-oxoG from the nucleotide pool. To investigate the importance of the 8-oxoG during parasite infection of mammalian cells and development in triatomine, two T. cruzi strains are being tested: wild type CL Brener clone, as well as CL Brener overexpressing the Escherichia coli MutT enzyme. This enzyme was cloned into pROCK expression plasmid and subsequently transfected in T. cruzi CL Brener epimastigotes. Afterwards, the epimastigotes were differentiated into infective metacyclic trypomastigote forms and maintained in tissue culture cells (Tissue Culture Trypomastigotes forms). Wild type and mutant TCT infectivity, as well as intracellular replication were tested by infection assays in murine fibroblasts. The mutant strain showed statistically significant increased growth after 48 hours of infection in fibroblasts compared to wild type cells, indicating that E. coli MutT enzyme overexpression can confer an advantage to T. cruzi during parasite intracellular replication. These T. cruzi strains are also being investigated in the insect vector and animal models.
Supported by:CNPq, FAPEMIG, HHMI

BM073 - **MULTIPLEX PCR DESIGN FOR THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS**

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Leishmaniasis caused by protozoan from the genus Leishmania, affects mainly people of low socioeconomic status from tropical and subtropical countries. Leishmaniasis are classified into three clinical manifestations: visceral, cutaneous and mucocutaneous. There are several species associated to the etiology of the Cutaneous Leishmaniasis, including Leishmania major, L. infantum and L. braziliensis. Although L. infantum is more associated to visceral leishmaniasis, it can also cause cutaneous manifestations in immune suppressed patients. Here we have performed an in silico analysis searching for species-specific microssatellites in the genomes of L. major, L. braziliensis and L. infantum to design set of primers for genotyping using Multiplex PCR. Eight pairs of primers were designed to amplify specie-specific microsatellites. All primers were checked for specificity by e-PCR. Based on in silico analysis, there was no amplification product of different size than expected in the three genomes. So far, three pair of primers were tested by PCR using as template the corresponding genomic DNA. Additional bands were detected in each system and it is likely due to the polymorphism in the alleles target 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 allow us to discriminate genomic DNA samples from the three species. After optimization of PCR conditions, the best primer combinations for each species will be applied in multiplex PCR's 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, which would enable a better prognosis and treatment of the patients.
Supported by:CNPq; FAPEMIG; CAPES

BM074 - **MAPPING OF MURINE LOCI PARTICIPATING IN MOUSE RESISTANCE TO INFECTION BY TRYPANOSOMA CRUZI**

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The use of forward genetic approaches offers an unbiased strategy to reveal unknown factors influencing the host-pathogen relationship. In this sense, murine models for whole genome screenings are invaluable tools for genetic investigations. For instance, differences in susceptibility to a pathogen between two mice strains may be explored using informative intercrossings between them and a strategy of Quantitative Trait Loci (QTL) analysis. We first show that A/J mice are susceptible to infection by Y strain of Trypanosoma cruzi as compared to the C57BL/6 strain. Therefore, we used an informative F2 population, (by intercrossing C57BL/6 and A/J mice), to map possible loci responsible for susceptibility to infection by Y strain of T. cruzi in mice. We analyzed survival and parasitemia on days 7, 9, 11 and 13 after infection to characterize the susceptibility. Genetic markers targeting microsatellite regions for each chromosome were used for genotyping samples. The correlation between genotype and phenotype was made using LOD score. We show that there is a putative QTL on chromosome 15 (LOD score 2.6), according to survival data. Mantel-Cox test showed differences between the survivals of the group that inherited parental A/J chromosome, with a shorter survival compared to the group inheriting a chromosome from C57BL/6 ($P < 0.01$). It was also observed differences related to inheritance of chromosome 7, with A/J chromosome reducing mice survival ($P = 0.02$). Albeit observed differences in survival between males and females, there is no evidence of a QTL on chromosome X. The data related to the average of parasitemia in the F2 population was also analyzed. However, we did not detected significant differences and did not identify genetic components accounting for control of parasite replication in the blood. From these results, we can speculate the existence of new genetic factors driving the host resistance in a lethal model of T. cruzi infection. Identification of such factors may provide relevant information for the understanding of host/pathogen interaction.
Supported by:FAPESP

BM075 - **MOLECULAR DIAGNOSIS USED IN AREAS OF AUTOCHTONOUS MALARIA IN THE STATE OF SAO PAULO AS A STRATEGY FOR RAPID AND ACCURATE DETECTION OF OUTBREAKS.**

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Introduction: Although not considered endemic for malaria, the region outside the Amazon basin reported 2,023 autochthonous cases from 1999 to 2007, with 88% occurring in the states of Parana, Sao Paulo and Espirito Santo. This scenario requires continuous surveillance, using appropriate tools for early detection and treatment, preventing the spread of outbreaks in receptive areas. Objectives: This study aimed to process samples of residents in area of autochthonous cases, using molecular methods for rapid and accurate detection of *Plasmodium*. Methods: We analyzed 100 samples collected in Bertioga, Ilhabela, São Sebastião and Ubatuba, located in Atlantic Forest of São Paulo. All samples were assayed by thick blood smear (TBS) for detection of parasites. DNA was extracted from whole blood with kit Invisorb ® (Invitek, Berlin) and from blood collected on filter paper with Chelex ® 100. Samples were processed by Real Time PCR (RT-PCR) with genus-specific primers for detection of *Plasmodium*. Positive samples were amplified by nested PCR (N-PCR) to determine the species. Results: According to TBS method, 10 samples were positive. TBS was positive in 12 samples while RT-PCR showed amplification in 23. N-PCR was not able to amplify all samples positive by RT-PCR. The extraction from whole blood was better than from filter paper, because of the low parasitemia profile of that area. The results show the need for revision of diagnostic tools in areas of low malaria transmission, since in this region asymptomatic cases are described. Malaria control programs consider the search for febrile individuals tested by TBS the main strategy in non-endemic areas. However, our results pointed out that molecular diagnosis including asymptomatic individuals should be applied in areas of autochthonous cases outside the Amazon region, as part of control programs, aiming the monitoring of outbreaks with more sensitive tools as RT-PCR, able to be used in large-scale sample processing.
Supported by:SUCEN

BM076 - **RNA GRANULES: THE ROLE OF TCDHH1 ON THE STABILITY AND TRANSLATION OF TARGET MRNAS IN TRYPANOSOMA CRUZI**

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In trypanosomatids, gene expression is regulated mainly post-transcriptionally by mechanisms involving changes in mRNA stability or access to polysomes. These mechanisms are important to the fast adaptations necessary for the parasite's survival in different environmental conditions of its life cycle. Recent studies have demonstrated that cytoplasmic RNA granules, present in several eukaryotes, have key role in regulation of gene expression. These granules are involved in mRNA sorting, storage and degradation and can be divided in different classes, including P-bodies and stress granules. P-bodies are sites where mRNA can be decapped and 5'-3' degraded or stored for subsequent return to polysomes. The highly conserved DEAD-box helicase Dhh1p is a marker protein of P-body functions. Defined cytoplasmic foci containing non-translating mRNPs and a homologue of Dhh1p have been described recently by our group in *T. cruzi.* TcDhh1 expression is not regulated through the parasite life cycle or under stress conditions. This protein is present in polysome-independent complexes and is localized to discrete cytoplasmic foci, resembling P-bodies. Several mRNA targets of TcDhh1p were identified by ribonomic microarray and confirmed by RT-PCR. These transcripts codify mainly proteins that are regulated in a stage-specific manner. These data indicate an important role of TcDhh1 granules in mRNA metabolism but don't confirm their actual function. The main focus of this study is to unravel if TcDhh1 granules have a major role on storage (similar to stress granule) or on degradation of transcripts (similar to P-bodies). In order to get further insides in TcDhh1 function, we are currently analyzing changes of target localization by FISH associated with TcDhh1 immunofluorescence, during *T.cruzi* differentiation and after drug treatments that block translation. In addition, we intend to estimate the half lives of target mRNAs and evaluate if the association with TcDhh1 granules interferes with the stability of mRNAs. Altogether, these data will elucidate the role of TcDhh1 granules and enhance the knowledge on the mechanisms of regulation of gene expression of the parasite.
Supported by:CNPq, Fiocruz, CAPES

BM077 - **FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION OF AN UDP-N-ACETYLGLUCOSAMINE TRANSPORTER IN TRYPANOSOMA CRUZI**

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Glycoconjugates play important roles in basic biological processes such as the development of multicellular organisms including plants and humans. In addition, the survival and infectivity of parasites such as Candida spp and Leishmania spp require these molecules. The synthesis of glycoconjugates occurs in the lumen of Endoplasmic Reticulum (ER) and Golgi apparatus, where they become glycosylated by glycosyltransferases, which use nucleotide-sugars as substrates. However, nucleotide-sugars are mostly synthesized in the cytoplasm and therefore must be transported into the lumen of the above organelles. This intracellular transport is crucial for glycosylation and it is mediated by specific transporters named Nucleotide-Sugar Transporters (NSTs). These are highly hydrophobic proteins displaying 6 to 10 transmembrane domains with the N- and C-termini facing the cytoplasm. In order to indentify and functionally characterize NSTs from trypanosomatid parasites we are using Trypanosoma cruzi, the etiologic agent of Chagas` disease, as a model organism. By perfoming Blastp searches we have identified 11 putative NSTs in the T. cruzi genome. Heterologous expression of these genes in a Kluyveromyces lactis mutant strain, deficient in UDP-N acetylglucosamine (UDP-GlcNAc) transport, revealed one gene able to rescue the wild type phenotype of the yeast cells, as evidenced by flow cytometry analysis. We are currently performing *in vivo* complementation and *in vitro* uptake assays to determine the kinetics parameters of the UDP-GlcNAc transport and to assess in more detail the substrate specificity of the transporter (named TcNST1). Expression of the gene during the parasite life cycle and metacyclogenesis – the differentiation of epimastigote forms into infective metacyclic trypomastigotes - is also being analyzed. The study of NSTs in trypanosomatids is relevant for a better understanding of glyconconjugates` biosynthesis and their importance in the life cycle and infectivity of these parasites.
Supported by:Fiocruz and CNPq

BM078 - **DEVELOPMENT OF A HOMOLOGOUS, IN VIVO PROTEIN-PROTEIN INTERACTION SYSTEM FOR TRYPANOSOMA CRUZI.**

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Functional genomics of Trypanosoma cruzi is a useful approach for studying complex molecular systems. Besides transcriptomics and proteomics analyses, we are studying networks of protein interactions. A comprehensive study of protein-protein interaction (PPI) networks in T. cruzi will be useful for functional characterization of proteins, also providing a framework for understanding the biology of this parasite as an integrated system. We are using yeast two-hybrid (Y2H) screens to study PPIs in T. cruzi, but it detects PPIs under heterologous conditions. Thus, our goal is to create a homologous in vivo system for PPI detection to complement the data obtained by Y2H. We are developing protein-fragment complementation assays based on two techniques: bimolecular fluorescence complementation (BIFC) and bimolecular luminescence complementation (BiLC). The first assay consists of structural complementation of two fragments of a fluorescent protein fused to a pair of interacting proteins, to investigate the formation of protein complexes and the subcellular localization of PPIs in vivo in T. cruzi. However, fluorescent proteins must be expressed at high levels to assure that signal is above background cellular fluorescence, being very laborious for large-scale screens. On the other hand, BiLC is based on luciferase complementation, being more sensitive and suitable to high-throughput analyses. The 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. For both approaches, two vectors containing different antibiotic resistance were modified to express the N- or C- terminus fragments of YFP or hGluc upstream the cloning site. We are currently selecting interacting candidates to test and validate these approaches. Our ultimate goal is to combine the Y2H system, BiFC and BiLC to obtain a comprehensive T. cruzi PPI network.
Supported by:CNPq

BM079 - **DEVELOPMENT OF A SYSTEM FOR HETEROLOGOUS EXPRESSION IN CRITHIDIA DEANEI, A NON PATHOGENIC TRYPANOSOMATID.**

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The Trypanosomatidae family comprises ancient parasitic protozoa with peculiar genetic features and also causes severe human diseases, such as Chagas disease, sleeping sickness, and leishmaniasis. Drug development against these diseases depends on genetic manipulation and protein expression in these protozoa. However, biosafety and time consuming methods are needed when manipulating these parasites, which represents a considerable difficulty during this process. For these reasons, we propose the construction of a heterologous expression system for eukaryotes in *C.deanei*, a monoxenic protozoan which harbors an endosymbiont bacterium. This protozoan grows in minimal medium to high cell density, represents no risk of infection for mammals, and is also able to realize posttranslational modification of target proteins, such as glycosylation. We have determined *C.deanei* drug sensibility to G418 and hygromycin as 0.25 mg/mL and 1.0 mg/mL in LIT medium, respectively. The G418 inhibitory concentration for *C.deane*i is similar to that used in *Trypanosoma cruzi*, however the Hygromicin dose is around 10 fold higher than that used with other trypanosomatids. We also tested the ability of *C.deanei* to be transfected with *T.cruzi* and *T.brucei* vectors, such as pTREX, pROCK and pLEW13, using standard electroporation protocols. This approach was not able to generate stable transfectants using these vectors. For this reason, a vector called pCdEX was constructed using *C.deanei* inter coding sequence (inter-CDS). The pCdEX vectors contain actin and GAPDH inter-CDS to regulate the GFP expression and alfa-tubulin sequences for processing of Neomycin resistance mRNA and vector integration. The vector evaluations are underway. Supported by Fundação Araucária, CNPq, and PPSUS.
Supported by:Fundação Araucária, CNPq, PPSUS

BM080 - **PROTEOMIC ANALYSIS OF LEISHMANIA (VIANNIA) BRAZILIENSIS RESISTANT TO POTASSIUM ANTIMONY TARTRATE**

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In this study we analyzed proteins differentially expressed in populations of *Leishmania braziliensis* susceptible (LbWTS) and resistant to potassium antimony tartrate SbIII (LbSbR) using two-dimensional gel electrophoresis followed by mass spectrometry (MS) for protein identification. These LbSbR parasites are 20-fold more resistant than its susceptible LbWTS. The differential expression analysis was performed comparing the quantity of matched spots in each pair of sample. A protein was considered differentially expressed when the ratio between the intensities of susceptible and resistant spots were ≥ 2-fold. Analysis of the gel images showed that *L. braziliensis* presented 98 and 30 spots more expressed in the susceptible and resistant populations, respectively. Out of the 92 spots analyzed through MS, 48 were identified as being 24 overexpressed in susceptible and 24 in Sb-resistant populations. Interestingly, a significant number of heat shock proteins (HSPs) and antioxidant enzymes are overexpressed in Sb-resistant population LbSbR. On the other hand, proteins associated with translation and cytoskeleton (actin, paraflagellar rod and beta tubulin) are increased in the susceptible parental parasite LbWTS. In addition, cyclofilin was identified as having reduced protein expression level in Sb-resistant LbSbR population. In order to confirm this result, protein expression was determined by western blot using polyclonal antibody raised against the TcCyP recombinant protein from *Trypanosoma cruzi*. Alignment analysis between the amino acid sequences this protein from *T. cruzi* showed 81% of identity with sequences from *L. braziliensis*. The antibody anti-TcCyP recognized a 19 kDa peptide in both *Leishmania* populations. The level of expression of this native protein in the LbSbR was 2-fold lower than in the LbWTS population. Studies are being performing to determine whether overexpressing LbCyP in the resistant population will confer antimony-resistant to these parasites.
Supported by:CNPq, PDTIS, FAPEMIG, CPqRR, UNIMONTES and UNICEF/UNDP/World Bank/WHO/TDR

BM081 - **CHARACTERISATION OF THE PUTATIVE SIALOGLYCO METALLOPEPTIDASE-LIKE GENE OF LEISHMANIA MAJOR**

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Peptidases of *Leishmania* are important virulence factors. The surface metallo peptidase GP63 contributes to the parasite-macrophage interaction. In the genome of *Leishmania major*, genes encoding putative metallopeptidases distributed in 16 families were identified. Apart from GP63 family, there are very few studies on the biological roles of metallopeptidases in *Leishmania*. We identified in *L. major* a single copy gene with similarity to the M22-family sialoglyco metallopeptidase (OSGEP) of *Pasteurella haemolytica*. Bacterial OSGEPs specifically hydrolyse peptide bonds between O-glycosylated sialylated aminoacids and process surface antigens of leukocytes such as CD34, CD43, CD44 and CD45, which are important for immunity in mammals. Other OSEGP-like proteins are found in bacteria and in eukaryotes. Among those, the bacterial Kae-1 putative peptidase shares 34% sequence identity with OSGEP, but does not have proteolytic activity. Kae-1 is an atypical DNA interacting protein that is important in the maintenance of genome integrity. In yeast, Kae is associated with the KEOPS complex, which is essential for telomere enlongation and for the transcription of essential genes. We set out to characterise the OSGEP of *L. major*. The gene encoding OSGEP was cloned by PCR using specific primers and genomic DNA as a template. *L. major* OSGEP showed 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. We expressed recombinant *L. major* OSGEP as fusion with a domain of thioredoxin and an N-terminus histidine tag and purified it in Ni-agarose resins. The protein was used to immunize BALBc mice to obtain the OSGEP anti-serum for studies of sub-cellular localisation. The generation of *L. major* lines overexpressing OSGEP is in progress.
Supported by:CNPq

BM082 - **LEISHMANIA (VIANNIA) BRAZILIENSIS TRANSCRIPTOME BY RNA-SEQ: NEW INSIGHTS ABOUT ADDITION OF POLY(A) AND POLY(A)-RICH TAILS IN RNA MOLECULES AND POSSIBLE RELATIONSHIP WITH POST-TRANSCRIPTIONAL GENE REGULATION AND PARASITE VIRULENCE.**

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Leishmaniasis represents an important global health problem for which there is no vaccine and few drugs are available. *Leishmania* and other Trypanosomatidae family members diverged early in eukaryotic evolution and consequently display unique cellular properties. Their apparent lack of transcriptional regulation is compensated by complex post-transcriptional control mechanisms, including the processing of polycistronic transcripts by means of coupled trans-splicing and poly-adenylation. Using high-throughput RNA-Seq (454/Roche) we have surveyed the transcriptomes from two life cycle stages, the infective metacyclic and the non infective procyclic forms of two *Leishmania (Viannia) braziliensis* isolates: ET, a strain with low virulence index and NSL, presenting higher virulence levels. Our results showed for the first time in *Leishmania* evidence of hetero- and homopolymeric poly-adenylation tracts truncating RNA molecules (rRNAs and mRNAs). It is known that poly-adenylation can play two opposite roles: site-specific stable poly-adenylation of mature RNA 3´-ends contributes to nuclear export, translation initiation and transcript longevity. Conversely, transient internal poly-adenylation targets RNA molecules to rapid exonucleolytic degradation. Interestingly, we observed higher frequency of internal poly(A)-tails truncating ribosomal RNA molecules in metacyclic forms from NSL than from ET. The observed poly(A)-truncated coding mRNAs in NSL and ET strains were quite distinct and include a transcription factor in metacyclic NSL strain. These results could be related to an eventually lower general protein translation rate in NSL strain and with its increased virulence. The internal poly(A)-tailed RNA truncation phenomenon can open new fields on post-transcriptional gene expression regulation in *Leishmania* and related organisms. It adds further complexity to the paradigm that almost the entire trypanosomatid genome is constitutively transcribed, in that the integrity of messages could play an important role in regulating the pool of transcripts that are effectively accumulated. Deciphering the heterogeneity in processed RNA molecules could potentially contribute to understanding the survival and success of the parasite population in the insect vector and in the mammalian host.
Supported by:FINEP/MCT; CAPES; FAPESP; CNPq

BM083 - **EXPRESSION AND PURIFICATION OF RECOMBINANT UROCANATE HYDRATASE FROM TRYPANOSOMA CRUZI**

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In many organisms, the amino acid L-histidine is involved in antioxidant and antiinflammatory processes, acting like a scavenger of hydroxyl radical and singlet oxygen. In addition, L-histidine was demonstrated to coordinate itself to some metal ions and, in high concentrations, modulate their absorption, thereby causing the decrease of their toxicity and the inhibition of the formation of products from the lipid peroxidation. L-histidine also participates in the formation of Ovotiol A, which is present in the mammalian stages of the life cycle of *Trypanosoma cruzi*. In spite of its participation in the mentioned process, it is interesting that histidine metabolism has not been studied yet in *T. cruzi*. The putative genes for the enzymes in the histidine - glutamate pathway were indentified in the *T. cruzi* genome database supporting the hypothesis that *T. cruzi* converts L-histidine into glutamate through a pathway involving four enzymes. The first enzyme, Histidine Ammonio Lyase (EC 4.3.1.3), is being characterized by our group. In the present work, we describe the experimental conditions for the expression and purification of the second enzyme, Urocanate Hydratase (EC 4.2.1.49), which will be used to determine its biochemical characteristics. The recombinant *Tc*UH was expressed in *Escherichia coli* BL21 Codon Plus (pGro7) strain using pET28a vector. The apparent molecular weight for *Tc*UH fused with a 6-histidine tag (N-terminus), was of 71.6 kDa as verified by SDS-PAGE. The recombinant protein was purified by affinity chromatography using NTA-Ni2+ resin. The kinetic characterization will be done, as well as the obtainment of specific antibodies against *Tc*UH for analysis of its expression and localization in *T. cruzi* by immunofluorescence.
Supported by:FAPESP, INBEQMEDI, CNPq and USP

BM084 - **PROTOZOADB 2.0: A DEDICATED DATABASE FOR STORAGE AND ANALYSIS OF PROTOZOAN GENOMES**

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One of the big problems in biology today is the storage and analysis of large amount of data that has been generated by the new generation of sequencers. We propose in this new version of ProtozoaDB (http://protozoadb.biowebdb.org) the use of technologies for Web services to integrate many public databases and allow a more comprehensive analysis of information related protozoa. ProtozoaDB is being developed to host both genomics and post-genomics data from Plasmodium, Entamoeba, Trypanosoma, Leishmania, Giardia, Theileria, Toxoplasma, Trichomonas and Cryptosporidium species, soon hosting other protozoan species genomes. Datasets publicly available at GenBank were semi-automatically analyzed and loaded into ProtozoaDB. All analyses results are available via AJAX-supported web pages linked to several external databases, allowing analysis and information download in a unique interface.
The new 2.0 version is made available containing data from twenty-two genomes of pathogenic protozoan species. Data from 193,559 genes and 218,100 protozoan proteins were analyzed and stored. Preliminary analysis includes the identification of orthologous groups with OrthoMCL and OrthoSearch systems. Among the several analysis options using Web services are: Conserved Domain Database, Superfamily, Human Proteome, PDB, KEGG and PubMed. Proteins can be retrieved based via many query fields (GI, accession number, keyword, organism, sequence type, and motif), also allowing queries based on BLAST similarity search. As preliminary results we have achieved together in a single interface many informations about protozoa.
Supported by CNPq, CAPES, FINEP, FIOCRUZ.

BM085 - **PHENOTYPIC CHARACTERISATION AND DIFFERENTIATION CAPACITY OF TRYPANOSOMA CRUZI EPIMASTIGOTES OVEREXPRESSING THE UDP-GLCNAC:THR-POLYPEPTIDE O-ALPHA-N-ACETYL-D-GLUCOSAMINYLTRANSFERASE (PPALPHAGLCNACT) TCOGNT2**

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Cells from all life cycle stages of *T. cruzi* are covered by highly O-glycosylated mucin-like glycoproteins. Biosynthesis of each O-glycan starts with the addition of alphaGlcNAc to Thr, a reaction catalyzed by a Golgi ppalphaGlcNAcT. Previously, we identified three genes encoding putative ppalphaGlcNAcTs in *T. cruzi* (TcOGNT-1, -2 and -L) and, after heterologous expression in *Leishmania tarentolae*, TcOGNT2 was proven a 'bona fide' ppalphaGlcNAcT. We also observed that TcOGNT2 is transcribed into a mature mRNA in epimastigotes with at least three 5'-UTR trans-splicing sites. We now show that mouse polyclonal antibodies, raised against recombinant TcOGNT2cat expressed in *E. coli*, are able to recognize the epimastigotes' endogenous ppalphaGlcNAcT by Western blotting (WB) and immunofluorescence, and that the protein is most likely associated with the Golgi. As a first attempt to test the importance of the enzyme in O-glycan biosynthesis and biology of *T. cruzi*, we overexpressed catalytically active (with 'DSH'-motif) and inactive (with 'ASH'-, 'NSH'- or 'DSD'-motif), c-myc-tagged versions of TcOGNT2 in epimastigotes using the pTEX vector. Initial analysis showed 10-fold higher levels of normal or mutant TcOGNT2 protein that was associated with microsomal membranes after differential centrifugation. Microsomes from cells overexpressing active TcOGNT2 exhibited 1.5x higher ppalphaGlcNAcT activity than equivalent fractions from controls, and 5-fold higher UDP-[3H]GlcNAc hydrolysis activity, signifying a 9:1 ratio of hydrolysis:transferase activity of the expressed relative to endogenous activity. WB using monoclonal antibodies showed no changes in quantity or size of parasites mucins, and flow cytometry analysis using fluorescent lectins showed unaltered binding of ConA and WGA, except for a 15% reduction in GS-IB4 binding that was only observed in parasites overexpressing TcOGNT2. Despite no difference in growth, parasites overexpressing wild-type or mutant TcOGNT2 exhibited strongly compromised metacyclogenesis, either in TAU-P medium or in Rhodnius prolixus. Thus overall Golgi protein burden may be a factor that regulates the ability of cells to differentiate under *in vitro* and *in vivo* conditions.

Supported by:CNPq, FAPERJ, NIH-FIRCA

BM086 - **FUNCTIONAL GENOMICS OF Δ-AMASTINS FROM TRYPANOSOMA CRUZI**

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There are few reports describing functional characterization of surface proteins of T.cruzi amastigotes. Therefore, studies regarding the membrane compounds are relevant because they vary during its life cycle to adapt to the host environments. Amastigotes differentially express on its surface a glycoprotein called δ-amastins, which may play a role on parasite virulence (unpublished results). The amastins have about 180 amino acids with 4 conserved transmembrane regions (including a probable signal peptide), and several potential O-glycosylation sites. Recently, the amastins have been stratified in 5 subfamilies, in which T.cruzi CL Brener strain contains 3 subfamilies (β, δ and pδ-amastins) encoded by 6 genes. Thus, to study the amastin function we decided to overerexpress δ-amastins containing potential dominant negative mutations, since it is encoded by multi copy genes and the RNAi is absent in T.cruzi. After sequence alignment of T.cruzi amastins, 5 threonines (37, 40, 44, 55 and 60) and 4 cysteines (42, 52, 68 and 139) residues were identified as candidates for mutations. Here, we generated constructions carrying mutated δ-amastins (5mT: T37A, T40A, T44A, T55A and T60A or 1mC: C42A) in fusion at GFP N-terminal using pTREXnGFP as a parental vector. In parallel, two truncated amastins were fused to GFP, one containing the probable signal peptide plus hydrophilic region (AmaSPGFP), and another carrying two hydrophobic regions (position 1 to 107: AmaBGFP). All constructs were electroporated into epimastigotes and analyzed by fluorescence microscopy. The subcellular location of amastin mutants 95MT AND 1mC) fused to GFP were similar to the wild type form. On the other hand, the AmasSPGFP and AmaBGFP were targeted to perinuclear localization, or in spherical organelles, respectively. Refined analysis of subcellular location by confocal microscopy and phenotypic changes will be performed using parasites expressing stably the fusion proteins constructed here.

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BM087 - **COMPARATIVE GENOMICS OF TWENTY-TWO PATHOGENIC PROTOZOA**

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Protozoa is the common name given to unicellular eukaryotic organisms, and comprises about 80,000 species, showing an extremely diversity and variety. Most species are free-living and only a few are pathogenic. While comparative studies among Protozoa can help to identify similarities and differences at the genomic level, identifying and studying their homologous genes (orthologs and paralogs) can help to figure out which genes are shared between these species, and which ones are specific to each organism, then improving our understanding of the biology of each of these species. For this study, *Plasmodium*, *Entamoeba*, *Trypanosoma*, *Leishmania*, *Giardia*, *Theileria*, *Toxoplasma*, *Trichomonas* and *Cryptosporidium* species, totalizing 346,468 proteins in 22 proteomes, were submitted to the program OrthoMCL to infer the relationship between these proteins. As results, 41,889 homologs groups (20,507 paralogs and 21,382 orthologs) were obtained, of these, 251 groups of orthologous proteins are shared by all the 22 organisms analyzed. Most of these proteins, representing the core proteome of Protozoa, belong to the functional category called “J” (Translation, ribosomal structure and biogenesis) according to KOG/NCBI, followed by the categories “O” (Posttranslational modification, protein turnover, chaperones) and “A” (RNA processing and modification), which is in agreement to the work of Ciccarelli et al (2006), that mapped orthologous genes common to all sequenced species, and called them Universal Orthologous Genes (UOG). Similarity analysis of the Protozoa core proteome (251 orthologous proteins) against prokaryote (COG) and eukaryote (KOG) orthologous groups showed that 85.6% is more similar to KOG proteins, 14% to COG proteins and 0,4% is equally similar to KOG and COG proteins. We are now performing phylogenomics analysis of the 22 Protozoa in order to obtain a more complete Protozoan species tree based on the methodology described by Dávila & Ocaña (2011).
Supported by:CNPq, CAPES, FAPERJ, FINEP, FIOCRUZ

BM088 - **TRYPANOSOMA CRUZI ORFEOME PROJECT V. 1.0**

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We have build a comprehensive collection of *Trypanosoma cruzi* protein coding regions in a suitable vector for high-throughput analyses, which is denominated an ORFeome. All 23,216 protein coding genes of *T. cruzi* were clustered with the MCL software, using specific criteria. The resulting 9,959 groups were called supergenes and were selected for inclusion in the ORFeome based on several rules (complete CDS, absence of pseudogenes, complexity of the coding region for hypothetical proteins etc). Nucleotide, protein and orthologous (*T. brucei*, *L. major*, *L. Infantum* and *L. braziliensis*) sequences were aligned and scrutinized to define the most probable start codon for each supergene. We have created a script to automatic design of primers pairs for more than 8,000 supergenes, in order to clone them in pDONR 221 vector (Gateway® platform). After high-fidelity PCR amplification from *T. cruzi* Dm28c strain DNA and bacterial transformation, we have selected four clones for each amplicon, totalling >32,000 elements in the ORFeome library. An important step in the construction of an ORFeome is sequence validation, which is both time and money consuming. We have used next generation sequencing, based in the SOLiD platform, to ameliorate this. Seven distinct pools of *T. cruzi* ORFeome clones were sequenced, comprising 512 clones from 128 supergenes, using 1/8 of a slide. We were able to identify the coding sequence for the majority of these clones, in a cheaper and faster way than traditional Sanger sequencing, validating our clones and also characterizing the variability between CL Brener and Dm28c strains. This ORFeome represents an excellent tool for high-througput functional characterization of *T. cruzi* genes and is being used in distinct projects of our group. We have also created a website to disponibilize the ORFeome data, as well as other datasets, to the whole community interested in *T. cruzi*.
Supported by:CNPq, FIOCRUZ.

BM089 - **TRYPANOSOMA CRUZI PHYLOGENETIC RECONSTRUTION BASED ON THE MITOCHONDRIAL GENES CYTB, COII AND ND1**

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Chagas Disease, a tropical disease caused by the protozoan *Trypanosoma cruzi*, still is a serious public health problem affecting 10 million individuals and causing 14,000 deaths per year in Latin America. *T. cruzi* presents extensive intra-specific variability and is classified into six different DTUs (TcI-TcVI). Despite important advances in biological and molecular characterization of these parasites the phylogenetic relationships among the different lineages are not completely understood. For example, it is unclear how many and which are ancestral DTUs and how many are hybrids. While one hypothesis postulates the existence of only two ancestral DTUs from which all the others were originated by hybridization events, the other postulates the existence of at least three ancestral DTUs. Herein, we addressed this question by analyzing DNA sequences of three mitochondrial genes (CYTB, COII and ND1) available on GeneBank of 16 *T.cruzi* strains belonging to the six different DTUs. Phylogenetic trees were inferred by both neighbor joining (Phylip) and maximum likelihood (Treepuzzle) methods using the three genes separately or in combination. The robustness of the tree topologies was evaluated by bootstrap analysis using 1000 replicates. *T. brucei*, *Leishmania tarentolae*, *L. major* and *T. vespertilionis* were used as out groups to root the trees. No identical tree was observed from our analyses. Modification in both the phylogenetic reconstruction methods and the analyzed gene produced significant alterations in the tree topologies. Despite these differences, however, the three previously identified mitochondrial clades (A, B and C) could be clearly recognized in all topologies confirming the existence of three completely distinct and non related mitochondrial haplotypes. However, until now is not possible to identify the most ancient mitochondrial haplotype since different haplotypes were set as the most ancient depending on the marker and the method of analysis used.
Supported by:FAPEMIG, CNPq and CAPES

BM090 - **POLYMERASE CHAIN REACTION (PCR) AS A PARASITOLOGICAL METHOD FOR DETECTING LEISHMANIA IN BLOOD OF DOGS LIVING IN ENDEMIC AREA OF VISCERAL LEISHMANIASIS**

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The diagnosis of *Leishmania* infection by serology is quite questionable, once low sensitivity to detect asymptomatic dogs and cross-reactions are frequently observed. PCR has markedly improved the VL diagnosis but as the number of copies of the DNA sequence is correlated with the PCR sensitivity, the choice of the target region to be amplified is crucial, especially when blood, a sample of easy collection but with lesser parasite burden than tissues, is investigated.
To inspect the PCR performance as a parasitological method for detecting *Leishmania* in blood of infected dogs and to compare the results with those obtained by ELISA.
Blood from 45 dogs (symptomatic=35; asymptomatic=7; uninfected=3) from the VL endemic area of Araçatuba, São Paulo-Brazil, were tested by conventional PCR, using primers targeting a 120 bp sequence of the kinetoplast DNA minicircles present in 10,000 copies per parasite. Sera were assayed by ELISA, using crude lysate of *Leishmania (L.) infantum chagasi* as antigen.
All samples of uninfected dogs tested negative by both PCR and serology. Nevertheless, positive results were found in 35 out of 42 infected animals (83.3%) by PCR whereas ELISA detected only 23 (54.8%). Of note is that blood-PCR diagnosed 6 out of 7 (85.7%) asymptomatic dogs while ELISA only 2 (28.6%).
Blood is routinely collected in endemic areas for serodiagnosing canine visceral leishmaniasis. Here, the same sample was used in conventional PCR that showed higher number of positive results compared to serology. Also, all asymptomatic animals, except one, tested positive by PCR in opposition when their sera were assayed by ELISA that presented only 28.6% of positive results. Therefore, blood-PCR could be used as a sole diagnostic tool or in addition to serology if titers are inconclusive and especially in endemic regions where asymptomatic animals are found in high number.
Supported by:Fapesp

BM091 - **MOLECULAR CHARACTERIZATION AND CELLULAR LOCALIZATION OF ANALOGOUS ENZYMES FROM ISOPRENOID BIOSYNTHETIC PATHWAY FROM TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is the etiological agent of the Chagas disease. There are some drugs that are being used to treat this disease,but they are active only against the acute phase. We used AnEnπ, a computational tool that possibilities the detection of analogous enzymes, for the identification of new possible drug targets, based on the structural differences of enzymatic activities shared by humans and *T. cruzi*. In other words, they have similar functions, but with differences on the 3D structure. Two of these enzymes are, isopentenyl diphosphate delta-isomerase (IPPI), that catalyzes the interconversion of isopentenyl diphosphate and dimethylallyl diphosphate, and phosphomevalonate kinase (PMK), that catalyzes the conversion of mevalonate-5-phosphate to mevalonate-5-diphosphate. These are essential step in the mevalonate entry into the isoprenoid biosynthetic pathway. The objective of this study is the molecular characterization and the cellular localization of the IPPI and PMK, a putative analogue enzyme that can lead us to develop new kinds of drugs that can inhibit or decrease the catalytic activity of this enzyme of the protozoan. In this study we have cloned and sequenced the gene of IPPI and PMK of T. cruzi. The amplicon from IPPI gene, was inserted in pBadThio-TOPO vector and cloned in *Escherichia coli* TOP10 strain. After induction with arabinose 0.02% (w/v), the expression of a protein with approximately 36 kDa was obtained in insoluble form. The amplicon from PMK gene, was inserted in pET-SUMO vector and cloned in E. coli Match1 and BL21 strains. After induction with IPTG 1mM, the expression of a protein with approximately 67.5 kDa was obtained in soluble and unsoluble form. These proteins were purified by Ni2+-HisTrap HP column on HPLC and after that utilized for susceptible BALB/c mice immunization to obtain anti-IPPI and anti-PMK polyclonal antibodies. These distinct antisera reacted with the respective proteins from *T. cruzi* epimastigote in Western blot and were used to localize IPPI and PMK in *T. cruzi* using immunofluorescence and immunoelectronmicroscopy. The images suggest that these enzyme are locate at cytosol. This work is of considerable relevance for the study of the parasite metabolism and for the development of new strategies for drug design against this pathogen.

Supported by:FAPERJ, PDTIS/FIOCRUZ and CNPq

BM092 - **THE IDENTIFICATION OF BLASTOCYSTIS HOMINIS SUBTYPES FOUND IN MEMBERS OF THE INDIGENOUS TAPIRAPÉ TRIBE, MATO GROSSO STATE, BRAZIL.**

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Numerous epidemiological surveys carried out in different countries identify Blastocystis as the most common eukaryotic organism reported in human fecal samples. However, its role as a cause of diarrhea is controversial since it can be found in both symptomatic and asymptomatic individuals. The morphological identification of Blastocystis is often challenging due to its presence in low numbers in faecal specimens. However, Blastocystis was identified in stool samples during a survey of the intestinal parasites of members of the Tapirapé tribe, who live in the Mato Grosso State in the Brazilian Amazon region. Faecal samples were preserved in ethanol and characterized using Blastocystis-specific primers targeting the SSU rRNA gene. The prevalence of this parasite in 378 stools was 21%. Three subtypes were identified (ST1, ST2 & ST3). ST1 was the commonest subtype and was found in 27 individuals (41%), followed by subtype ST2 found in 21 individuals (32%) and ST3 in 11 individuals (17%). Five mixed infection of ST1 and ST2 was identified (7%), one single mixed infection of ST1 and ST3 and another mixed infection of ST2 and ST3. The relative proportion of the different subtypes is different from that seen in Europe and North America where ST3 is the commonest subtype. Also ST4 which occurs in the above mentioned regions was not found. Our results are the first molecular characterizations of Blastocystis hominis in Brazil and in Indian communities from Latin American.

Supported by:CNPq, DECIT, FAPEMAT, FUNASA

BM093 - **EXPLORING PROTOZOAN ORTHOLOGOUS GENES AS MARKERS FOR GENOTYPING**

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Universal orthologous genes (UOG) were identified in pathogenic protozoan genomes and tested as markers for genotyping, aiming to obtain a more accurate characterization at the inter- and intraspecific level. For this study, thirty three UOG present in Protozoa were used to build multiple alignments then used for primer design with Primer3 and "in house" software. After preliminary testing of the designed primers, PCR reactions were performed and the products obtained were cloned. PCR reactions were successful for 19 UOG in *Leishmania major*, *L. braziliensis*, *L. infantum*, *L. mexicana*, *T. cruzi*, *T. vivax* and *Plasmodium vivax*, and other 5 UOG were successful for *Leishmania braziliensis*, *L. amazonensis*, *L. guyanensis*, *L. infantum*, *L. mexicana*, *L. major*, *Trypanosoma cruzi*, *T. evansi*, *T. vivax*, *Plasmodium vivax* and *P. falciparum*. All PCR products obtained showed the expected size then were submitted to sequencing after cloning. After preliminary phylogeny analysis (NJ with 1000 bootstrap), “GETP-binding protein DRG1”, “Leucyl-tRNA synthetase”, methionyl-tRNA synthetase, seryltRNA synthetase showed good resolution for the inter-specific genotyping of parasitic protozoa, indicating that these genes could be good markers.
Supported by:CNPq, CAPES, FAPERJ, FIOCRUZ.

BM094 - **CHARACTERIZATION OF TRYPANOSOMA CRUZI RESPONSE TO IONIZING RADIATION THROUGH TRANSLATION INHIBITION AND EVALUATION OF MITOCHONDRIAL ACTIVITY**

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Trypanosoma cruzi is extremely resistant to ionizing radiation withstanding doses of 1000Gy gamma rays. Ionizing radiation can cause several kinds of damages but the hazardous is DNA double-strand breaks. Previous studies showed that the growth of irradiated T. cruzi arrests up to 240h before returning to normal cell growth rate. The pattern of chromosomal bands is completely restored 48 hours after irradiation. In an attempt to understand how T. cruzi can recognize and repair such damage, we developed different strategies to investigate this phenomenon. Irradiated (500Gy) and non-irradiated epimastigotes were treated with the translation inhibitors cycloheximide or puromycin for six hours after irradiation (drugs were added at different time points: 0h, 24h or 48h). The treatment caused a long growth arrest of irradiated cells. This delay was even longer if drugs were added immediately after irradiation and puromycin treated cultures returned earlier to normal growth than cycloheximide treated parasites. This could be explained by early degradation of mRNAs coding for proteins responsible for trigging cell proliferation. Transcript degradation could be due to translation blockage caused by cycloheximide treatment that stalls mRNA in the ribosomes and reduces the number and size of cytoplasmic stress granules. Moreover, mitochondrial activity of irradiated T. cruzi was assessed through the measurement of oxygen consumption and ATP synthesis. Because gamma rays induce oxidative stress via reactive oxygen species production, we also evaluated H2O2 release. Preliminaries results show that irradiated parasites have higher oxygen consumption rates, slightly higher ATP levels and release less H2O2 than non-irradiated parasites. Irradiated T. cruzi seems to have higher mitochondrial activity, giving support to an increased energy demand to overcome radiation damage. Finally, irradiated T. cruzi can also have better ability to resist to oxidative stress.
Supported by:CNPQ, FAPEMIG

BM095 - **GENOTIPIC CHARACTERIZATION OF GIARDIA INTESTINALIS OBTAINED FROM INDIGENOUS TAPIRAPÉ TRIBE THAT IS LOCATED IN THE CONFRESA MUNICIPALITY, MATO GROSSO, BRAZIL.**

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The indigenous Tapirapé community, composed of 542 individuals living in 6 villages of the Mato Grosso Amazonia, has a history of gastrointestinal problems associated with diarrhea. As part of a study to determine the aetiological agents associated with this pathology we studied the genotypic variation of samples of Giardia intestinalis obtained from members of the tribe. DNA was isolated from 20 faecal samples. A PCR using primers coding for glutamate dehydrogenase (gdh) gave a fragment of approximately 600 bp to Giardia intestinalis. The PCR proved to be highly sensitive for the locus as the 14 positive were also positive in microscopic examinations. The generated products were sequenced and the results were compared to those deposited in GenBank. By this method 10 genotypes sequencing were identified: 2 belonged to Assemblage AII (non zoonotic) and 8 Assemblage B (zoonotic). The nucleotide sequences showed a high level of similarity to those described in previous studies and no new genotype was found. These results suggest that parasites of the zoonotic genotypes are important in the epidemiology of giardiasis amongst the Tapirapé.
Supported by:CNPq, DECIT, FAPEMAT, FUNASA

BM096 - **MOLECULAR CHARACTERIZATION AND CATALYTIC ACTIVITY OF THE MEVALONATE KINASE FROM TRYPANOSOMA CRUZI**

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In eukaryotic cells, sterols are important determinants of membrane fluidity and permeability and serve as precursors for bioactive molecules, which function as regulators of cell cycle and development. The importance of sterols for trypanosomatids has been proved and there are some inhibitors that act at different points of the pathway. Mevalonate Kinase (MK) plays a central role in the cholesterol biosynthesis pathway, catalyzing the phosphorylation of mevalonic acid to form mevalonate 5-phosphate. Recently, MK study showed that when trypanosomatids invasion occurs in the host, this enzyme is overexpressed. This suggests the importance of this enzyme as a target for the development of future drugs. Although structural characterization for some of the MK from trypanosomatids is known, there have been no studies that characterize the MK from Trypanosoma cruzi (TcMK). Herein, we report biochemical and structural preliminary studies of the TcMK. TcMK was cloned into pet28a(+) vector, overexpressed in E. coli at 22 °C in auto-induction medium (ZYM 5052) and purified by affinity chromatography. Size exclusion chromatography (SEC) was performed in order to determine the oligomeric state. The SEC result revealed that the protein has two different oligomeric states. These oligomers of TcMK protein were used for activity assays suggesting that the enzyme is active mainly in the dimeric form. The specific activity was determined for the dimer at pH 9.0 resulting in (80±20) microM of mevalonate 5-phosphate per min and per mg enzyme. The monomeric sample presented an activity 10 timer lower and we did not have evaluation of the change of oligomeric form in the assay. At pH 7.0 the activity decreases by a factor of 5. Crystallization experiments are been realized aiming to obtain suitable crystals for diffraction measurements.
Supported by:CNPQ

BM097 - **CHARACTERIZATION OF NON-CLASSICAL AMASTINS FROM TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is a human pathogen that uses different molecular mechanisms to survive at the insect gastrointestinal tract and to circumvent the mammalian defenses. The knowledge of the T.cruzi-host cell interaction mechanisms can be further used to identify new drug targets for Chagas disease treatment. The amastins are known as surface glicoproteins, initially described as amastigote-specific proteins in T.cruzi, and are promising drug targets candidates since they do not share homology to human proteins and are located at the parasite plasma membrane. This group of proteins can be stratified in at least five subfamilies (α, β, γ, δ and p-δ). The analysis of the T.cruzi, CLBrener genome revealed that this parasite contains six amastin genes belonging to three subfamilies (3 δ-, 1 pδ and 2 β-amastins). To understand the amastin function we decided to determinate de subcellular localization and verify the expression profile of pδ and β-amastins during its life cycle. First of all, the two genes encoding β-amastins from CLBrener (called here as Ama390 and Ama394) and pδ-amastin (Ama40) were obtained by PCR and cloned at the N-terminal of GFP, using the pTREX vector. These constructs were introduced into *T. cruzi* epimastigotes and the preliminary analyses have shown that pδ-amastin, different from δ-amastins, localizes at the membrane and some spherical organelles. To determine the expression profile of each subfamily of amastin, the 3' inter-CDS of pδ-(Ama40) and β-(Ama390) amastins were cloned downstream firefly luciferase(FLUC) in pTcDUALuc vector. After drug selection, the parasites carrying FLUC regulated by Ama40-3'UTR showed FLUC activity in trypomastigotes 100x higher than in epimastigotes which was 10X higher than amastigotes. On the other hand, Ama390-3'UTR down regulated the FLUC activity 1000X in amastigotes compared to epi- and trypomastigotes. These data suggest that each stage requires an specific set of amastins. Financial support: CAPES/REUNI, CNPq, Fundação Araucária.
Supported by:CAPES/Reuni

BM098 - **INSIGHT INTO MRNA EXPORT PATHWAY IN TRYPANOSOMA CRUZI: FUNCTIONAL CHARACTERIZATION OF TCDBP5**

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Mechanisms of post-transcriptional regulation are very important to gene expression in trypanosomatids, however some questions about it still needs to be elucidate, as the case of nucleocytoplasmic export of mRNA. Recently, we have demonstrated that this process is the least conserved along eukaryotes lineages. To understand this pathway in Trypanosoma cruzi, among the few conserved proteins, we have studied the DEAD-box helicase TcDbp5, homologous protein of Dbp5 (Saccharomyces cerevisiae). Dbp5 functions in the late step of mRNA export, releasing the transcripts to translation after interaction with Nuclear Pore Complex (NPC). By western blotting of cellular fractions and imunnofluorescence assays, we have observed that TcDbp5 is present in both fractions with enrichment at cytoplasmic site. Besides, ultrastructural analysis confirmed that TcDbp5 has cytoplasmic localization near to NPC. However, the presence in cytoplasmic site does not reflect the association with translational process, as observed by analysis of polysomal association using sucrose gradient fractions. The electronic microscopy data also demonstrated that the presence of protein in the nucleus is associated with chromatin regions related to transcription sites, it suggests that the interaction of TcDbp5 with mRNA can occur already into the nucleus. Furthermore, c-terminal fusion of the protein with GFP resulted in accumulation of TcDbp5 in the nucleus. Since the c-terminal domain of TcDbp5 has RNA-binding motifs, we have speculated that TcDbp5 shuttling would be dependent of the interaction with RNA. To investigate the functional activity of this protein in mRNA export pathway, we will treat the parasite with drugs that affect transcription and processing of mRNA to evaluate if it could change the localization. To get further insight into TcDbp5 function we are getting efforts to develop reverse genetic and proteomic approaches that will be essential for these studies.
Supported by:CAPES, CNPq, Fundação Araucária, Fiocruz

BM099 - **COMPARATIVE CHARACTERIZATION OF *TRYPANOSOMA RANGELI* GP82 AND GP85 GLYCOPROTEIN GENES**

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Trypanosoma cruzi and *T. rangeli*, a non-pathogenic protozoan parasite of mammals, share surface glycoproteins (gp) of the trans-sialidase superfamily. The gp82 has been implicated in host cell invasion and gp85 in adhesion and/or internalization of the parasite to host cells. Due its avirulent nature, we have characterized *T. rangeli* gp82 and gp85 ORFs encoding for sialidases using genomic and cDNA libraries. Comparative sequence analysis revealed 8 complete ORFs highly similar to the homologous *T. cruzi* genes. Similar to *T. cruzi* gp85, a single *T. rangeli* ORF present two conserved copies of the motif SxDxGxTW (Asp box) and one VTV motif (VTVxNVFLYNR). However, most of the *T. rangeli* ORFs showing similarity to *T. cruzi* gp82 revealed a partial conservation of the VTV motif, a single conserved a one partial copy of the Asp motif. Since *T. cruzi* gp82 and gp85 are GPI-anchored, analysis of the presence of cleaving sites and GPI-anchoring signals on *T. rangeli* orthologs was carried out. Differently from the cleavage site located between residues 26/27 on *T. cruzi* gp85, *T. rangeli* does not presented a conserved position for this signal, varying from residues 19-33 in all orthologs presenting a potential GPI-anchoring site. Comparative similarity assays reveled that *T. cruzi* gp82 and gp85 are quite similar to each other (66.4%) and revealed distinct similarity values to *T. rangeli* gp82 (48.3-61.1%) and gp85 (47.6-58.5%). Despite such analysis, the reduced conservation among *T. rangeli* gp82 and gp85 not allowed the clear characterization of each gp type.

Supported by:CAPES, FINEP, CNPq and UFSC

BM100 - **EVALUATION OF TBSUB2 AS A SPLICING FACTOR IN TRYPANOSOMA BRUCEI**

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In eukaryotes, the expression of coding genes is regulated by several molecular mechanisms, including pre-mRNA processing and mRNA export from the nucleus to cytoplasm. The RNA helicase Sub2/UAP56 is an essential protein for pre-mRNA splicing, nuclear mRNA export and cytoplasmic mRNA localization in different eukaryotes. However, nothing is known about this protein in trypanosomatidae family. Recently, our group showed that trypanosome protein (Tryp-Sub2) is an essential protein for cell viability and is also involved in transcription/export of mRNA in these parasites. To get further insight in the function of this protein, we are currently evaluating if Tryp-Sub2 would be involved with trans and cis splicing process, since Sub2/UAP56 regulates pre-mRNA splicing in various ways in yeast/human. For that, we have been executing genetic analysis using an inducible RNA interference system in Trypanosoma brucei. The protein (TbSub2) level strongly reduced as consequence of decreasing of around 90% in the RNA levels after 12 hours of RNAi induction. By primer extension analysis, we could verify that the knocking down of TbSub2 increased the SL RNA level and decreased the Y structure intermediate of trans splicing, mainly after 36 hours of TbSub2 silencing. These results strongly indicate that Sub2 is related to trans splicing in trypanosomatids. However, we are currently performing quantitative PCR to confirm the function of TbSub2 in pre-mRNA processing and run on assays to evaluate if the knockdown of TbSub2 affects the amount of pre-mRNA in cell. Furthermore, the interaction of TbSub2 with snRNA or splicing factors is being analyzed by immuneprecipitation assay followed by primer extension and mass spectrometry, respectively. We intend to sequence nuclear transcripts from parasites before and after RNAi induction by SOLiD to identify which pre-mRNA and mRNA have the transcription/processing and export affected by the knockdown of the protein. Taking together, the data from this study will evaluate if TbSub2 is involved with pre-mRNA processing in T. brucei and will contribute to the knowledge about the mechanism of processing and export of mRNA in these parasites.

Supported by:CAPES, CNPq, Fundação Araucária, Fiocruz

BM101 - **KNOCKOUT OF THE PROLYL OLIGOPEPTIDASE GENE FROM TRYPANOSOMA CRUZI AFFECTS PARASITE GROWTH**

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Our group had already demonstrated that the prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80) could be involved in the infection process by facilitating *T. cruzi* migration through the extracellular matrix. In order to elucidate the role of this enzyme in the pathogenesis of Chagas disease its knockout was outlined knowing that *poptc80* is a single copy gene per haploid genome. G418 (neomycin)-resistant *T. cruzi* epimastigotes (CL-Brener strain) were obtained after transfection and homologous recombination of a fragment containing 470 pb of the 5'UTR and 574 pb of the 3'UTR of *poptc80* interconnected by neomycin phosphotransferase (*neo*) gene. The poptc80 gene was amplified in all G418-resistant parasites. The PCRs using primers of *poptc80* flanking genes corroborate the correct insertion of neo gene in the parasite's genome. These results suggest that we were able to produce a *poptc80* single-allele knockout. These mutants showed a reduce in parasite growth. Immunoblotting assays using POP Tc80 antibodies revealed a significant decrease in the amount of the protein. This decrease was corroborated with activity tests using POP Tc80 specific substrate, Gly-Pro-Leu-Gly-Pro-AMC. Other analysis of these single-allele knockout parasites are under investigation.
Supported by:CNPq, FAP-DF, Finep and CAPES

BM102 - **EVALUATION OF ALLELIC FORMS OF THE ERYTHROCYTE BINDING ANTIGEN 175 (EBA-175) IN PLASMODIUM FALCIPARUM FIELD ISOLATES FROM BRAZILIAN ENDEMIC AREA**

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P. falciparum Erythrocyte Binding Antigen 175 (EBA-175) plays a central role in erythrocytes invasion being considered a target for malaria vaccine development. Eba-175 presents two well characterized regions, II and III. The region II is conserved and immunogenic, and contains two cysteine-rich segments (F1 and F2), which are involved in binding to glycophorin-A on the surface of erythrocytes. The region III contains mutually exclusive C (strain CAMP) and F (strain FCR3) fragments, which defines the two allelic families of EBA-175. Several studies performed in highly endemic malaria areas in Africa have shown the influence of this dimorphism on the outcome of clinical disease. The differences observed in different endemic areas concerning the circulating parasites are important factors in terms of vaccine strategies since the efficacy of a potential vaccine may vary in different epidemiological scenarios. The goal of this study was to evaluate the genetic diversity of regions II and III of EBA-175 in P. falciparum isolates circulating in Porto Velho (RO), a region of unstable malaria transmission. The blood samples were collected in three time points: 1994 (PV94, n=101), 2002 (PV02, n=57) and 2007 (PV07, n=30). The genetic polymorphism was analyzed by PCR and sequencing. We observed in the region II only one 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. The region II sequencing revealed 5 nucleotide changes in 3 of 15 isolates, leading to 2 amino acids replacements. The region III sequencing 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. Our results showed limited genetic diversity of EBA-175 in *P. falciparum* isolates circulating in Porto Velho, predominance of C-fragment and temporal stability of allelic dimorphism of the EBA-175.
Supported by:FIOCRUZ, FAPERJ, CNPq

BM103 - **CONSERVATION AND DIVERSITY OF INTERGENIC REGIONS OF TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is an old species, with a number of well defined evolutionary lineages. Knowledge of its genome plasticity is important to understand regulatory processes at several levels (transcriptional, post-transcriptional, translational). The genome of Trypanosoma cruzi was derived from a hybrid strain (CL-Brener) and contains information from two divergent parental genomes. In this study we analyzed the sequence diversity of intergenic regions (IGRs) in the Trypanosoma cruzi genome by aligning the assembled sequences of both Esmeraldo-like and Non Esmeraldo-like alleles of the Cl Brener genome. We found a conserved group of 2,948 IGRs, whose upstream and downstream coding sequences (CDSs) were orthologous, syntenic and had expected start and stop codons. We also identified 10 groups of variable size corresponding to IGRs associated with unrelated loci (i.e. non-allelic, non-homologous). These IGRs showed significant portions of sequence similarity. In some cases, the copies of this region were more conserved in one parental allele (e.g. Esmeraldo-like) but exhibited high sequence variation in the other (e.g. non Esmeraldo-like). In other grouped IGRs, the conserved region was shared by both alleles, highlighting a different genomic arrangement of CDS and IGRs. We also analyzed the distribution of size, composition and sequence variation of every pair of allelic IGRs, and CDSs. We found a strong correlation between paired CDS and IGR alleles according to their length. As expected, there was less variation for coding sequences, having highly similar mean and standard deviation values, and a strictly identical length in 68.22% of all pairs, with 3.64% of outliers. On the contrary, only 3.15% of paired IGRs were of identical length, and this distribution showed 6.9% of outliers. The global mean coverage of the alignments was 88.1%, with a mean difference in length of
8.9% indicating not only length similarity but also a relative large sequence conservation. Overall the number of variable sites was nearly 5% with a transition/transversion ratio of 1.97, suggesting preferential nucleotide substitution. The mean base frequencies were similar between haplotypes, revealing a dispproportion of T and C nucleotides usage.
Supported by: