BM.001 - NEW RECOMBINANT ANTIGENS FOR VISCERAL LEISHMANIASIS DIAGNOSIS

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Visceral Leishmaniasis (VL) in Brazil is caused by *Leishmania chagasi* and is a major public health problem. In the last two years we have developed a proteomic study of *L. chagasi* for identification of new antigens with potential use in diagnosis and vaccine. These tests allowed us to identify over 40 promising protein. In this study the goal was to select the proteins by bioinformatics the most promising for use as recombinant antigen in the diagnosis of VL. Therefore, initially, we made the prediction for B cell epitopes using the programs ABCPRED, BCPREDS and BEPIPRED. It was observed that the HIP400 protein had 12, 6, 6 epitopes predicted by the respective programs, with an overlap of 93 amino acids (approximately 47% protein) amongst at least two programs simultaneously and HIP1000 protein showed 27, 12 and 12 predicted epitopes, with 214 amino acids (approximately 60% protein) overlapped at least two programs simultaneously. Moreover, they are proteins with a size smaller than 1kDa without signal peptide which may indicate hydrophobicity. For the production of recombinant proteins, we used the pGEM cloning vector and *E. coli* - XL1-Blue as host cell. The host cell was grown in liquid environment and then plated on agar containing ampicillin. The positive colonies confirmed by PCR, were lysed and digested for obtaining the fragment. This was inserted into the expression vector (pET-Tev) and incorporated by another host (*E. coli* - BL-21). The host cell was grown and then plated on agar containing kanamycin. The positive colonies confirmed by PCR, were incubated with an inducing factor expression (IPTG). After the process of expression, cells were lysed to obtain a purified protein that was bound to the column on the Akta Histrap. The purified proteins are going to be used in standardized testing and validation of VL diagnosis by ELISA.
Supported by: FAPEMIG and CNPq

BM.002 - SEARCH FOR PHOSPHATIDYLSERINE FLOPPASES IN *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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In its life cycle the protozoan *Leishmania* alternates the two forms between the insect and the mammalian hosts. However some aspects of the infection of the mammalian macrophage remain to be elucidated. It has been suggested that lipid organisation of the plasmatic membrane of the parasite can play a role in the phagocytic process as well as in the ability to survive in mammal host. Phosphatidylserine (PS) exposure at the exoplasmic leaflet of the plasma membrane could be one of the signals delivered by amastigotes promotes the phagocytosis but inhibits the macrophages activation (Balanco et al., 2001). Floppase is a translocase that is responsible for the transport of PS from the cytoplasmic to the exoplasmic leaflet. In the present comunication we described a strategy to isolate genes responsible for the externalisation of PS in *L. (L.) amazonensis*. Cells, originated from a transfection with a genomic library of *L. (L.) amazonensis* (Uliana et. al., 1999), were selected with Annexin-V coupled to magnetic microbeads. The cosmids from the selected cells were recovered and DNA was cloned into *E. coli*. *Bam*HI restriction profiles of those cosmids were analyzed showing the existence of two distinct cosmids that were then used on a new transfection of wild type *L. (L.) amazonensis*. The produced cells were labeled with Annexin-V-FITC and their fluorescence was assessed by flow citometry (FACS). The culture produced by the transfection with one of the cosmid showed a differential fluorescence, suggesting a change in the fenotipe, probably induced by the expression of cosmid information. We intend to analyze the genes present in the cosmid to isolate the one responsible for this differential fluorescence. Supported by FAPESP and CNPq.

BM.003 - MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF A GENE ENCODING A LEUCINE RICH PROTEIN IN *LEISHMANIA*

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Leucine-rich repeats (LRRs) are versatile binding motifs found in a variety of proteins involved in protein-protein interactions. During the process of characterizing the *Leishmania amazonensis META1* genomic region, we identified a 2.03 Kb ORF encoding a protein with 6 LRRs in its central region and presenting similarity with the human NOD3 protein. The *LaLRR17* protein is found in increased abundance in amastigotes and is also secreted to the cytoplasm of *L. amazonensis*-infected macrophages. where it might interact with macrophage proteins and modulate the cell's response to infection. In *L. braziliensis* we identified an orthologue to *LaLRR17* gene in chromosome 17 (*Lbr*M17_V2.0920, http://www.genedb.org). A more detailed study of this genomic region identified a second, truncated, copy of this gene, encoding the first 84 amino acids of the protein. However, hybridization analysis and attempts to characterise this second gene copy indicated that there is an assembly error in this region of the genome. To study the function of the LRR17 protein in *L. braziliensis* and *L. amazonensis* we developed specific policlonal antibodies against peptides derived from amino and carboxyl regions conserved between *L. braziliensis, L. major, L. amazonensis* and *L infantum* LRR17 proteins. We found that *LbLRR17* gene expression is differentially regulated during the life cycle of *L. braziliensis* and the protein is found with increased abundance in promastigotes. We also obtained mutant lines of *L. amazonensis* overexpressing the *LaLRR17* gene and of *L. braziliensis* overexpressing the *LbLRR17* gene. These mutant strains were more infective to macrophages *in vitro* when compared with the wild type strains, indicating that the LRR17 protein may interact with macrophage molecules through their LRRs, modulating the cellular response to increase parasite survival. The phenotype of these mutants will be analyzed both *in vitro,* and *in vivo* to test for increased virulence in experimental models of infection. Support: FAPESP, CNPq, CAPES.

BM.004 - FUNCTIONAL CHARACTERIZATION OF A LEUCINE RICH PROTEIN OF *LEISHMANIA MAJOR*

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Proteins containing leucine rich repeats (LRR) are known to be involved in macromolecular interactions in many processes such as bacterial colonization of host cells, immune response in plants and inhibition of RNA binding. A previous study in our laboratory identified a *L. amazonensis* gene encoding a protein containing 6 LRRs (*LaLRR17*). *LaLRR17* is a stage-regulated gene expressed with increased abundance in the amastigote stage. Additionally, the LaLRR17 protein has been shown to be secreted to the macrophage's cytoplasm. Highly conserved homologues of LaLRR17 were found in all *Leishmania* species analyzed. Therefore, the aim of this study was to characterize the homologous protein of *L. major* (LmLRR17). Antibodies raised against peptide sequences common to LaLRR17 and LmLRR17 allowed the study of the steady-state protein abundance. Interestingly, LmLRR17 protein was found to be up-regulated in procyclic promastigotes, instead of amastigotes. To investigate the function of this protein in *L. major*, mutants over expressing a myc-tagged version of LmLRR17 or of LaLRR17 protein were obtained by stable transfection. Chimeric proteins in mutant strains were expressed following the same pattern of expression observed in the wild type parasites, in spite of the increase in gene copy number. The phenotype of these mutants was assessed *in vitro* through macrophage infections. Overexpression of LmLRR17 protein in *L. major* resulted in a decrease in the percentage of infected cells as well as in the number of intracellular amastigotes. On the other hand, overexpression of LaLRR17 in *L. major* induced an increase in virulence with a higher number of infected cells and intracellular parasites. These results indicate that the expression of LmLRR17 protein in *L. major* is tightly regulated and an increase in LmLRR17 protein levels seems to be detrimental to the parasite, while the expression of the heterologous LaLRR17 protein increased infectivity *in vitro*. Support: FAPESP, CNPq.

BM.005 - INVESTIGATION OF AN EXTRA-RIBOSOMAL FUNCTION FOR THE RIBOSOMAL PROTEIN L19 OF *LEISHMANIA*.

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Several ribosomal proteins are reported to play diverse extra-ribosomal functions. However, little is known about the ribosomal proteins in trypanosomatids. Thus, we decided to investigate a possible extra-ribosomal role in gene expression for RPL19, a protein whose transcript is present at variable levels during the parasite life cycle. We generated a transfectant of *L. major* (LV39) that overexpresses the transcript and L19 protein. Promastigotes of independent clones showed an impaired growth. To verify if the phenotype was due to the L19 overexpression, we cured clones and observed that they rescue the wild type growth curves patterns. We have previously observed that the endogenous and overexpressed L19 are dispersed throughout the cytoplasm in a granular appearance. We are further investigating whether the L19 excess leads to an increment of ribosomes in the cell or if the overexpressed L19 remains free in the cytosol. We investigated proteome profile differences between the *L. major* transfectants with parental line. The differentially expressed proteins detected were identified by mass spectrometry. Analysis of identified genes did not reveal any common or complementary function for them, neither allowed to rescue conserved motifs (from the untranslated regions) potentially involved in control of gene expression. Regarding a possible protein or RNA interaction with L19 immunoprecipitation assays with anti-L19 have been unsuccessful. Pull-down experiments are in course to investigate L19 interaction molecules.
Supported by FAPESP.

BM.006 - MOLECULAR AND FUNCTIONALCHARACTERIZATION OF *Leishmania amazonensis* CERAMIDE SYNTHASE

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Sphingolipids are involved in fundamental aspects of celullar biology, including growth, differentiation, apoptosis and oncogenesis and are essential membrane components of eukariotes, including Kinetoplastidae protozoans such as *Leishmania*. Ceramide synthase (CerS) catalyzes the acetylation of sphinganine and sphingosine to form the N-acyl derivates dihydroceramide and ceramide, respectively, which constitute the sphingolipid backbone. Due to the fundamental role of this enzyme we decided to identify and characterize the CerS of *L. amazonensis*. The identification of consensus elements in the alignment of CerS sequences of several organisms allowed the search for a *Leishmania* homologue in genome databanks. The nucleotide sequence of a putative *L. major* CerS gene (LmjF31.1780) identified in this search was used to design oligonucleotides that were then applied to identify the *L. amazonensis* corresponding sequence. The *L. amazonensis* putative CerS gene encodes a 460 aminoacid protein with six potential transmembrane domains. The translated sequence contains a TRAM_LAG1_CLN8 motif (PF03798), found in all CerS previously characterized. Yeast complementation tests were used to determine gene function. The *L. amazonensis* putative CerS gene was able to rescue *Saccharomyces cerevisiae* YPK9 strains with a deletion in the endogenous CerS gene. Since CerS activity is essential in this yeast strain, functional complementation showed that the *L. amazonensis* encoded protein possesses CerS activity. In conclusion, we have identified and characterized the CerS gene of *L. amazonensis*. The characterization of sphingolipid biosynthesis in *Leishmania* may prove important to identify new targets for the chemotherapy of leishmaniasis.
Funding: FAPESPand CNPq.

BM.007 - GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF *Leishmania (Viannia) braziliensis* RESISTANT TO POTASSIUM ANTIMONY TARTRATE

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Mechanisms of drug resistance in New World *Leishmania* species are poorly understood. Recently, we selected *in vitro* populations of *Leishmania braziliensis,* which are 20-fold more resistant to potassium antimony tartrate Sb III (Lb SbR) than its susceptible counterpart (Lb WTS) (Liarte & Murta, 2010). In this study we analyzed gene amplification and deletion and identified transcripts differentially expressed in Lb WTS and Lb SbR populations using DNA microarray methodology. The final array consisted of 13,321 *Leishmania* oligonucleotides: 13,011 from *L. major*, 112 from *L. infantum*, 66 control oligos. The oligonucleotide sequences were clustered by similarity in 12,146 contigs. The genomic analysis (GCH – comparative Genomic hybridization) allowed the identification of 124 sequences amplified 2 to 21-fold (1.78%) and 128 deleted 2 to 9-fold (1.71%) in the Lb SbR population compared to Lb WTS. The amplification of the H region and regions from chromosomes 17, 20 and 31 was observed in the SbIII-resistant population from *L. braziliensis*. The transcriptomic analysis showed 560 up-regulated transcripts and 397 down-regulated transcripts in SbIII-resistant *L. braziliensis* population. Functional annotation data suggest an increased expression of transcripts related to DNA replication and transcription and a decreased expression of transcripts associated with metabolism of lipids and carbohydrates and transport of proteins. Taking into account only the genes indentified by two techniques simultaneously, we observed that 46 genes are up-regulated/amplified in Lb SbR and 15 genes are down-regulated/deleted in this population. Sequence analysis showed that 60% of those 61 genes encode hypothetical proteins. The other genes encode proteins involved with metabolism, replication, DNA repair, transcription, glycoconjugate biosynthesis and others. The identification of genes that are differentially expressed in SbIII-resistant and -susceptible *L. braziliensis* populations may help our understanding of the molecular basis of drug resistance in this parasite and also provide information on drug targets for chemotherapy.Financial Support: CNPq, FAPEMIG, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR.

BM.008 - PROTEOMIC ANALYSIS OF MILTEFOSINE RESISTANCE IN *LEISHMANIA (VIANNIA) PANAMENSIS*

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Miltefosine is the first orally effective drug for the treatment of visceral and cutaneous leishmaniasis. However, despite its recent approval (2002) failures in miltefosine treatment have been reported, which indicates that there is probability of finding drug-resistant field isolates of *Leishmania*. In Colombia, miltefosine is used as therapeutic alternative in the treatment of cutaneous leishmaniasis, which is mainly caused by *L. (V.) panamensis*. In this work we conducted a comparative proteomics study using two-dimensional electrophoresis and mass spectrometry to identify proteins differentially expressed between wild-type and miltefosine-resistant *L. (V.) panamensis* promastigotes. Five gels from five different sample preparations for both sensitive and resistant lines were processed, and were detected 572 ± 60 y 526 ± 48 proteins, respectively, in two-dimensional proteome maps. Comparative analysis of the two proteomes allowed the identification of seven differentially expressed protein spots between wild-type and miltefosine-resistant *L. (V.) panamensis* promastigotes, among of proteins we found heat shock proteins (Hsp60, Hsp70), ribosomal proteins (P2, S12), and proteins involved in cellular metabolic pathways (glutamine synthetase, peroxidoxin, p-nitrophenylphosphatase). This is the first study of miltefosine resistance in *Leishmania* parasites belonging to the *Viannia* subgenus using a proteomic approach, in which was achieved the identification of proteins that had not been previously implicated in miltefosine resistance. This comparative proteomic analysis highlights the variety of cellular responses required for the acquisition and/or maintenance of miltefosine resistance in *Leishmania*, which besides being mediated by membrane transporters, also can be modulated directly or indirectly by cytoplasmic and mitochondrial proteins.

BM.009 - ASF1 (*Anti Silencing Factor 1*) OF *LEISHMANIA MAJOR*

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The mechanisms and genes involved in chromatin remodeling in trypanosomatid have been the subject of intense investigation in recent years. Studied in different organisms, ASF1 (Anti-Silencing Factor 1) was identified as a histone chaperone that contributes to histones deposition during nucleosome assembly in newly replicated DNA. This protein is also involved in cellular response to DNA damage and transcriptional silencing. We are investigating a putative ASF1 from *L. major*, which seems to be involved in chromatin packaging and preservation of DNA integrity. In an attempt to evaluate the involvement of ASF1 in the control of gene expression in *L. major*, we analyzed the proteome of a *L. major* transfectant that overexpresses ASF1 (*Lm[pXNeo-ASF1]*) by comparison with the one of a control line (*Lm[pXNeo]*). We analyzed the protein profile differences by two-dimensional gel electrophoresis and we have detected 19 differentially expressed proteins that were identified by mass spectrometry. To specifically investigate a possible role of LmASF1 to control levels of expression of genes present at the telomeric ends, as it is the case of the *Saccharomyces cerevisiae* ASF1, we compared transcript levels of 42 genes in *Lm[pXNeo-ASF1]* with *Lm[pXNeo]*, by Real time PCR. Our results suggest that LmASF1 does not modify expression of these genes. To evaluate the role of LmASF1 in DNA repair, we submitted *Lm[pXNeo-ASF1]* and *Lm[pXNeo]* to different genotoxic agents that trigger distinct DNA repair pathways. Our results suggest that overexpression of ASF1 contributes significantly to the resistance of the cells to oxidative stress since *Lm[pXNeo-ASF1]* is more resistant to damage caused by the hydrogen peroxide (H_2O_2). In addition, after gamma irradiation transfectants overexpressing LmASF1 recovers more slowly than the control line, which may suggest that LmASF1 is involved in cell cycle control. This set of complementary approaches allows understanding ASF1 roles in *Leishmania* parasites. Supported by FAPESP.

BM.010 - EVALUATION OF ASSOCIATIONS BETWEEN POLYMORPHISMS IN THE NRAMP1 GENE (natural resistance-associated macrophage protein 1) AND DOGS SUSCEPTIBILITY TO LEISHMANIASIS

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Studies have shown that polymorphisms in some genes are involved in genetic predisposition and/or susceptibility to infections by *Leishmania* spp in dogs. The aim of this work is to analyze the relationship between polymorphisms of a microsatellite in intron 1 of the nramp1 gene and susceptibility of dogs from canine visceral leishmaniasis endemic areas to infection by *Leishmania* (*Leishmania*) *chagasi*. Previously. 191 dogs with serology (IFAT and ELISA) and PCR positive for *L.* (*L.*) *chagasi* (infected group - IG) and 61 dogs with serology and PCR negative (uninfected group – UG) were examined. All animals participating in this study were crossbreeds. We identified seven alleles that differ to each other by four base pairs (bp) and ranged from 133-157 bp in length. The allele 145 was the most frequent in IG (0.35 vs. 0.28 in UG) and allele 141 was the most frequent in UG (0.33 versus 0.28 in IG). Allele 157 appeared only in IG (0.005). The homozygous genotype 149/149 was more frequent in IG group (0.33) and 145/145 in the UG group (0.35). Differences in allelic and genotypic frequencies between case and control groups were not statistically significant ($p > 0.05$) but the presence of alleles such as 157 can suggest a protective effect. Altet et al. (2002), in a study conducted in Spain using breed dogs, observed a significantly higher frequency of the allele 145 in IG ($p < 0.025$), which appeared exclusively in homozygosis. Although preliminary, our data suggest a positive association between allelic frequencies and dog susceptibility or resistance to infection by *L.* (*L.*) *chagasi*.

BM.011 - UV IRRADIATION INDUCES ALTERATIONS IN THE EXPRESSION OF LEISHMANIA SPP. TELOMERIC PROTEIN RBP38 (LARBP38)

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Recent results demonstrated that Rbp38 is a protein exclusively expressed in trypanosomatid parasites, including *Leishmania* spp. Rbp38 is probably a multifunctional protein since it seems to exert different functions in the kinetoplast and in the nucleus of the parasites. The *L. amazonensis* Rbp38 (LaRbp38) protein was co-purified with LaRPA-1 from nuclear extracts positive for telomerase activity. LaRbp38 can also bind in vitro and in vivo with telomeric DNA and with kDNA, although its biological function is yet unknown. We recently demonstrated that LaRbp38 forms part of a minimum telomeric complex at *Leishmania* telomeres where we can also find LaRPA-1 and telomerase. Using different protein extracts obtained from total and subcellular fractions of promastigote forms, we were able to show by Western Blot, that under normal conditions, LaRBP38 can be found in both nuclear and mitochondrial fractions. By indirect immunofluorescence using anti-LaRbp38 serum, the protein was mainly localized in the kinetoplast, showing an antipodal distribution, but it was also present to a lesser extent, in the nucleus and in some cells it was possible to visualize the protein in both compartments. We further exposed *L. major* promastigotes to 1200J/m^2 of UV radiation for 2-10 min and let parasites recover in culture in the dark, for 1h-24h. Protein extracts were obtained from irradiated and control parasites at all time points and then submitted to Western blot analysis. The results showed a gradual increase in LaRbp38 expression in irradiated parasites and during recovery in culture. No changes were seen in alfa-tubulin expression, used as the loading control. When we analysed the expression of LaRBP38 in a nuclear fraction from irradiated parasites, we observed a slight increase in protein expression after 8-10 min of UV exposition and that after 6h of recovery no protein was detected in the extracts. The expression of LaRbp38 in the nucleus did not return to the normal levels even after 24h, when compared to non-irradiated parasites. Curiously, the expression of LaRBP38 was unaltered in the mitochondrial fraction even after UV irradiation and recovery. We are currently checking if alterations in LaRBP38 expression are associated with the recruitment of DNA repair proteins and other telomeric proteins as well.

BM.012 - CLONING OF GENES FOR PUTATIVES PROLINE DEHYDROGENASE AND Δ^1-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE FROM *LEISHMANIA* (*LEISHMANIA*) *AMAZONENSIS* AND FUNCTIONAL CHARACTERIZATION OF THEIR PRODUCTS

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It is known that trypanosomatids use amino acids, particularly proline, as a carbon and energy sources. In addition, it was involved in other physiological processes such as metacyclogenesis, osmorregulation or resistance to different stress conditions. The proline-glutamate redox pathway is being studied in *Leishmania (Leishmania) amazonensis* by our group. Two enzymes are involved in this route: 1. proline dehydrogenase (PRODH) which converts L-proline into Δ^1-pyrroline-5-carboxylate (P5C) and 2. Δ^1-pyrroline-5-carboxylate dehydrogenase (P5CDh) which converts P5C into L-glutamate. Although, the presence of this pathway in some species of the *Leishmania* genus is strongly supported by their genome project data, no biochemical evidences of their activities were reported up to now. In this work we focus on some aspects of the proline-glutamate pathway in *L. (L.) amazonensis.* The genome sequence of this specie has not been completed yet. Using the protein sequences available on databases from *L.* (*L.*) *major, L.* (*V.*) *braziliensis, L.* (*L.*) *infantun* and *L.* (*L.*) *mexicana*, we have obtained a consensus sequence for both genes. Degenerated oligonucleotides from conserved domains were designed and used to amplify by PCR, clone and sequence the corresponding DNA fragments for *La*PRODH and *La*P5CDh. The ORFs sizes were 1,7 Kb and 1,68 Kb for *La*PRODH and *La*P5CDh, respectively. For protein analysis, the recombinant enzymes were cloned in the pRSET-C vector and expressed fused to an N-terminus his-tag. The purified recombinant proteins showed an apparent molecular weights of 62 and 66 kDa, as expected. The functionality of these genes is being analyzed by using yeast strains lacking the orthologues genes (Δput1 and Δput2). The Δput2 mutant transformed with *La*P5CDh regained the ability to grow in selective medium with proline as a sole nitrogen source. Our data suggest the presence and functionality of this pathway in *L. (L.) amazonensis*. Supported by FAPESP, USP and CNPq.

BM.013 - THE BEHAVIOUR OF TELOMERIC AND REPAIR PROTEINS UPON DNA DAMAGE IN *LEISHMANIA*

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Replication protein A is a complex of single-stranded DNA-binding proteins implicated in DNA metabolism, including DNA repair and telomere maintenance. In *Leishmania* the subunit RPA-1 (LaRPA-1) binds and co-localizes *in vivo* with telomeres. In yeast and human telomeres RPA also works as a telomerase recruiter and as a DNA damage sensor. Its involvement with the DNA damage response triggers its hyperphosphorylation and the recruitment of proteins from the RAD51 group. We intend to determine if in *Leishmania*, RPA is also required to protect chromosome ends from being detected by the DNA damage machinery. Our first results showed that even high doses (~500 ug/ml) of the DNA damaging agent phleomycin was unable to kill *L. amazonensis* promastigotes. In contrast, low doses of the drug (~20-40 ug/ml) induced G1/S cell cycle arrest. Then, we verified if phleomycin treatment triggered changes in expression of telomeric (RPA, RBP38 and telomerase) and repair proteins (RAD51, MRE11 and H2Ax). The results indicated that the expression of LaRPA-1 and other telomeric proteins slightly diminished in parasites treated with phleomycin whereas a gradual increase in the expression of RAD51 occurred probably in response to DNA damage. Moreover, it was possible to see by chromatin immunoprecipitation that more LaRPA-1 was immediately recruited to the G-rich telomere strand upon phleomycin-induced damage, compared with the C-rich telomeric strand, suggesting that the presence of LaRPA-1 may prevent loss of single-stranded telomeric DNA or elicit activation of a local DNA damage response. We are currently checking if double strand breaks on DNA alters the known protein:protein interactions at parasite telomeres.
Supported by CNPq.

BM.014 - *Leishmania infantum chagasi* NTPDase as New Antigen in Canine Leishmaniasis Diagnosis

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Leishmaniasis are tropical neglected diseases caused by parasites from the genus *Leishmania*. *Leishmania infantum chagasi* is aetiological agent of American Visceral Leishmaniasis and canine Leishmaniasis. Actually dogs had been pointed as the main epidemiological reservoir for Leishmania and fast and effective epidemiological vigilance is crucial to the control of this antropozoonosis. The main goal of this work is the development of a strategy to do rapid canine leishmaniasis diagnosis using the new recombinant *L. infantum chagasi* (NTPDase) as antigen (LicNTPDase) an lateral flow immunocromatography. NTPDase is a leishmania ecto-localized virulence molecule. The soluble portion of NTPDase gene was cloned in pET21b vector. This construction pET21b-NTPDase was used to transform *Escherichia coli* BL21-DE3 strain. Transformed bacteria were isolated and used to express the recombinant *L. infantum chagasi* NTPDase. The LicNTPDase was expressed after 1 hour of IPTG induction, recovered and purified from inclusion bodies using Ni-NTA agarose affinity chromatography. The recombinant protein was tested as antigen in a direct ELISA assay using standard dog sera and presented good differentiation of negative and positive sera. These results showed the potential use of LicNTPDase as new antigen to be used in canine Leishmaniasis immune diagnosis. Our perspectives include the development of a new rapid immunochromatography kit to be used in epidemiological inquiries helping in the control of this important tropical disease.
Supported: FAPEMIG, UFV.

BM.015 - CELL CYCLE DETERMINATION AND CHARACTERIZATION OF THE PROMASTIGOTE STAGE OF *LEISHMANIA (L.) AMAZONENSIS*.

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The eukaryotic cell cycle is a precisely controlled phenomena involving the replication and segregation of organelles culminating in the production of two identical daughter cells. In kinetoplastid protozoa, the cell cycle is characterized by the duplication of the kinetoplast (mitochondria), the nucleus and growth of an extra flagellum prior to cytokinesis. We present in this study the morphological events and the timing of these events during the cell cycle of *Leishmania amazonensis* promastigotes. *L. amazonensis* is the protozoan parasite that causes tegumentar leishmaniasis, an important neglected disease in Brazil. Cell cycle characterization was done using culture synchronization with hrydroxyurea treatment followed by flow cytometry analysis, DAPI DNA staining, flagellum labeling, bromodeoxyuridine incorporation, and indirect immunofluorescence analysis. Results showed that DNA replication takes around 6-7 hours with the nucleus generally completing division shortly after the kinetoplast. The new flagellum is formed during late S phase and G2, reaching its final size during the final stages of the cycle (G2/M). Complete separation of daughter cells may take up to 2 hours after the cycle is completed. During S phase, replication protein A subunit 1 colocalized with bromodeoxyuridine incorporation confirming that this protein participates in the DNA replication machinery in the nucleus. Successful synchronization using hydroxyurea treatment allowed us to determine for the first time the timing and order of the various events that compose the cell cycle in *L. amazonensis*. The determination of telomere and kinetoplast DNA replication in synchronized parasites is currently under way.
Supported by FAPESP, CNPQ and PROPe (UNESP).

BM.016 - UNVEILING PROTEIN: PROTEIN INTERACTIONS AT *LEISHMANIA* SPP. TELOMERES

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In most eukaryotes, telomere binding proteins such as POT1 and TRF2 play crucial roles in telomere biology by interacting with several other telomere regulators to ensure proper telomere maintenance and to form high order complexes known as telosome or shelterin. *Leishmania* spp. telomeres are composed by the conserved TTAGGG repeats which are maintained by telomerase. The basic *Leishmania* telomeric protein complex is formed by the proteins LaRPA-1 and LaRbp38, which bind *in vitro* and *in vivo*, with high affinity, to the G-rich single-stranded DNA, and by proteins that interact with the double-stranded region of telomeres such as the recently described TRF homologue. The *Leishmania* spp. genome, like other trypanosomatid, lacks many of the conserved single-stranded telomeric proteins found in other eukaryotes, such as the CDC13 and POT1 protein homologues. Thus, we speculate that the *Leishmania* RPA-1 homologue may play the same roles as POT1/CDC13 at parasite telomeres, although it can also bind to other single-stranded DNA with high affinity and in a sequence-independent manner. LaRPA-1 together with the multifunctional LaRbp38 protein, which also interacts with a wide range of GT-rich sequences, including telomeres, seems to form part of a parasite telomeric complex that resembles the recently described CST complex. The CST complex is being considered a second telomere capping mode occurring in a broad variety of species, except budding yeast, and is mainly formed by RPA-like proteins. In this report we used different approaches to show that LaRPA-1 interacts with both LaRbp38 and with telomerase, and that these protein:protein interactions seem to occur in a cell-cycle independent manner. In addition, LaRPA-1 partially co-localizes with both proteins, probably reflecting its functions in DNA metabolism. We speculate whether these protein interactions reflect the entire telomeric complex or the presence of functionally distinct subcomplexes at parasite telomeres. Agência Financiadora: FAPESP

BM.017 - SELECTION OF *L. (L.) AMAZONENSIS* AND *L. (L.) MAJOR* MUTANTS RESISTANT TO PURINE ANALOGUE TUBERCIDIN

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Species of the protozoan Leishmania are the causative agents of a wide range of pathologies called leishmaniasis. Gene amplification is a common phenomenon observed in Leishmania cell lines subjected to drug pressure. Tubercidin (TUB), a toxic purine analogue has already been described as a potent antiparasite action. It is incorporated in nucleic acids of microorganism and mammalian cells, inhibiting DNA synthesis and repair. In this study, we obtained two resistant lines, *L. (L.) amazonensis* (La) and *L. (L.) major* (Lm) resistant to TUB (TUB^r). First, we selected $LaTUB^r$ by increasing drug pressure from the wild type IC_{50} value concentration (0.23μM) to 5μM of TUB, conferring to the $LaTUB^r$ 5 a resistance level greater than 43 fold when compared to La wild type cells (IC_{50} value of approximately 10μM). As expected, after 2 months without TUB pressure, $LaTUB^r$ 5 (-2months) cells showed a declined resistance ratio (from 43 to 5 fold TUB resistance; an IC_{50} value of approximately 1.0μM). Curiously, when the same experiment was carried out for Lm cells, during the same period, we just reached 0.5μM of TUB, a concentration 10 fold smaller than the TUB concentration reached for La cells (5μM). To understand why such a marked difference in the TUB sensibility occurs among these two leishmania species, we analyzed the presence of circular DNAs in both resistant lines, by a plasmid alkaline lysis preparation and analysis in agarose gel. We also analyzed the presence of some restriction fragment amplified with digestion of total DNA with *BamH*I and *Hind*III restriction enzyme and no fragment was observed. Cross resistance with allopurinol, glucantime and pentamidine was done, and again no cross-resistance was observed. Further analysis within these mutants must be performed to clarify these different characteristics among these leishmania species in order to find new markers for the inter-specific differentiation. Supported by CNPq, CAPES, FAPESP and LIM48-FMUSP.

BM.018 - IDENTIFICATION OF ONE LOCUS OF LEISHMANIA (LEISHMANIA) MAJOR RELATED TO TUBERCIDIN RESISTANCE

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A rational strategy for therapeutic exploitation of parasitic diseases can be based on identification of fundamental differences between the parasite and its mammalian host. An important pathway is the purine metabolism. Leishmania and other protozoans are unable to synthesize purine nucleotides by *de novo* and must *salvage* them from the host. Tubercidin (TUB), a purine toxic compound can be considered a potential antiparasite agent by inhibition of purine transport for the Leishmania. Gene identification involving drug resistance has contributed to a better understanding of the action mechanisms of antiparasite compounds. Using a transfection and overexpression selection strategy, we isolated a 31kb *locus* of *L. (L.) major* (cosTUB2) capable to confer resistance to TUB, with resistance ratio 4 times greater than the wild type. After a couple of deletion sets we obtained a 3kb fragment containing a gene probably involved with TUB resistance, according to GenBank related as a hypothetical protein. A realtime PCR demonstrated that the hypothetical protein mRNA was about 30 times more expressed than cosTUB2. However, functional analysis showed that overexpression of hypothetical protein alone presented a 2 fold smaller TUB resistance value than cosTUB2. These results suggested that the upstream region can be involved as a regulation factor in this hypothetical protein. A new construction with the hypothetical protein containing the upstream region was done and it is being submitted to amplification copy number. Cross resistance analysis with the allopurinol showed that both, cosTUB2 and wild type cells presented comparables IC_{50} values of approximately 10μg/mL. This indicated that the mechanism used by cosTUB2 cells is selectively of purine pathway and not for pirimidines. Thus, this study can explain better the purine metabolism in Leishmania and can suggest this important pathway as a new target for antileishmania agents.
Supported by: CAPES, CNPq, FAPESP and LIM48-FMUSP.

BM.019 - IFN-1 SIGNALING AND TLR2 ARE REQUIRED IN *LEISHMANIA AMAZONENSIS* – INDUCED PKR GENE EXPRESSION.

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Leishmania parasites are able to modulate the suppressor and microbicidal macrophage functions. Many macrophage functions and signaling pathways can be regulated by PKR (dsRNA-activated protein kinase R), a host anti-viral protein that also favors *Leishmania* infection. PKR is also involved in the modulation of cytokine signaling and transcriptional. We demonstrated in a *previous study that Leishmania amazonensis induced the PKR protein levels* and can modulated the expression of the *pkr* gene. The TATA-less promoter of *pkr* gene drives the basal and inducible expression through the KCS and ISRE elements of the *pkr* promoter. Toll-Like Receptor 2 (TLR2) also contributes to the innate immunity in *Leishmania* infection, mainly through the binding of LPG (lipophosphoglycan), which trigger several signaling pathways. To investigate the possible role of *Leishmania amazonensis* in the modulation of *pkr* gene expression in infected macrophages, and the putative role of IFN-type I and TLR2, we used a pkr-promoter luciferase gene reporter and RT-PCR/Real-time assays in HEK 293T cell and RAW 264.7 macrophages. Moreover, we also assayed wild-type and knockout TLR2 and IFN-I receptors macrophages. We demonstrated that *L. amazonensis* and LPG_{amaz} modulated the expression of *pkr* gene in wild-type cells, but failed in TLR2 and IFN-I-R-Ko macrophages. Luciferase reporter assays with HEK 293T cells co-transfected with the pkr promoter construction and TLR2 demonstrated that *L. amazonensis* and LPG_{amaz} induced the expression of *pkr* gene. In addition, the Luc activity is dependent on the presence of the TLR2. RT-PCR assays revealed that *L. amazonensis* or LPG_{amaz} induced the expression of *pkr* gene in wild-type cells but not in IFN-I-R knockout cells. Taken together, these data indicate that *L. amazonensis* infection or LPG induces the expression of the *pkr* gene through TLR2 and IFN-type I pathways. Financial support: CNPq, FAPERJ and INCT-Amazônia.

BM.20 - THE ROLE OF TOLL LIKE RECEPTORS AND THE PROTEIN PACT IN PKR ACTIVATION DURING *LEISHMANIA AMAZONENSIS* INFECTION

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The mammalian protein kinase PKR (ds-RNA dependent protein kinase) is a known as a critical component of the innate immune response against viral infection and modulates the expression of immunomodulatory mediators. To be enzymatically active, latent PKR needs to be induced by binding to one of its activators, dsRNA or the PACT protein. We have previously shown that *Leishmania amazonensis* is able to activate and increase the PKR levels in infected macrophages. PKR activation leads to intracellular proliferation in a IL-10-dependent fashion. The signaling pathways triggered by toll like receptors induce the production of innate immune mediators and may rely on PKR activation. In proliferation assays, we report that peritoneal macrophages of TLR2 or TLR4-KO B6 mice, similar to the observed to PKR-KO macrophages, showed a decreased *L. amazonensis* intracellular proliferation compared with wt macrophages. Our data suggest that toll receptors pathways are important for *L. amazonensis* infection. Additionally, western blotting assays showed that infected macrophages from TLR2-KO and TLR4-KO mice showed weaker PKR activation than wild-type macrophages, suggesting that PKR phosphorylation seems to be partially dependent on TLR. We also showed that the supressor cytokine IL-10, is reduced in macrophages from TLR2 and TLR4-KO mice compared with wt macrophages. Finally, we also observed that *L. amazonensis* is able to induce PACT mRNA, suggesting that PACT may activate PKR in presence of *L. amazonensis* infection. Taken together, these data indicate that PKR-dependent intracellular proliferation of *L. amazonensis* may depend on TLR2 and 4 activation. Additional experiments are in progress to determine the signalling pathways connecting TLRs and PKR, as well as the parasite molecules involved with this activation. Supported by FAPERJ, CNPq and INCT – Amazônia.

BM.021 - HETEROLOGOUE EXPRESSION OF RECOMBINANT SCAVENGER RECEPTOR MARCO BY CHO CELLS INCREASE THE BINDING OF *Leishmania major*

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CBA mice are resistant to *Leishmania major* yet permissive to *L. amazonensis* infection. CBA macrophages control infection by *L. major,* but not *L. amazonensis, in vitro*. Previous studies have demonstrated that the MARCO scavenger receptor gene is upregulated in CBA macrophages infected with *L. major,* but not with *L. amazonensis*. MARCO is blocked by Mab ED31, reducing macrophage infection by *L. major* by 30%, which suggests that MARCO plays a role in *L. major* recognition by host macrophages. This study aimed to identify the proteins associated with MARCO on the surfaces of macrophages during *L. major* recognition. The MARCO receptor gene was cloned into a pTAPC2 vector containing the TAPTAG sequence, which allowed for the use of a dual-tagging purification method. CHO cells were transfected with pTAPC2-MARCO, the cells were lysed, MARCO expression was identified by western blot and the parasite binding ability of recombinant MARCO-TAPTAG was evaluated. *L. major* promastigotes were allowed to bind to transfected CHO cells at 4°C for 30 minutes and then submitted to immunofluorescence. CHO cells that express MARCO-TAPTAG bind to *L. major* to a greater extent than control cells, indicating that MARCO-TAPTAG is functional and appears to directly interact with this parasite. Furthermore, MARCO-TAPTAG-expressing CHO cells presented lamellipodia-like protrusions, as has been previously demonstrated. Protein complexes from the pTAPC2-MARCO stably transfected-CHO cells that bound to *L. major* were purified using the tandem affinity purification method and identified by western blot. Similar studies will be performed using pTAPC2-MARCO stably transfected RAW cells to identify the protein complexes involved in *L. major* recognition using mass spectrometry. The identification of proteins involved in *L. major* parasite binding and phagocytosis will contribute to the understanding of the mechanisms that influence infection outcome.
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BM.022 - USING PROTEOMIC ANALYSIS TO IDENTIFY MOLECULAR EXPRESSION IN MACROPHAGES INFECTED WITH *Leishmania amazonensis* OR *Leishmania major*

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CBA mice are resistant to *Leishmania major* yet susceptible to *Leishmania amazonensis*. In addition, CBA macrophages control infection by *L. major* and are permissive to *L. amazonensis*, which suggests that macrophages play an important role in the outcome of *Leishmania* infection. In order to evaluate the global macrophage response to *Leishmania* infection, proteomic studies were carried out. Protein expression was identified six and 24 hours after infection with *L. major* or *L. amazonensis*. Protein extracts were obtained from infected cells to identify peptides using LC-MS/MS with a MudPIT approach. The results from six independent experiments were analyzed and 382 proteins were found to be expressed differently, in accordance with infection by *L. amazonensis* or *L. major*. These proteins are involved in a variety of cell functions, including cell death, post-translational modification, lipid metabolism, molecular transport, amino acid metabolism, small molecule biochemistry, cell signaling, cell cycle and cell-mediated immune response. Using IPA software, ten protein networks were constructed. The proteins related to lipid metabolism and small molecule biochemistry were grouped into one network and exhibited higher expression levels in *L. amazonensis*-infected cells compared to *L. major*. Proteins related to cell signaling and cellular assembly, organization and movement formed another network exhibiting higher expression levels in *L. major*-infected cells compared to *L. amazonensis*. These results clearly demonstrate that *L. amazonensis* and *L. major* modulate macrophage functions in different ways. In conclusion, macrophage response to *L. amazonensis*, did not establish a definite activation profile. However, *L. major* activates cell-signaling networks, with respect to cell activation. Taken together, the data indicate that parasites play important roles affecting cell activation related to infection outcome. Currently, western-blot analyses are being performed to corroborate differences in protein expression levels detected by high throughput proteomic analysis. Supported by CNPq (306672/2008-1).

BM.023 - FUNCTIONAL CHARACTERIZATION OF THREE *LEISHMANIA* PABP HOMOLOGUES WITH DISTINCT BINDING PROPERTIES TO RNA AND PROTEIN PARTNERS

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The Poly(A) binding protein (PABP) is a conserved eukaryotic protein involved in many aspects of mRNA metabolism such as mRNA biogenesis, processing, transport and degradation. It also performs major roles during translation initiation, through an interaction with the translation initiation factor eIF4G, and also possibly during termination. Here we describe the study of the three *Leishmania major* PABP homologues (*Lm*PABP1 through 3) whose genes were found within available genomic sequences. The three proteins are abundantly expressed and cytoplasmic but only *Lm*PABP1 is represented as multiple isoforms. Upon transcription inhibition with actinomicyn D a major shift in protein localization was observed, with both *Lm*PABP2 and 3 migrating to the nucleus, whilst *Lm*PABP1 remained predominantly cytoplasmic. This differential localization of *Lm*PABP2 and 3 is not induced upon inhibition of mRNA processing or translation using the inhibitors sinefungin and cycloheximide, respectively. Through pull-down assays we observed that all three proteins bind to a *Leishmania* eIF4G homologue (*Lm*EIF4G3), with the binding of *Lm*PABP1 being the most efficient. Immunoprecipitation assays (IP) confirmed the binding between *Lm*EIF4G3 and *Lm*PABP1 although we were not able to detect any interaction between *Lm*EIF4G3 and *Lm*PABP2 and 3 or between a second eIF4G homologue, *Lm*EIF4G4, and any of the three PABPs. IP assays also showed an interaction between *Lm*PABP2 and *Lm*PABP3 homologues, in a RNA-independent manner. Through RNA binding assays *Lm*PABP1 was found to bind specifically to A-rich sequences whilst *Lm*PABP2, despite overall strong affinity to RNA, lacked specificity and *Lm*PABP3 displayed an intermediate phenotype. Complementary RNAi analyses of *T. brucei* prociclic forms with the *Lm*PABP1 and 2 orthologues indicate that both proteins are required for cellular viability. Our results imply that the *Lm*PABP1 homologue is the major candidate to play a role in translation and that the *Lm*PABP2 and 3 may participate in novel functions.
Supported by FACEPE, FIOCRUZ, CNPq

BM.024 - DIAGNOSIS OF AMERICAN TEGUMENTARY LEISHMANIASIS AND *LEISHMANIA* SUBGENUS IDENTIFICATION USING PCR-RFLP IN THE WEST-CENTRAL REGION OF BRAZIL

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American Tegumentary Leishmaniasis (ATL) is a human disease with a broad spectrum of clinical manifestations depending on parasite species and host immune responses. The aim of this study was to evaluate the contribution of PCR-RFLP to the diagnosis and parasite subgenus identification in biopsy fragments from cutaneous and mucosal lesions. Patients clinically suspected of having cutaneous (CL, n = 31) or mucosal (ML, n = 6) leishmaniasis were assisted at the Tropical Disease Hospital, in the West-Central Region of Brazil. Montenegro skin test (MST), indirect immunofluorescence (IFI), and histopathological exam followed by immunohistochemistry analysis to confirm parasite presence were performed. Genomic DNA was extracted from fragment biopsies and a PCR to amplify conserved region of the kDNA minicircles was done. PCR products were further submitted to PCR-restriction fragment length polymorphism (PCR-RFLP) analysis, using Hae III or AVA I enzymes. Among 37 patients, only 33 were confirmed ATL (CL, 29; ML, 4). Confirmed cases of ATL were detected with similar sensitivity by histopathological analysis (60.7%), MST (73.1%), and PCR (72.7%). However, IFI presented low sensitivity (36.6%). Although high level of agreement between results of histopathological and PCR tests to diagnosis of ATL, for ML all histopathological analysis did not detect parasites on lesions while PCR was positive in two samples. PCR-RFLP analysis identified 91.7% of parasites as *L. (Viannia)* (CL, 20; ML. 2) and 8.3% as *L. (L.) amazonensis* parasites (CL, 2). In conclusion, our data support the value of PCR-RFLP as a reliable method for the diagnosis of ATL. Our data from ML suggest that PCR-RFLP can be more sensitive than other methods for diagnosis when very low numbers of parasites are present in lesions. Parasite genus/species identification is helpful to improve treatment schedule, to define the epidemiology of the leishmaniasis in Brazil, and to understand the pathogeny of ATL. Supported by CNPq and FAPEG.

BM.025 - SEARCHING FOR SIGNALS IN THE TRITRYPS GENOMES

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The protozoans *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major* (Tritryps), are evolutionarily ancient eukaryotes which cause worldwide human parasitosis. Probably due to the early branching in eukaryotic evolution, they present unique biological features. Particularly, RNA polymerases (RNAP) are distinctive. For RNAPII transcribed genes, the search for transcription initiation sites has been elusive so far. Polycistrons strand switch regions have been implicated in this process and evidence favoring this hypothesis has been accumulating lately. Besides, *Trypanosoma brucei* ARNPI is capable of synthesizing the pre-mRNAm coding for VSG and procyclin proteins. In eukaryotes, RNAPI promoters are characterized by the conservation of conformational elements but no sequence conservation.We aim to determine the structural characteristics of transcription initiation sites in Tritryps to better describe putative binding sites. In this regard, we have collected the sequences corresponding to the RNAPI transcription start sites of about 25 eukaryotes (including the ones for Tritryps) and constructed databases of their conformational characteristics. We found interesting conformational similarities among RNAPI promoter sites from Tritryps and other eukaryotes. We also performed a genome wide analysis for the Tritryps of intrinsic curvature and constructed chromosome specific files that allow visualization of curvature in a global scale using the Artemis genome browser. Apart from this, we plan to carry out an experimental approach including the characterization of each polymerase transcriptome. We are carrying out *run-on* assays in the presence of α-amanitin to establish the transcriptome of RNAPI. We conducted these experiments with *T. brucei* procyclic forms. qRT-PCR analysis of the sub-population of RNAs obtained established that the methodology is able to separate RNAs synthesized *de novo* but the conditions for RNAPI inhibition should be adjusted.

BM.026 - INVESTIGATION OF MECHANISMS AND ELEMENTS INVOLVED IN REGULATION OF GENE EXPRESSION IN *LEISHMANIA*

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Untranslated regions (UTR) of genes may contain elements that interact with protein machineries that will modify mRNA steady state levels or initiation of translation activity of that given gene. This mode of postranscriptional regulation of gene expression in regulatory effects of gene expression in trypanosomatids such as mRNA maturation, mRNA abundance and translational efficiency. We developed an in silico pipeline to search for conserved sequences motifs within the flanking regions of all annotated CDSs of the *Leishmania braziliensis*, *L. major* and *L. infantum* genomes. These conserved sequences (CICEs) were grouped using *L. major* Gene Ontology Family and we selected four conserved elements that flank genes LmjF21.0725, LmjF22.1060, LmjF22.1630 and LmjF33.3190. The elements are oligonucleotides varying in size from 34 to 41 nucleotides. Preliminary results of Electrophoretic Mobility Shift Assays (EMSA) suggest that these RNA sequences interact with proteins. To test the potential role of these elements in modifying transcript stability or level of translation of the controlled gene we generated versions of a construct in which the neomycin phosphotransferase (*NEO*) gene is flanked by the the Dihydrofolate Reductase Thymidilate Synthase (*DHFR-TS*) sequences and each CICE was inserted within the *DHFRTS* 3'UTR . Targeting fragments of these constructs have been transfected in *Leishmania major* to replace one DHFRTS allele. After selection of positive clones, no drug of selection is used and *NEO* works as a reporter gene. Mutants and control cells were compared regarding growth behavior, reporter gene expression levels and transcript stability, results will be presented.
Supported by FAPESP.

BM.027 - COMPARATIVE ANALYSIS OF THE EXPRESSED GENOME OF *LEISHMANIA BRAZILIENSIS* ISOLATED FROM TWO DISTINCT CLINICAL MANIFESTATIONS

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Leishmania braziliensis is a major ethiological agent of cutaneous leishmaniasis in Brazil. The mucosal form of the disease affects about 5% of patients which may present progressive destruction of cartilage or bones of the face, pharynx and larynx. To investigate possible phenotypic modifications between parasites from mucosal and cutaneous sites we evaluated the proteome profile differences of paired mucosal and cutaneous isolates of *L. braziliensis*. Parasites recovered from patients with concomitant mucosal (LBM) and cutaneous lesions (LBC) were investigated. We have previously shown that the molecular karyotypes of all isolates were indiscernible. The proteome comparative analysis revealed among other genes, the differential pattern of expression of LbrM31_V2.2410 (prostaglandin f2-alpha synthase) which was uniquely expressed in LBC^1 (patient 1) and was 1.7 times more expressed in LBC^2 (patient 2) when compared with LBM^1 and LBM^2, respectively. To further investigate this gene and the biological significance of the finding we conducted Northern Blotting experiments and we constructed a plasmid bearing LbrM31_V2.2410 to be expressed in *Escherichia coli*. The overexpressed soluble protein has been injected in rabbit and chicken for the production of polyclonal antibodies. Additionally, we have observed in one of the *in vivo* infection experiments a mucosal lesion in one hamster infected with LBM[1]. We could not rescue parasites from the lesion. Next, we conducted *in vivo* infections to reproduce such mucosal lesions, but they were never observed. Histological sections from mucosal and cutaneous tissues of 16 hamsters infected with LBM^1, LBM^2, LBC^1 and LBC^2 were examined in search for tissue infection signs, or inflammatory infiltrate. Parasites and inflammation were only detected in the cutaneous lesions of all animals evaluated.
Supported by: FAPESP and CNPq.

BM.028 - *IN SILICO* WORKFLOW TO MAP AND TO CHARACTERIZE CONSERVED INTERCODING SEQUENCES OF *LEISHMANIA* SPP. GENOMES

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After obtaining the complete genomes of different *Leishmania* species, *in silico* analyses of generated data are now widely used to improve the understanding of these pathogens' biology. *Leishmania spp.,* such as other trypanosomatids, have their protein-coding genes grouped in long polycistronic units of functionally unrelated genes and accumulating evidence indicates that control of gene expression happens by diverse mechanisms at the posttranscriptional level. The high degree of synteny among *Leishmania* species is accompanied by highly conserved coding sequences (CDS) and poorly conserved non-coding sequences (intercoding). Having as our main goal the identification of elements involved in control of gene expression, an *in silico* investigation of conserved sequences in the intercoding regions of *L. major, L. infantum and L. braziliensis* genomes was conducted. A combination of computational tools Linux-Shell[1], PERL[2] and R[3] languages, BLAST[4], MSPcrunch[5] and SSAKE[6] algorithms were used to construct a workflow for: formatting data files (1,2); searching for conservation in the target-regions and creating a redundant FASTA file with millions of conserved intercoding sequences (2, 4, 5), clustering this FASTA file to eliminate redundancy (6), assigning GO biological process and KEGG pathways classification for the generated contigs using *L. major* neighbouring CDS as template (1, 2) and giving statistical support for the gene-enrichment annotation (3). The computational workflow will be presented together with some identified motifs specifically associated (enriched) to GO and/or KEGG terms. These findings may contribute to the prediction of putative regulons in the *Leishmania spp.* genomes. Supported by FAPESP and CNPq

BM.029 - INVESTIGATION OF THE LEISHMANIA MAJOR HUS1 GENE AND ITS ROLE IN ATM/ATR-MEDIATED RESISTANCE TO REPLICATIVE AND OXIDATIVE STRESS.

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The parasite *Leishmania* has a dynamic and plastic genome in which gene amplification and chromosome translocations are common phenomena. Such plasticity hints at the necessity of dependable genome maintenance pathways. In other eukaryotes, PIK-related kinases ATR and ATM act as principal sensors that govern DNA damage response. Also, the Rad9-Rad1-Hus1 (9-1-1) trimeric complex has a central role in sensing and signaling DNA damage. The binding of 9-1-1 complex at DNA lesion stabilizes ATR allowing an effective activation of effector kinase Chk1. We have investigated the 9-1-1 subunit Hus1 in *Leishmania major.* LmHus1 is a nuclear protein that improves the cell capability to cope with replicative and oxidative stress. Overexpression of LmHus1 mediated resistance to the genotoxic drugs such as hydroxyurea (HU), methyl methanesulfonate (MMS) and Oxigen peroxide (H_2O_2). On the other hand, an increase in LmHus1 expression did not confer resistance to phleomycin. Inhibition of ATR and ATM kinases by caffeine abrogates the resistance phenotype associated to LmHus1 overexpression.The LmHus1 involvement in double strand breaks (DSB) repair is being further investigated using gamma-ray assay. Also, a possible homologue of the effector kinase Chk1 has been identified in the parasite genome e is being investigated. Current work is focused not only in the generation of an *LmHUS1* disrupt cell line, but also in the investigation of the LmHus1 expression along parasite life. Preliminary results suggested that the gene is not expressed in the metacyclic form of the parasite. These studies will provide a better understanding of the DNA repair pathways and mechanisms of genome maintenance in the protozoan parasite *Leishmania*.
Financial support: FAPESP and CNPq

BM.030 - WORKING ON THE CHARACTERIZATION OF THE PSEUDOURIDINE SYNTHASE 7 FROM *TRYPANOSOMATIDS*

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Transfer RNAs (tRNAs) 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, U2 small nuclear RNA and rRNA. In pre-$tRNA^{Tyr}$, it acts on U35, catalyzing the formation of pseudouridine (ψ), a highly phylogenetically conserved modification. We found homologues of the yeast Pus7 enzyme in the genomes of the tritryps (*Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*). In order to functionally characterize these enzymes in trypanosomatids we cloned the *T. cruzi pus7*-like gene to produce the recombinant enzyme and test its activity towards different tRNA transcripts as substrates. Furthermore, we have cloned and sequenced an internal region of the *pus7*-like gene from *T. brucei* and generated a RNAi construct to evaluate the importance of this enzyme for the different life stages of the parasite.
Supported by: FAPERJ, OMS, CNPq
Key words: Trypanosoma, tRNA, Pseudouridine, trypanosomatids

BM.031 - SELENOCYSTEINE INCORPORATION MACHINERY IN *NAEGLERIA GRUBERI*

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Selenium (Se) is an essential trace element *in vivo,* which is mainly found in selenoproteins, that contains the 21st amino acid (Selenocystein – Sec – U). The selenoproteins generally works in the balance of redox state of the cell, playing an important role on cell growth and proliferation. Selenocystein is encoded by a TGA codon in phase in an open reading frame (ORF) when a specific stem-loop structure, designated Sec insertion sequence (SECIS) element, is located in the 3'-untranslated region (UTR) directs the insertion of Sec. The publication of *Naegleria gruberi* (ATCC 30224) genome allowed us to investigate the presence of the Sec-incorporation pathway in a primitive eukaryote. Using a throughly bioinformatics approach, we identified the following genes involved in Sec-incorporation: Phosphoseril tRNA kinase (PSTK), Selenocysteine Synthase (SepSecS), Selenophosphase synthase (SelD or SPS), elongation factor EFSec (SelB) and a SECIS binding protein (SBP). Also, we found two potential tRNASec and one selenoprotein that is homologue to a mitochondrial thioredoxin redutase (TR3). Our predictions were confirmed by total RNA extraction, RT-PCR and sequencing, showing that the selenocysteine incorporation machinery is indeed present in *N. gruberi.* Interestingly, the SelD of *N. gruberi* has two distinct domains. The N-terminal has a predicted methiltransferase activity and the C-terminal is homologue to SPS/SelD. The SelD domain is phylogenetically of prokaryote origin, which could indicate an event of lateral gene transfer to *N. gruberi*. This is also observed in *Spironucleus barkhanus,* a member of the basal eukaryotic infrakingdom Excavata. Such observations suggest the possibility of finding completely new selenoproteins, and it has interesting features that may help to elucidate the evolutionary history of this pathway. For this purpose, a careful search for conserved domains and SECIS using hidden Markov and covariance models is currently underway. Supported by CAPES, CNPq and FAPESP

BM.032 - CHARACTERIZATION OF KINETOPLAST PROTEINS FROM *Crithidia deanei*

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Trypanosomatids are unicellular organisms that parasitize plants, animals and insects. Many species are monoxenic with single invertebrate host, usually an insect, during life cycle. Among these monoxenic species, *Crithidia deanei, C. oncopleti, C. desouzai, Blastocrithidia culicis* and *Herpetomonas roitmani* harbor a symbiotic bacterium in the cytoplasm. This mutualism is an excellent model to study the origin of organelles and cellular evolution. The mitochondrial DNA in trypanosomatids is localized in the structure named kinetoplast (kDNA). The presence of the endosymbiont causes ultrastructural modification toward a larger unwind kDNA network. Studies of kDNA on protozoans that harbor symbiotic bacteria are unknown. In collaboration with the National Laboratory of the Scientific Computational (LNCC), we have sequenced the genomes of the *C. deanei* and *B. culicis* using the 454 Roche platform. These are the first genomes sequenced from parasite with endosymbiont and they are being annotated in the SABIA system (http://www.sabia.lncc.br). Our objective is to identify kinetoplastid-associated proteins (KAPs) and proteins of the replication mechanism of the kDNA in the genome of *C. deanei*. Approximately 51 million of base pairs were analyzed with 23X genome coverage. The genome has 48% of G+C content and 17,762 ORFs with an average length of 1320 bp. We were able to find sequences coding for proteins involved in the kDNA structure and in the replication mechanism. The universal minicircle sequence binding protein (UMSBP), which has 9 zinc-fingers domains and 70% of the identity with UMSBP of the *T. cruzi*, and the KAPs of C. deanei, with 60% of identity with the *T. cruzi* and *C. fasciculata* orthologs, are examples. The information generated in this project will contribute to a better understanding of the biology of this monoxenic protozoan.
Supported by CNPq, FAPERJ, LNCC.

BM.033 - COMPARATIVE GENOMICS AND FUNCTIONAL ANALYSIS OF PROTEINS INVOLVED IN MRNA NUCLEOCYTOPLASMATIC EXPORT IN TRYPANOSOMES

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Different RNAs are exported from the nucleus by specialized pathways. It is not known whether RNA export pathways are conserved in deeply diverging eukaryotic lineage, and the question of how complex the RNA nucleocytoplasmic export was in the last eukaryotic common ancestor (LECA) remains open. One of our objectives was to reconstruct the evolutionary history of RNA export pathways across eukaryotes by screening genomes for the presence of homologs in metazoa and fungi, using human and yeast as queries. Our comparisons indicate that several key proteins involved in RNA export pathways are conserved across most eukaryotic lineages, inferring that orthologs were already present in LECA. The mRNA export pathway is the most complex and the least conserved one, suggesting that among deeply diverging eukaryotic lineages is different from what is observed in extant “higher” eukaryotes. To better understand mRNA export in deeply diverging eukaryote lineages, we are investigating the function of well conserved proteins in trypanosomatid protozoa, causative agents of deadly human diseases. The most conserved protein across eukaryotes was Sub2/UAP56, a component of TREX complex that connects transcription with mRNA export. It is a nuclear protein in *L.major*, *T.brucei* and *T.cruzi*. Ultrastructural analysis showed that *T.cruzi* Sub2 localizes in foci, excluding nucleolus, at the interface between dense and non-dense chromatin areas indicating its association to transcription sites. This result was further analyzed by BrUTP incorporation assays and TcSub2 colocalizes with RNA pol. II transcription sites. These evidences strongly suggest that TcSub2 participates in nuclear mRNA metabolism. We are currently investigating the role of Sub2 to elucidate if it’s component of mRNA export in trypanosomatids. Besides, the double knockout of the TcSub2 gene is lethal in *T. cruzi* and TbSub2 iRNA causes a growth defect in *T. brucei*, indicating that it is an essential protein for trypanosomes. Supported by CAPES, CNPq, Fundação Araucária and FIOCRUZ.

BM.034 - KNOCKOUT OF THE GENE ENCODING THE METACYCLIN II PROTEIN IN *Trypanosoma cruzi*

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The protozoan, *Trypanosoma cruzi* is the causative agent of Chagas disease, the most important parasitic infection in South America. The life-cycle of this parasite involves two intermediate hosts (triatomine insects and mammals) and three well-defined morphological and functional developmental stages: epimastigotes, trypomastigotes and amastigotes. The differentiation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) involves the transformation of a replicative non-infective form of *T. cruzi* into a non-replicative infective stage. Several major phenotypic changes occur during metacyclogenesis, including the kinetoplast morphology and kinetoplast DNA (kDNA) compaction. The kDNA is an unusual form of mitochondrial DNA consisting of a catenated network of several thousand minicircles and a smaller number of maxicircles. The kDNA is associated with H1 histone-like proteins, known as kinetoplast-associated proteins (KAPs), which condense the kDNA network. Little is known about the role that KAPs play in kDNA remodeling that occurs during the differentiation of *T. cruzi*. Three KAPs (KAP3, KAP4 and KAP6) were identified in all developmental stages of the parasite and present a differential distribution within the kinetoplasts of epimastigote, amastigote and trypomastigote forms (Cavalcanti *et al*., 2009, De Souza *et al*., 2010). Another protein (metacyclin II), which is encoded by the gene *Tcmet2*, was localized mainly at the kinetoplast of the parasite and is up-regulated in metacyclic trypomastigote (Yamada-Ogatta *et al*., 2004). In this work we used gene deletion to investigate the function of TcMET2. *Tcmet2* null mutants are viable and we are investigating possible changes in kinetoplast morphology by light, confocal and electron microscopy. We are also analyzing its fitness during proliferation, differentiation and infectivity. It is still unclear how the compact kinetoplast disk of epimastigotes is converted into a globular structure in the infective trypomastigotes, we believe that TcMET2 could play a role in the kinetoplast morphology and kDNA compaction. Supported by CNPq, CAPES and Fundação Araucária

BM.035 - *TRYPANOSOMA CRUZI* SUBTELOMERIC GENOMIC CONTEXT AND ITS ROLE IN RECOMBINATION EVENTS

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GP85 gene family, related to cell invasion, display great sequence diversity and is often associated with subtelomeric regions. We previously suggested that the preferred telomeric location of GP85 genes could hold up recombination events further the generation of new variants. The role of subtelomeric regions in DNA recombination has been analyzed with a *T. cruzi* artificial chromosome (pTAC) carrying a GP85 pseudogene. No recombination events involving displacement of pTAC sequences could be detected. Reduced recombination rate has been described in T. brucei laboratory-adapted strains (Boothroyd et al., 2009). Authors demonstrated that the introduction of DNA double strand breaks (DSB) adjacent to the 70-bp repeats upstream of the transcribed VSG increases switching in vitro 250-fold. Our proposal is introduce DSB adjacent to repetitive telomeric sequences near GP85 and verify if there is an increase in rearrangements in T. cruzi genome. To induce DSB, we performed a system established by Taylor MC and Kelly JM, involving plasmids pLEW13/pTcINDEX. T. cruzi (G, CL and Y strains) were transfected with pLEW13 to express T7 RNA polymerase and tetracycline repressor protein constitutively. Afterwards, these clones were transfected with luciferase enzyme cloned in pT7LUC. The system was tested after induction with tetracycline 24h, 48h, 15 and 30 days after transfection. Luciferase activity could be induced up to 75-fold when compared with control, within 48h of tetracycline addition. We selected few clones from each strain and transfected with pTcINDEX-RFP. Preliminary results shown CL clones die in presence of hygromycin, the pTcINDEX resistance gene. Therefore, just clones from G and Y strain were transfected with pTcINDEX- I-SceI. We hope pLEW13/pTcINDEX system can represent a valuable genetic tool for studying recombination process involving telomeric sequences in T. cruzi. Although, recombination events are very rare, artificial DSB in specific points can induce rearrangements in T. cruzi genome. Supported by FAPESP, CNPq and CAPES.

BM.036 - IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NUCLEOTIDE-SUGAR TRANSPORTERS OF *TRYPANOSOMA CRUZI* BY HETEROLOGOUS EXPRESSION IN YEAST AND MAMMALIAN CELLS

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Glycoconjugates play a fundamental role in many different biological processes such as the development of multicellular organisms and the survival and infectivity of parasites. A variety of human diseases are caused by defects in glycosylation and key processes for parasite infection such as cell invasion and modulation of the host immune system depend on glycoconjugates. The addition of sugars to proteins and lipids occurs in the lumen of the endoplasmic reticulum (ER) and Golgi apparatus and is catalyzed by glycosyltransferases located in these organelles. Nucleotide sugars, the activated sugar donors, are mostly synthesized in the cytosol and therefore must be transported across the ER and Golgi membranes. The intracellular transport of nucleotide sugars by multi-spanning transmembrane proteins known as nucleotide-sugar transporters (NSTs) is essential for glycoconjugate biosynthesis. To functionally characterize nucleotide-sugar transporters from *Trypanosoma cruzi* we initially searched for putative NSTs in the *T. cruzi* genome. By performing Blast searches in the GeneDB and TriTrypDB databases using characterized NSTs of different organisms as queries we have identified a family of 11 putative NSTs. Sequence analyses of the *T. cruzi* genes revealed hydrophobic profiles and characteristic motifs found in other members of the NST family. Heterologous expression of these genes in *Saccharomyces cerevisiae* and *Kluyveromyces lactis* mutants - deficient in GDP-mannose and UDP-N-acetylglucosamine transport, respectively – to rescue the wild-type phenotype were unsuccessful suggesting that trypanosomatid NSTs may not be functional in yeast. Interestingly it has recently been shown that *Leishmania* NSTs are able to rescue glycosylation defects of mammalian mutants deficient in UDP-galactose transport. We are thus using a similar approach to identify and characterize *T. cruzi* NSTs responsible for the uptake of this substrate. Furthermore gene knockout experiments are currently being performed to evaluate the role of glycoconjugates and specific NSTs in the parasite infectious cycle. Supported by Fiocruz and CNPq.

BM.037 - EXPRESSION AND EPITOPE MAPPING OF SAP (SERINE-, ALANINE-, AND PROLINE-RICH PROTEIN) OF *TRYPANOSOMA CRUZI*

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Some members of SAP (serine-, alanine-, and proline-rich proteins) family are secreted/excreted by metacyclic trypomastigotes in the extracellular medium. The central domain of SAP (SAP-CD, 155 amino acids) binds to mammalian cell in a dose-dependent and saturable manner, inhibiting the cell adhesion and invasion by metacyclic forms. In the present study, to determine the important amino acid sequences for the adhesion, epitope mapping was performed using overlapping recombinant peptides corresponding to the SAP-CD. Monoclonal antibodies (MAb SAP4 and SAP5) and polyclonal monospecific antiserum were generated against the SAP-CD expressed as GST fusion protein. In immunofluorescence assays, MAbs and the monospecific antiserum reacted with cytoplasmic components of epimastigotes and metacyclic trypomastigotes permeabilized with saponin and fixed with formaldehyde. Murine MAbs and polyclonal rabbit antiserum reacted with a 55-kDa SAP shed into the extra-cellular medium by metacyclic forms. The 55-kDa SAP was also detected by the MAbs in the protein extracts of epimastigote and metacyclic forms. To map continous epitopes of SAP, overlapping sequences from the SAP-CD were expressed as GST fusion proteins. All anti-SAP antibodies recognized a peptide of 54 amino acids (aa) located in the middle of SAP-CD. A major B-cell epitope spanning residues 36 to 46 (GSPSPPPPATP) has been predicted in the 54-aa peptide. Recombinant proteins (in fusion with GST) spanning the 54-aa region were generated and tested with MAbs and polyclonal anti-SAP antibodies. Interestingly, a 22-aa peptide (MGAA GSPSPPPPATPGSAGANS) carrying the predicted epitope reacted with the rabbit antiserum but failed to be recognized by the MAbs. This could suggest that the antigenic site recognized by MAbs is defined by a conformation-dependent structure rather than a linear sequence. Recombinant proteins containing additional sequences larger than the 22-aa peptide will be constructed to define the main antigenic site of MAbs.
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BM.038 - CHARACTERIZATION OF DIFFERENT MEMBERS OF AMASTINS AND THEIR ROLE IN *TRYPANOSOMA CRUZI* HOST CELL INTERACTION

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Amastins are 170-200 amino acids, highly hydrophobic surface glycoproteins expressed not only in *T. cruzi* amastigotes but also in amastigotes from several species of *Leishmania*. The *T. cruzi* genome contains several copies of amastins, which can be subdivided in two groups in this parasite. The δ amastins are organized in a cluster containing alternated copies of tuzin genes, differently from β amastins which are not. Likewise δ amastins, mRNA from members of the β group is more abundant in amastigotes than in the others stages of the parasite. Analyses of amastin genes present in the genome of various strains of *T. cruzi* showed that they encode highly divergent proteins with increased amino acid variability in the protein domain that is likely in contact with the host cell cytoplasm. In order to gain new insights of their function, parasites over expressing amastin were generated and yeast two hybrid (Y2H) experiments were performed to identify human proteins able to interact with amastins. Epimastigotes over expressing a δ amastin gene in fusion with GFP showed a higher multiplication rate in comparison to wild type parasites. From the Y2H experiments, ten positive clones were identified and IL-15 cDNA was present in two of them. IL-15 is a cytokine which modulates different cell populations and has numerous immune-related functions, as well as antiapoptotic effect in various cell types. In contrast to other cytokines, IL-15 exhibits two isoforms: a secreted and a cytoplasmic one. Moreover, elevated levels of this cytokine were found in heart lesions from people infected with *T. cruzi*. We observed that IL-15 interacts with the surface of intracellular amastigotes and, to confirm a direct interaction of amastins with IL-15, *in vitro* pull-down experiments and co-immunoprecipitation assays are being conducted. Supported by: CNPq, FAPEMIG and HHMI.

BM.039 - PROTEASOMAL ATPUBIQUITIN-DEPENDENT AND -INDEPENDENT PROTEOLYSIS DURING THE *IN VITRO* METACYCLOGENESIS OF *TRYPANOSOMA CRUZI*

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Proteasomes are large protein complexes, the main function of which is to degrade unneeded or damaged proteins. The inhibition of proteasome activity in *Trypanosoma cruzi* blocks parasite replication and cellular differentiation. We analyzed proteasome-mediated proteolytic degradation during the cellular differentiation of *T. cruzi* from replicative non infectious epimastigotes to non replicative and infectious trypomastigotes (metacyclogenesis). We provide the first demonstration that proteasome-dependent proteolysis occurs during metacyclogenesis. We also present biochemical evidence for the coexistence of proteasomal degradation processes dependent on and independent of ubiquitin. During *in vitro* metacyclogenesis, no increase in protein degradation was observed following the addition of ubiquitin and ATP. No peaks of ubiquitin-mediated degradation were observed and the profile of ubiquitinated conjugates was similar at all stages of differentiation. An analysis of carbonylated proteins showed significant variation in oxidized protein levels at the various stages of differentiation. Proteasome inhibition also increased oxidized protein levels. Proteasomes may therefore be involved in the degradation of oxidized proteins, because oxidized or misfolded proteins are the natural substrates of ubiquitin-independent proteolysis. During metacyclogenesis, several proteasome complexes may act together, and the 20S proteasome may be free or linked to regulatory particles (PA700, PA28/PA26). We hypothesize that the coordinated action of these complexes controls the coexistence or degradation of different proteasomes responsible for degrading ubiquitin-tagged or oxidized proteins.
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BM.040 - UBIQUITIN-RELATED PROTEOME OF *Trypanosoma cruzi*

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Trypanosoma cruzi metacyclogenesis is a differentiation process driven mainly by post-transcriptional changes in gene expression, that may be controlled at protein level by modulation of the activity, location or amount of stage-specific proteins. This modulation involves a complex combination of signaling systems, in which ubiquitination – modification of target-proteins by ubiquitin (Ub) - plays an important role. Aiming the identification of Ub-related proteins, the present work has generated some important tools for this study, such as the production of antibodies against *T.cruzi* Ub and functional recombinant Ub, besides transfected *T.cruzi* cells that express recombinant Ub fused to different molecular tags. In this way, different ubiquitin-affinity proteins could be identified: an hypothetical protein (4764.t00006) with an ubiquitin-binding domain (UBA); DNA and RNA binding proteins, such as Alba (4859.t00001) and ribosomal subunits; proteins that regulate the translation initiation, such as the initiation factor eIF4a (8728.t00019) and a small GTP-binding protein (8128.t00005) from the endocytic pathway. All above-mentioned mechanisms are regulated by the ubiquitination process in other organisms. We have also identified other proteins with still not described function, such as some hypothetical proteins with no known domains (4718.t00003 and 8445.t00002, among others). Furthermore, we have also identified proteins involved in cellular processes where, up to now, no ubiquitination is known to occur, such as proteins involved in the Krebs cycle (7146.t00001), in the pentoses pathway (8033.t00005), or even the gluconeogenesis (4917.t00002). The present work has initiated investigations on the molecular and cellular mechanisms of ubiquitination in this primitive organism. Identification of ubiquitin-interacting proteins and of targets for this signalization in *T.cruzi* will help to understand the mechanism of regulation of gene expression in this pathogenic protozoan.
Supported by CAPES and CNPq.

BM.041 - CLONING, EXPRESSION CHARACTERIZATION AND CELLULAR LOCALIZATION OF COMPONENTS OF UBIQUITINATION SYSTEM IN *T. CRUZI* METACYCLOGENESIS

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Ubiquitination is a post-translational mechanism that modifies target-proteins by another protein known as ubiquitin. At least three groups of enzymes are required for ubiquitination: Ub-activating (E1), Ub-conjugating (E2) and Ub-ligase (E3) enzymes. E3 is generally considered to be most important in controlling target specificity, despite E2 also being responsible for target recognition. This mechanism plays an important role in most cellular processes by modulation of the activity, location or amount of many proteins. *Trypanosoma cruzi* metacyclogenesis is a differentiation process driven by environmental stimuli, like population and nutritive stress, and for adaptation to this new environment is necessary a gene expression reprogramming. Control of gene expression in this organism occurs at post-transcriptional level and ubiquitination may be involved tagging stage-specific proteins. In this work, *in vitro* metacyclogenesis was used as an experimental model to investigate the expression of enzymes involved in ubiquitination system. The expressed recombinant proteins from these genes were used to produce polyclonal antibodies, which were subsequently used to characterize the expression (by immunoblot assays) and localization (by immunolocalization using confocal laser microscopy) of these enzymes during metacyclogenesis. The expression analysis disclosed two patterns: decreased (two E2, one E3) and unchanged (one E1, one E3) protein levels during metacyclogenesis. Cellular localization of all enzymes showed a cytoplasmic distribution, some of them with accumulation around the nucleus (one E3), other with a strong localization at flagellar adhesion zone (one E2). These results are the first attempt for the characterization of the ubiquitin system in *T. cruzi*. Supported by CAPES and CNPq.

BM.042 - GENOME-WIDE ANALYSIS OF TcRBP40 AND ITS ASSOCIATED mRNAs IN *Trypanosoma cruzi*

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Beyond the medical interest on Chagas disease, *Trypanosoma cruzi* is considered a model organism for studying the posttranscriptional mechanisms that regulate gene expression in eukaryotes. These mechanisms are mediated and facilitated by RNA binding proteins (RBPs) and regulatory elements present in the untranslated regions (UTR) of mRNAs. mRNAs associate in functionally related ribonucleoprotein complexes (RNPs) to define regulatory networks known as RNA Regulons. Characterizing the proteins present in the different RNPs and their target mRNAs may contribute to the comprehension of posttranscriptional mechanisms in eukaryotes. We have selected several proteins that are expressed in the epimastigote forms of *T. cruzi* and show some degree of regulation during differentiation, focusing on those containing the RNA Recognition Motif (RRM). We characterized the function of one of these proteins, TcRBP40, using a ribonomic approach. His- and TAP-tagged recombinant TcRBP40 were used in pull-down assays with epimastigote RNA. Recovered RNAs were amplified and analyzed by *T. cruzi* oligonucleotide microarray. 70% of the identified mRNAs correspond to hypothetical proteins, and half had a signal for transmembrane localization. The TcRBP40 recognition element was investigated by EMSA and results point to an A- and G-rich region as the strongest putative element. The expression profile of TcRBP40 targets indicates that they are more abundant in the metacyclic life stage of the trypanosome. On the other hand, TcRBP40 expression was not detected on this same life stage. Overexpression resulted in reduction of the accumulation of its targets. These results suggest an mRNA destabilization function of TcRBP40. The protein was located at the posterior region, in specific cytoplasmic foci. Further analyses will help to fully characterize the mechanisms of target regulation by TcRBP40 and determine its relevance on cell differentiation process.
Supported by FIOCRUZ, CNPq and CAPES.

BM.043 - INFECTIVITY AND PATHOGENICITY OF TRYPANOSOMATIDS ISOLATED FROM BATS

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The trypanosomes of bats are difficult to be distinguished by morphological examination and have significant genetic diversity. Biological tests, such as inoculation in laboratory animals, assess characteristics of infectivity and pathogenicity of the strains, contributing to the characterization of the species. The purpose of this study was to investigate the parasitemia and tissue parasitism in recent experimental acute infection (5 days) and late (40 days) of mice inoculated with trypanosomes isolated from bat species *Artibeus planirostris* (EM437) and *Phyllostomus discolor* (EM465). Non-isogenic mice were inoculated intradermally in front of their thigh, with 2 x 106 culture trypomastigotes/mL. The investigation of parasitemia and tissue parasitism was made on the 5th and 40th days after infection. Parasitemia was assessed by microhematocrit and blood culture and tissue parasitism by histological analysis by Hematoxilin-Eosin staining and PCR (kDNA) with primers 121 and 122. Mice inoculated with the isolates showed no parasitemia (microhematocrit and blood culture) or tissue parasitism (HE). However, PCR analysis detected the presence of 330bp kDNA band corresponding to *Trypanosoma cruzi* in the following organs and tissues: Fifth day: EM437 - lymphnode, intercostal and psoas muscles; EM465 - lymphnode, heart, ileo-caecal junction and psoas muscle; Forth day: EM437 - lymphnode, diaphragm, intercostal and psoas muscles; EM465 - heart and psoas muscle. It is concluded that assessment of parasitemia and tissue parasitism is variable and insufficient to discriminate and identify trypanosomes of bats. In addition, PCR can indicate the presence of parasite DNA or that the DNA of the parasite was not completely eliminated after infection, it is recommended to carry out additional studies.
Supported by CAPES, CNPq, FAPEMIG and FUNEPU.

BM.044 - mRNA EXPORT IN *TRYPANOSOMA CRUZI:* CHARACTERIZATION OF THE DEAD-BOX HELICASE TcDbp5

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Transport of mRNA from the nucleus to the cytoplasm is an essential step of gene expression and in trypanossomatids little was described so far. Previous data from our group demonstrated that the mRNA export pathway is the least conserved along eukaryotes lineages. To better understand mRNA export in deeply diverging eukaryote lineages, we start investigating the function of well conserved proteins in *Trypanosoma cruzi*. Dbp5/DDX19 (*Saccharomyces cerevisiae*/Human) is among the most conserved and functions in the final step of mRNA export, releasing the mRNA to translation after crossing the Nuclear Pore Complex (NPC). The ortholog protein in *T. cruzi* is a DEAD-box helicase with 59% of similarity to Dbp5. Molecular modeling analysis showed that TcDbp5 structure is very similar to eIF4AIII and DDX19. Basically, TcDbp5 presents all typical motifs from DEAD-Box family members with major differences in the N-terminal region. Even similar to eIF4AIII, the *T. cruzi* protein is not associated with polysome fractions. Western blot using cytoplasmic and nuclear fractions showed that TcDbp5 is mainly present in the cytoplasm. It was further confirmed by immunofluorescence microscopy, showing that TcDbp5 is disperse in the cytoplasm and more concentrated around the nucleus. To confirm the association of TcDbp5 with nuclear pore proteins, specific antibodies were raised against nucleoporins to be used in immunoprecipitation assays. Besides, the RNA helicase activity, essential for its function, will be tested by in vitro assays. Since Dbp5/DDX19 associates to the mRNA inside the nuclei and shuttles to cytoplasm where it is activated, we intend to evaluate the mobility of the TcDbp5 in *T. cruzi* by blocking the protein transport from nuclei by treatment with Leptomicyn-B and observing if TcDbp5 accumulates in the nucleus. Taking together, our results will provide evidences for the role of TcDbp5 in *T. cruzi* mRNA export. Supported by CNPq, Fundação Araucária, Fiocruz.

BM.045 - STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A NEW FAMILY OF MEMBRANE PROTEINS OF *TRYPANOSOMA CRUZI* THAT SHARES SIMILARITY WITH THE *TRYPANOSOMA BRUCEI* PROCYCLIC FORM SURFACE GLYCOPROTEINS

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We identified a new family of membrane proteins that share 40% identity with the procyclic-form surface glycoproteins from *Trypanosoma brucei.* ***T. brucei*** procyclic-form surface glycoprotein is a stage-specific antigen with features of a typical transmembrane glycoprotein but with unusual cytoplasmic tail composed of proline-rich tandem repeats. The putative *T. cruzi* procyclic-form membrane proteins (Tc-pmp) have conserved 2−3 transmembrane helices. In addition, some of these glycoproteins (i.e., EAN98004, EAN98005, and EAN98006) seem to contain an uncleaved signal anchor sequence, suggesting that they are located on the cell surface. Tc-pmp gene has expanded into a family of duplicated paralogous genes located in the chromosomal band XX of clone CL Brener. Southern hybridization analysis of *T. cruzi* isolates provides evidence for the strikingly conservation of the Tc-pmp gene family, but also for some chromosomal duplication events in isolates from Tc group I. Tc-pmp transcripts and peptides are expressed in epimatigotes and metacyclic trypomastigotes. The subcellular distribution of Tc-pmp will be analyzed using antibodies against the recombinant. Supported by FAPESP, CNPq and CAPES

BM.046 - EVALUATION OF OXIDATIVE METABOLISM IN NATURAL BENZNIDAZOLE-RESISTANT *Trypanosoma cruzi* STRAINS

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The mechanism of drug resistance of *Trypanosoma cruzi* is poorly understood. Studies have searched potential targets for trypanocidal substances reporting an increase on the production of enzymes involved in the oxidative stress which probably could be responsible for the benznidazole resistance of certain strains. Such enzymes have important roles in the survival and growth of the parasites as: superoxide dismutase (SOD), a metaloenzyme which eliminates superoxide radicals converting them into hydrogen peroxide and molecular oxygen; old yellow enzyme (OYE), an NADPH-flavin oxidoreductase which may be involved in the reduction of some trypanocidal substances and, peroxiredoxin (Prx), which catalyzes the reduction of peroxides. This work aims to evaluate the susceptibility of some different *T. cruzi* strains to benznidazole and also analyse these three enzymes expression. The susceptibility of epimastigotas forms to benznidazole was performed using colorimetric MTT and IC_{50} values of each strain were, respectively: Y = 34.62 µM, Bolivia = 96.06 µM, Santo Inácio (1) = 27.28 µM, Santo Inácio (3) = 105.28 µM, Santo Inácio (8) = 58.40 µM and Quaraí (II) = 63.78 µM. The ORFs (Open Reading Frame) of the three enzymes were cloned and all recombinant proteins were purified. At this moment, the Prx polyclonal antibody was produced in rabbit and probed by Western blot to characterize Prx protein production in benznidazole-treated *T. cruzi* strains. The results showed an increase on Prx-expression in some resistant strains. The anti-Prx antibody also reacted with other isoforms of the enzyme; however, it seems that the higher expression is related to the oxidized form of the enzyme. When the parasites were treated with hydrogen peroxide as a control, the expression of Prx was inhibited. According to other studies, a previous induction may be necessary to improve the resistance of the parasite to oxidative substances.
Supported by FAPESP.

BM.047 - THE ABC TRANSPORTER SUPERFAMILY IN *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 goal of this study was to identify and classify ABC transporters in the *T. cruzi* genome. The putative ABC genes were retrieved from the TriTrypDB database with the ABC protein signature motif as query. 56 ORFs coding for putative ABC proteins were identified. This represents 0.22% of the total number of *T. cruzi* genes (~25,000). Of the 56 ORFs, 36 are intrinsic membrane proteins and 20 do not show any transmembrane domain. The two haplotypes of the same ABC gene were identified for most ORFs. However, 21 haplotypes were not encountered, suggesting genic loss or incomplete sequencing/assembly of CL Brener genome. Three pseudogenes were also disclosed. The ABC genes are largely dispersed in the genome and are found on 17 different CL Brener chromosomes. Sequences were assigned as orthologues with *Leishmania* and *Trypanosoma* species if they showed the highest score in BLAST search analyses using OrthoMCL Database. Multiple sequence alignments were performed on the amino acid sequences of the ATP-binding domains by using CLUSTAL W with the default settings. The two Walker A and B nucleotide-binding domains of full-length ABC proteins were treated independently for alignments. The resulting multiple sequence alignments were subjected to analyses using neighbor-joining and bootstrapped maximum parsimony methods. *T. cruzi* ABC proteins were classified into ABCA to ABCH subfamilies, following the HUGO nomenclature adopted for eukaryotes ABC proteins. The present study provides a phylogenic classification of *T. cruzi* ABC proteins and sets the basis for further functional studies on this important class of proteins, some of which are associated to multidrug resistance. Support: FAPESP; CAPES; CNPq.

BM.048 - GLOBAL MAPPING OF CHROMOSOME-SIZED SCAFFOLDS ON CHROMOSOMAL BANDS OF TRYPANOSOMA CRUZI CLONE CL BRENER

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The genome of *T. cruzi* (clone CL Brener) has been sequenced and compiled as contigs and scaffolds, but specific chromosome location of all scaffolds is unknown. Recently, the genome sequence was assembled in 41 chromosome-sized scaffolds (TcChr) and the sequence deposited in the TriTrypDatabase (Weatherly et al., 2009, BMC Genomics 10:255; http://tritrypdb.org/tritrypdb). To determine which chromosome-sized scaffolds belong to the chromosomal bands, we have selected 123 genetic markers, reaching an average density of one marker every 300 kb. The probes were hybridized to chromosomal bands from CL Brener and G strain separated by PFGE (pulsed field gel electrophoresis). Until now, 31 chromosome-sized scaffolds have been already assigned to the chromosomal bands. Most of chromosome-size scaffolds were ordered and oriented according to in silico assembly. This approach allows the identification of several homologous chromosomes of different sizes, and chromosomal duplication and translocation in *T. cruzi* genome. Several anomalies were addressed and several contigs including subtelomeric sequences were reassigned. For instance, markers at 5´ end of chromosome-sized scaffold 35 (TcChr-35) hybridized with chromosomal bands XI and I, while a third marker distant 442 kb hybridized with band XI and the last marker positioned at 3´ end of TcChr-35 hybridized with chromosomal band XVIII. These misassembled contigs were easily identified by chromoblot hybridization. Our work addresses erroneous computer-based assignment of a few numbers of contigs and emphasizes the need for alternative and confirmatory methods of scaffold construction. Taken together, our results can be useful to the final assembly of *T. cruzi* chromosomes and provide important information about the genomic organization of the parasite.
Supported by FAPESP, CNPq and CAPES.

BM.049 - PCR AMPLIFICATION, cDNA CLONING AND EXPRESSION OF BRANCHED CHAIN AMINO ACID 2-OXOISOVALERATE DEHYDROGENASE COMPLEX IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi, is the etiological agent of Chagas´ disease affecting about 10 million individuals in the endemic areas of Americas and with about 40 million people at risk of acquiring the disease. *T. cruzi* has the ability to use carbohydrates and amino acids as carbon and energy sources. Beyond their participation as protein components, amino acids are also involved in osmoregulation, metacyclogenesis and management of nutritional and oxidative stress in *T. cruzi.* 2-oxoisovalerate dehydrogenase complex consists of three polypeptides coded by three genes: 2-oxoisovalerate dehydrogenase E1 component (EC 1.2.4.4), 2-oxoisovalerate dehydrogenase E2 component (EC 2.3.1.168) and dihydro lipoamide dehydrogenase (EC 1.8.1.4), being the whole complex responsible for the catabolism of second step of leucine, isoleucine and valine degradative pathways. In the present work, the three genes were identified in the *T. cruzi* genome and further amplified from *T. cruzi* genomic DNA, rendering amplicons of 1107, 1311 and 1434 bp for E1 component, E2 component and dihydro lipoamide dehydrogenase respectively. Furthermore, the obtained DNA fragments were cloned into vector pGEMT Easy and sub-cloned into expression vector pET28a+. Identities of all genes were confirmed by sequencing. The cDNAs were expressed in different *Escherichia coli* strains, fused to an N-terminal His-tag. 2-oxoisovalerate dehydrogenase E1 component was successfully expressed in BL 21 Codon Plus by the induction of 0.5 mM IPTG for 20 hours at 37˚C, 2-oxoisovalerate dehydrogenase E2 component was successfully expressed in Rosetta pLysS by the induction of 0.1 mM IPTG for 20 hours at 28˚C and the expression of dihydro lipoamide dehydrogenase was achieved by using Rosetta pLysS and induction with 0.1 mM IPTG for 20 hours at 28˚C. In all cases, soluble proteins were obtained, having the predicted sizes (40.18, 47.36 and 50.51 kDA for E1 component, E2 component and dihydro lipoamide dehydrogenase respectively). This research project has financial support from TWAS (The Academy of Sciences for the Developing World), FAPESP and CNPq.

BM.050 - A PLATFORM FOR *TRYPANOSOMA CRUZI* REVERSE GENETICS: A STEP CLOSER FOR HIGH-THROUGHPUT GENE CHARACTERIZATION

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The sequencing of the *Trypanosoma cruzi* genome revealed the high percentage of genes encoding hypothetical proteins, being necessary a functional characterization of these proteins to better understand the biology of the parasite. Reverse genetics-based tools have been largely employed for many purposes to obtain biological information on genes of unknown function. Actually, there are technical limitations in protein expression in heterologous systems, being of great need the development of a high-throughput reverse genetics platform for *T. cruzi*. We previously constructed plasmid vectors carrying genes for N-terminal fusions, as fluorescent proteins (green, cyan and yellow), and sequences for the *c-myc* epitope, tandem affinity purification (TAP) or poly-histidine tags. These vectors have neomycin or hygromycin as antibiotic resistance marker and to ensure a fast and efficient cloning system the platform is based on Gateway® technology. Here we show some applicabilities of these vectors and modifications made to improve the platform. Using vectors containing N-terminal GFP and CFP tags, we success co-localized two previously characterized proteins (*Tc*Rab7 and PAR2) and using the TAP tag vector we purified two-protein complex (*Tcr*L27-ribosome and *Tc*pr29A-proteasome) validating this platform. We made the exchange of one restriction site, allowing each restriction enzyme to cut only once in the vector. Such modifications become the platform fully flexible allowing the exchange of all elements, such as promoters, fusion tags, intergenic regions or antibiotic resistance markers. In the new version of the vectors, we have add phleomycin as an option for antibiotic resistance marker and created a C-terminal fusion tag vector. The development of this platform is an important step towards improving available methodologies for the characterization of thousands genes whose function remain unknown in *T. cruzi*. Supported by NIH, CNPq and FIOCRUZ.

BM.051 - MULTIPLEX TAQMAN REAL TIME PCR PROTOCOLFOR IDENTIFICATION OF *TRYPANOSOMA CRUZI* DISCRETE TYPING UNITS

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Diversity of *Trypanosoma cruzi* extensively demonstrated using different biological, biochemical and molecular strategies targeting different genetic markers, allowing the identification of six discrete typing units (DTUs) designated as Tc I – Tc VI (Zingales et al., 2009). In this presentation, we propose a novel algorithm for identifying DTUs, based on two consecutive multiplex real time PCRs using DTUs´specific TaqMan probes. The first PCR strategy involves one common forward primer and four differential reverse primers for amplification of the intergenic region of the spliced leader (SL) genes, and specific LNA Taqman probes for detection of four groups of DTUs: Tc I, Tc III, Tc IV and Tc II/V/VI. This strategy was tested using 27 *T. cruzi* reference strains from Argentina, Chile, Brazil, Colombia, Mexico and USA, belonging to the six DTUs. SL-multiplex PCR was used to characterize *T. cruzi* isolates from faeces of sylvatic triatomines (*Triatoma gerstaeckeri*, *T. protacta*, *T. indictiva*, *T. sanguisuga* and *T. lecticularia*), and from isolates from blood cultures of opossums and raccoons from Southern USA, allowing DTU identification in 31/39 samples, 4 of them being characterized as mixed infections composed of Tc I and Tc IV DTUs. The second PCR further discriminates among Tc II, Tc V and Tc VI DTUs by amplifying 18s rRNA and cytochrome oxidase subunit II (COII) genes using 3 differential TaqMan probes. It was tested using 6 *T. cruzi* reference strains. The protocol herein described, currently under standardization, may constitute a methodological improvement in DTU identification, increasing specificity and reducing costs due to the incorporation of TaqMan probes in multiplex reactions and enabling detection of mixed DTU infections, which have been difficult to be detected by conventional PCR assays.
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BM.052 - INSIGHTS INTO THE CHROMOSOME ARCHITECTURE AND EVOLUTION IN *TRYPANOSOMA CRUZI*

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We compared the level of synteny between *Trypanosoma cruzi* and *Trypanosoma brucei* by looking at homologous chromosomal segments. We chose genetic markers previously mapped on megabase chromosomal bands XX and XVI of *T. cruzi* (clone CL Brener). Recently, *T. cruzi* genomic sequences were assembled in platforms here named as chromosome-sized scaffolds (TcChr). Taking into consideration that *T. brucei* and *T. cruzi* exhibit a striking conservation of gene order, we compared chromosomes XX and XVI to their homologous regions in *T. brucei.* TcChr37 and TcChr4 were assigned to chromosome XX of CL Brener and they are homologous to *T. brucei* chromosome Tb10. The comparison between the sequences of large chromosomal fragments from each species showed that there is a large segment inversion involving TcChr37. The region located between two chromosome inversion segments in Tb10 corresponds to TcChr4. TcChr39 was assigned to chromosome XVI in CL Brener. It is homologous to *T. brucei* chromosomes Tb9 and Tb11. Trypanosomatid common ancestor seems to present smaller chromosomes and a more fragmented genomic organization. This is in agreement with bioinformatics analysis of TcChr39 where two different fragments have joined to form one single chromosome in *T. cruzi* (TcChr39) while these same fragments have joined to others fragments to form different chromosomes in *T. brucei* (Tb11 and Tb9). Comparative genome analysis showed evidence that there appear to be several cases of chromosome fusions in *T. brucei*. TcChr14, TcChr30 and TcChr35 were assigned to different regions of Tb11 chromosome. We showed that specific markers along Tb11 chromosome hybridized with distinct chromosomal bands in CL Brener. We found overwhelming evidence that many fragments corresponding to entire chromosomes in *T. cruzi* appear to have joined to form a single *T. brucei* chromosome (Tb11). A detailed study of each chromosome must be done to understand chromosome evolution in trypanosomes. Supported by FAPESP, CNPq and CAPES.

BM.053 - *TRYPANOSOMA CRUZI* TRANSCRIPTOMICS IN RESPONSE TO STEROL BIOSYNTHESIS INHIBITORS

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Similar to fungi, and unlike mammals, *T. cruzi* produces mainly ergosterol. Therefore, its biosynthesis route represents a potential chemotherapy target for Chagas disease treatment. The present work aims to analyze the transcriptome of this parasite in response to two drugs acting at different points of this pathway: Ketoconazole and Lovastatin. First we calculated the drug doses capable of inhibiting the epimastigote culture growth by 50% after 3 days of exposure (IC50), or kill all cells in 24 hours (LD100). Polissomal RNA samples were extracted in short exposure times (30 and 60 minutes) to the LD100 (in triplicate) and in long times (1 to 5 days) to IC50 (in duplicate). The cDNAs generated from purified poly A^+ fractions were analyzed by massive parallel sequencing (RNA-Seq) with the SOLiD 3 platform. We generated about 320 million reads of 50 bases for the LD100 experiments, and a preliminary analysis showed more than 1000 genes differentially expressed in response to Lovastatin and about 400 in response to ketoconazole. These genes include heat shock proteins, kinases, cytoskeletal proteins and several RNA binding proteins. Further characterization was performed by transmission electron microscopy and flow cytometry, where high doses of both drugs induced a process similar to autophagy,. This was not corroborated by modulation at the transcriptional level, suggesting a possible role for post-translational regulation. For the IC50 doses, microscopy and flow cytometry experiments showed an increased number of reservosomes, organelles possibly involved in storage and synthesis of sterols. Currently, we are analyzing 220 millions reads produced for the IC50 experiments. The data generated constitute the first global assessment of the transcriptomic regulation of *T. cruzi* to these drugs, leading to a better understanding of the molecular changes in response to the inhibition of the sterol biosynthesis. Keywords: *Trypanosoma cruzi*; transcriptomics; sterol biosynthesis. Financial support: CNPq, CAPES, NIH, Fundação Araucária and FIOCRUZ

BM.054 - FUNCTIONAL CHARACTERIZATION OF MYOSINS COMMON TO TRYPANOSOMATIDS IN *Trypanosoma cruzi*

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The cytoskeleton of eukaryotic cells consists of three types of polymers that control the shape and mechanics of cells: actin filaments, microtubules and a group of polymers collectively known as intermediate filaments. The cytoskeleton of the Trypanosomatidae family organisms is still very poorly studied. This family includes some organisms that cause human diseases, such as Chagas disease and sleeping sickness, caused by species of the *Trypanosoma* genus, and leishmaniasis, caused by species of *Leishmania* genus. While microtubules are the main filament of the trypanosomatids, it had not been detected yet the presence of the actin filaments, although it has been shown the presence of this protein in its monomeric form by immunoassays. Both microtubules and actin filaments may function as tracks for molecular motors, responsible for the cargo transport. Myosins are motor proteins that move along the actin filaments and are present in almost all eukaryotic organisms; they are classified into classes according to their similarities. Trypanosomatids possess Class I myosin, present in almost all organisms, and a myosin present only in this family. The presence of a myosin exclusive to trypanosomatids is quite intriguing, mainly because little is known about the actin cytoskeleton of these organisms. This work aims the functional characterization of the two myosins of *T. cruzi* common to the trypanosomatids and has as specific purposes: protein expression analysis in different stages of the life cycle and cellular localization using antibodies produced in mice; localization through expression of a GFP-tagged myosin in *T. cruzi*; dominant negative assays and inhibition of protein expression in *T. brucei* by RNA interference. Genes were cloned and proteins were expressed in *Escherichia coli*, antibodies anti-myosin were produced in mice and immunoassays, dominant negative and interference assays are in progress. Supported by CNPq and Fundação Araucária.

BM.055 - CHARACTERIZATION OF TWO *T.cruzi* PROTEINS: INVOLVEMENT IN REGULATION OF GENE EXPRESSION

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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 are an important pathway of posttranscriptional regulation in eukaryotes, and recently was shown that mRNA granules are present in *T. cruzi*. TIA1/TIAR are nuclear proteins involved in splicing, apoptosis, posttranscriptional regulation and also play an important role in eukaryote´s stress response, since they migrate to the cytoplasm under stress conditions acting in the assembly of stress granules. Two *T. cruzi* proteins similar to the human TIA, named TRRM2 and DRBD9, were chosen for this study. The genes encoding these proteins were cloned using the Gateway® technology, recombinant proteins were obtained and polyclonal antibodies were produced, in order to perform immunoassays to characterize these proteins. The immunofluorescence assay showed that TRRM2 presents nuclear localization and DRBD9 appears in cytoplasmic granules. Further colocalization assays will be performed to analyze the composition of the DRBD9-containing granules. Both proteins are expressed throughout the parasite's life cycle and sucrose gradient assays showed that DRBD9 is associated to non-polysomal heavy complexes, while TRRM2 only appears in the light fractions, suggesting that it is not associated with large protein complexes. The characterization of these proteins will help to elucidate the mechanisms of posttranscriptional regulation in *T. cruzi*.
Supported by FIOCRUZ and CNPq.

BM.056 - GENE EXPRESSION IN *TRYPANOSOMA CRUZI* PARASITES EXPRESSING A TcBDF2 DOMINANT NEGATIVE MUTANT

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Histones postranslational modifications, their role in gene expression regulation and others nuclear events in trypanosomatids are currently an active field of research. Recently it has been proposed a model for RNA Polymerase II transcription initiation in *Trypanosoma brucei* which involves histone posttranslational modifications that would serve as binding site for a bromodomain factor (BDF). In turn, this BDF would recruit chromatin remodelling proteins involved in incorporating variants of histones into nucleosomes, resulting in less stable nucleosomes, permissive for polymerase binding and transcription initiation. We have reported that *Trypanosma cruzi* Bromodomain Factor2 TcBDF2 is expressed in discrete regions inside the nucleus, between dense and less dense chromatin regions. It binds H4, with preference for K10 and K14 acetylated residues. In order to analyze its function, TcBDF2 fused to c-myc tag and deleted at its C-term (TcBDF2ΔC) were expressed in epimastigotes from a tetracycline regulated promoter. TcBDF2ΔC mutant acts as a dominant negative, inhibiting parasite growth and enhancing their sensitivity to UV irradiation. Global transcriptome from TcBDF2ΔC, TcBDF2-c-myc and control parasites was evaluated 48 h after tetracycline addition by using a 10 K oligonucleotide microarray. Limma software (R) was used to determine mRNA relative abundances (LFC>1.5 and p value =0.01) among the three strains. Just 25 genes were up- and down-regulated between TcBDF2myc and control parasites. However, 100 genes were differentially expressed in TcBDF2ΔC respect to control parasites and this quantity increased to more than 1000 when mRNA relative abundances of TcBDF2ΔC and TcBDF2myc parasites were compared. RT-PCR assays are in progress to corroborate these data. Neither up-regulated nor down-regulated genes showed a pattern of localization in chromosomes respect to strand switch region. Our results support the model proposed and the hypothesis that acetylated histones and BDF2 are taking part in gene expression process throw chromatin remodeling. Supported by ANPCyT and CONICET

BM.057 - GENE EXPRESSION AND REGULATION OF HSP10 CHAPERONIN GENES OF *TRYPANOSOMA CRUZI*

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The heat shock proteins (HSPs) are a group of chaperone proteins that have their gene expression increased in response to heat shock and other stressing agents. Some HSP families have received more attention because of their important function in the cell. Among these is the chaperonin (HSP60 and HSP10) families. In our study, we are investigating how gene expression of HSP10 is regulated in *Trypanosoma cruzi*, that cause Chagas disease, and also how it is coordinated with HSP60 gene expression. It was shown in earlier studies by our lab that the gene is more similar to *the T. brucei* ortholog, and that HSP10 gene mRNA level remains unchanged after heat shock. In the present study we investigated the mRNA processing sites. A single trans-splicing acceptor site was identified 110 nucleotides upstream of the HSP10 coding region, which apparently is used in each of the three gene copies. We are currently mapping the polyadenylation site(s). After identifying the lengths of both 5' and 3' UTRs, reporter plasmids will be contructed in which both UTRs of the central copy of the HSP10 gene will be placed flanking the reporter gene, chloramphenicol acetyltransferase (CAT). Stable cell lines will be generated by transfection of epimastigotes and used to investigate the presence of heat shock-responsive elements in the CAT mRNA. We are also investigating the stability of the endogenous HSP10 mRNA by measuring its half-life using real-time RT-PCR and northern blots. Finally, with the purpose of obtaining HSP10-specific antibodies, we produced a GST-HSP10 fusion protein after cloning the coding region in an expression vector. The HSP10-specific antibodies will be used for western blot and intracellular localizations analysis. Suported by CNPq and FAPERJ.

BM.058 - EVALUATION OF PCR ASSAYS SENSITIVITY FOR MOLECULAR MARKERS EMPLOYING ARTIFICIAL MIXTURES OF DIFFERENT *Trypanosoma cruzi* Strains

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Chagas disease is a tropical disease caused by the protozoan *Trypanosoma cruzi* that is endemic in Latin America and affects approximately 10 million individuals. Patients in endemic areas may be infected by multiple contacts with different triatomines, and these, in turn, may feed on different infected individuals. This scenario propitiates the formation of multiclonal populations in hosts and vectors. By using microsatellite analyses it was demonstrated that the percentage of multiclonal populations decrease progressively when we compare strains isolated from the sylvatic cycle with those isolated from man in acute or chronic phase of the infection. However, the identification of mixed populations in a host depends primarily on the sensitivity of the molecular markers used. Thus, the major goal of this work was investigate the sensibility degree of PCR assays for different molecular markers using artificial polyclonal populations. Twenty and seven DNA mixtures from Sílvio X10 cl1 (TcI) and CL Brener (TcVI) in different percentages ranging from 99,9% Silvio/0,1% CL Brener to 0,1% Silvio/99,9% CL Brener, were initially analyzed. These mixtures containing a total of 1 ng of parasite DNA were submitted to PCR assays for 24Sα rDNA and Cytochrome Oxidase II (CO II) genes and seven microsatellite polymorphic loci: SCLE10, SCLE11, MCLF10, TcAAAT6, TcATT14, TcTAT20 and TcCAA10. Preliminary results indicated that PCR assays for 24Sα rDNA and COII genes presented more sensitive in detecting mixture of different parasite populations than those accessed by microsatellite analyses. The detection limits found for 24Sα rDNA and COII were 0,5 and 5% for Silvio and 2 and 8% for CL Brener, respectively. The microsatellite loci more sensitive were TcAAAT6, SCLE10 and SCLE11 with sensitivity of 1, 4 and 10% for Silvio and 2, 3 and 5% for CL Brener, respectively. However, to confirm these results additional experiments will be performed using new combinations of DNA from *T. cruzi* strains belonging to the six different phylogenetic lineages.
Supported by FAPEMIG, CNPq, CAPES.

BM.059 - GENOMIC MARKERS FOR THE DIFFERENTIAL PCR DIAGNOSIS OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* INFECTIONS IN HUMAN BLOOD SAMPLES

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Recent reports of human infections by *Trypanosoma rangeli*, a protozoan parasite non-pathogenic to humans, and of outbreaks caused by *Trypanosoma cruzi* infections point out the necessity of new approaches for the reliable differential diagnosis of the infections caused by these trypanosomatid species. The aim of this work is to develop PCR protocols for detection of *T. rangeli* and *T. cruzi* DNA in culture and in experimentally infected human blood samples. Primers used belonged to three classes: (i) conserved *T. rangeli* and *T. cruzi* telomeric (T189Fw2/Tc189Rv3 and TrF3/TrR8) and Chaperone (Tr3/Tr4) sequences were used as controls; (ii) species-specific amplification of *T. rangeli* DNA (Tr1/Tr2, Tr5/Tr6 and Tr7/Tr8, no predicted function); and (iii) species-specific amplification of *T. cruzi* DNA (Serine Carboxipeptidase e Ubiquitin intergenic region). PCR was conducted in DNA samples obtained from axenic cultures of *T. cruzi* (four strains), *T. rangeli* (four KP1+ strains and two KP1- strains), and *Leishmania sp*. (*L. major*, *L. infantum* and *L. braziliensis*) and in DNA samples obtained from human blood experimentally contaminated with serial dilutions of *T. rangeli* P07 and *T. cruzi* Y parasite strains (0; 0,1; 1; 10; 100 e 1.000 parasites/mL). Control reactions allowed the species confirmation and the assessment of DNA integrity. Expected amplicons were specifically observed for all targets. Primers Tr05/Tr06 allowed the observation of a differential amplification pattern in *T. rangeli* samples, entirely coincident with the parasite genotypes defined by kDNA classification. Considering the experimental conditions used, each reaction permitted the detection of five genomic copies of *T. rangeli* or *T. cruzi*, a detection limit which can be improved with the optimization of PCR protocols. Furthermore, the use of primers Tr05/Tr06 allows the determination of intra-specific polymorphism in *T. rangeli* suggesting the possibility of being used as a marker for diagnosis and testing of new genotyping and phylogenetic relationships.
Supported by FAPEMIG and CNPq.

BM.060 - *Trypanosoma cruzi* GENES INVOLVED IN THE GPI BIOSYNTHETIC PATHWAY: CELLULAR LOCALIZATION, FUNCTIONAL COMPLEMENTATION AND GENE DELETION

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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 and evasion of the host immune response. Moreover, 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 is an important aspect of the study of the parasite cell biology and offers potential targets for drug development towards treatment of Chagas disease. Here we present the characterization of nine *T.cruzi* genes encoding homologs of proteins involved in GPI biosynthesis: DPM1, PIG-A, GPI1, PIG-L, PIG-M, PIG-V, GPI10, GAA-1, and GPI8. To verify whether these genes encode protein homologues of *Saccharomyces cerevisiae* enzymes, we transformed yeast mutants defective in each GPI biosynthesis gene and showed that DPM1, PIG-A, GPI1, PIG-L, and GPI10 from *T.cruzi* restore the growth of mutant yeasts in non-permissive condition. Sequences corresponding to PIG-A and PIG-L 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, PIG-A, PIG-L, and GPI8 genes in fusion with GFP, which were found to co-localize with a red fluorescent reporter protein containing an endoplasmic reticulum localization signal. 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. After transfection with the knockout construct and selection for neomycin resistant parasites, characterization of the mutants was realized by PCR, which confirmed the integration of the NEO gene and disruption of one allele of GPI8 gene. Further characterization of the single allele mutant, as well as deletion of the second allele, are currently underway.
Supported by CNPq, FAPEMIG and HHMI.

BM.061 - PURIFICATION OF ACTIVE RECOMBINANT *Trypanosoma cruzi* APYRASE (NTPDase 1): REFOLDING, STABILITY AND EVALUATION OF OLIGOMERIZATION

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An ecto-NTP diphosphohydrolase activity was previously characterized on the surface of live *T. cruzi* parasites. Additionally, trypomastigotes (infective form) were shown to have a 2:1 ATP/ADP hydrolysis ratio, while epimastigotes (non-infective form) presented a 1:1 ratio, suggesting a possible role for the NTPDase in the parasite's virulence mechanisms. Nowadays, it's known that E-NTPDases act as virulence factor and infectivity facilitators. Therefore, these enzymes appear as good targets for the development of new approaches to chemotherapy, immunotherapy and diagnosis of Chagas disease. Using the soluble portion of *T. cruzi* NTPDase-1 cloned in pET21b (Novagen), we performed its heterologous expression in *E. coli* BL21-DE3 system, purified it from inclusion bodies, renaturated and analyzed the stability and oligomerization state of the recombinant protein. The stability of the purified protein was tested at -22 °C, 4 ° C and 22 ° C for 20 days. The enzyme activity was stable at 4 °C for 11 days, with active peak in 48h. The oligomerization state was evaluated by molecular exclusion chromatography in Sephadex G200 column in AKTA-Purifier system (GE). This analysis showed that recombinant NTPDase-1 present more than one protein peak. One of them is the monomeric protein and another fraction of the protein is in an agglomerated or oligomerizated state. Only the monomeric protein remains active after the chromatographic separation. Now, the active protein is been tested with adenosine analogs as possible inhibitors. These studies will open a new range of possibilities, leading us in the search for new drugs, to be applied in Chagas disease chemotherapy.
Supported by: Capes, FAPESP, UFV, FAPEMIG and CNPq

BM.062 - mRNA CIS-ACTING ELEMENTS INVOLVED IN STAGE-SPECIFIC REGULATION OF GENE EXPRESSION IN *TRYPANOSOMA CRUZI*

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Several studies have shown that transcription in trypanosomatids is policistronic and that sequences present in untranslated regions of *T.cruzi* transcripts are involved in post-transcription regulation of gene expression. Proteins bound to specific sequences in the 3'UTR of mRNAs are known to affect mRNA stability, thus controlling individual mRNA steady levels during the parasite life cycle. In order to evaluate the role of 3'UTRs from stage-specific mRNAs of *T cruzi*, we generate a new vector, named pTcDuaLuc, in which sequences present in the 3 UTR can be inserted downstream from the firefly-luciferase reporter gene. Because the *Renilla*-luciferase gene is also cloned in this vector, it can be used to normalize the values, avoiding the requirement of co-transfection with a control plasmid. In transient transfection assays, using the circular vector, we showed that luciferase activity is higher in epimastigotes transfected with pTcDuaLuc containing the 3'UTR of alpha tubulin, whose mRNA is up-regulated in epimastigotes. After generating stable transfected cell lines from epimastigote cultures transfected with pTcDuaLuc containing 3´UTR from the alpha tubulin gene, we showed that the spliced leader sequence and poly-A tail were inserted in the predicted sites in the firefly luciferase mRNA, thus indicating the correct processing of the transcripts. We also showed that deletion of a U-rich region in the same 3'UTR results in decreased luciferase activity and mRNA levels because it affects poly-A addition. More importantly, we showed that luciferase activity as well mRNA levels changed during the *T. cruzi* life cycle according with the 3'UTR sequences inserted downstream from the luciferase coding region: they are higher in epimastigote when the 3´UTR is derived from the alpha tubulin mRNA, in amastigote when the 3´UTR is derived from the amastin mRNA and in trypomastigotes when the 3´UTR is derived from the trans-sialidase and MASP mRNAs. Financial support:CNPq, FAPEMIG, HHMI.

BM.063 - A SENSITIVE MULTIPLEX PCR SYSTEM FOR DOUBLE INVESTIGATION IN *Trypanosoma cruzi* POPULATION STUDIES: CLASSIFICATION INTO SIX DISCRETE TAXONOMIC UNITS AND INDIVIDUAL TYPING

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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 to identify the *T. cruzi* DTUs and simultaneously characterize intraspecific variability among the parasite strains. Herein, we propose a sensitive multiplex PCR system based on a Full Nested strategy 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 the second round of PCR assays. All DNA 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, CAPES, CNPq.

BM.064 - *TRYPANOSOMA CRUZI* : FUNCTIONAL CHARACTERIZATION OF A POLY-Q RICH PROTEIN ORTHOLOGOUS TO *T. BRUCEI* GAP2

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In trypanosomatids, regulation of gene expression occurs mainly at the post-transcriptional level. The stability of mRNA and the access to polysomes must be tightly regulated, allowing *Trypanosoma cruzi* to adapt to the different environmental conditions during its life cycle. Post-transcriptional regulation requires the association between mRNAs and certain proteins to form mRNP complexes. To characterize protein complexes associated with non-translated or translated mRNAs, 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). Among these proteins, a hypothetical poly-Q rich protein was identified in the polysomal fraction of epimastigotes under nutritional stress. The aim of this study is to characterize this protein and to determine if it is involved with regulation of gene expression. The gene encoding this protein was cloned in an expression vector and the recombinant protein was used for the production of polyclonal antiserum. Western blot analysis was performed and showed that this protein is downregulated in metacyclic forms. When epimastigotes and nutritionally stressed epimastigotes lysates were loaded onto a 15-55% sucrose gradient and the fractions were analysed by immunoblot, we observed that the protein was present in polysome-dependent complexes only in the latter. Immunofluorescence analysis showed that the protein is localized mostly in the kinetoplast in both forms. *In situ* labeling of the nicks and gaps in network minicircles with terminal deoxynucleotidyl transferase (TdT) and fluorescent dUTP followed by immunofluorescence showed partial colocalization indicating some involvement with minicircle replication. These data suggest that this protein can be part of mitochondrial mRNPs or can have different roles depending on its localization. Supported by CNPq and CAPES.

BM.065 - MOLECULAR KARYOTYPE MAINTENANCE IN *TRYPANOSOMA CRUZI* AFTER DNA DOUBLE STRAND BREAKS (DSBs) INDUCED BY IONIZING RADIATION.

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T. cruzi displays a pronounced resistance to lethal effects of ionizing radiation and exhibit high DNA repair capabilities. Gamma radiation induces cell growth arrest and chromosomal fragmentation, which can be associated with DNA double-strand breaks (DSBs). However, 48h after irradiation, normal sized chromosomal bands have been detected by pulsed field gel electrophoresis (PFGE) (Silva et al. Mol Biochem Parasitol 149:191, 2009). Although very effective recombination repair mediated by Rad51gene has been proposed as a contributing factor, a detailed understanding of *T. cruzi* irradiation responses has not yet been obtained. Therefore, our study was carried out to understand the chromosome reconstruction after exposition of epimastigotes to gamma radiation. Exponentially growing epimastigotes (clone CL Brener and G strain) were exposed to gamma-radiation doses of 500 to 2000 Gy. Cell survival, growth inhibition, DNA damage and chromosomal recovering were studied at various post-irradiation time intervals. Growth inhibition starting immediately after irradiation and extending up to 96h was observed at dose of 500 Gy, while a large delay (12 days) was observed at 1000 Gy. Parasites irradiated at higher doses (1500 and 2000 Gy) did not survive. Using assays detecting DSBs (TUNEL) and chromosomal fragmentation (PFGE), we evaluated the genotoxic effect of irradiation on epimastigotes. Six days after irradiation, the percentage of TUNEL-positive cells in samples irradiated with 500 Gy is near to that found in non irradiated parasites and 3-fold higher than those cells receiving 1000 to 2000 Gy. We found that TUNEL positivity and chromosomal fragmentation seems to exist in unison. We compared the level of synteny between irradiated and non irradiated parasites by looking at homologous chromosomal segments. Irradiated cells exhibit a striking conservation of gene order when compared to non treated parasites. Factors causing the recovery of chromosomes from radiation-induced damage are presently being investigated.
Support: FAPESP, CNPq and CAPES.

BM.066 - FURTHER MOLECULAR CHARACTERIZATION OF *TRYPANOSOMA CRUZI* STRAINS ISOLATED IN SANTA CATARINA STATE – BRAZIL, AFTER AN OUTBREAK OF ORAL TRANSMISSION IN 2005.

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In March 2005, several cases of acute Chagas disease occurred in Navegantes, Santa Catarina-BR, related to the ingestion of *Trypanosoma cruzi* in sugar cane juice, which during its preparation was contaminated by the naturally-infected insect vectors. All the infected individuals had ingested the juice in February 13 afternoon, in the same place. A total of 10 strains were isolated from different hosts next to the outbreak area or from the infected patients: SC90 (isolated from *Didelphis aurita*), SC93 (isolated from *Triatomma tibiamaculata)*, SC94, SC95, SC96, SC97, SC98, SC99, SC101 e SC102 (isolated from humans) and were characterized as *T. cruzi* I and *T. cruzi* II populations at that time. Herein, we characterized these populations by analyzing three polymorphic genes (Cytochrome Oxidase –COII, spliced leader intergenic region - SL-IR, and 24Sα rRNA genes and six microsatellite loci (SCLE10, SCLE11, MCLF10, TcAAAT6, TcTAC15 and TcTAT20). The main goal was not only to determine the major lineages of the strains in according to the classification proposed in the Second Satellite Meeting held in Búzios-RJ, but also to characterize the intra-lineage variability identifying eventual polyclonal or mixed populations. As expected, the majority of the isolates were classified into *T. cruzi* II lineage, but 3 isolates were composed by population mixtures (*T. cruzi* I + *T. cruzi* II or *T. cruzi* II + *T. cruzi* VI). In addition to the expected fragments of 110 and 125bp, the 24Sα rDNA analysis revealed anomalous fragments of 117 and 119bp demonstrating the great variability for this marker in these isolates. Furthermore, the presence of identical microsatellites profiles among isolates obtained from different vectors and hosts suggests the presence of characteristic genotypes circulating in this endemic region. Further work is ongoing to analyze single cells derived of these polyclonal populations using FACS Cell Sorter apparatus. Supported by FAPEMIG, CNPq, and CAPES.

BM.067 - IDENTIFICATION OF DNA TARGETS OF THE NUCLEAR PERIPHERY COILED-COIL PROTEIN TcNUP-1 IN *Trypanosoma cruzi*.

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The nuclear lamina is a structure that lines the inner nuclear membrane. In metazoans, lamins are the primary structural components of the nuclear lamina and are involved in several processes. Eukaryotes that lack lamins have distinct proteins with homologous functions. Some years ago, a coiled-coil protein in *Trypanosoma brucei*, NUP-1, was identified as the major filamentous component of its nuclear lamina. However, its precise role has not been determined. We characterized a homologous protein in *Trypanosoma cruzi*, TcNUP-1, and identified its *in vivo* DNA binding sites using a chromatin immunoprecipitation assay. We demonstrate for the first time that TcNUP-1 associates with chromosomal regions containing large non-tandem arrays of genes encoding surface proteins. We therefore suggest that TcNUP-1 is a structural protein that plays an essential role in nuclear organization by anchoring *T. cruzi* chromosomes to the nuclear envelope.
Supported by CNPq and Fundação Araucaria.

BM.068 - THE GTPase RJL IS INVOLVED IN METACYCLOGENESIS OF *Trypanosoma cruzi*

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RJL proteins belong to the Ras superfamily of small GTPases. The cellular function of this new family is still uncertain. We have previously showed the sequence conservation of RJL genes in the genome of trypanosomatids and the expression in the three evolutive stages of *Trypanosoma cruzi*. In this work we aimed to uncover the function of TcRJL in *T. cruzi*. The wild type (TcRJL) and the mutant (TcRJL-S37N), a dominant-negative mutation in the GTPase, were cloned in the trypanosomatid expression vector (pTEX-GFP) and transfected in epimastigotes. After selection we pursued a number of observations in order to reveal a specific phenotype. Our results with fluorescent microscopy showed that RJL is dispersed in the cytoplasm. Growth curve analysis did not reveal any significant differences compared to the control DM28c strain. However, metacyclogenesis assays showed that cells overexpressing RJL did not complete the differentiation process and were arrested in the intermediate stage. Pull down and co-immunoprecipitation assays revealed an associated 20kDa protein which is under characterization. Moreover, TLC experiments showed a poor hydrolysis of GTP by RJL. In conclusion, our results suggest an important involvement of RJL in metacyclogenesis and a poor GTPase activity. Experiments of protein-protein interaction has showed a putative associated protein.
Supported by CAPES,FAPERJ and CNPq

BM.069 - EXPRESSION AND IMMUNOCYTOLOCALIZATION OF THE COHESIN SUBUNIT SCC1 IN *TRYPANOSOMA CRUZI*

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The segregation of sister chromatids to opposite poles of the cell during division is the most complex and, at the same time, the most important event during the life cycle of a eukaryotic cell. Both in mitosis and meiosis cohesion between sister chromatids is essential for the occurrence of the correct chromosomal segregation. The protein complex responsible for cohesion between chromatids is called Cohesin. The Cohesin complex is well known in yeast and mammals, consisting of two SMC (structural maintenance of chromosomes) proteins, SMC1 and SMC3, and two proteins SCC (sister chromatid cohesion) proteins, the SCC1 and SCC3 (SA1 and SA2 in mammalian cells). The Cohesin keeps sister chromatids together from S phase until the transition between metaphase and anaphase in cell cycle, when sister chromatids separate to the opposite poles of the cell. In trypanosomatids, there are few studies about this complex and the genome project revealed the presence of all Cohesin complex genes in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*. In this work we proposed the analysis of the expression of the Cohesin TcSCC1 subunit and its imunocytolocalization in *T. cruzi* cells. For this, TcSCC1 gene was amplified by PCR, cloned and expressed in *E. coli* cells. The protein TcSCC1 was then used for production of polyclonal anti-TcSCC1 antibody in rabbit. This antibody was used in Western blots and immunofluorescence confocal microscopy analyses of amastigote, epimastigote and trypomastigote forms of *T. cruzi*. These analyses indicate that the TcSCC1 protein is detected mainly in the amastigote forms with distinct nucleus localization. Epimastigote form presented a weak signal for anti-SCC1 antibody and trypomastigote form presented no signal. These results suggest that the SCC1 subunit of the Cohesin complex is present in *T. cruzi* and it is mainly evident in the nucleus of amastigote form of this parasite. Supported by FUB and CAPES.

BM.070 - FUNCTIONAL CHARACTERIZATION OF mRNA-PROTEIN COMPLEXES (mRNPs) IN *Trypanosoma cruzi*

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Gene regulation is mainly posttranscriptional in trypanosomatids. The stability of mRNA and access to polysomes are thought to be tightly regulated, allowing *Trypanosoma cruzi* to adapt to different environmental conditions during its life cycle. Posttranscriptional regulation requires the association between mRNAs and some proteins to form mRNP complexes. We investigated the dynamic association between proteins and mRNAs, using poli(T) beads to isolate and characterize proteins and protein complexes bound to poli-A+ mRNAs by mass spectrometry. We identified 542 protein components of the mRNP complexes associated with mRNAs. Twenty-four of these proteins were present in all fractions, whereas some other proteins were exclusive to a particular fraction: epimastigote polysomal (0.37%) and postpolysomal (2.95%) fractions; stress polysomal (13.8%) and postpolysomal (40.78%) fractions. We also identified the mRNAs present in each fraction by microarray analysis, showing that proteins expressed mostly in metacyclic form such as mucin II and MASP were present in the postpolysomal fraction from both epimastigote and stressed parasites, hence indicating that these mRNAS that are not being expressed in epimastigotes are stored somewhere in the cytoplasm to be farther expressed in the infective form. We selected five proteins for further characterization: elongation factor 1-alpha (EF1-α), zinc finger RNA binding protein (ZF-211.70), RNA binding protein with a cold-shock domain (CD-33.60), prostaglandin F 2 alfa synthase (PF2αS) and prostaglandin F synthase (PFS). Immunofluorescence assays, polysome profile in sucrose density gradient and the expression pattern through the parasite's life cycle with the selected proteins allowed a preliminary characterization of specific mRNPs and additional studies will help to elucidate the posttranscriptional regulation mechanisms and dynamics during stress as well as the formation of RNA regulons in *T. cruzi.*
Financial support: CNPq, CAPES (Fiocruz).

BM.071 - BIOINFORMATIC ANALYSIS OF KDNA MINICIRCLE INTEGRATION SITES IN HUMAN GENOME

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Interspecies DNA transfer is a major biological process leading to the accumulation of mutations among eukaryotes. In a previous study it was demonstrated (Hecht et al, 2010) that *Trypanosoma cruzi* mitochondrial DNA (kDNA) integrated into various human chromosomes, mainly in retrotransposable elements. Most kDNA mutations were found in chromosome X (28%), but any chromosome can be targeted by the minicircle sequences. The kDNA integrations ruptured open reading frames (ORFs) of several genes. Microhomology mediated end-joining of 6-22 AC-rich nucleotide repeats in the minicircles and host DNA seems to mediate foreign DNA integrations. In this work, we performed bioinformatics analysis to investigate the presence of putative chimera proteins in sequenced clones. Sixty-four new ORFs were identified. Most of these putative transcripts bore no similarity with genes/proteins previously described. However, six chimera proteins had already been described in our lab (Nitz et al, 2004; Simões-Barbosa et al, 2006), and other nine chimeras were similar to previously identified proteins. High homology was observed between one chimera protein and the secretory carrier membrane protein SCAMP. The *in silico* analysis suggests that kDNA mutations may generate new genes, pseudogenes and modify pre-existing genes expression. We suggest that resulting genotype-phenotype alterations could be associated with the pathogenesis of Chagas disease. Further functional studies are necessary to r explain the role kDNA mutations play in the intrasignaling events among distant regions in the human genome.

Supported by CNPq and FAPDF.

BM.072 - MOLECULAR DIAGNOSIS IN THE INFECTIONS BY *TRYPANOSOMA CRUZI* IN PATIENTS CONTAMINATED BY ORAL MODE AND THEIR FAMILY IN THE BRAZILIAN AMAZON REGION

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The epidemiology of Chagas disease is conditioned by the triatomine insect vectors, the parasite *Trypanosoma cruzi* and the mammalian reservoirs. Chagas disease affects about 18 million people in endemic areas in Latin America and 100 million people present a strong potential for contamination by *T. cruzi*. In Brazil, although control measures have succeeded in decreasing the vectorial transmission of the disease seems that there are suspected cases of oral transmission in some areas. The Amazon region, which has been considered low risk area, in the present shows a significant increase in the number of acute and chronic cases of *T. cruzi* infections and Chagas disease. These cases may be related to oral transmission by ingestion of contaminated food typical of the region, for example, the açaí palm fruit. There are few studies describing the morbidity of Chagas disease transmitted orally. The epidemiology of sylvatic *T. cruzi* and oral transmission in Amazon region can cause distinct symptoms in particular cardiac abnormalities with peculiar characteristics. In this report, we investigated 4 families from Breves and Barcarena, Pará State, Brazil, which suspicion of infection seems to be related to ingestion of contaminated food with *T. cruzi*. The objective was to detect the presence of parasite mitochondrial DNA (kDNA) and nuclear DNA (nDNA) in the genomes of Chagas patients and their descendants and correlate with the results obtained by serology.

Supported by Pronex FAPDF, CNPq/MCT

BM.073 - COMPARATIVE ANALYSIS AMONG THE LEVELS OF PARASITAEMIA, TISSUE DAMAGE AND LYTIC ANTIBODIES CORRELATING WITH THE PRESENCE OF THE *T. CRUZI* COMPLEMENT REGULATORY PROTEIN (TC-CRP) GENE IN BALB/C AND C57BL/6 MICE INFECTED WITH HELENA *T. CRUZI* STRAIN

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The better understanding of the host-*Trypanosoma cruzi* interactions in experimental infections has been extensively studied with genetically different hosts infected by *T. cruzi* strains. Resistant hosts (C57BL/6) present higher production of lytic antibodies (LA), decreased tissue lesions and prolonged survival, whereas the opposite is beheld for susceptible ones (BALB/c). Although the Tc-CRP represents one of the main targets for LA, the antibody levels against this antigen have not been measured during *T. cruzi* experimental infections. So, the aim of this study was to evaluate the presence the Tc-CRP coding gene in different mice organs and its correlation with humoral immune response and the course of infection with Helena strain. A total of fifteen BALB/c and fifteen C57BL/6 mice were infected intra-peritonially with the mentioned strain and their parasitaemia was monitored daily. Five animals of each group were sacrificed in three points of infection; the following organs were collected for histological and molecular analysis: heart, liver, spleen, skeletal muscle, diaphragm, bladder and three gastrointestinal junctions. Sera from these animals were collected for LA evaluation by LMCo and Tc-CRP ELISA tests. Tissues samples were submitted to total genomic DNA extraction followed by the amplification using Tc-CRP specific primers. As expected, C57BL/6 mice produced significantly lower parasitaemia and higher levels of anti-Tc-CRP antibodies during the infection. Moreover, both groups presented higher levels of antibodies in the later phases, which corroborate our previous studies. In the acute phase of infection few alterations were observed only on heart and intestinal junctions' tissues while in the later phases, samples from cardiac, skeletal and smooth muscles presented considerable alterations. The PCR reaction detected the Tc-CRP DNA in five out of nine examined tissues proving the presence of this gene in these samples. Altogether, these findings suggest that either the host or the parasite genetic could influence on the resistance or susceptibility to infection by *T. cruzi* and on the immune response generated against the parasite. Supported by FAPEMIG, CAPES, CNPq, REUNI

BM.074 - A CLEAN MODEL TO STUDY THE KDNA TRANSFER AND CHAGAS-LIKE INFLAMMATORY CARDIOMYOPATHY

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Birds are refractory to *Trypanosoma cruzi*, but infection can be established in the embryo during the first week of incubation. The chicks hatched from *T. cruzi* infected eggs retained the kinetoplast DNA (kDNA) in the genome. The PCR assays with specific primer sets revealed kDNA and the absence of nuclear DNA, thus showing the birds were parasite-free. The *Gallus gallus* proved to be a clean model for the study of the pathogenesis of Chagas disease. The identification of the minicircle kDNA sequences integrated in the bird`s genome was made using the tpTAIL-PCR technique, and the chimera sequences showed conserved and variable kDNA regions in several chromosomes. The crossbreedings revealed that the kDNA mutations were vertically transferred to progeny. The kDNA-mutated birds showed cardiomegaly and the histopathology analysis documented typical Chagas disease lesions, whereby parasite-free target heart cells were rejected by the immune system mononuclear cells. These lesions are not present in control non-kDNA mutated birds. These findings highlight the association between the inflammatory heart disease with rejection of the target self-tissue and the genotype alterations resulting from the kDNA integrations in the chicken genome. These results suggest that kDNA mutations in the chicken genome induce autoimmunity and, consequently, the heart lesions in the host. The genetic-driven autoimmune heart rejection would explain the pathogenesis and clinical manifestations of this disease in the parasite-free chicken, which is similar to other genetic-driven idiopathic Chagas-like cardiomyopathies. Supported by CAPES and CNPq.

BM.075 - GENETIC HETEROGENEITY OF *Trypanosoma cruzi* POPULATIONS ISOLATED FROM STATE OF RIO GRANDE DO NORTE, BRAZIL

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Twenty seven *Trypanosoma cruzi* stocks from the state of Rio Grande do Norte, Brazil, isolated from man and triatomine bugs were studied by random amplified polymorphic DNA. This procedure was performed essentially as described (Steindel *et al*., 1993) and the analysis of the genetic profiles of the *T*. *cruzi* isolates obtained by polymerase chain reaction (PCR) with three primers (L15996, M13-40, and λgt11-F) showed a clear amplification and good reproducibility. The results showed RAPD profiles with an average of 66%, 75% and 80% of shared bands for L15996, λgt11-F e M-1340, respectively. When the RAPD profiles were used to build a phenetic tree by UPGMA and obtained a phenogram consisting of great similarity and grouping between the isolates in the general topology of the trees. The genetic distance analysis revealed three main clusters distinguishing the genetic groups TcI, TcII and TcIII. TcI isolated from the humans; TcII from the humans and *Triatoma brasiliensis* while TcIII only isolated from triatomines-*Panstrongylus lutzi* and *T*. *brasiliensis*. The genetic variability these *T*. *cruzi* populations do not depend on localities origin, but of the host and their genetic group, evidencing that isolates of same group were genetically well correlated. These data reinforce that *T*. *cruzi* populations corresponding to TcI, TcII and TcIII groups are circulating among humans and vector species *P*. *lutzi* and *T*. *brasiliensis* suggesting transmission cycles complexes in different municipalities of this state, and showed a notable genotypic and phylogenetic diversity. Supported by CNPq-Editais Universal e MCT/CNPq/CT-Saúde/MS-SCTIE-DECIT N° 034/2008, FAPERN/PPSUS and DCR/CNPq/FAPERN fellowship.

BM.076 - CLONING, EXPRESSION AND PURIFICATION OF MEMBERS OF THE MULTIGENE FAMILY MASP OF *Trypanosoma cruzi* DERIVED FROM DIFFERENT SUB-GROUPS

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The surface of pathogens is the major interface with their hosts, and therefore participates in important events related with survival and proliferation. The parasite *Trypanosoma cruzi* has several multigene families encoding surface proteins, many of them already well characterized, such as TcMUC Mucins and Trans-sialidase. A new multigene family, named MASP (Mucin Associated Surface Protein), was identified with the parasite genome sequencing. This family has 1377 genes, 814 of which are full-length genes and 563 are partial and/or pseudogenes. MASP is mainly expressed in the tripomastigote stage of the parasite, has N- and C-terminal conserved domains and a central hypervariable region. These N- and C-terminal sequences encode, respectively, the signal peptide and the GPI anchor addition site, suggesting a surface location of the mature protein. Despite of its extensive variability, this family was divided into 6 sub-groups (Freitas et al., in preparation). Since no member of this family was characterized to date, we decided to generate MASP recombinant proteins representatives of each one of the 6 sub-subgroups to produce monoclonal antibodies and perform structural studies. To this end, MASP sequences were amplified by PCR, cloned in the pGEM-T system, sub-cloned into the pET28a-TEV expression vector and several clones were sequenced to confirm MASP identity. The recombinant proteins were expressed in *Escherichia coli* BL-21 star bacteria and purified by affinity column. To date, three MASPs from distict sub-groups, were already expressed and purified. These three recombinant proteins are already being used to generate monoclonal antibodies and one of them will be used for structural studies.
Supported by: CNPq, FAPEMIG, WHO

BM.077 - IMPLICATIONS OF *TRYPANOSOMA CRUZI* INTRASPECIFIC DIVERSITY IN THE PATHOGENESIS OF CHAGAS HEART DISEASE

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Chagas disease has a variable clinical course, ranging from symptomless infection to severe chronic disease. Cardiac involvement is the most serious and frequent manifestation of chronic infection and may result from cardiac arrhythmias to sudden cardiac death. Heart transplantation (HTx) is a useful therapy for end-stage Chagas heart disease (CHD), but infection reactivation remains the major complication after the necessary immunosuppression. The factors determining clinical variability and disease reactivation have not been elucidated, but it is reasonably assumed that both host and parasite aspects are involved. Herein, our main goal is to correlate the parasite genetics and the CHD manifestations by molecular profiling of *T. cruzi* populations directly in infected tissues. Retrospective and follow-up studies have been conducted on CHD patients submitted to HTx or pacemaker implantation (PI) in Minas Gerais, Brazil. Parasitological diagnoses were conducted by hemoculture and kDNA-PCR. Fifteen HTx patients were investigated totalizing 43 different samples with 37% of positivity to *T.cruzi* DNA (11/25 fresh cardiac tissues, 2/5 paraffined tissues, 1/8 endomyocardical biopsies after Tx, 1/2 blood samples and 1/1 skin sample from reactivated patients). Blood samples before Tx (2) were negative. *T. cruzi* was detected in 30% of the pericardial fat tissue collected from 10 patients who underwent PI. Positive samples were subjected to strain typing by a triple step assay comprising PCR-RFLP of COII gene, amplification of ITS leader and 24Sα rRNA genes. So far, we detected *T. cruzi* II in all kDNA positive samples. Mixed *T. cruzi* II/VI infection was detected in one PI patient. These results reinforce the current idea that *T. cruzi* II is the major lineage associated to CHD, at least in Minas Gerais. Further studies are ongoing to enlarge the number of analyzed CHD patients and to discriminate the intraspecific variability of *T. cruzi* populations by microsatellites and LSSP-PCR analyses. Supported by FAPEMIG, CNPq and CAPES.

BM.078 - DIVERSITY AND FUNCTIONALITY ANALYSIS OF GP82 SURFACE GLYCOPROTEIN GENES IN *Trypanosoma cruzi*

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T. cruzi improves the likelihood of invading or adapting to the host through their capacity to present a large repertoire of surface molecules. The metacyclic stage-specific surface glycoprotein gp82 identified by monoclonal antibody (MAb) 3F6 has been implicated in host cell invasion. GP82 is encoded by multiple genes from the trans-sialidase superfamily. However, apart the Mab 3F6-reactive GP82, little information is available on members of this protein family. The central domain of GP82 containing the mammalian cell binding site which is contiguous to and partially overlaps the epitope for MAb 3F6, and appears to be conformational, being possibly formed by juxtaposition of two sequences separated in the linear molecule by a hydrophobic stretch (Yoshida, An Acad Bras Ciênc 78:87, 2006). Here we investigate the genetic divergence and polymorphism among gp82 multigene family members in the strains G, CL, Guatemala and Peru, and clone CL Brener. When we focused on the Mab 3F6 and cell binding motifs, we found differences among the isolates and phylogenetic analysis revealed that sequences from each isolate clustered together, suggesting that GP82 genes may be structured in a strain-specific manner. We are developing a gene expression cassette in Escherichia coli that can be used to readily generate short GP82 peptides carrying the Mab 3F6 and mammalian cell binding sites. This system will reduce time and effort to confirm whether the annotated GP82 genes in the genome are functionally active. A comparison of synonymous (dS) and nonsynonymous (dN) substitutions frequency in GP82 genes detected selective pressure. The reason *dS*/*dN* estimated by SLAC and FEL methods are 0.86 and 0.63 ($\leq$ 0.05 significance), respectively, suggesting a negative selection acting on GP82 genes. Next we will identify specific amino acid sites that are likely targets of selection using both maximum likelihood approaches and patterns of parallel amino acid change.
Supported by FAPESP, CNPq and CAPES.

BM.079 - CHARACTERIZATION OF TRNA-DERIVED SMALL RNAS IN THE METACYCLIC *TRYPANOSOMA CRUZI*

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Trypanosomatid genes are organized into long policistronic units that are transcribed constitutively, leaving the control of gene expression post transcriptionally. In eukaryotes small non coding RNAs participate in a variety of cellular processes, including gene silencing. Recently, a new subfamily tRNA-derived small RNAs was described in plants, fungi and animals. Experiments revealed that in stressed cells there was an increased amount of small RNAs derived from tRNAs and they were thought to participate in translational repression. When the small RNAs from *Trypanosoma cruzi* epimastigotes were isolated and sequenced, it was noticed that tRNA-derived fragments were not only present but represented 26% of the population of small RNAs. Interestingly, this percentage seemed to increase upon nutritional stress. The objective of this work was to characterize the fraction of short RNAs from metacyclic *T. cruzi*. Not surprisingly, in these infective forms the levels of tRNA-derived fragments increased to 66%, lowering the amount of rRNA-fragments. While fragments of $tRNA^{Asp}$ and $tRNA^{Glu}$ were more significative in epimastigotes, fragments of the last appeared significantly increased in metacyclics. Looking for their location in the cell by FISH using an antisense DNA probe to $tRNA^{Glu}$, the tRNA-fragments were observed in cytoplasmic granules whereas in metacyclics they were dispersed in the cytoplasm. These results confirm the fact that tRNA-derived fragments increase in amount in stressed cells and suggest that the biological function of these fragments may be associated with their location
This project has financial support from CNPq, Fundação Araucária and CAPES

BM.080 - INTEGRATION OF KDNA MINICIRCLE FROM *TRYPANOSOMA CRUZI* INTO THE MOUSE GENOME

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The lateral DNA transfer (LDT) is now recognized as a major driving force of prokaryotic and eukaryotic genome evolution. Despite the difficulties to document such phenomenon between far distant organisms in the phylum, the growing number of LDT reports in the last five years was driven by robust new sequencing technologies and improved algorithms. The transfer of minicircles of kDNA from *Trypanosoma cruzi* (LkDT) to various vertebrate host genomes has been documented. The insertions of sequences of kDNA minicircles were often associated with retrotransposable LINE-1. In addition, *in vitro* treatment of macrophages previously infected with *T. cruzi* with drugs that block important eukaryote cells signaling pathways was effective to prevent the kDNA integration. We evaluated the impact of treatment of chagasic BALB/C with ciprofloxacin, zidovudine, topoisomerase II and reverse transcriptase inhibitors in association with benznidazole, aiming at to determine the abrogation of minicircle integrations into the animal host genome. To document the structure of the kDNA minicircles integrations at the host's DNA junction sites, the tpTAIL-PCR technique was performed, using sets of primers for *Mus muscullus* LINE-1 ORF2 and for the kDNA minicircles. The investigation revealed that the animals subjected to the therapeutic regimes had the kDNA-insertions in the genomes. A possible decrease in the ratios of kDNA insertions in groups of mice treated with benznidazole awaits further analyses. No difference was observed among groups of mice receiving ciprofloxacin- or zidovudine-benznidazole associations with that obtained in the *T. cruzi*-infected but untreated group. Furthermore, kDNA insertions were always associated with LINE-1 sequences. Due to the huge quantity and similarity of LINEs widely spread in the murine genome, it was not possible to determine yet in which chromosomes the kDNA was integrated. Supported by CAPES and CNPq.

BM.081 - MOLECULAR DIAGNOSIS IN THE ACUTE PHASE OF CHAGAS DISEASE IN THE PARÁ STATE, BRAZIL

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The *Trypanosoma cruzi* infections and endemic Chagas disease represent a serious public health problem in Latin America. The insect-vector transmitted infection acquisitions via the feces of tritomines are responsible for the endemicity. However, other modes of transmission via blood transfusion and congenitally from mother to offsprings are described. In the northern Pará State, Brazil, oral transmission of the *T. cruzi* infections by contaminated food was suspected. In this work cases of the acute Chagas disease, showing patent parasitemia are reported. The patients with the acute infections often showed nonspecific symptoms, which could be interpreted as any other acute infectious ailment. However in a minority of cases the symptoms were fever, myalgias, arthralgias asthenia, edema and shortness of breath in severely ill people, showing signs of ECG alterations, echocardiograph abnormalities and increased heart silhouette in the X-Rays. The acutely infected cases were subjected to the PCR with specific kDNA and nuclear DNA primer sets to confirming the serological diagnosis by indirect hemagglutination, indirect immunofluorescence and enzyme-linked Immunosorbent assay (ELISA). Our data suggest that PCR can be a solution to the problem related to lack of specificity of serologic tests due to co-infections and/or immunosuppressions in absence of anti-*T. cruzi* antibodies. The discrepancy between the results of serological tests with those obtained by PCR suggests that the onset of acute cases of Chagas disease in the Amazon may well be underestimated. In conclusion, further studies will confirm the importance of genomic technology to performing the diagnosis of the acute *T. cruzi* infections and Chagas disease in the Greater Amazon. Supported by Pronex FAPDF, CNPq/MCT

BM.082 - *Trypanosoma cruzi* MSH2: MULTIPLE ROLES IN DNA REPAIR AND OXIDATIVE STRESS RESPONSE

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The study of *Trypanosoma cruzi* MSH2, the main protein of DNA Mismatch Repair Pathway (MMR), has led to the discovery of three distinct isoforms, named TcMSH2 A, B and C. Based on the *Tcmsh2* sequence present in their genomes, *T. cruzi* strains can be classified in haplogroups A, B or C. Experimental evidences indicated that strains presenting a TcMSH2 A isoform have a more efficient MMR compared to TcMSH2 B and C. To further characterize the TcMSH2 protein, we generated single knockout parasites and demonstrated that they are more susceptible to hydrogen peroxide treatment and accumulate more 8-oxoguanine in mitochondrial DNA than wild type parasites. This second role of TcMSH2 in *T. cruzi* may explain why it seems to be an essential gene, since it was not possible to generate double knockout parasites. In order to verify whether MSH6 is also involved in the oxidative stress response, we generated *Tcmsh6* knockout, which are currently being characterized. These parasites are being tested for their ability to grow in the presence of hydrogen peroxide. MSH2 and MSH6 proteins have also been expressed in fusion with GFP and RFP, respectively. To investigate its subcellular localization, polyclonal antibodies raised against a recombinant form of TcMSH2 were used in immunolocalization assays, which suggest that TcMSH2 has an unusual cytoplasmic location.
Supported by CNPq, FAPEMIG, HHMI

BM.083 - RNA-BINDING PROTEINS INTERACTOME IN *Trypanosoma cruzi*

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Trypanosoma cruzi is a protozoan parasite that causes Chagas' disease, affecting more than 15 million people in Latin America and having no cure. Besides the problem of public health, this parasite has peculiar molecular aspects which make it an interesting study object for understanding biology; also, it is an ancient eukaryote, and its study can provide clues to cellular mechanisms distinct from those of other model organisms, such as new mechanisms or different controls. One of its major features is the control of gene expression, which occurs post-transcriptionally, and studying the processing, storage and degradation of mRNA is extremely important for understanding the gene expression control mechanisms of this organism. RNA-binding proteins are extremely important in regulating these processes, as through their interaction with specific mRNAs they can define mRNA fate. We are conducting several large scale analyses of *T. cruzi* molecular biology, including microarrays, RNA-seq and mass spectrometry; these results are providing important clues about *T. cruzi* biology, but their integration is expected to provide an even better picture, including new ideas that are not apparent from each dataset. However, integration of distinct types of high throughput data is not trivial; a possible way to improve the initial integration analysis is creating a large map of protein-protein interaction (PPI), over which we can plot the other datasets. Aiming to create an initial map of RNA-binding PPI, we have constructed a *T. cruzi* ORFeome, which consists in cloning all protein coding regions in a suitable vector. Today, we have ~4,000 proteins cloned in a pDONR221 vector (50% of the whole ORFeome), which can be used in downstream applications. We have selected ~300 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 vectors, containing the DNA-binding domain (BD) and activation domain (AD), in order to test their interaction in a yeast two-hybrid (Y2H) system using selective media, as HIS-, URA- and lacZ. 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. Supported by CNPq, FIOCRUZ, NIH, Fundação Araucária.

BM.084 - IDENTIFICATION OF CONSERVED SEQUENCES WITHIN THE 3' UNTRANSLATED REGIONS OF CO-EXPRESSED TRANSCRIPTS IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi, the etiologic agent of Chagas disease, belongs to the Trypanosomatidae family, a group of organisms that has unusual mechanisms of gene expression. There is no identifiable RNA polymerase II promoter, the protein-coding sequences are transcribed as large polycistronic units by through trans-splicing, and cis-splicing is a rare event because only four genes have introns and most mitochondrial mRNAs undergo extensive RNA editing. Because the genes are transcribed constitutively, most of control of gene regulation in these organisms occurs post-transcriptionally. Processing of polycistronic transcripts to generate monocistronic mRNAs involves two coupled co-transcriptional RNA-processing reactions: SL trans-splicing that result in the addition of the splice leader (SL) sequence at the 5'-UTR region and a polyadenylation at the 3'-UTR region of each mRNA. Beside this, mRNA stabilization and translational control are important steps that modulate gene expression in these parasites. It is known that mRNA degradation and translation efficiency may be mediated by the presence of regulatory elements within 3'UTRs of the transcripts. Therefore, the aim of our work is to identify, describe and compare conserved regions within *T. cruzi* stage-specific mRNA sequences that may regulate gene expression in this organism. ESTs from each life stage were downloaded from Genbank and parsed by Seqclean to extract vectors strings, polyA-tail and low-quality sequences. Those parsed-ESTs were aligned against the whole *T. cruzi* genome with Megablast. Perl scripts were developed to select only the best hits from Megablast outputs and to generate a database of 3'-UTR sequences of the parasite. These sequences are been analyzed using clustering methods and compared with data derived from the expression levels of the corresponding mRNAs. Preliminary results using 3´UTR sequences from MASP and trans-sialidases indicated the existence of conserved regions as well as secondary structures that may be related to the trypomastigote-specific expression of those genes. Supported by CAPES, CNPq, HHMI and FAPEMIG

BM.085 - CARACTERIZATION OF A TPPP/P25 PROTEIN IN *TRYPANOSOMA CRUZI*

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Proteins from TPPP/p25 (*Tubulin Polymerization-Promoting Protein*) family were initially identified and described as a brain-specific protein. The genome analysis from different organisms made possible the identification of genes homologous to the mammalian TPPP/p25 in ciliated eukaryotes indicating a possible role in function or structural organization of such structures. The proteins from TPPP/p25 family have a strong association to tubulin bundles being capable to induce tubulin polymerization. However, even with their conservation in eukaryote evolution, issues like the function and regulation of TPPP/p25 family of proteins have yet to be proven. Recently our group started the characterization of a gene from *Trypanosoma cruzi* genome annotated as 'hypothetical conserved' gene which sequence analysis revealed to be a member from the TPPP/p25 family. Comparisons between TcTPPP and the mammalian homologous protein revealed similarities like the presence of unordered regions and differences as the absence of the N-terminal region. By DNA microarray analyses and Western blot using polyclonal serum, we observed that both RNA and protein expression patterns reach the highest levels in the metacyclic trypomastigote stage. Strategies based in optical and electron microscopy and the use of transfectant cells porting a GFP-fused protein determined the basal body as the cellular localization of the TcTPPP. Aiming new functional evidences of this protein, we performed the silencing of the ortholog gene in *Trypanosoma brucei* and we observed cells with difficulties in the finalization of the cell division process and cells with an aberrant shape and motility. New evidences about TcTPPP function will be achieved by the gene knockout in *T. cruzi* genome.
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BM.086 - CTLA-4 +49 and PD-1.3 polymorphisms can contribute for digestive form in chagasic patients.

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CTLA-4 and PD-1 molecules are related to T lymphocytes negative regulation, and could constitute a potential mechanism by which *Trypanosoma cruzi* can evade the immune response. Polymorphisms described into CTLA-4 and PDCD1 genes were associated to autoimmune diseases and can be related to magnitude of expression, or associated in their inhibitory function. In this study we analyzed CTLA-4 +49 and PD-1.3 single nucleotide polymorphisms in subjects infected by *T. cruzi* stratified according to clinical presentation and healthy controls by PCR-RFLP. With regard to CTLA-4 polymorphism, AA and AG were more prevalent in both chagasic patients (43.22% each one; GG=13.56%) and healthy controls (AA=39.89%; AG=47.87%; GG=12.24%), thereby suggesting that CTLA-4 +49 polymorphism was not associated with Chagas' disease development susceptibility (p=0,8031). When we compared clinical status with healthy controls, we observed that CTLA-4 gene polymorphism was associated only with digestive form development (p=0.0054) in chagasic patients. The most frequent genotype detected in digestive form group was AA (62.50%), followed by AG (31.25%) and GG (6.25%) genotypes. Genotype frequencies observed in cardiac, indeterminate and mixed forms was similar than in healthy controls. Regarding to PD-1.3 polymorphism, GG was more prevalent in both chagasic patients (AA=2.45%; AG=8.82%; GG=88.73%) and healthy controls (AA=1.58%; AG=15.26%; GG=83.16%), and there was no statistical difference between these groups, suggesting that PDCD1 polymorphism was not associated with Chagas' disease development (p=0,3476). When we compared clinical status with healthy controls, we observed that PDCD1 polymorphism was also associated with digestive form development (p=0.0205) in chagasic patients. The most frequent genotype detected in digestive form group was GG (80.64%), followed by AG (9.68%) and GG (9.68%) genotypes. Genotype frequencies observed in other forms have no statistical difference when compared with healthy controls. Therefore, both polymorphisms analyzed can influence for Chagas' disease digestive form. Supported by CAPES, CNPq and FAPESP.

BM.087 - GENETIC VARIABILITY OF TRYPANOSOMA CRUZI II BY CORRELATIONS BETWEEN NUCLEAR AND MITOCHONDRIAL GENOME (KDNA) ASSOCIATED WITH CLINICAL MANIFESTATIONS OF CHAGAS DISEASE

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, presents a wide spectrum of clinical manifestations varying between individuals and geographical regions. In the chronic phase, around 70% of the individuals are asymptomatic (IF), whereas ~30% develop the cardiac (CF) or digestive forms of the disease. The factors that determine the outcome of the infection are unknown, but certainly depend on complex interactions amongst the genetic make-up of the parasite, the host immunogenetic background and environment. In this study, we analyzed nuclear and Mitochondrial genes as well as microsatellite markers and the structure of cytochrome oxidase subunit I (*COI*), II (*COII*), III (*COIII*), *Cyb, NADH Dehydrogenase subunit 4(ND4) and 7(ND7)* genes by PCR assays and sequencing in *Trypanosoma cruzi* isolates obtained from 61 chronic patients with well-characterized clinical forms of this disease and belonging to *T. cruzi* II genotype. To analyse our results we decide to group these markers by nuclear and mitochondrial haplotypes to compare and search for correlations between them and their clinical manifestations. For microsatellite analysis we use a software for haplotype reconstruction, and recombination rate estimation from population data (PHASE) and one that generates evolutionary trees and networks (Network). After these analyses we observed that there is no correlation between mitochondrial and nuclear markers, and our network microsatellite tree shows that different isolates from *T.cruzi* II have different steps of mutation. We conclude that there is no correlation between these markers and the clinical manifestation, but we can demonstrate that there are subgroups in *T.cruzi* II genotype which probably are correlated to hybridization events. Supported by CAPES, FAPEMIG and CNPq.

BM.088 - TCRBP19, A mRNA DOWNREGULATING PROTEIN IN *TRYPANOSOMA CRUZI* AMASTIGOTES?

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In eukaryotic organisms, the relevance of the steps of gene expression regulation that link transcriptional and translational processes is being widely recognized. Though an inactive role has been traditionally ascribed to messenger RNAs along this pathway, it is now clear that its actual fate is defined through the interaction with trans-acting factors. RNA-binding proteins (RBPs) play an important role in controlling gene expression at the post-transcriptional level. Besides, post-transcriptional regulation of gene expression is considered to be the main point for control of transcript abundance and functionality in trypanosomes. For that reason, those parasites are considered proper models for the study of post-transcriptional regulation mechanisms. Here we describe the functional characterization of TcRBP19, an RNA-binding protein from *Trypanosoma cruzi* that is preferentially expressed in amastigotes in association to polysomes. Orthologous genes have only been detected in trypanosome genomes preventing the *in silico* assignment of the functional role. Through ribonomic analysis we assessed that this protein affects the steady state of a set of polysomal mRNA. Further analysis of TcRBP19-RNA interaction network allowed the identification of a group of RNAs containing U rich regions as TcRBP19 targets. Among those messengers, we found TcRBP19 messenger, suggesting an auto-regulation event. In addition, in TcRBP19 over-expressing parasites, a reduced mRNA level of target genes is observed. Globally these data suggest the participation of TcRBP19 in the down regulation of a specific set of genes. Supported by PEDECIBA, ANII, CSIC.

BM.089 - MUTAGENESIS EXPERIMENTS AND MOLECULAR SIMULATIONS PROVIDE EVIDENCE OF THE CATALYTIC MECHANISM OF POP Tc80 FROM *Trypanosoma cruzi*

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We have previously demonstrated that the *Trypanosoma cruzi* parasite responsible for Chagas' disease expresses a secreted prolyl oligopeptidase (POP Tc80) and suggested that this proteinase could be involved in the infection process by facilitating *T. cruzi* migration through the extracellular matrix. A 3D model of POP Tc80 was obtained by homology modelling, suggesting a structure formed by two domains: a catalytic α/β hydrolase and a β propeller. The collagen ligand, upon docking on this model, formed an interface between the two domains, promoting a gating access mechanism involving a "jaw opening" of Tc80, for which the collagen binding site encompasses both the hydrolase catalytic triad and a hinge at the domains' junction. To validate the model, three hydrolase residues were identified as candidates for forming a disulfide bridge with propeller Cys255 upon Cys mutation, therefore locking Tc80 into its model predicted closed state, and blocking the enzyme catalytic reactivation. Molecular dynamics simulations of the three Tc80 mutants were conducted in parallel to site-directed mutagenesis and binding assays; the simulation predictions were found in both qualitative and quantitative agreement with experimental results. The Ser591Cys and Asn471Cys mutants showed significantly decreased biological activity (lowest for the former) correlated with the observation of the disulfide bridge (strongest for the former). Surprisingly, the Ala588Cys mutant was more active – this was shown to be related to Cys588 interacting with Arg633 rather than with propeller Cys255, facilitating the interdomain gating mechanism instead of obstructing it. Therefore we consider that our POP Tc80 model structure is validated, and thus provides a strong basis for virtual screening of chemical libraries in parallel with new binding assays. We will now adopt this structure-based drug design strategy in order to detect novel putative anti-Chagas compounds. Supported by: CNPq, CAPES, Finep and FAP-DF.

BM.090 - EXPRESSION OF ARGININE TRANSPORTER IN *Leishmania (Leishmania) amazonensis* IS SENSITIVE TO THE PRESENCE OF THE AMINO ACID

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In the mammal host, the intracellular form of *L. (L.) amazonensis* needs arginine as substrate for the synthesis of polyamines to support its multiplication. In this study we evaluated the control of gene expression of arginine transporter by the parasite. The transporter is codified by a gene present twice in the genome arranged in tandem. They show identical ORFs, but both 5' and 3' UTR's present distinct regions. Then, targeting those regions by quantitative RT-PCR enables the evaluation of mRNA expression of each copy. We observed that the amount of mRNA of copy B (the second in the tandem array) did not change along a promastigote culture curve. However, copy A presents a large amount of mRNA than copy B, mainly in late log phase, accompanying Meta1, a marker of metacyclogenesis expression. Then, 10^7 log phase parasites were deprived of nutrients, with the exception of glucose, for 4 hours. After that, the mRNA amount and the arginine uptake were determined after supplementation or not with 400μM arginine. Copy B mRNA number, in the absence of arginine, was similar to the control that received the amino acid, both normalized by GAPDH mRNA. When deprived of nutrients, copy A mRNA number increased, but not in the presence of arginine ($p < 0.05$). The amount of mRNA correlates with amino acid uptake, indicating that the transporter physiological function correlates with mRNA amount. Both Meta1 and arginase mRNA remained constant with or without supplementation. We concluded that parasites have mechanisms to detect arginine and regulate its uptake by the amount of mRNA coding the transporter. Experiments inhibiting mRNA transcription or/and mRNA maturation will determine if the transcription is enhanced, if there is an increase in trans-splicing or a stabilization of the transporter mRNA allowing its translation in a higher level when arginine is at low concentration. Supported by FAPESP and CNPq.

BM.091 - A SPECIES-DISCRIMINATING DIAGNOSTIC QUANTITATIVE PCR FOR LEISHMANIASIS: PARASITE DETECTION AND ENUMERATION IN HUMAN BLOOD AND IN SAND FLIES

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A rapid method to diagnose leishmaniasis and to discriminate among *Leishmania* species in human blood and environmental samples could be of tremendous use clinically and in assessing the risk of parasite acquisition in endemic regions. Many diagnostic tests depend upon detection of kinetoplast DNA, which contains maxicircle and minicircle DNA sequences unique to the *Trypanosomatidae* family protozoa. There is not a standard systematic application of these methods to distinguish between *Leishmania* species, and to quantify parasite numbers. We developed a qPCR based method to detect, quantify, and distinguish among the different *Leishmania* spp. in human or experimental animal blood and tissue specimens. The method entails first a SYBR green quantitative PCR assay that couples amplification of parasite DNA with the melting temperature of the qPCR product for parasite detection and species screening. A Taqman assay is then employed to validate detection and species identification, using primer sets and Taqman probes specific to the prevalent *Leishmania* species. qPCR assays and a web-based flow chart were developed to detect, quantify and distinguish between *Leishmania* species. DNA extracted from both serum and buffy coat of 2 individuals with symptomatic visceral leishmaniasis (VL) from northeast Brazil tested positive for *Leishmania* spp. Serum from two additional persons with VL tested positive while symptomatic, but resolved to negative after successful treatment. Human serum samples from 5 out of 5 symptomatic VL patients from Bangladesh tested positive. Sand flies were captured from the walls of houses of individuals who had recently contracted VL. Sand flies that were engorged, i.e. had experienced a recent blood meal, were analyzed for Leishmania DNA. 27.2% of sand flies collected from houses in an endemic peri-urban area outside of Natal, Brazil were positive for *L. infantum chagasi* DNA. The range of parasite loads per infected sand fly collected in these endemic households ranged from 17 to 323 parasites per fly. The step-wise diagnostic qPCR assay can address basic questions of parasite species and numbers that are relevant to clinical and environmental studies of leishmaniasis.

BM.092 - LmHus1 CO-LOCALIZES WITH LmRPA-1 UPON DNA DAMAGE AND ASSOCIATES WITH TELOMERES.

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Leishmania genome stability is constantly challenged by its plasticity as well as by regular DNA damage factors. The ability to deal with such variability denotes the existence of robust machineries evolved to finely control genome maintenance. We have identified the LmHus1 protein, which in other organisms forms the 911 complex with Rad9 and Rad1, involved in DNA damage repair. We raised specific antibodies and observed that LmHus1 is a nuclear protein. In cells submitted to replicative stress by hydroxyurea LmHus1 relocates to the nuclear periphery. Hydroxyurea treatment also promotes the association of LmHus1 to chromatin, a pattern observed for RPA-1, which is known to acts at the early steps of DNA damage repair. We found that LmHus1 co-localizes with LmRPA-1 and hydroxyurea exposure significantly increased this co-localization. Considering that RPA-1 is a telomeric protein in Leishmania, we investigated the possible involvement of LmHus1 with telomeres. We detect telomeric sequences in chromatin immunopreciptation experiments using a-LmHus1 antibody. Also, the overexpression of LmHus1 affected the expression of a resistance marker located at a subtelomere. These data indicate that LmHus1 participates in DNA damage repair and is also a telomere component. We further investigated the existence of the other 911 complex subunits. We were unable to find Rad1 or Rad9 in the genome dataset. However, we found a particular ORF which doubles the size of Rad1 or Rad9 from other organisms and may possess both Rad1 and Rad9 motifs, as showed by secondary structure prediction. This indicates that this protein plays Rad9/Rad1 function. Current work is focused in the generation not only of the antibody a-LmRad9/Rad1, but also the LmRad9/Rad1 overexpressor and KO cell lines in order to investigate the association of this protein with LmHus1 and their potential role in Leishmania telomere maintenance and DNA damage repair. Supported by FAPESP and CNPq.

BM.093 - MECHANISMS OF ACTIVATION OF p50/p50 NF-κB COMPLEX AND THE MODULATION OF HDACs DURING *Leishmania amazonensis* INFECTION

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Leishmania parasites subvert important macrophage signaling pathways involved in the control of the infection. NF-kappaB is an important transcriptional factor which modulates the expression of genes involved in the immunological response. Besides the activation of transcriptional factors, chromatin epigenetic modifications are pivotal regulators of gene transcription. Chromatin remodeling proteins such as deacetylase histones (HDAC) are involved with transcriptional repression while acetyltransferases histones (HAT) are involved with transcriptional activation. In this work, we sought to study the mechanisms of NF-kappaB activation and the participation of HDAC during *L. amazonensis* infection. We have demonstrated by EMSA that *L. amazonensis* infection activates the p50/p50 NF-kappaB complex. We have observed by western blot assay that *L. amazonensis* induces the augment of p50 subunit into the nucleus. Accordingly, we observed a reduction of p105 serine 907 phosphorylation which is thought to be important for p105 stability. In agreement with the activation of p50/p50 NF-kappaB complex, a classic transcriptional repressor, we have demonstrated by gene-reporter assays a dependent-NF-kappaB transcriptional repression of the iNOS promoter due to *L. amazonensis* infection. Consistent with this transcription repression, we have detected an increase of HDAC 1 mRNA and protein levels in early hours of *L. amazonensis* infection. Interestingly, we have observed a reduction of HDAC 11 levels and an increase of lysine 9 acetylated histone 3 in *L. amazonensis* infected macrophages. Our data also revealed that total histone deacetylase activity is increased in *L. amazonensis* infected macrophages. These results suggest a possible mechanism of p50/p50 NF-kappaB activation induced by *L. amazonensis* and indicate that important epigenetic modifications are taking place in infected cells. The relevance of these findings is under investigation.
Financial support: CNPq, INCT-CNPq, FAPERJ.

BM.094 - INTERGENIC POLYMORPHIC REGION OF THE TCUMSBP LOCUS OF TRYPANOSOMA CRUZI CLBRENER: STUDIES ON THE RNA PROCESSING AND STABILIZATION.

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Gene expression in *Trypanosoma cruzi* is carried out through polycistronic transcripts, which are processed by trans-splicing to mature mRNAs. The control of gene expression is mostly post-transcriptional. In our previous work, we have characterized an intergenic polymorphic region of the TcUMSBP locus, due to an indel of 62 bp, in which the derived transcripts have differential efficiency of processing and two distinct polyadenylation sites. After polycistronic RNA processing the indel remains as the 3'UTR of the beta-5 proteasome subunit (B5PS) gene. Results from the use of chloramphenicol acetyl transferase (CAT) gene in transient constructs corroborated the quantification of endogenous polycistronic RNA, that is, the presence of the 62bp indel in the 3'UTR reporter gene increased production of the CAT enzyme in relation to the absence of the indel. To better characterize this process we have built permanent strains of *T. cruzi* CLBrener in which a plasmid containing the CAT reporter gene is flank by the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) intergenic region at 5' end and the polymorphic intergenic region of TcUMSBP locus at the 3' end. These plasmids are maintained as episomal using G418 selection. We have characterized these strains for further experiments. Assays to confirm the sites of processing of RNAs derived from the transfected plasmid are also being conducted. The process described above may result from increased efficiency of translation or an increased mRNA half-life, resulting from the stabilization of the RNA by the 62pb indel. To address this issue we are measuring the half-life of the mRNA produced by the endogenous polymorphic alleles and the ones originate from the transfection. Supported by FAPERJ and CNPq

BM.095 - PROLYL OLIGOPEPTIDASE SINGLE-ALLELE KNOCKOUT IN *Trypanosoma cruzi*.

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We have previously demonstrated that the secreted prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80) could be involved in the infection process by facilitating *T. cruzi* migration through the extracellular matrix. Since *poptc80* is a single copy gene per haploid genome, its knockout was outlined in order to elucidate the role of this enzyme in the pathogenesis of Chagas disease. G418 (neomycin)-resistant *T. cruzi* epimastigotes (CL-Brener strain) were obtained after transfection and 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. Several PCRs were carried out to verify both the presence of *poptc80* and *neo* in the parasite genome. *poptc80 was* amplified in all G418-resistant parasite. 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. A detailed analysis of this single-allele knockout is under investigation; however the morphological phenotypes already observed included slow growth and atypical cytokinesis.
Supported by: CNPq, FAP-DF, Finep and CAPES.

BM.096 - STRUCTURAL PROPERTIES OF OLIGOPEPTIDASE B FROM *Trypanosoma cruzi*

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Oligopeptidase B (OpdB, EC3.4.21.83) belongs to the prolyl oligopeptidase family of serine protease (clan SC, family S9). It is increasingly being implicated as an important virulence factor in trypanosomiasis. The drugs most commonly used in sleeping sickness treatment reduce the activity of *T. brucei* oligopeptidase B. *T. cruzi* oligopeptidase B (OPBTc), the focus of this work, is involved in cell invasion by generating a Ca^{2+} agonist necessary for recruitment and fusion of host lysosomes at the site of parasite attachment. This scenario indicates that further structural and functional characterization of OPBTc should help clarifying its physiological function and lead to the development of therapeutic targets for Chagas' disease. In the present work, we report that OPBTc has a dimeric structure confirmed by different methods: Exclusion size chromatography, Dynamic Light Scattering (DLS) and analytical ultracentrifugation (AU) assays. The dimer association is not due to intermolecular disulfide bonds and it is salt-resistant. The enzyme retains its dimeric structure and it is fully active until 42 °C. The structural stability of the fully active recombinant OPBTc was investigated through thermal unfolding processes monitored by circular dichroism. Far UV CD experiments showed that OPBTc has a highly stable secondary structure at different pHs and less stable at moderate ionic strength condition. On the other hand, near UV CD spectra demonstrated that the tertiary structure of OPBTc is completely lost when the enzyme is heated at temperatures above 45°C, which correlates well with temperatures-dependent activity assays.
Supported by: CNPq, FAP-DF, Finep and CAPES.

BM.097 - CONSTRUCTION OF CRE RECOMBINASE SYSTEM IN TRYPANOSOMAS

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Trypanosomatids arouse great interest among researchers, since they use particular mechanisms of controlling gene expression to adapt at different environments and to circumvent the human defenses. To study gene function in these organisms, we decided to develop a system for conditional gene knockout because the RNAi based strategies cannot be applied to all trypanosomes and several genes are encoded by multi copy families. Here, we describe the first steps to transfer a CRE recombinase regulated by ligand (Dimerizable Cre – DiCre) to Trypanosomes. The CRE is a site-specific recombinase that catalyzes the recombination between two sequences called LoxP to excise or invert an intervening sequence or create intermolecular recombinations. Due to its intracellular toxicity and the necessity of a tight regulation of its activity, the DiCre system was chosen to obtain conditional knockouts in *T.cruzi* and *T.brucei*. To create a construct to express DiCre in both parasite species, the DiCre59 and DiCre60 genes from the pCDNA vectors were cloned into pROCK-TK-Hygro generating the pROCK-DiCre-Hygro. A second plasmid, pROCKET-FEKOPur-Neo, was generated to test the DiCre activity in *T.cruzi*. The FEKO Pur cassette from the pyrFEKO-PUR plasmid (Scahill et al 2008) consists in a puromycin resistance gene flanked by two LoxP sites. These linearized plasmids were transfected into *T.cruzi* epimastigotes and the parasite selection is in progress. In parallel, the pROCK-DiCre-Hygro plasmid was also introduced into *T.brucei* procyclic forms, and preliminary PCR analysis of hygromycin-resistant parasites showed that they contain the DiCre gene. Experiments to assess the presence of DiCre activity are being performed. Using this system, the multi copy genes will be deleted by insertion of LoxP sites followed by DiCre excision. Financial support: CAPES/REUNI, CNPq, Fundação Araucária.

BM.098 - 152 1 MOLECULAR CHARACTERIZATION OF *TRYPANOSOMA EVANSI* MEVALONATE KINASE

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The synthesis of isoprenoids precursors through mevalonate is an important metabolic pathway in eukaryotes. The biosynthesis of isoprenoids is essential for the viability of trypanosomatids, as part of the formation of glycosylphosphatidyl inositol (GPI)-anchored variant surface glycoproteins (VSGs). *Trypanosoma evansi* is the etiological agent of a disease in horses and other livestock, popularly known as "derrengadera" or “surra”, causing considerable economical losses in endemic regions. This parasite displays only a trypomastigote form, requiring constant modifications to their VSGs coat to protect from attack of the host immune system. Mevalonate kinase (MK) is a ATP-dependent phosphotransferase essential to isoprenoids metabolic route. This study aimed to identify and characterize the mevalonate kinase gene of *Trypanosoma evansi* (TeMK) and purify the recombinant protein for further enzymatic and structural characterization. In order to obtain a purified genomic DNA (gDNA), the blood of a Wistar rat infected with *T. evansi* was first purified by Percoll® gradient and ion exchange chromatography with DEAE-cellulose. The gDNA was then obtained by extraction with phenol-chloroform. The open reading frame encoding TeMK was obtained by using specific degenerated primers. A fragment of 990 base pairs was amplified by polymerase chain reaction, extracted, purified and cloned into a commercial vector. TeMK displays a high homology with *T. brucei* mevalonate kinase (TbMK). The TeMK gene was inserted in pQTEV expression vector (protein structure factory, Berlin), transformed into *E. coli* BL21(DE3) and was induced with 1mM IPTG at 20ºC. A protein band around 35 kDa was observed in SDS-PAGE gels. Western blots assays were performed with *T. evansi* extracts using a polyclonal anti-TbMK, in which a band with the expected molecular weight was observed. Indirect immunofluorescence showed that TeMK colocalized with aldolase in glycosomes, as already observed in other kinetoplastids studied. The recombinant protein is being processed for further biochemical and structural characterizations. Supported by: FAPESP, CNPq, UDESC.

BM.099 - PROTEOMIC PROFILE OF *TRYPANOSOMA EVANSI*

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Trypanosoma evansi is the most widespread of the pathogenic salivarian trypanosomes and affects most livestock and wild animals mainly in endemic regions. The *T. evansi* infections are popularly know as “surra” or “mal de cadeiras” and there are no drugs or vaccines to cure or prevent the disease. Knowledge about molecular and cell biology of the parasite is limited, being most part of the studies linked to *Trypanosoma brucei*, phylogenetically close to *T. evansi*. The study of proteins through proteomics based approach is an important reference for biochemical, molecular and epidemiological studies in order to identificate species-specific proteins, new drugs targets and development of diagnostic tools. Recently the proteomic composition of *T. evansi* was characterized using liquid chromatography (LC/MS). The main goal in the present study is to develop an optimized and reproducible protocol using two-dimensional gel electrophoresis to determine *T. evansi* protein expression profile and post-translational modifications. The parasite was collected from infected Wistar rat and was subjected to purification in Percoll® gradient and ion exchange chromatography on DEAE-cellulose. Trypomastigote protein extracts were obtained with lysis solution containing 7.7 M urea, 2.2 M thiourea, protease inhibitor cocktail (Sigma) and 4% CHAPS. The samples were measured by Bradford method and it was used 220 µg of sample for the elaboration of the 2D gel. The isoeletric focalization was performed using pH 3-11 (GE Healthcare) gradient strips on Ettan IPGphor III (GE Healthcare). The SDS-PAGE was carried out by 15% polyacrilamide gel and was visualized by Coomassie Brilliant Blue G-250 staining. It was observed and marked over 165 spots. The spots were excised from gel and are being processed through the MALDI-TOF mass spectrometer analysis. These preliminar results are the first step towards the generation of proteome profiles for use in future studies on protein expression and comparison with other trypanosomatids. Supported by: CNPq, FAPESC, UDESC.

BM.100 - 187 1 GENOMIC ORGANIZATION AND EXPRESSION OF MAJOR SURFACE PROTEASES (MSP) IN *TRYPANOSOMA RANGELI*

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Members of Major Surface Proteases (MSP) family were initially described in parasites of the genus Leishmania, and later in other trypanosomatids. MSPs are considered virulence factors in *Leishmania* and have key roles in establishing infection of the host cell. We selected twelve recombinant clones from a *Trypanosoma rangeli* genomic library, which have high sequence identity with *Trypanosoma cruzi* MSPs. The objective of this work is to characterize the recombinant clones carrying the sequences of *T. rangeli* MSPs and determine the genomic organization and expression of the MSPs in different genotypes of the parasite. The sizes of the inserts of recombinant clones ranged from the 2,200bp 3,500bp. The analysis of BLASTX (Basic Local Alignment Search Tool) of the twelve *T. rangeli* sequences showed an identity of 37% to 62% with the sequences of MSPs from other trypanosomatids, and the best results were achieved with the sequences of *T. cruzi*. Of the twelve sequences generated, nine correspond to the carboxy-terminus region of *T. cruzi* MSPs and five sequences to the amino-terminus region, and one of them contains the 5' untranslated region. The multiple sequence alignment showed extensive conservation between sequences of *T. rangeli* and other trypanosomatids, including the catalytic site of MSP (HEXXH), which is represented by three clones in the library. Southern hybridization analysis, at high stringency conditions, revealed the presence of a single band of 4kb in samples of strain P07. The evaluation of the expression of MSP, performed by RT-PCR, employing primers MSP-1 and MSP-2, revealed a fragment of 212bp in the strains PO7 and SO18, representing genotypes KP1 (+) and KP1 (-), respectively. 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: CNPq, FAPEMIG and CAPES.

BM.101 - CHARACTERIZATION OF RIBOSOMAL P0 PROTEIN OF *Phytomonas serpens*, A TOMATE PARASITE THAT SHARES ANTIGEN WITH *Trypanosoma cruzi*

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Trypanosomatids of the genus *Phytomonas* alternate their life cycle between plants and phytophagous hemipterans. *Phytomonas* spp. can parasitize the phloem, lactiferous tubes, fruits and seeds of some plants with a wide geographic distribution and of great agriculture importance. Previous results from our research group showed that *P. serpens* 15T, a tomato parasite, shares antigens with *Trypanosoma cruzi*, among them, the ribosomal P0 protein (P0ribPs). Humoral immune response against P ribosomal proteins of *T. cruzi* is prevalent in patients with chronic Chagas' heart disease. These antibodies recognize mainly the C-terminal domain of the polypeptides, a highly conserved region between P proteins of eukaryotic organisms. It was also observed that there was partial protection against infection of BALB/c mice immunized with living *P. serpens* 15T by the intraperitoneal or oral route and later challenged with a lethal inoculum of blood trypomastigotes of *T. cruzi*. Taking all together, the aim of this study was to characterize the gene that encodes the ribosomal P0 protein of *P. serpens* 15T. The coding sequence of the gene was cloned and sequenced. Northern blotting analyses, using total RNA of *P. serpens* 15T and specific *P0ribPs* probe, detected only one transcript of 1.28 kb. Recombinant protein was expressed in *Escherichia coli*, purified and inoculated in BALB/c mice to raise antibodies against the P0ribPs. The polyclonal antibodies recognize a polypeptide of approximately 40 kDa in a total protein extract of *P. serpens* 15T. And did not cross-react with other ribosomal P proteins. Indirect immunofluorescense assay using these antibodies showed that the P0 ribosomal protein is localized in the cytoplasm of the parasite. Further studies are warranted to determine the role of P0ribPs during the experimental Chagas' disease, and such investigations are currently underway in our laboratory. Supported by: Fundação Araucária – Paraná and CNPq.

BM.102 - CHARACTERIZATION OF SINGLE MYOSINS OF *Trypanosoma cruzi*

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Myosins are molecular motors involved with various forms of cell movement like phagocytosis, cytokinesis, muscle contraction, beating of cilia and trafficking of organelles and particles. They use energy derived from ATP hydrolysis to perform these movements and have a common motor domain. The diversity of the family members enables us to classify them in classes. The muscle myosin was defined as conventional (class II), whereas other types are collectively referred to as unconventional myosins (grouped in many classes). Myosins that phylogenetically do not group to any other myosin are termed orphan myosins. *T. cruzi* has, besides the genes coding for 2 myosins (one present in almost all organisms - class I - and the other present only in trypanosomatids), seven other genes coding for orphan myosins, called TrcMyoA, TrcMyoB, TrcMyoC, TrcMyoD, TrcMyoE, TrcMyoF, TrcMyoG, which are the focus of this work. The study of *T. cruzi* myosins, their functions and their interactions within the cell will contribute to the evaluation of the cytoskeleton role in *T. cruzi*. To start the characterization of these myosins, experiments are being conducted to elucidate the cellular localization and the proteins that interact with each myosin, and to achieve this goal it was necessary to produce recombinant proteins for antibody production that will be used for immunolocalization and immunoprecipitation assays. In parallel, transfection of the parasite to the expression of protein fused to GFP and identification of their location is being performed. To date, with the exception of one myosin, all the recombinant proteins were inoculated in mice and the antibodies are being analyzed. The transfection assays are in progress.
Supported by CAPES and Fundação Araucária.

BM.103 - EXPRESSION LEVELS AND SITES OF A *Trypanosoma rangeli* PROTEIN TYROSINE PHOSPHATASE (*Tr*PTP2)

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Protein tyrosine phosphatases (PTP) play an essential role on the control of crucial cellular processes such as division and differentiation. Kinetoplastid-specific PTPs are associated with these parasites' life cycle and, due to their differences from human PTPs, are considered potential drug targets. The focus of this work was to comparatively characterize the expression sites and levels of a *Trypanosoma rangeli* PTP (*Tr*PTP2). The *Tr*PTP2 gene ORF has 987bp and predicts a protein of 36kDa that revealed to be highly similar to PTPs from phylogenetically related organisms (*T. cruzi*, *T. brucei* and *Leishmania* spp.). *Tr*PTP2 is 72% identical to *T. cruzi Tc*PTP2 and 58% to *T. brucei Tb*PTP1. Among the highly conserved regions between *Tr*PTP2 and these proteins are PTP active sites and trypanosome-specific PTP domains. The *Tr*PTP2 gene was cloned in pET14b, expressed in *E. coli*, purified by electroelution and used for polyclonal antiserum production in Balb/C mice. Western blot assays using anti-*Tr*PTP2 antiserum revealed an ~60kDa protein in the soluble protein extract of *T. rangeli* epimastigote and trypomastigote forms. This unexpected molecular mass is higher than the predicted 36kDa for *Tr*PTP2, possibly due to glycosylation sites observed in its deduced aminoacidic sequence. Immunofluorescence assays indicated a distinct cellular localization pattern for *Tr*PTP2 between *T. rangeli* epimastigotes and trypomastigotes, being spread throughout the cellular membrane in epimastigote forms and concentrated at the flagellar membrane in the infective forms. A disparate distribution for kinetoplastid PTP between biological forms has already been observed in *L. major* and *T. brucei*, in which the association of this protein to the membrane suggests a mean of interaction with these parasites' hosts. Supported by CNPq, FINEP and UFSC.

BM.104 - COMPARATIVE CHARACTERIZATION OF *Trypanosoma rangeli* GP63 METALLOPROTEASES CODING GENES

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Gp63 is a GPI-anchored Zn^{2+}-dependent metalloprotease protein originally described in *Leishmania* promastigotes. In *T. cruzi*, Gp63 is expressed in all stages and has been implicated in the infection of mammalian cells *in vitro*. In this work, Gp63 ORFs encoding for metalloproteases were identified and characterized in *Trypanosoma rangeli* genomic and cDNA libraries. Comparative sequence analysis revealed 13 complete ORFs highly similar to the *T. cruzi* homologous genes. *T. rangeli* Gp63 genes seem to be composed by at least two groups (Gp63-A and Gp63-B) in a family-like organization, reinforcing the phylogenetic proximity to *T. cruzi*. The presence of multiple copies of the Gp63 genes for both groups was confirmed by *Southern blot*, indicating a probable tandem repeat arrangement. Gp63-A and Gp63-B encode deduced proteins of approximately 605 and 587 amino acid (aa) residues (39% identical). Gp63-A showed His and Glu residues in the HEXXH motif, the most important residues associated with metalloprotease activity, while Gp63-B showed a replacement of a Glu residue for an Ala (HAXXH), suggesting the loss of catalytic activity in this group. The most important structural characteristics in both groups are the presence of (i) potencial N-glycosylation sites in Gp63-A (7) and Gp63-B (5), (ii) GPI anchor addition signals of 26aa for Gp63-A and 25aa for Gp63-B on the C-terminal portion of both groups, (iii) a predicted cleavage signal in the N-terminal region, consisting of 20 and 27 amino acids for Gp63-A and Gp63-B, respectively. Since the presence of mRNA for both Gp63-A and Gp63-B gene groups was detected in epimatigote and trypomastigote forms of Choachí and SC-58 strains, we are assessing the expression of Gp63 by *T. rangeli* and the activity of these proteins in both epimastigote and trypomastigote forms. Supported by CNPq, FINEP and UFSC.

BM.105 - COMPARATIVE PROTEOMIC ANALYSIS OF *Trypanosoma rangeli* EPIMASTIGOTE AND TRYPOMASTIGOTE FORMS

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Trypanosoma rangeli is a hemoflagellate parasite that infects humans as well as a variety of domestic and wild mammalian species. The infection in humans is harmless but induces an immune response that leads to serological cross-reactions with *Trypanosoma cruzi*, the agent of the Chagas disease. *T. rangeli* has a complex life cycle alternating between replicative and non-infective (epimastigotes) forms to non-replicative and infective forms (trypomastigotes). In this study, proteomic maps of both *T. rangeli* stages were obtained by unidimensional and two-dimensional (2D) electrophoresis following peptide mass fingerprinting identification by MALDI-TOF mass spectrometry (MS). Resolution of 2D gels using a 3-10 pH range showed that most of the proteins were focused between pH 4-7, where 282 and 205 spots could be identified for epimatigotes and trypomastigotes, respectively. Several proteins revealed multiple isoforms on 2D gels, some displaying differential expression levels between forms. Interestingly, 36% of the spots were found to differ between epimastigotes and trypomastigotes. Despite of the absence of *T. rangeli* genomic database for comparisons, some proteins could be identified by MS based on other trypanosomes databases. Among the identified polypeptides there were heat shock proteins (HSP) like HSP 60, HSP 70, HSP 83 and HSP 85, elongation factors, metabolic pathway enzymes, structural proteins and some virulence factors, including mucin-associated surface proteins (MASP). These results represent the first analysis of the parasite proteome, offering a draft of the protein expression profile of both epimastigotes and trypomastigotes. Upon finishing the ongoing genome sequencing, further studies on identification and molecular characterization of proteins related to the *T. rangeli* metacyclogenesis will allow the understanding of unknown biological aspects of this parasite. Acknowledgement: CDC/CCEHIP/NCEH. Suported by CNPq, FINEP and UFSC.

BM.106 - THE FOUR TRYPANOSOMATID eIF4E HOMOLOGUES FALL INTO TWO DISTINCT GROUPS, WITH FUNCTIONS IN TRANSLATION AND OTHER CRITICAL PROCESSES

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Translation initiation in eukaryotes requires eIF4E, the cap binding protein, which mediates its function through an interaction with the scaffolding protein eIF4G, as part of the eIF4F complex. In trypanosomatids, four eIF4E homologues have been described but the specific function of each is not well characterized. The functional analysis of these proteins in *Trypanosoma brucei* (*Tb*EIF4E1 through 4) has been initiated and it was seen that, at the sequence level, they can be assigned into two groups; *Tb*EIF4E1 and 2, similar in size to metazoan eIF4E1; and *Tb*EIF4E3 and 4, with long N-terminal extensions. All were constitutively expressed, but whilst *Tb*EIF4E1 and 2 localise to both nucleus and cytoplasm, *Tb*EIF4E3 and 4 are strictly cytoplasmic and are more abundant. *Tb*EIF4E3, despite its inability of binding to 7-methyl-GTP Sepharose, was the only one confirmed to be essential for viability of procyclic form through RNAi assay. For *Tb*EIF4E1, a reduction in the rate of cellular growth was observed, but cells viability was not impaired. We also identified that *Tb*EIF4E3 and 4 were all essential for the bloodstream form. It's worthwhile to note that despite *Tb*EIF4E4 is essential for viability in bloodstream, it is not in the procyclic form. Simultaneous knockdown of *Tb*EIF4E1 and 2 caused cessation of growth and death in procyclics, although with a delayed impact on translation, whilst knockdown of *Tb*EIF4E3 alone or a combined *Tb*EIF4E1 and 4 knockdown led to substantial translation inhibition preceding cessation of growth. Through HA-tagged TbEIF4Es imunoprecipitation and pulldown assays we observed that only *Tb*EIF4E3 and 4 interacted with *T. brucei* eIF4G homologues. *Tb*EIF4E3 bound to both *Tb*EIF4G3 and 4 whilst *Tb*EIF4E4 bound only to *Tb*EIF4G3. These results are consistent with *Tb*EIF4E3 and 4 having distinct but relevant roles in translation initiation while *Tb*EIF4E1 and 2 may be involved in other critical processes.
Supported by CAPES, UFPE, FIOCRUZ and FACEPE.

BM.107 - MOLECULAR CHARACTERIZATION OF TWO BRAZILIAN STOCKS OF *TRYPANOSOMA EVANSI*

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Trypanosoma evansi is the most widespread of the pathogenic salivarian trypanosomes due to its ability to be mechanically trasmitted by blood-sucking flies. Several studies have shown that *T. evansi* is genetically related to *T. brucei* with some differences, such as kinetoplast absence or deletion. In this work, the RAPD technique, hypervariable minisatellite and microsatellite loci were employed to analyze the genetic variability of two brazilian *T. evansi* isolates. The presence of kinetoplast was also verified. To obtain genomic DNA, the blood of a Wistar rat infected with *T. evansi* was purified by Percoll® gradient and ion exchange chromatography with DEAE-cellulose. It was used six primers to RAPD reactions with *T. evansi* stocks and a *T. brucei brucei* strain. Close RAPD profiles, among *T. brucei* and *T. evansi,* were demostrated. The primers showed that the *T. evansi* isolates are highly homologous. Minisatellite and microsatellite markers 292-locus and MORF2 also proved high homology. To detect specific *T. evansi* VSG sequence RoTat 1.2, described primers were employed to assert that both isolates have the VSG sequence. Immunofluorescence staining assay with DAPI was used to observe the Kinetoplast. Primers derived from the sequence of the maxicircle of *T. brucei* encoding NADH dehydrogenase (nadh5) were used to assert the absence of maxicircles. Amplification of 464bp minicircle kDNA sequence were used to certify the loss of minicircles. All methods indicate the total absence of kDNA. The high homogeneity of isolates around the world is characteristic of *T. evansi* stocks, which there is no recombination of the parasite in the vector. The clonal population in the host also contributes to reduce the genetic variability of *T. evansi*. However, some remarkable differences are observed, as presence of kDNA minicircles of african strains and total absence of kinetoplast in american strains. Supported by: CNPq, FAPESC, UDESC.

BM.108 - IDENTIFICATION OF *Trypanosoma evansi* SELB AND SELD GENES

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Selenoproteins are mainly related to prevent oxidative stress in all kingdoms of life. These proteins contains the amino acid selenocysteine (Sec), encoded by an in frame UGA “stop codon”. In Eukaryotes, there are five main genes involved in the biosynthesis of Sec. In this work, two of these genes in *Trypanosoma evansi* were cloned and characterized: *selB*, a specific elongation factor involved in the translation process; and *selD*, which catalyzes the conversion of selenide and ATP in monoselenophosphate, the active selenium donor compound for Sec biosynthesis. These genes were amplified from genomic DNA, cloned and sequenced. The theoretical pI found was 7.19 and 5.46 for *selB* and *selD*. Analysis of the amino acid sequence showed that both proteins present specific structural characteristics that correlates with their biological function, (i) a Cys residue in position 42 for *selD*; and (ii) EF-Tu domains for *selB.* Since a high similarity in the amino acid composition with *Trypanosoma brucei* was observed, 99.54% for *selB* and 99.74% for *selD*, an antibody of *Trypanosoma* brucei (kindly granted by Dr. Otávio H. Thiemann) was used to determine the presence and cellular localization of *selD*. A cytoplasmatic localization was observed by indirect immunofluorescence and an expected 43 Kda band was detected in a Western blotting assay. Studies have been performed to identify other elements of this metabolic route and their role in *Trypanosoma evansi*. Supported by CNPq, FAPESC, FINEP, UDESC.

BM.109 - PROTEOMIC DATA SUGGEST A MULTI-PROTEIC COMPLEX RESPONSIBLE FOR SELENOCYSTEINE INCORPORATION IN KINETOPLASTIDS.

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The discovery of new amino acids such as selenocysteine and pirrolisine, resulting in the expansion of the genetic code from the traditional 20 amino acids to the currently recognized 22 amino acids, have attracted the interest of several research groups. The synthesis pathway of selenocysteine (Sec - U) represents the major biological form of selenium, whose incorporation occurs co-translational into selenoproteins and depends is determined by an in-frame stop codon UGA and a tertiary structure in the messenger RNA known as SECIS element (Sec insertion sequence). We identified the existence of the selenocysteine synthesis route in kinetoplastid and characterized the genes of this pathway: SELB (Elongation Factor EFSec), SELD (Selenophosphate sintetase), PSTK (Fosfoseril tRNA kinase), SECSEPS (Selenocysteine Sintase) and SELC ($tRNA^{[ser]sec}$). Furthermore, three selenoproteins were identified, SELK, SELT and SELTryp. The last member has no identity with other mammalian selenoproteins. However, details about composition and structure of the Sec incorporation complex are not known, and the purpose of project is the identification of proteins that participate in the Sec incorporation complex, using techniques of complex purification such as PTP (ProtC – TEV – ProtA)-tag, using trypanosome PSTK as a bait. The PSTK coding-gene was cloned into the pN-Puro-PTP vector, linearized with *Xcm* I and transfected into *Trypanosoma brucei* cells (strain 427). Positive clones were selected with puromycin and western blotting experiments using anti-protein A antibodies confirmed the expression of PSTK fused with PTP-tag. A total *T. brucei* cell protein extract was obtained and immunoprecipitation assays confirmed the ability of PSTK-PTP to interact with IgG sepharose, the first step of PTP-tag purification. After the complete PTP-tag purification process, at least four proteins were observed, with approximately 66, 60, 45 and 29 kDa. These data are awaiting confirmation by mass spectrometry, but strongly suggest the presence a multi-protein complex, responsible for incorporation of selenocysteine in *Trypanosoma* cells. This observation is consistent with previous immunofluorescense experiments using polyclonal antibodies against SELB that indicated the formation of cytoplasmic granules, corroborating a possible protein complex been formed. Supported by: FAPESP and CNPq

BM.110 - 214 1 CHARACTERIZATION OF A LARGE TFIIA-ASSOCIATED PROTEIN COMPLEX IN *TRYPANOSOMA BRUCEI*

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TFIIA is a basal factor of RNA polymerase (pol) II transcription initiation interacting with the TATA-binding protein (TBP) and stabilizing the TBP-core promoter interaction. TFIIA consists of two subunits in yeast and, due to proteolytic cleavage of the larger protein, of three subunits in higher eukaryotes. In *Trypanosoma brucei*, a clear ortholog of the small TFIIA subunit (TFIIA-2) was characterized as part of a larger complex consisting of three SNAPc subunits, the trypanosome TBP homolog TRF4, and a sixth protein whose sequence conservation is too weak for unambiguous annotation. Interestingly, tandem affinity purification of TFIIA-2 co-purified not only the SNAPc/TFIIA/TRF4 components but specifically also several proteins of minor abundance indicating that TFIIA-2 is assembled into a second protein complex. Since we could identify one of the minor proteins by mass spectrometry, we expressed the protein in trypanosomes as a C-terminal fusion to the composite PTP tag and tandem affinity-purified it from extract. At least eight proteins were co-purified; mass spectrometry identified them as TFIIA-2 and seven *conserved hypotheticals* that lack sequence similarity to proteins outside of trypanosomatids. Since all nine proteins co-sedimented in a sucrose gradient, they appear to form a single complex which we termed TFIIA-associated complex (AAC). While the *AAC-3* subunit is localized in the nucleus, it is, unlike subunits of SNAPc/TFIIA/TRF4, dispensable for RNA pol II transcription of spliced leader RNA genes. Preliminary data indicate that silencing of *AAC-3* is lethal. Interestingly, first RNA analyses revealed that this knockdown affects the abundance of RNA pol II- but not of RNA pol I-synthesized mRNA suggesting that AAC-3 functions specifically in RNA pol II transcription of protein coding genes.

BM.111 - MOLECULAR CHARACTERIZATION OF A *Trypanosoma brucei* POLY(A)-BINDING PROTEIN.

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Trypanosomatids are a major cause of mortality in tropical regions of the world and are affected by the lack of effective treatments. Several intriguing molecular pathways are found in these parasites also, rendering them particularly attractive for biochemical investigation. This unique eukaryotic cell lacks mechanisms to control gene expression at the transcriptional level, as protein synthesis is mostly controlled by posttranscriptional regulation processes. Hundreds of protein-coding genes of unrelated function have to be separated cotranscriptionally by trans-splicing at the 5' end and polyadenylated at the 3' end. Several RNAs and proteins are central to this process, including poly(A) binding proteins. The poly(A)-binding protein of eukaryotes is the major cytoplasmic mRNA binding protein and also plays a role in polyadenylation of nuclear transcripts. In the cytoplasm, PAB_1 has been implicated in translational initiation and termination, and in mRNA turnover. When complexed to mRNA, the poly(A) tail interacts with cap, enhancing translational initiation and stabilizing mRNA. In addition, this protein is important in mRNA decay. In our work a novel poly(A) binding protein ($PABP_1$) was identified in *Trypanosoma brucei*. Transcriptional silencing using the RNA interference technique revealed that the absence of its transcript is lethal to the procyclic form of the parasite. Protein immunolocalization experiments indicate a cytoplasmatic localization. We are presently investigating the domain organization of the protein and its involvement in the polyadenylation/trans-splicing coupled events. Supported by FAPESP, CNPq, NIH

BM.112 - *Trypanosoma brucei* SELENOCYSTEINE PATHWAY VALIDATION BY INTERFERENCE RNA.

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Selenoproteins, proteins containing the 21st amino acid selenocysteine (Sec – U) are present in the three domains of life and require a complex molecular biosynthesis and incorporation machinery. Genes coding for components of the Sec insertion machinery were identified in the Kinetoplastida genomes of *Trypanosoma cruzi, Trypanosoma brucei* and *Leishmania*. We focused our work on *Trypanosoma brucei*, the etiologic agent of Nagana and Sleeping Sickness. To produce selenocysteine, this organism obtains the biologically active selenium donor compound through the catalysis of selenide and ATP by Selenophosphate Synthetase (SPS2). RNA interference experiments of SPS2 in procyclic *T. brucei* form shows significant growth inhibition and an apoptotic-like phenotype. Also, an increased susceptibility to oxidative stress induced by hydrogen peroxide suggests a possible role of selenoproteins on oxidative pathways and protection to oxydative stress. The selenoproteome of these flagellated protozoa consists of distant homologs mammalian SelK and SelT, and a novel selenoprotein designated SelTryp, that has neither Sec- nor cysteine-containing homologs in the human host, appearing to be a Kinetoplastida-specific protein (Lobanov, 2006). Since these proteins have predicted redox motifs, we cloned them into a vector for RNAi ($p2T7^{TABlue}$) to individually investigate their function in different life stages of *T. brucei.* Complementary, polyclonal antibodies are been used to estimate the expression levels of these proteins, giving a better understanding of the role of selenoproteins in this organism.
Supported by FAPESP.

BM.113 - IMPROVEMENT OF THE HETEROLOGOUS GENE EXPRESSION IN *Trypanosoma brucei* USING POST-TRANSCRIPTIONAL AND POST-TRANSLATIONAL MECHANISMS

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The functional genomics is a growing field due to the increasing number of published genomes, a fact which tends to improve with the advent of new sequencing platforms. To elucidate the gene function in *T.brucei*, there was a large range of techniques and methodologies to be employed. One of these is the heterologous expression using inducible promoters regulated by tetracycline repressor. However, some reports have shown that there is some weakness on controlling gene expression, and the leak in the expression of toxic gene products in the absence of the inductor limits the advances of various post genomic approaches. On this assumption, we designed a *T.brucei* expression vector (based on pLEW100) regulated by exogenous molecules: transcriptionally by GPEET promoter with tetracycline (tet) operators, post-transcriptionally by a riboswitch molecule (Ribo), and post-translationally by fusioning the target protein to FKBP destabilizing domain (DD). The constructs were created using pLEW100 as a parental vector. Our first attempts, using procyclics transfected with two pLEW100 vectors containing *Renilla* luciferase controlled by the inducible promoter and a tet-sensitive riboswitch at 3'UTR, or an always active ribozyme, showed that this new system regulates over a thousand times the luciferase activity in the presence of the ligand in relation to its absence. To obtain a higher efficiency of gene regulation, we are generating constructs carrying GFP fused to DD polypeptide wich will be also regulated by tet repressor and riboswitch.
Financial support: Fundação Araucária, CAPES/REUNI, FAPEMIG and CNPq

BM.114 - STANDARDIZATION OF A SINGLE MOLECULE ANALYSIS OF REPLICATED DNA (SMARD) OF *Trypanosoma brucei* CHROMOSOME I

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DNA replication is a crucial step during the cell cycle and little is known so far about the DNA replication of *Trypanosoma brucei*. Single Molecule Analysis of Replicated DNA (SMARD) allows the visualization by fluorescence microscopy of single molecules of replicated DNA stretched on microscope slides. Using this method is possible to determine the number of replication origins, the fork direction and the DNA replication rate (kb/min) of a DNA fragment. Here we want to define how replication occurs on chromosome 1 from *T. brucei* (1.85 and 3.6 Mbp). Since the technique limits the molecules analyzed to a maximal length of 500 kbp, two different approaches are being developed: the analysis of chromosome 1 (1.85 and 3.6 Mbp) fragments smaller then 500 kb and the entire chromosome 1. For the fragment analysis, DNA was digested with two different enzymes, Fse I and Asc I. After running the pulsed field gel electrophoresis, the fragments were analyzed using specific probes and they showed length differences compared to the prediction because the strain sequenced (*T, brucei* TREU 972) was different from that used in this experiment (*T. brucei* 427). Although the fragments were still smaller then 500 kbp and they can be identified on slides through specific probes that are being developed. In order to analyze the entire chromosome 1 (1.85 Mbp), a PFEG program will be developed to separate and extract chromosome 1 from the genome. Probes are also being developed to determine the chromosome 1 orientation (5' – 3'). Once all these probes are available, chromosome 1 and the fragments can be analyzed and then it will be possible to know how many origins are present in chromosome 1, and also the fork direction and speed. Supported by FAPESP.

BM.115 - **A NEW *T. BRUCEI* CELL LINE THAT IS USEFUL FOR ACCESSING FUNCTION OF PROTEINS INVOLVED IN TRANSLATION**

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Trypanosoma brucei is a privileged model for studies of gene expression mainly because of its feasible cultivation and the development of several tools involving genetic manipulation and inducible expression. Many plasmids developed for *T. brucei* are based on tet-responsive elements; however the evaluation of individual proteins involved in basic processes, such as translation, would require the use of a constitutive gene reporter that could be easily detected in these parasites. We aimed to generate new *T. brucei* cell lines expressing the luciferase as a monocistronic reporter to access, in a direct manner, the translation-related functions of some trypanosomatid initiation factor (eIF) homologues. The constructs used in transfection experiments were derived from pLew20 and pLew82. The modifications were designed for a constitutive expression of firefly luciferase and a puromycin drug resistance marker under the action of a single promoter. Cultures of procyclic host cell line 29-13, coexpressing T7RNAP e TetR, were harvested and transfected with *Not*I-linearised plasmids pLEW20ΔtetOpac and pLEW82ΔtetOpac. Puromycin-resistant clones were evaluated for constitutive luciferase expression and the expression driven by an endogenous (pLew20) or a T7 promoter (pLew82) was also compared. Initially the clones examined expressed continuous levels during a growth curve, with T7RNAP leading to luciferase activities exceeding those produced by the endogenous promoter. The cell lines were then designated 29-13-20m and 29-13-82m. However, after several passages, the cell line 29-13-82m showed much reduced levels of expression, similar to the parental 29-13, but it maintained the puromycin-resistance showing that the cells turned into revertant forms. The cell line 29-13-20m maintained high levels of luciferase expression and it proved to be useful for new rounds of transfection with tet-responsive plasmids for functional assays involving the eIF homologues and luciferase activity which will be able to serve as parameter to determine their influence in translation.
Supported by CAPES and CNPq

BM.116 - IDENTIFICATION OF POST-TRANSLATIONAL MODIFICATIONS ON HOMOLOGUES OF TRANSLATION INITIATION FACTORS IN *Trypanosoma brucei*

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Trypanosoma brucei is a unicellular protozoan which in humans causes the sleeping sickness and whose life cycle alternates between two hosts, its procyclic form being found in insect vectors and its bloodstream form in susceptible mammals. Genomic analysis identified a considerably large number of protein kinases, leading to speculation that protein phosphorylation, among the post-translational modifications, may also be a key mechanism for such regulation events. In eukaryotes, the eIF4F complex (formed by subunits eIF4A, eIF4E and eIF4G), assisted by the poly(A)-binding protein (PABP), works in translation initiation and has its activity regulated by modifications such as phosphorylation. This study sought to analyze the expression of selected eIF4E, eIF4G and PABP homologues in *T. brucei* (*Tb*EIF4E1, 3 and 4; *Tb*EIF4G4 and 5) and to investigate the occurrence of post-translational modifications which may be associated with control of their function. First, growth curves were generated and protein extracts made from cells derived from selected time points. All the chosen proteins were observed to be expressed throughout the curves in both forms of the parasite's life cycle, but with distinct expression patterns. *Tb*EIF4E1 and 3 are the least expressed and the most expressed proteins, respectively, in both procyclic and bloodstream forms. *Tb*EIF4E3 and 4 and *Tb*EIF4G4 were represented by more than one isoform, unlike the other proteins studied, and the three proteins were found in phosphorylated forms. Through two-dimentional electrophoresis it was possible to visualize 2, 5 and 4 isoforms for *Tb*PABP1, *Tb*EIF4E3 and *Tb*EIF4E4, respectively, whilst only one non-phosphorylated isoform was observed for the *Tb*EIF4AI. In addition, it was shown that phosphorylation of *Tb*EIF4G4 does not prevent its interaction with *Tb*EIF4E3. These results indicate that phosphorylation is a mechanism that may have a predominant role in regulating the function of the translation factors studied.
Supported by FACEPE and CNPq.

BM.117 - EVALUATION OF THREE METHODS FOR DNA EXTRACTION FROM STOOL SAMPLES FOR DETECTION OF *ENTAMOEBA* BY REAL-TIME PCR

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Amebiasis is an infection caused by *Entamoeba histolytica.* Differentiation between *E. histolytica* and *E. dispar*, a morphologically identical species, is fundamental for precise diagnosis in order to base public health and therapeutic conducts to prevent and control the occurrence of invasive disease. Parasite demonstration by microscopy continues to be the technique more frequently used for the diagnosis of amebiasis. However, this method cannot differentiate *E.histolytica* from *E. dispar*. Real-time Polymerase Chain Reaction (RT-PCR) was shown to be a high specific and sensitive method for diagnosis and species differentiation. DNA extraction is required for an efficient PCR reaction and should be adequate to each type of clinical sample and microorganism. In this study, the efficiency of three different methods for extraction of DNA from stools was evaluated: two commercial kits, FastDNA® (MP Biomedicals) and QIAamp® DNA Stool (QIAGEN), and one "in house" technique. The SYBR Green reaction was used for all the tests. Stool samples negative for the *E. histolytica/E. dispar* complex were spiked with 5.5 x 10^7 trophozoites of *E. histolytica* and stool samples with cysts of *E. dispar* were used for DNA extraction. Amplified DNA sequences of 132 base-pairs (bp) and 96 bp were specific for *E.histolytica* and *E.dispar*, respectively. Four different concentrations of DNA obtained from samples containing trophozoites were assess. The cycle numbers (C_t) detected were: 19.0, 21.0, 24.3 and 28.1 for the "in house" extraction; 19.9, 24.2, 26.4 and 29.0 for the QIAamp® method, and 22.1, 24.6, 27,5 and 28.4 for the FastDNA® method. The results indicate that the "in house" extraction method was the best to detect for trophozoites, while detection of cysts was better achieved by using the FastDNA® method. Supported by FAPERJ and CNPq.

BM.118 - MULTIPLEX REAL-TIME PCR FOR THE IDENTIFICATION AND DIFFERENTIATION OF *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* BY USING THE SYBR GREEN TECHNOLOGY

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Entamoeba histolytica is a protozoan that causes amoebiasis, a infection that can result in serious disease. The microscopic examination of fresh or fixed stools is the method more frequently used for *Entamoeba* detection. Two morphologically indistinguishable species, the potentially pathogenic *E. histolytica* and the non-pathogenic *E. dispar*, are established. Then, new diagnostic methods are necessary for the identification and differentiation of these two species. Polymerase Chain Reaction (PCR) - based methods have been proposed by several groups. The development of real-time PCR contributed to circumvent sensitivity and contamination problems observed with conventional PCR, improving these techniques in order to be used in clinical laboratories. The objective of this study was to standardize a multiplex real-time PCR protocol using the SYBR Green technology to identify and differentiate the species belonging to the *E. histolytica*/*E. dispar* complex. A standard curve was obtained using 10 1:2 DNA serial dilutions. DNA amplification was detected for all the dilutions, expressing the sensitivity of 0.0143 pg for *E. histolytica* and 0.5156 pg for *E. dispar.* In order to differentiate the *Entamoeba* species with the SYBR Green technology, the dissociation stage after the cycling conditions was included. This additional step produces a melting curve, which indicates specific melting temperatures (Tm) for each species sequences. The mean melting temperature (Tm) observed for the DNA sequence amplified from *E. histolytica* was 73.6ºC while *E. dispar* DNA sequence was 70.6ºC. The results demonstrate that the SYBR Green multiplex real-time PCR offers a new and improved alternative method for the differentiation of the *E. histolytica*/*E. dispar* complex.
Supported by CNPq and FAPERJ.

BM.119 - CHARACTERIZATION OF THREE APPLE CONTAINING DOMAINS PROTEINS FROM THE APICOMPLEXAN *Neospora caninum*

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Neospora caninum, an Apicomplexan parasite, has been increasingly recognized as an important cause of bovine fetal loss worldwide due to the abortion it causes in cattle. The host cell invasion process of this obligatory intracellular parasite is very conserved among the phylum and involves secretory organelles like micronemes, roptries and dense granules. Pan/Apple domains, which are known to be related to adhesion functions, were found in microneme proteins from other Apicomplexan parasites, like EtMIC5 from *Eimeria tenella* and TgMIC4 from *Toxoplasma gondii*, probably mediating binding interactions between parasite and host cell. The aim of this work is the identification and characterization of proteins containing Apple domains in *N. caninum*. Based on ESTs containing Apple domain sequences, three flanking fragments were found in a single contig after screening the non-annotated genomic database of *N. caninum.* Sequencing data revealed a high level of similarity among the three fragments and each of them was predicted to encode one protein containing three Apple domains (PAN1), and one of these fragments also had one Apple-like domain (PAN3). Two fragments were chosen to be cloned in pET28 and expressed in *E. coli* BL21 cells, resulting in two recombinant proteins with molecular weight of 37 KDa and 41 KDa, called PAN domain-containing protein1 (PANdcp1) and PANdcp2. Two New Zealand rabbits were immunized with each recombinant protein and sera raised were collected after each of four immunizations. ELISA assays were made to verify serum conversion and recognition of native and recombinant Apple proteins. We are currently investigating by 2D Western Blot for the Isoeletric point and Molecular Weight of the native proteins in total extract from *N. caninum*. Next steps include *in vitro* invasion assays and localization of these Apple proteins in the tachyzoite of *N. caninum*. Supported by CNPq (project grant 480039/2009-7) and CAPES (fellowship to LPO).

BM.120 - TRANSIENT CONTROL OF EXPRESSION BY TETR/TETO SYSTEM ADAPTED FOR *N.CANINUM*

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Neospora caninum is an Apicomplexan protozoan, obligatory intracellular parasite that has the dogs as definitive hosts and especially cattle as intermediate hosts, causing in the first ones encephalopathy and the last ones abortion with fertility impairment. Because of its intracellular cycle, several strategies have been developed to characterize the invasion process in the phylum, such as knock-out or expression control of proteins involved in active invasion. The operon TetR/tetO system is present in gram-negative bacteria that gain the ability to expulse tetracycline from periplasmic region. The system is composed by TetR, a dimmer protein that bind to the tetO nucleotide sequence, repressing expression on tetracycline operon. The expression is activated when tetracycline interact to dimmer TetR liberating it from tetO sequence and allow the expression of operon. An inducible system based on TetR/tetO in *Toxoplasma gondii*, where TetR is expressed by a tubuline vector and tetO is downstream a RPS13 promoter controlling the expression of β-galactosidase. Thus, TetR can be incorporated in the parasite genome by a chloramphenicol cassette resistance flanked by the promoter and the 3' region of *T. gondii* SAG gene (TgSAG1). In our work we have substituted the *T. gondii* promoters to *N. caninum* in TetR and tetO (tubulin 1990 pb and RPS13 651pb, respectively) and the promoter and 3' region of the *Tg*SAG to *N. caninum* SAG1 gene. In a transient assay the NctetO vector expressed β-galactosidase 700% over control (for 10 days). Tachyzoites were transiently transfected with NcTetR followed by NctetO and treated with 0,1; 1 and 10 μg/ml of tetracycline and the presence of 1 μg/ml improved the expression of β-galactosidase by 14% The experiments with stable transfection and control of β-galactosidase expression are being performed aiming to future functional assays with *N. caninum* genes involved with invasion.
Supported by CAPES (fellowship) and FAPESP (project grant 2005/53785-9).

BM.121 - GENOMIC AND PROTEOMIC CHARACTERIZATION OF *TOXOPLASMA GONDII* BRAIN CYSTS FROM MICE INFECTED WITH GENETICALLY DISTINCT STRAINS

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Toxoplasmosis, caused by the coccidian *Toxoplasma gondii,* is transmitted by ingestion of raw or undercooked meat or by ingestion of food and water contaminated with oocysts from infected cat faeces. Although asymptomatic in most cases, the agent causes ocular disease or severe disease in immunocompromised patients or fetus. Several strains of *T.gondii* have been described in recent years, with variable virulence that could be associated with severity of human disease, including the reinfection of a previously immune host. The primary infection by *T.gondii* usually confers reinfection protection by a new strain but there are anecdotal reports of successful reinfection. We devised to study reinfection and co-infection models in experimental mouse toxoplasmosis using genetically defined strains for study specific cyst survival in the brain, looking for evidence of recolonization after primary infection. We used two genetically defined strains, ME49 (genotype II) and VEG (genotype III), which promotes cyst brain infection. Brain cyst genotype was determined by SAG2 gene RFLP on infected brains. Whole anti-*T.gondii* IgG and peptide specific strain IgG was determined by ELISA. Strain specific immunohistochemistry of infected brains was performed with anti-peptide antibodies. ME49 primary infection give higher cysts number than VEG primary infection both with strain specific IgG production. Co-infection with both strains presented early predominance of ME49 cysts with subsequent VEG cysts with both strains specific IgG. Primary infection with subsequent challenge with another strain showed that the primary infection colonizes the brain and block subsequent challenge strain. Specific strain antibody production is also from primary strain despite evidence of virulent infection. Immunohistochemistry confirms those data. Our findings show that primary strain infection protects the brain from subsequent colonization from a new strain of *Toxoplasma* but without evidence of adaptive specific immune response to the new strain. Supported by CNPq and LIM49HCFMUSP.

BM.122 - IMMUNOLOCALIZATION BY ELECTRON MICROSCOPY OF PROTEINS INVOLVED IN *Toxoplasma gondii INVASION*

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The parasite *Toxoplasma gondii* belongs to the Phylum Apicomplexa and is able to invade all types of nucleated cells. This process is mainly coordinated by the parasite, and depends on three types of secretory organelles: rhoptries, micronemes and dense granules. It is common sense that at the region of entry in host plasma membrane, parasite secreted proteins give rise to the moving junction. That is crucial for the formation of the parasitophorus vacuole, a “safe heaven” inside the host cell for the parasite’s development. In this work, we have used high resolution scanning and transmission electron microscopy to immunolocalize toxoplasma’s secreted proteins involved in the moving junction. Our model is *T. gondii* RH strain and as host, the epithelial cell line LLC-MK2. The interaction was for 15 minutes at 4^{o}C and 5 minutes at 37^{o}C before the fixation. Standard protocols for immunolocalization were employed for visualization by scanning and transmission electron microscopy and imunoflurescence (IFA). IFA assays for RON-4, a protein from rhoptry neck, had a ring shape distribution, and was a marker for the moving junction region. However, not all invasion points presented this stain. Using scanning electron microscopy, we confirmed the presence of moving junction, and the hourglass shape of the parasites but the labeling at this point had only a few Au particles. The immunocytochemical labeling by TEM confirmed these data. Comparing this model with macrophage invasion, the same pattern was observed using both FE-SEM and IFA assays. These results indicate that invasion of host cells may result from more than one process of interaction, and that the moving junction may not be present in all of them. The RON-4 labeling in moving junction was visualized at first time by SEM.
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BM.123 - CONGENITAL TOXOPLASMOSIS IN MINAS GERAIS, BRAZIL: ISOLATION, VIRULENCE AND MOLECULAR DIAGNOSIS OF *Toxoplasma gondii*

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Toxoplasma gondii is a widely distributed apicomplexan parasite of great medical importance. Most primary infections are asymptomatic in immunocompetent hosts. However, immunosuppressed individuals like fetuses, organ graft recipients or patients with acquired immunodeficiency syndrome develop a severe disease. The objective of this work was to carry out a study of congenital toxoplasmosis in the State of Minas Gerais, Brazil. In an attempt to samples isolate and characterize *T. gondii*, blood from 220 newborns were collected. Of the total children screened, 81% (178/220) had the diagnosis confirmed with toxoplasmosis congenital by the persistence of IgG in ELISA test after the 12th month of life. *T. gondii* was isolated from peripheral blood of 27 infected newborns by mouse bioassay. Our experiments showed that 15.2% (27/178) of the newborns with congenital toxoplasmosis showed parasitemia. Isolates were divided into three groups according to the virulence phenotype. Ten isolates (38%) were characterized into virulent for mice (LD100 equivalent to a single viable tachyzoite). Two (8%) were characterized into non-virulent, as all mice survived after the 30-day-period of observation. All surviving mice showed to have anti-*T. gondii* antibodies. Fourteen isolates (54%) showed to have intermediate virulence (virulence phenotype varying among those described above). Gene amplification was performed for B1 gene in all blood samples verifying a total of 64/220 (29.0%) children were positive by PCR (sensitivity 31.46% and specificity 80.85%). Our research corroborates that nested PCR using the primer B1, is not sufficiently sensitive and specific to be a useful diagnostic tool for congenital toxoplasmosis diagnosis in peripheral blood samples from newborns.
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BM.124 - LABORATORIAL DIAGNOSE OF CRYPTOSPORIDIOSIS USING REAL-TIME PCR

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Cryptosporidium spp. is an important diarrhea-causing parasitic protozoan found in both humans and animals. *Cryptosporidium hominis* and *Cryptosporidium parvum* are responsible for the most cases of cryptosporidiosis in human beings. In developing countries, *Cryptosporidium spp* infections occur mostly in children younger than 5 years of age. In immunodeficient human (HIV / AIDS), cryptosporidiosis can be associated with chronic potentially life-threatening diarrhea. The conventional method for detecting oocysts in fecal specimens in HUAP laboratorial routine, involve microscopy and safranin-methylene blue staining technique. However, this method cannot identify *Cryptosporidium* at the species level. Real-time PCR with specific primers and probes represents an alternative to conventional PCR for increasing the sensitivity, specificity and speed of sample analysis. The objective of this study was to evaluate the TaqPCR in relation to the safranin-methylene stain in clinical specimens examined in the HUAP laboratory. From June of 2009 to July of 2010, 19 stool samples from HIV serology-positive patients examined for *Cryptosporidium*. Of these stool samples, 4 were positive for *Cryptosporidium spp.* by microscopy and by TaqPCR (3 - *C. hominis* and 1 - *Cryptosporidium spp.*). One sample was positive only by microscopy and 4 samples were positive only by TaqPCR (2 - *C. hominis* and 2 - *Cryptosporidium spp.*). These results showed the advantage in the association of these two techniques which allowed the detection of *Cryptosporidium* in nine samples. Species discrimination is important for molecular epidemiological purposes in order to evaluate potential sources of infections.
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BM.125 - CHARACTERIZATION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM *Phytomonas serpens* 15T

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The enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyses the oxidative phosphorilation of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate. This reaction plays an important role in controlling ATP production in pathogenic parasites. GAPDH enzymes from human pathogenic trypanosomatids species have been extensively studied at both the enzymological and gene level. Overall, at least two isoenzymes, glycosomal and cytoplasmic, of GAPDH have been described for these trypanosomatids. The glycosomal enzymes are encoded by two tandemly arranged genes of identical sequence and encode polypeptides with approximately 38 kDa which present around 55% identity with the amino acid sequences of cytosolic enzymes. In the case of other trypanosomatids there are little informations about the GAPDH enzymes. In view of this, here we describe the characterization of glycosomal GAPDH from *P. serpens* 15 T, a heteroxenic tomato parasite. Northern blot employing total RNA from *P. serpens* exponential promastigotes was hybridised with *gapdh* probe, and a transcript of around 1.5 kb was observed. GAPDH was overexpressed in *E. coli* BL21(DE3) strain carrying the recombinant plasmid pET28a-GAPDH and the recombinant protein was used as antigen to immunize BALB/c mice. Western blot analysis of log-phase promastigotes with this antiserum recognized a protein with an estimated molecular weight of 38 kDa. The localization of GAPDH was further investigated. The protein was detected mainly at the cytoplasmic region of *P. serpens* promastigotes by confocal immunofluorescence microscopy. Transmission electron microscopy showed the localization of GAPDH gold particles in membrane-bound cytoplasmic structures of the plant parasite. No background staining was observed when secondary antibodies were incubated alone with the *P. serpens* promastigotes in both microscope assays. Supported by: Fundação Araucária – PR and CAPES.

BM.126 - 422 1 DOCKING STUDIES OF NADH ANALOGUES AS POTENTIAL INHIBITORS OF *PLASMODIUM FALCIPARUM* LACTATE DEHYDROGENASE

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The enzyme lactate dehydrogenase from *Plasmodium falciparum* (*Pf*LDH) is considered a potential molecular target to the development of new chemotherapics against malaria based on its importance in the final step of the parasite energy obtention process. It has been suggested that the mechanism of action of the quinolinic antimalarials could be related to the inhibition of this enzyme by competition with its binding pocket for NADH (Read et al, J. Bio. Chem., 1999). Based on this assumption, we have now performed a screening on the Drug Bank for analogues of NADH and further computational studies on the binding mode of these compounds in the *Pf*LDH active site. The docking energies of each compound were calculated with the software Molegro Virtual Docker (MVD)® which permitted the selection of 50 compounds with interactions on the active site similar to that of NADH. In addition, we also analyzed the main interactions between the *Pf*LDH active site and the selected compounds in order to evaluate if the residues involved are specific of *Pf*LDH, as compared to human LDH. Among the compounds studied three (posaconazole, itraconazole and atorvastatin) were chosen for further experimental studies on *Pf*LDH. Since they presented very good theoretical results and, also, because are commercially available for use in human beings, these compounds were forwarded to be tested in experimental protocols (see abstract by Penna-Coutinho et al.).
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BM.127 - THE KINASES SECRETED IN SALIVARY GLANDS FROM TRIATOMINAE (HEMIPTERA: REDUVIIDAE)

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The transmission of Chagas disease by triatominae insects across South and Central America has imposed enormous economic and health burdens to the human populations. The kissing bugs in the subfamily Triatominae rely on the chemical properties of the saliva constituents to inhibit the host's blood homeostasis and to prevent harmful proliferation of microorganisms. The investigations about the saliva contents from various triatomine insects suggested the presence of protein kinases in the insect's saliva. This existing enzymatic function, which had never been reported on triatomines saliva, could elucidate some unexplained peculiarities noticed during triatomines blood feeding. Mass spectrometry LC-MS/MS assays performed with salivary gland proteins from *Panstrongylus megistus* and *Rhodnius brethesi* revealed a gamut of different proteins with reported kinase activities in salivary glands from those triatomine species. The analyses of transcript from salivary gland of *Rhodnius robustus* and *Triatoma infestans* also indicated the presence of three main kinases, two of which had been identified by LC-MS/MS assays. The databank searches showed that some of the kinases involved with the blood feeding possibly evolved from genes acquisitions in the course of natural selection. In this regard, kinase genes differentiated to display different functions. Our results suggest that protein kinases in the kissing bugs salivary glands might play a role in hematophagy. Further experiments will determine the functional roles of the secreted protein kinases in the triatomines salivary glands.
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