Biologia Molecular - Molecular Biology

BM001 - Generation of *Leishmania* mutants to be used in large-scale drug testing

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Pentavalent antimonials have been the drug of choice against leishmaniasis, a spectral disease caused by the protozoan parasite *Leishmania*, for more than 60 years. Nevertheless, the search for novel effective compounds to combat leishmaniasis is relevant given the toxicity, resistance and high costs of current treatment schemes. Combinatorial libraries of compounds are made available for testing *Leishmania* susceptibility. Nevertheless, high throughput assays are hampered by the lack of *Leishmania* mutants that allow large-scale screening approaches. The objective of this work is the generation of *Leishmania* mutants to overexpress constitutively a reporter gene inserted in the rDNA locus. To avoid potential interference with tested compounds, these transfectants must lack a drug resistance marker. Initially, we generated Green Fluorescent Protein-expressing mutants (GFP+) bearing neomycin phosphotransferase (NEO+) to evaluate levels of expression and variations associated with different species using a vector developed by others (Misslitz et al, MBP107:251, 2000). Transfectants of *Leishmania braziliensis*, *Leishmania donovani* and *Leishmania major* were generated and FACS analysis revealed differences of GFP expression levels between species and individual clones analyzed. Furthermore, we conducted transfection experiments with the same backbone without NEO, and alternative approaches of selection based on fluorescence failed to rescue GFP positive clones. We are currently testing alternative transfection and rescuing approaches. In parallel, we are constructing other vectors to use the hit-and-run strategy (LeBowitz et al, MBP51:321, 1992 and Denise et al, FEMS Microbiol Lett. 235:89, 2004). These under-construction vectors bear either GFP or Alkaline Phosphatase as reporter genes, a positive (HYG - hygromycin phosphotransferase) and a negative selection markers (HSV*tk* - Herpes simplex virus thymidine kinase). Financial support: UNICEF-UNDP/World Bank/Special Programme for Research and Training in Tropical Disease and FAPESP

BM002 - FUNCTIONAL GENOMIC ANALYSIS OF *Trypanosoma cruzi* SUBMITTED TO DIFFERENT TYPES OF STRESS

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An essential event during the in vitro metacyclogenesis of *T. cruzi* is the nutritional stress mimicking the conditions faced by the parasites at the mid-gut of the insect host. Important changes in the gene expression program occur during metacyclogenesis and it is likely that they might play an important role in the physiological and morphological changes observed during parasite differentiation. However, little information is available concerning the effect of different stress conditions on metacyclogenesis triggering and also, about the genes involved in the regulation of these responses. We performed a comparative analysis of different stress conditions (temperature, pH and nutritional stress) in triggering metacyclogenesis in vitro. To compare the biological properties of metacyclic trypomastigotes originated from each stress condition, infection assays were done on cultured Vero cells and Swiss mice. In addition, microarray analysis was used to investigate the expressed genes. Concomitantly, shotgun proteome LC-MS/MS analysis has been carried out in order to gain further insight into the relative abundance of *T. cruzi* proteins when different stress conditions were established. Quantitative PCR analysis (qPCR) has been performed and the results obtained were in agreement with the data obtained using microarrays. Financial support from CNPq (Prosul and Pronex), Fiocruz, NIH

BM003 - HIGH-THROUGHPUT PROTEOME ANALYSIS OF *Trypanosoma cruzi* METACYCLOGENESIS

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The study of *T. cruzi* metacyclogenesis is of great interest since it comprises the morphogenetic transformation of a non-pathogenic form (epimastigote) to a pathogenic form (metacyclic trypomastigote). Metacyclogenesis can be mimicked in vitro by incubating epimastigotes in a chemically defined medium (TAU3AAG medium), rendering possible obtaining parasites at various time points of the differentiation process. The fact that most trypanosomatid mRNAs

result from processing of polycistronic transcripts suggests that their expression is regulated at the post-transcriptional level. Whole-organism, shotgun proteome LC-MS/MS analysis have been carried out in order to gain further insight into the relative abundance of proteins of *T. cruzi* during metacyclogenesis. The proteomic approach is an important tool for studying global protein expression and is being applied for obtaining protozoan parasite protein maps. In this work, proteomic maps of epimastigotes, metacyclic trypomastigotes and four intermediate forms during the parasite differentiation were performed to identify proteins and to understand the dynamics of protein synthesis during this differentiation process. Proteins were identified by searching the MS/MS spectra against our *T. cruzi* database and its reverse complement using Bioworks v3.2. Peptide and protein hits were scored and ranked using the probability-based scoring algorithm incorporated in Bioworks v3.2 and adjusted to a false positive rate of $< 1\%$. Only peptides identified as possessing fully tryptic termini with cross-correlation scores (Xcorr) greater than 1.9 for singly charged peptides, 2.3 for doubly charged peptides and 3.75 for triply charged peptides were used for peptide identification. In addition, the delta correlation scores were required to be greater than 0.1 and for increased stringency, proteins were accepted only if their probability scored was <0.00001. Therefore, approximately 800 proteins were identified after searching databases. These proteins have been grouped functionally in order to identify the proteins categories present during the cellular differentiation of *T. cruzi*.

BM004 - Proteomics analysis of the *Crithidia deanei* symbiotic bacteria

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Endosymbiosis is an interesting model for the study of the origins of symbiosys and cellular evolution. We are sequencing the endosymbiont genome of *C. deanei*, with around 700 genes. In addition we are conducting a proteome analysis using a LC-MS/MS approach (MudPIT), to provide a broader picture of its physiology and relationship with the trypanosomatidae host. Briefly, protein extracts purified from three different samples of isolated endosymbionts were digested with trypsin, submited to a LCQ Deca Plus and the resulting spectrum data was compared against three databases: endosymbiont, kinetoplastida and betaproteobacteria, using the Sequest algorithm. We identified 204 proteins (28.7% of total amount of endosymbiont predicted protein) in at least one experiment, with a p-value of 1e10-5;. Using no p-value threshold, 353 proteins (49.6%) were identified. The KEGG functional annotation of these 204 proteins is related mainly to aminoacid metabolism ($n=\tilde{3}0$), purine metabolism (n=15), aminoacyl-tRNA biosynthesis (n=12), glycolysis/gluconeogenesis (n=9), ribosome (n=8), oxidative phosphorilation (n=7), pyrimidine metabolism (n=7) and TCA cycle (n=6). We have also compared the protein spectrum data with kinetoplastid and betaproteobacteria database, in order to evaluate protein contamination of host origin and unpredicted genes due to the unfinished status of the genome sequencing. We were able to identify as much as 49 proteins using the same criteria as described above, mainly from kinetoplast and glycosome origin, reinforcing the quality of the endosymbiont purification. The betaproteobacteria comparison identified 66 proteins, all of them present in the endosymbiont database, suggesting that the actual genome coverage comprises most, if not all, endosymbiont genes.

BM005 - EGene 2 – A platform for automated sequence processing and annotation – using *Eimeria* spp. as a model

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EGene is an open-source, generic and modular system developed by our group (Durham *et al.*, *Bioinformatics* 21: 2182-2183, 2005) to construct pipelines for sequence pre-processing. The initially developed components perform tasks such as base calling and quality evaluation, vector masking, end-trimming, quality filtering, contaminant filtering and DNA assembly. The system is highly portable and generic, and has been applied for many different large-scale DNA sequencing projects, including some protozoan parasites. Here we report the enrichment of the data model and the development of a new set of components destined for automated annotation. We developed in total 21 new components, comprising an ORF finder and translator, modules for seven gene prediction programs (ESTScan, GENSCAN, GlimmerM, GlimmerHMM, Phat, SNAP and Twinscan), tandem repeats finders (TRF, String and mreps), tRNA gene prediction (tRNAscan-SE), cDNAs mapping onto genomic sequences (SIM4 and exonerate), similarity searching (BLAST), protein motif finding (HMMER/Pfam, RPS-BLAST and InterProScan), transmembrane domain and signal peptide finding (SignalP, TMHMM and Phobius). Finally, we have developed components that automatically generate feature annotation exchange files in several formats such as Feature Table (as defined by DDBJ/EMBL/NCBI), GFF3 (Generic Feature Format) and an extended Feature Table format which contains Artemis custom tags. These files allow the user to perform manual inspection and curation using standard annotation viewers and editors, or

can be used for sequence submission to public databases. EGene2 has been used and validated with clusters of 45,000 ORESTES cDNA reads of three distinct *Eimeria* species. The results presented here indicate that the annotation process is quick and generates a reliable annotation. CoEd, a visual tool to facilitate pipeline construction and documentation is also provided in the package. We foresee that EGene2 will be very useful for large-scale sequencing and annotation projects, including those of parasitic organisms.

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BM006 - FUNCTIONAL CARACTERIZATION OF DIFFERENTIAL EXPRESSED PROTEIN DURING *T. CRUZI* METACYCLOGENESIS.

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Microarray analysis of *T. cruzi* metacyclogenesis resulted in the identification of approximately 1,000 differentially expressed genes and several of them encode hypothetical proteins. Aiming to produce functional information about these genes, an entry vector bank based on the Gateway platform was constructed allowing the functional characterization of several genes of interest through the use of specifically developed destination vectors (*T. cruzi*DEG-ORFeome). Up till now, 400 genes were inserted into entry vectors (pDONR) and 200 in an *E. coli* expression system vector (pDEST17). As a first step towards the functional characterization of the hypothetical genes, 27 clones were selected based on their expression pattern and from these 18 recombinant proteins were obtained. After inoculation in mice, 17 antisera were raised against the recombinant proteins, and 11 recognized the *T. cruzi* native protein as well. Western Blot analysis with protein extracts from epimastigotes, epimastigotes at 24 hours of differentiation, metacyclic trypomastigotes and amastigotes showed that the protein and mRNA differential expression data corroborated each other. Sub-cellular localization was obtained for eight proteins in epimastigotes, allowing the identification of robust patterns of functional modulation, as for instance the intracellular vesicle transport pathway. This work represents the first systematic evaluation on the influence of transcriptome modulation on protein levels, an important question due to the importance of the post-transcriptional regulation on gene expression of this parasite. Financial support: CNPq, CAPES,NIH, Fundação Araucária and FIOCRUZ

BM007 - Functional genomic characterization of Trypanosoma cruzi life cycle

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A functional genomic analysis was performed to study the main four stages of Trypanosoma cruzi life cycle: epimastigotes (Epi), metacyclic trypomastigotes (Meta), amastigotes (Ama), and cell culture trypomastigotes (Trypo), using a whole genome T. cruzi microarray with 10720 probes. Polysomal RNA was obtained from 4 different biological samples of each form and was amplified in vitro. Eight hybridizations were performed among the four stages in each group, totalizing 32 hybridizations. Differentially expressed genes (DEGs) were detected by the significance analysis of microarray (SAM). Preliminary analysis of hybridizations of one group, considering a differential expression level of 2-fold, revealed that, comparing Epi with Meta, 770 DEGs were detected with a FDR of 10%, being 401 up regulated in Meta and 369 in Epi. The comparison between Epi and Ama showed 1355 DEGs (FDR 5%), 732 up regulated in Epi and 623 in Ama. The comparison between Trypo and Meta showed 430 DEGs (FDR 10%), 204 up regulated in Meta and 226 in Trypo. Comparing Epi with Meta and Epi with Ama, ~48% of DEGs corresponds to hypothetical proteins. Comparing Trypo with Meta, ~64% corresponds to hypothetical proteins. Ten per cent of DEGs up regulated in Meta comparing to Epi are probes corresponding to the major surface proteins (mucins, mucin-associated surface protein - MASP and trans-sialidase), and 9% of DEGs up regulated in Ama comparing to Epi corresponds to these some genes. The analysis of the remaining 3 experiments is in process. Financial support from CNPq, Pronex (CNPq-Fundação Araucária), Fiocruz, NIH.

BM008 - Sequence and evolutionary analysis of selected genes of the *Trypanosoma cruzi* Structural Proteomics Project

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We have initiated a *Trypanosoma cruzi* Structural Proteomics project aiming to produce three dimensional structures of differentially expressed genes, many of them encoding hypothetical proteins. The main goals of this project are to produce folding information aiming to rationalize drug discovery and to attribute function by structure comparison. We have initially selected 192 proteins, whose coding regions were cloned in the Gateway platform (Invitrogen), based on *T.cruzi* Dm28c genome. There are two main lineages in *T. cruzi*, represented by the strain used in this project and CL Brener, the genome project reference strain. In order to provide a correct primary amino acid sequence for all proteins in study, a requirement for 3D structure prediction, the main objective of this work was to determine the sequence of all selected genes in Dm28c. These genes are representative of several distinct biological pathways and provide an extensive dataset suitable for evolutionary studies on lineage separation. Hence we have conducted an analysis aiming to identify distinct patterns among *T. cruzi* lineages and to correlate these evolutionary patterns with gene function. We have sequenced the complete CDS for all 192 genes, aligned the Dm28c sequences with the orthologs of CL Brener, *T. brucei, Leishmania major, L. infantum, L. braziliensis* and *L. donovani* using the ClustalW software. We are presently analyzing multiple sequence alignment with several evolutionary models and methods, such as maximum likelihood and dn/ds ratios.

BM009 - Proteomic identification of molecules involved on establishment and control of cutaneous Leishmaniasis

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In our laboratoratory, it was demonstrated that CBA/J mice are resistant to *Leishmania major* and susceptible to *Leishmania amazonensis* infection. Also, CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*, suggesting an important role for macrophages on the determination of *Leishmania* infection outcome. Next, using short oligonucleotide microarray analyses we showed that *L. amazonensis*- and *L. major*-infected macrophages express different sets of genes related to early cell-*Leishmania* interaction and to the immune-inflammatory response. Microarray technology are useful to identify molecules at transcriptional level, however it is necessary to associate expressed genes to identification of theirs respective proteins. Besides that, several important modulation occuring during the infection are only seen at the proteome level. With a proteomic approach, we compared 6 and 24 hours of *L. major* and *L. amazonensis* infection on CBA/J macrophages. Proteins extracts were obtained to characterize peptides by LC-MS/MS in a MudPIT approach. Preliminary qualitative analyses demonstrated that only 18 macrophages proteins are exclusive found during *L. amazonensis* infection. However different 50 proteins are expressed on macrophages infected with *L. major*. On *L. amazonensis*-infected macrophages, proteins are related to apoptosis and to metabolic stress, while macrophages infected by *L. major* express a large set of proteins related to phagolysosome formation and to MHC expression and antigen presentation. Future quantitative proteomic analyses will be performed. These data point to a pivotal role for the parasite on determining the subsequent immune response and on the course of infection. The identification of protective or susceptible markers of infection could help design new therapeutic or prophylactic drugs against leishmaniasis.
Financial support: CNPq - 478804/2004-0 and FAPESB - ET 64/2004.

BM010 - Molecular and functional characterization of the acyl-CoA dependent ceramide synthase from *Trypanosoma cruzi.*

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The biologically active ceramide is a simplified sphingolipid synthesized by the condensation of a fatty acyl-CoA with a sphingoid base, a reaction catalyzed by the acyl CoA-dependent ceramide synthase (CerS). Three genes, *LAG1*, *LAC1* and *LIP1*, have been shown to be required for CerS activity in *Saccharomyces cerevisiae*. Deletion of either *LAG1*

or *LAC1* does not seems to cause growth defects; however, the double δ *lag1* δ *lac1* mutant is severely crippled and displays cell wall defects in the genetic background of W303 cells, and the same double mutation is lethal in the background of YPK9 cells. Here we demonstrated that lethality of YPK9 δ *lag1* δ *lac1* can be overcome by expression of a CerS gene ortholog from *T. cruzi* (*TcCerS*), a gene that shows 22% identity to yeast *LAG1*. No orthologs of Lip1p, which forms a heteromeric complex with Lag1p and Lac1p, were found in animals, plants or *T. cruzi*. Southern blot analysis suggests that *TcCerS* has more than one copy in the genome. Northern blot analysis shows two mRNA species of 1.8 Kb and a 3.0 Kb in epimastigote cells. In addition, a *TcCerS* 5' *trans*-splicing acceptor site was mapped by RT-PCR. The ceramide synthase activity from *T. cruzi* was detected in crude microsomal membranes and it exhibited a preference for fatty acyl-CoA rather than free fatty acid as donor substrate. In addition, the activity was blocked by Fumonisin B1, a known inhibitor of this activity in fungi, plants and mammals. Using the cell-free assay system described here the CerS activity was characterized using different chain length fatty acyl-CoAs, protein concentrations, pH, cofactor and detergents. The results suggest that TcCerS is an authentic acyl-CoA dependent ceramide synthase from *T. cruzi*. Support: CAPES, CNPq, FAPERJ, IFS.

BM011 - Molecular Identification of Casein Kinase 2 (CK2): Amplification, Cloning and Heterologous Expression.

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The leishmaniasis is caused by parasites of the genus *Leishmania*. These parasites are transmitted by the bite of phlebotomine sandflies and infect cells of mononuclear phagocyte lineage of their vertebrate hosts, promoting a spectrum of diseases which can be cutaneous, mucosal or visceral. Several molecular mechanisms have been described in trypanosomatids life cycle in the last few years, which ensure the parasite infection despite the host efforts. Protein phosphorylation-dephosphorylation is one of the most powerful mechanisms able to control cell activities. Studies have demonstrated the role protein phosphorylation may play in the coordination of parasite defenses. Therefore, the studies of enzymes related to phosphorylation and dephosphorylation of proteins present on external surface of these parasites have fundamental importance. Several ecto-enzymes have been described in trypanosomatids, including ecto-phosphatases and ecto-kinases. Casein kinase 2 (CK2) activities have been described both on cell surface and as secreted enzymes of *L. major*, *L. amazonensis*, *L. tropica* and *L. braziliensis*. These enzymes seem to be involved with cell growth, differentiation and infectivity. In this study we have cloned and sequenced the CK2 gene of *L. braziliensis* infective strain. The amplicon with about 1,116 bp, was inserted in pBad-Thio-TOPO vector and cloned in *Escherichia coli* TOP10 strain. The CK2 sequence from *L. braziliensis*, have high homology with other CK2 genes. After induction with arabinose 0,02 % (w/v), the expression of a protein with approximately 55 kDa was obtained. The protein was purified by Cu^{2+}-IMAC column on FPLC and after that utilized for susceptible BALB/c mice immunization to obtain polyclonal antibodies. These results will aid the development of genetic modified parasite, that can be used to new vaccinations protocols. Supported by: Faperj, CNPq

BM012 - The *Leishmania major HTBF* gene belongs to the YIP family of proteins.

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The *HTBF* gene from *Leishmania major* is a member of the YIP family of proteins, which participate in the targeting and fusion of vesicles as recruiters of Ypt-Rab proteins. Some YIP proteins are characterized by a DLYGP motif and four transmembrane domains. In *Saccharomyces cerevisiae* Yip1 is part of a trimeric complex further formed by Yos1p and Yif1p. The formation of the complex is necessary for its function. Human homologues for these proteins were identified and a phylogenetic comparison places HTBF as close to the yeast Yip1 as the human protein (HsYip1). Assuming these proteins form a conserved Rab interacting protein family, we tested the hypothesis that HTBF from the *L. major* is included in this family and thus is involved in the formation and/or redirection of vesicles, improving mechanisms of drug extrusion or membrane repair. HTBF has already been shown to mediate terbinafine resistance. The Rab protein LmYPT1, which was shown to localize to the Golgi complex, negatively interferes with terbinafine resistance in overexpressing cell lines. These results suggested that HTBF participates in resistance through a mechanism that involves vesicle trafficking. The human homologues HsYip1A and HsYif1 were characterized and shown to localize to the Golgi complex. Similarly, the parasite HTBF protein was found in the Golgi complex when expressed in mammalian cells. Within the parasite, the correct localization of an HTBF::GFP fusion was dependent on the transmembrane domains across the protein. When these domains were ablated the fusion protein was found diffuse in the cytoplasm. On the other hand, the fusion containing an intact HTBF was localized to the Golgi complex and the flagellar pocket. Supported by FAPESP, CNPq and CAPES

BM013 - Molecular characterization of ribonucleoproteic antigens from *Trypanosoma cruzi*

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Ribonucleoproteins and proteins containing repetitive amino acid sequences are among the most prominent antigens expressed in *Trypanosoma cruzi* amastigotes. We characterized two proteins carrying similar repetitive amino acid motifs, which share homology to a RNA binding protein or eukaryotic L7a ribosomal protein, named TcRBP28 and TcRpL7a respectively. Western blots using recombinant proteins, including truncated forms of TcRpL7a containing only the repetitive and the non-repetitive domains, showed that the humoral chagasic response is mainly directed to the TcRpL7a repetitive region. ELISA assays showed that 73% of chagasic patients sera (n=59) reacted with the TcRpL7a repetitive domain, 71% reacted with the TcRBP28 antigen and 80% of 30 sera reacted with 1:1 mixture of both antigens. Both proteins were localized in parasites transiently transfected with the cDNAs cloned in fusion with GFP. Whereas GFP::TcRBP28 was found dispersed in the cytoplasm, GFP::TcRpL7a accumulates in the nucleus where the biogenesis of the ribosome takes place. Antibodies raised against both recombinant antigens identified equivalent amounts of the native proteins in cell extracts from all three stages of the parasite life cycle. Western blot analyses of subcellular fractions also indicated that TcRBP28 is present in the cytoplasm and TcRpL7a, in spite of the presence of the large repetitive amino acid domain, co-fractionates with polysomes. Current experiments are being carried out to evaluate whether these antigens may be used as vaccine candidates and the influence of the repetitive amino acids in the capacity of TcRpL7a to generate a protective immune response during the *T. cruzi* infection. SUPPORT: CNPq, FAPEMIG and *Howard Hughes Medical Institute.*

BM014 - MOLECULAR CHARACTERIZATION OF CHAPERONE ClpA/HSP100 OF TRYPANOSOMA CRUZI

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Members of the HSP100/ClpA family of chaperones promote disaggregations of proteins complexes and protein degradation. Being a heat-inducible protein, HSP100 is a good model for studying gene regulation. We aim to study the ClpA/HSP100 gene structure, expression and regulation in Trypanosoma cruzi. The predicted aminoacid sequence of HSP100 of Trypanosoma brucei was used to search for orthologous sequences in the draft genome of T. cruzi clone CL Brener. A coding region of 1810pb was identified, and an internal segment was selected for PCR-based amplification and used as a probe in genomic southern blots. The hybridization pattern is compatible with HSP100 genes being present in low copy number in the genome. Protein similarity analysis and inspection of the relevant region of the draft genomic sequence showed that the coding region obtained is missing a large portion at the 3‘ end. We found most of the missing sequence which, due to an assembly error, is located elsewhere in T. cruzi draft genome sequence. This sequence, with approximately 300 aminoacids, shows the same degree of similarity with T. brucei and Leishmania sp. orthologs as rest of the sequence. We are currently performing experiments to obtain the remaining aminoacid sequence and complete the coding region. We are also performing northern blot analysis to determine HSP100 mRNA levels and half-life in normal and elevated temperatures. The complete coding region will be cloned into expression vectors and the recombinant protein used to generate HSP100-specific antibodies in order to characterize the subcellular localization of the protein. Key-words: HSP100; ClpA/HSP100; Chaperones. Supported by CNPq and FAPERJ.

BM015 - Characterization of a family of cytoskeleton-associated calpain-like proteins in *Trypanosoma cruzi*

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In a previous work, Galetovic *et al.* 2006 (XXII Annual Meeting SBPz, p.104) have described a subset of *T. cruzi* calpain-like cysteine peptidases associated with 68-amino acids (aa) repeats which are tandemly arranged in the central domain of the molecule. The 68-aa repeats were previously found in an immunodominant antigen which is involved in the attachment of the flagellum to the cell body of the parasite. For this reason we called this protein H49/calpain-like protein. Here we present further characterization of the structure and cellular location of H49/calpain-like protein. To study the occurrence and subcellular distribution of this protein in epimastigotes, we have cloned and overexpressed different domains of the calpain and the 68-aa repeats in bacteria, purified the proteins, and employed them to immunize rabbits and mice. The affinity purified polyclonal antibodies were used as probes to

study the organization and subcellular distribution of 68-aa repeats and calpain in epimastigotes. Antibodies raised against the calpain domains and 68-aa repeats reacted, by immunoblot analysis, with a >240 kDa in the whole epimastigote extracts. Anti-calpain antibodies reacted with additional bands of ~ 180 and ~ 60 kDa which may correspond to the calpain proteins without 68-aa repeats. Immunofluorescence analysis showed that H49/calpain-like protein is only localized in the region of adhesion between the cell body and flagellum. Calpain was present not only in the flagella, but it was also localized in the cytoplasm. Further, the cytoskeleton-associated H49/calpain-like was colocalized with markers of the flagellar attachment zone. We describe here a novel family of cytoskeleton-associated proteins in *T. cruzi*. These proteins are characterized by their similarity to the catalytic region of calpain, and also by the presence of tandemly arranged amino acid repeats.

Support: Beca Presidente de la República-Chile, FAPESP, CNPq, CAPES.

BM016 - Identification of the gene encoding an inorganic pyrophosphatase in *Trypanosoma rangeli*

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Inorganic pyrophosphatases (IPP) catalyzes hydrolysis of the phosphoanhydride bound in inorganic pyrophosphate (PPi), a compound produced in various reversible nucleoside 5 triphosphate-dependent reactions. These proteins play an important role in distinct cell metabolisms such as DNA, RNA and protein synthesis. In this work, the IPP gene encoding a putative soluble inorganic pyrophosphatase was identified in a *Trypanosoma rangeli* genomic library. The full-length coding sequence was obtained from mRNA by PCR amplification using primers directed to the conserved intergenic regions of the gene exon, cloned in pGEM-T-easy vectors and sequenced. After quality analysis and clustering, the resultant contig was analyzed by Blastx to confirm the sequence identity. The open reading frame is of 819bp, encoding a putative protein of 273 aminoacids, with predict molecular mass of 30,51kDa. In a recent study, sequence analysis by alignment with homologous proteins of this family indicates that active-sites residues are highly conserved, even though the overall sequence similarity is quite reduced. Comparisons with orthologous of other Kinetoplastida protozoan species with *T. rangeli* IPP gene showed high inter-specific similarity, mainly with *T. cruzi* (93%), *T. brucei* (72%) and *Leishmania* sp. (70%). The high degree of conservation between *T. rangeli* and *T. cruzi* IPP genes reinforce their genetic, biological and biochemistry proximity. In addition, analysis of the genomic organization showed a Peter Pan-like protein located at the 3 end of the *T. rangeli* IPP gene. Comparative sequence analysis revealed a strong conservation of these gene order in all kinetoplastid species which have this region sequenced, confirming the high syntheny of this group. Phylogenetics analysis carried out with the obtained sequence alignment of kinetoplastid species and mammalian genes showed a strong topology, allowing a clear inter-specific differentiation. Supported by CNPq, Fiocruz and UFSC.

BM017 - Studies on the type II DNA topoisomerases in *Trypanosoma rangeli*

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Topoisomerases are enzymes involved in cellular functions directed to topological manipulation of DNA strands. Type II topoisomerases (topoII) catalyze changes by introducing transient double-stranded cuts. Along with their role in nuclear DNA metabolism, these enzymes are also involved in replication and organization of the kinetoplast DNA in trypanosomatids. Since DNA topoisomerases are inhibited by antimicrobial and antitumoral agents, they are promising tagets for drug therapy for Chagas disease and leishmaniasis. We have cloned and sequenced two genes encoding topoII from *T. rangeli* (TrTOP2mt e TrTOP2α), both showing high similarity with topoII genes of other trypanosomatid species. Selected fragments were cloned in pGEXB and expressed in E. coli, including the carboxy domain of TrTOP2mt and the central portion of TrTOP2α. These regions were especifically choosen due their sequence not present high conservation among these type II DNA topoisomerases. The recombinant proteins fused with GST were purified through gluthathione sepharose columns and used for antiserum production in mice aiming to address the expression levels and cytolocalization of topoII in *T. rangeli*. Poly-clonal antisera against TrTOP2mt and TrTOP2α fused fragments (38,1kDa and 48,7kDa, respectively) were obtained and Western blot analysis of T. rangeli protein extracts with these antisera revealed polypeptides of 138 kDa and 165kDa, compatible with the expected size based on the deduced amino acid sequences of *T. rangeli* topo II genes. Supported by CNPq, Fiocruz and UFSC.

BM018 - Cloning and characterization of a *Trypanosoma rangeli* galactofuranosyl transferase gene.

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The cell surface glycoconjugates of trypanosomatid parasites are intimately involved in parasite survival, infectivity and virulence for their insect vectors and mammalian hosts. The galactofuranose is a glycoside usually absent in mammalian cells and *Trypanosoma brucei*, but found in glycoconjugates of several pathogenic species such as *T. cruzi* and *Leishmania sp.*, for which genes coding to β-galactofuranosyl transferase (β-gt) were described. During the *T. rangeli* Transcriptome Project carried out by our group, cDNA fragments with high similarity with the *T. cruzi* β-gt gene were clusterized in a 552pb sequence, representing the 3' end termini of the gene. Based on these findings, the aim of this work was to amplify and characterize the *T. rangeli* galactofuranosyl transferase gene. For that, *T. rangeli* SC-58 strain cDNA was used for PCR amplification using primers designed to the conserved gene region and to the spliced-leader. The obtained fragments of 914bp were cloned into pGEM-T easy vectors and sequenced. After quality analysis (Phred >=20), a new 1,248bp contig was obtained resulting in an ORF of 1,142pb, which represents $\tilde{8}7\%$ of the predicted gene to *T. cruzi* and *Leishmania sp.* Inter-specific comparisons with other Kinetoplastid species revealed 65% identity with *T. cruzi* and 34% with *Leishmania sp.* The search for conserved domains showed a protein with 380aa covering the "Transferase Galactofuranosyl transferase LPG1-like domain"(aa 54-174) and the "Endoplasmic Beta-Galactofuranosyl transferase Reticulum Signal-Anchor Transmembrane domain"(aa 214-378). These results describe, for the first time, a galactofuranosyl transferase gene in T. rangeli. Additional studies are in progress to determine the complete gene sequence, the copy number, the expression level and to determine the intra/inter-specific variability. Supported by CNPq and UFSC.

BM019 - CLONING OF DNA SEQUENCES THAT CODIFY HEMOLYSINS IN LEISHMANIA MAJOR

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Previous works from our group had shown that Leishmania amazonensis has a pore forming protein (PFP), named leishporin, that lyses erythrocytes and damages macrophages. L. major also exhibit a hemolytic activity, although we have not yet characterized a PFP in this species. L. amazonensis leishporin has already been studied in a number of aspects. However, its molecular identity is still unknown. Since the genome of the L. major is now completely sequenced, we started a search for hemolysins in this species, in attempt to identify putative PFPs. We found four sequences that were annotated as possible hemolysins type III. These sequences showed a significant homology to other deposited PFPs sequences and, moreover, presented the same Pfam (PF03006) that characterizes this gene family. The information deposited in the database corresponds to the complete codifying sequences of these genes. Therefore, using specific primers, we amplified by PCR from the genomic DNA of L.major and cloned one of these genes (LmjF36.5520) in TOPO2.1. DNA sequence analysis revealed that the majority of clones in which the sequence was inserted in the correct direction showed nucleotide substitutions or deletions. The clones encoding the complete fragment was subcloned into a vector with regulated expression pGEX 4T1 plasmid (GE Healthcare). Bacterial protein extracts was assayed by its hemolitic activity, however,this activity was not observed. Two hypotesis were raised to explain these results: the protein is either not being expressed at detectable levels or is toxic to the bacteria. We are currently working in the cloning of the sequence LmjF36.5520 in the vector pBAD/gIII (Invitrogen), which allows a strong regulation of the expression and the secretion of the recombinant protein in the periplasmatic space. Financial support:WHO; FAPEMIG; CNPq.

BM020 - Comparative intra-specific analysis of the *Trypanosoma rangeli* Histone H2A intergenic region

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Trypanosoma rangeli is a serious concern for the epidemiology and diagnosis of Chagas disease in spite of its lack of pathogenecity to man. Mostly of the research on *T. rangeli* has been focused on the genotypic characterization of this parasite using DNA fingerprinting, isoenzymes, RAPD profiles and PCR variants, such as LSSP-PCR, which can distinguish KP1(+) and KP1(-) subpopulations based on the intergenic region of histone H2A gene. In this work we have sequenced the H2A gene intergenic region from distinct *T. rangeli* strains aiming to assess the intra-specific sequence variability. After quality analysis and alignment, the sequences were submitted to bootstrapped maximum parsimony methods using MEGA software. PCR amplification of the H2A intergenic region from 17 *T. rangeli* strains revealed a discrete size polymorphism, varying from 402bp for strains 5048, Tre and C-23 to 420bp in strains H9 and Ma-

cias. Detailed comparative analysis showed the existence of several single nucleotide polymorphisms and two regions of major variability among the studied strains, being major responsible for the observed size polymorphism. The first, located between positions 194 and 217, contains a variable number of timines (6 to 12) and guanines (2 to 15) for each strain. The second, between positions 304 and 319, is characterized by variable number of timines (9 to 19). These regions appears to be related to KP1(+) and KP1(-) groups and were not observed in any other kinetoplastid species. Among *T. rangeli* KP1(-) strains insertions at positions 33, 193, 206, 207, 306-315 and substitutions at positions 159 and 370 permitted the distinction of Southern Brazil and Colombian strains. The same conclusions were obtained through phylogenetic analysis, which allowed inter-specific distinction (*T. rangeli/T. cruzi*), but intra-specific differentiation does not showed strong topological support. Supported by COLCIENCIAS, CNPQ, PUJ and UFSC.

BM021 - THE PHOSPHOGLUCOMUTASE (PGM) OF *Trypanosoma cruzi*: MAPPING THE IMPORT MOTIFS RESPONSIBLE FOR THE INTRACELLULAR SORTING TO GLYCOSOMES.

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Phosphoglucomutase (PGM) catalyses the interconversion of Glucose-6-phosphate into Glucose-1-P, an intermediate required for the synthesis of UDP-Galactopyranose. Since *T. cruzi* is incapable of transporting Gal from the extracellular milieu, PGM is thought to be essential for the synthesis of Gal-containing glycoconjugates. In trypanosomatids, enzymes that participate in sugar metabolism are confined to peroxisome-like organelles called glycosomes. Although in most eukaryotes PGM is found exclusively in the cytoplasm, we observed that it is partially localised in *T. cruzi* glysosomes. Glycosomal location was further confirmed by epimastigote sub-cellular fractionation in sucrose gradients, which revealed the presence of PGM in fractions enriched in glycosomes, but also in the cytoplasm. Protein targeting to glycosomes is usually mediated by peroxisome-import motifs comprising tri-peptidyl PTS1-type motifs, located at the C-terminal extremity of the protein, or degenerated nona-peptidyl PTS2-type motifs at the N-terminus. However, no classical PTS1 or PTS2 motifs are present in the *T. cruzi* PGM sequence. Nonetheless, three distinct tri-peptides containing PTS1-type motifs: SSL, HHL and PNA, are present dispersed throughout PGM. In order to investigate if internal PTS motifs of PGM govern protein targeting to glysosomes, we generated constructs containing the green fluorescent protein (GFP) fused at the 3' end of a series of truncated *PGM* gene fragments, which were transfected into epimastigotes. The expression levels of GFP-tagged proteins in transfected parasites were assessed by Western Blot using anti-GFP and anti-PGM antibodies. Immunofluorescence revealed punctate vesicular distribution resembling glycosomal location of fusion proteins bearing full length PGM or PGM-truncated at the C-terminal 40 aminoacid residues. However, the GFP-fusion containing PGM truncated at the last 280 residues and lacking the PNA motif was distributed throughout the cytoplasm, similarly to parasites expressing control GFP. Our results indicate that internal motifs present between residues 380-560 of PGM mediate enzyme targeting to *T. cruzi* glycosomes. Support: CNPQ, FAPERJ.

BM022 - Characterization of the gene encoding the dihydrolipoamide dehydrogenase (LipDH) enzyme in *Trypanosoma cruzi* strains susceptible and resistant to benznidazole

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Dihydrolipoamide dehydrogenase (LipDH) is a FAD-dependent enzyme which catalyses the reversible conversion of lipoamide, NADH and H^+ into dihydrolipoamide and NAD^+. This enzyme in *T. cruzi* (TcLipDH) has an important role in the parasite's redox metabolism and presents an identity of less than 53% to the human LipDH, which makes it a potential target for Chagas' disease chemotherapy. In an attempt to understand the mechanism of drug-resistance in *T. cruzi*, Murta *et al.* (2002) used the Representation of Differential Expression and the Differential Display methodologies to select genes differentially expressed in *T. cruzi* strains susceptible and resistant to benznidazole (BZ). Both techniques selected the *TcLipDH* gene which was over-expressed in an *in vitro*-induced BZ-resistant *T. cruzi* population (17LER) when compared to its BZ-susceptible counterpart (17WTS). In the present study, the level of mRNA expression, amplification, chromosomal location and level of protein expression of the *TcLipDH* gene have been investigated in 10 *T. cruzi* populations susceptible and resistant to BZ. Northern blot analysis probed with the *TcLipDH* gene showed a 2.3 Kb transcript for all *T. cruzi* samples. This transcript was more expressed in 17LER population when compared to its susceptible pair. Real time RT-PCR assays confirmed this data. The levels of *TcLipDH* mRNA were similar among the other *T. cruzi* samples analyzed. No amplification of *TcLipDH* gene was observed in the parasite's genome. Western blot analysis showed that a 50 KDa protein encoded by *TcLipDH* was present in all *T. cruzi* samples. The intensity of this polypeptide from the 17LER population was 2-fold lower than that of the 17WTS. The *TcLipDH* gene is located on four chromosomal bands that vary from of 1.2 to 2.2 Mb. Further studies are underway to investigate

the involvement of the TcLipDH with drug-resistance phenotype in *T. cruzi.* Supported by: CNPq, FAPEMIG, Fundep and PAPES3/Fiocruz.

BM023 - FUNCTIONAL CHARACTERIZATION OF A LEUCINE RICH PROTEIN IN *LEISHMANIA* SPECIES

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Leucine-rich repeats (LRRs) are sequence motifs present in a number of proteins with diverse functions that provide a versatile structural framework for the formation of protein-protein interactions (Curr Opin Struct Biol. 11(6):725-32, 2001). The La17LRR gene, identified initially in the *L. amazonensis* genome (FEMS Microbiol Lett. 238(1):213-9, 2004), encodes a protein with 6 LRRs in its central region. The initial characterization of the La17LRR gene of *L. amazonensis* allowed the identification of a transcript with abundant expression in the amastigote form of the parasite. The approximately 70 kDa La17LRR protein was shown to be present in total extracts of *L. amazonensis* promastigotes but was not detected in extracts obtained from lesion-derived amastigotes (Franco et al., XXII Meeting of the Brazilian Society for Protozoology, Caxambu MG, 2005). With a polyclonal antibody obtained against the recombinant protein, we now show by immunohystochemistry and electronic microscopy that the La17LRR protein is secreted to the cytoplasm of *L. amazonensis*-infected macrophages. These results suggest that the La17LRR protein can interact with macrophage proteins and modulate the cell‘s immune response. We have also characterized the *L. major*-L17LRR orthologue (Lm17LRR), which encodes a protein with 94 % similarity with LA17LRR, and showed that it does not have the same regulation pattern observed in *L. amazonensis.* Northern blots with total RNA indicated that, in *L. major*, the transcript is more abundant in log phase promastigotes and barely expressed in amastigotes. Constructs for the overexpression of the Lm17LRR gene have been obtained and transfected into *L. amazonensis* and *L. major.* The phenotype of these mutants will be analyzed in vitro, through macrophage infections, and in vivo, by experimental infection of susceptible and resistant mice, allowing the investigation of a possible role of this protein in the parasite‘s virulence and interaction with the host cell. Financial support: CNPq and FAPESP.

BM024 - Characterization of ubiquitination pathway enzymes in *Trypanosoma cruzi* and their expression during the metacyclogenesis

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Metacyclogenesis is a process driven mainly by post-transcriptional changes in gene expression. Ubiquitin-mediated proteolysis should play a role in the remarkable adaptative protein turnover which leads to new morphology, physiology and behavioral features. At least three enzymes are required for ubiquitination: Ub-activating (E1), Ub-conjugating (E2) and Ub-ligase (E3). The E3 is generally considered to be most important in controlling target specificity because it is responsible for recruiting the target protein and positioning it for optimal transfer of the ubiquitin moiety from the E2. To gain further insight into the mechanisms involved in *T. cruzi* ubiquitination and to investigate its possible role during metacyclogenesis, three E1, six E2 and three E3 were selected for cloning and characterization. Metacyclogenesis *in vitro* was used as experimental model to investigate the expression of these enzymes. Selected genes were amplified, cloned and expressed. In addition new bioinformatics approaches were conducted to investigate the trustworthiness of the genome annotation. Using this analysis the number of proteins with characteristic domains of ubiquitination enzymes found in *T. cruzi* genome was: 4 UBACT (E1), 14 Uq-con (E2), 10 HECT, 1 U-box and 35 zn-C3HC4 (E3). The recombinant proteins were used to produce polyclonal antibodies for the characterization of the expression and cell localization of these enzymes during metacyclogenesis. Also, cellular mRNA levels of the same genes were analyzed by microarray and real-time PCR quantification assays. The expression analysis disclosed three trends: increased (one E2, two E3), decreased (one E1, one E2) and non-changed (one E1, three E2, one E3) mRNA levels during metacyclogenesis. One E3 seems to localize in the reservosome and another in the cytostome indicating a variety of functions for these enzymes in the parasite. Financial support: CNPq and Fundação Araucária

BM025 - Characterization of the ubiquitin-proteasome pathway during *Trypanosoma cruzi* metacyclogenesis

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The differentiation of *T. cruzi* epimastigotes into metacyclic trypomastigotes involves the transformation of a replicating, non-infectious form into a non-replicating, infectious stage. During metacyclogenesis the ubiquitin-mediated proteolysis should play a role in the remarkable protein turnover associated with the modifications on its morphology, physiology and behavior. The use of lactacystin, a specific irreversible inhibitor of the 20S proteasome, showed that the metacyclogenesis process is strongly repressed in more than 95%. Concomitant with this differentiation inhibition, important morphological changes were observed including alterations in the mitochondrion and nucleus. In order to identify proteins specifically degraded by the ubiqutin-proteasome pathway during the cellular differentiation of *T.cruzi*, 2D-MS and LC/MSMS proteomics approaches were used to compare parasite cultures treated with lactacystin and the control population. We are also performing the quantification of proteolysis during the metacyclogenesis to quantify the participation of ubiquitin-proteasome proteolysis in different time points of *T.cruzi* cellular differentiation. Financial support: CNPq and Fundação Aráucaria

BM026 - Characterization of a midgut-specific chitinase gene from *Lutzomyia longipalpis*, the main visceral leishmaniasis vector in Brazil

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Leishmaniasis are caused by *Leishmania* parasites, transmitted by the bite of infected sand-fly vectors. Current methods for fighting this disease are inefficient and the development of new techniques for vector control are necessary. Little is known about the physiology of digestion and about the sand-fly/leishmania interaction mechanisms in *Lutzomyia longipalpis*, the main visceral Leishmaniasis vector in Brazil. In our laboratory we are studying differentially expressed molecules after blood-feeding and infection by *Leishmania*. To identify these molecules, we have used EST sequencing of gut cDNA under various conditions, and applied DDRT-PCR to identify differentially expressed gut genes after blood-feeding or infection. One cDNA of interest identified by DDRT-PCR codifies a chitinase with high levels of transcription after 72 hours of blood ingestion that may have a role in peritrophic matrix degradation. A genomic clone containing the chitinase gene was isolated and partially sequenced, revealing three introns that interrupt the codifying region. A putative promoter was found in the flanking region of the gene and probably is involved in the expression of chitinase after a blood meal. The nucleotides starting at the transcription initiation site and continuing immediately downstream from the promoter identified in *L. longipalpis* are also present in the 5'UTR of a orthologous chitinase gene from *Phlebotomus papatasi*. Both enzymes are transcribed after blood feeding and are potentially responsible for peritrophic matrix degradation. It would be interesting to sequence the corresponding complete promoter sequence of *P. papatasi*, since this promoter may be part of a conserved system able to induce protein expression in the sand-fly midgut post-blood meal. During RT-PCR analyses, a non expected PCR product was found, which was cloned and sequenced revealing a new chitinase splicing form that seems to be expressed only in the larval stages of the sand-fly.

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BM027 - TcBDF2, a bromodomain factor, in *Trypanosoma cruzi*

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Little is known about chromatin structure and epigenetic regulation in trypanosomatids. *T. cruzi* chromatin is organized in nucleosomes and it condenses as fibbers during cell division. *T. cruzi* histones are among the most divergent known and their posttranslational modifications and their role in gene expression regulation, are poorly understanded. Bromodomain-containing proteins bind acetylated lysine residues and this motif is present in many transcriptional regulators, participating in chromatin structure remodelling and transcription control. Several chromatin remodelling factors and Histone Acetyl Transferase complexes contain at least one bromodomain motif. Here, proteins containing the bromodomain motif found in *T. cruzi* were studied. By using bioinformatic tools it was found three sequences that codify for bromodomain proteins (BDF1 to 3) and two sequences with far similitude to bromodomain (BDF4 and 5). One of them, TcBDF2, was detected by immunoblots (WB) in protein extracts of epimastigotes, tripomastigotes and amastigotes using specific antibodies raised against recombinant TcBDF2. Indirect immunofluorescence assays with this antibody revealed it was present only at

the nucleus, showing a granular staining pattern in all parasites forms. Nuclear localization was confirmed by WB using nuclear protein extracts. In parasites submitted to agents that produce DNA damage, only UV treatment increased TcBDF2 expression. Maximal expression of TcBDF2 was observed 18 hour after treatment, with decrease to normal levels at 48 hours. It is known that protein-complexes containing bromodomain factors are taking part or DNA repair mechanisms and recombination in eukaryotes. Probably these mechanisms are also present in this parasite. More studies are underway to confirm these results. Supported by FAPESP, CNPq (Prosul) and ANPCyT.

BM028 - CHARACTERIZATION OF THE Tcp28, THE INTERCALATED GENE OF THE RNA BINDING PROTEIN GENES (TCRRM) IN *TRYPANOSOMA CRUZI.*

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The function of Tcp28 is unknown and is found in *Trypanosoma cruzi* genome located at the TcRRM locus as a multicopy gene. Tcp28 is intercalated between genes of TcRRM1 and TcRRM2. The Tcp28 transcripts analyzed by Northern Blot assays accumulate in trypomastigotes when compared with other forms. These results suggest that Tcp28 may be regulated through different cell forms and may play a specific function in these cells. The control of gene expression in trypanosomatids is usually exerted posttranscriptionally. The objective of this work is to analyze the intergenic regions of the gene Tcp28 of *Trypanosoma cruzi* in order to find sequences that regulate the Tcp28 gene expression in different cell forms of *T. cruzi.* Total RNA from epimastigote forms extracted by the GuSCN/GUHCI were used to produce the cDNA using the 3'RACE kit with specific initiators in order to map the polyadenylation and trans-splicing sites in 3'UTR and 5'UTR region. The 3' UTR amplification was done with initiators homologue to the polyA tail and to the carboxyl-terminal region of Tcp28. The product of this amplification was cloned into plasmid, transformed into bacteria and sequenced.The same procedure was realized for the 5' UTR region using initiators homologue to the miniexon and to the N-terminal region of Tcp28. Results from sequencing of the 3'UTR region showed only one site of polyadenylation and one type of sequence of 3'UTR region. We are currently analyzing the 5'UTR sequences. We have determined that half-life of Tcp28 and TcRRM mRNAs is about 50 minutes in epimastigote. Supported by CNPq and Faperj.

BM029 - Expression of WARP, a putative target for Transmission Blocking Vaccines, during *Plasmodium gallinaceum* sexual development

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During the life cycle of malaria parasites, one of the most crucial stages is the midgut invasion in the invertebrate host, when the number of invading parasites steadily decreases and reaches the lowest count throughout its cycle. Paradoxically, the elements and mechanisms involved in such significant bottleneck are yet poorly understood. One of the micronemal proteins believed to play a part on the invasion is the von Willebrand Factor A Related Protein (WARP), a secreted, strongly conserved protein that has already been shown as a promising target for inhibiting oocysts development. The goals of this study are to determine how and when WARP is expressed as zygotes transform into ookinetes and correlate its expression pattern to its putative function. New findings on elements involved in midgut invasion mechanisms may develop into novel transmission blocking strategies based on effector molecules capable of disturbing parasite development. The vWA domain sequence was produced as a recombinant protein using an expression prone plasmid, purified by affinity chromatography by using Ni resin, and the protein was used to immunize rabbits for the production of polyclonal monoespecific antibodies. Confocal microscopy was carried out using the polyclonal antibodies to detect WARP in cultured *Plasmodium gallinaceum* sexual stages. WARP can be detected from the early stages of ookinete development up to the mature palmate-shaped forms. It presents an intracellular granular distribution with focal concentration towards the apical end in mature ookinetes, corroborating its micronemal localization. Currently, experiments involving confocal imaging of infected midguts and expression profiling through RT-PCR are also underway. Financial support: FAPEMIG, FIOCRUZ, CNPq

BM030 - Cloning and expression a ribose-5-phosphate isomerase gene from *Leishmania major.*

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Leishmaniasis is a widespread disease that is endemic in several parts of the world and is caused by some species of *Leishmania* genera. These parasites are responsible for several clinical forms of the disease that includes cutaneous, mucosal and visceral leishmaniasis. It is known that 12 million people world-wide are infected with this parasite. There are several

drugs that are being used to treate this disease, like pentavalent antimony and pentamidine, but the parasite is becoming resistent to many of them. Them, it is essential importance the development of new effective drugs against this parasite. We used AnEnPi, a computational tool that possibilities the detection of analogous enzymes, for the identification of possible cases of drug targets, based on the structural differences of enzymatic activities shared by humans and *Leishmania major*. One of these enzymes is ribose-5-phosphate isomerase that catalyzes an important step of pentose phosphate pathway. The objective of this study is the molecular and biochemical characterization of the ribose-5-phosphate isomerase, a putative analogue enzyme that can lead us to develop new kinds of drug that can inhibit or decrease the catalytic activity of this enzyme of the protozoan, but do not do the same with the human form. In other words, they have similar functions, but with differences on the 3D structure. In this study we have cloned and sequenced the gene of ribose-5-phosphate isomerase of *L. major*. The amplicon with about 519 bp, was inserted in pBad-Thio-TOPO vector and cloned in *Escherichia coli* TOP10 strain. After induction with arabinose 0.02% (w/v), the expression of a protein with approximately 48 kDa was obtained in insoluble form. This polypeptide will be used for mouse polyclonal antibody development and immunocitochemical studies. Supported by: PDTIS - FIOCRUZ; CNPq, FAPERJ.

BM031 - Molecular cloning and protein expression of Oligopeptidase B-Like from *L. amazonensis*

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Oligopeptidase B is an important virulence factor and therapeutic target in trypanosome infections. The *L. major* Genome Project characterized a new oligopeptidase B, denominated oligopeptidase B-Like. In this study, a complete open reading frame of Oligopeptidase B-like from *L. amazonensis* (PH8 strain) was amplified with PCR using primers designed for the Oligopeptidase B-Like gene from *L. major*. The 2715 bp fragment coded for a protein of 905 amino acids with a predicted molecular mass of 103,918.9 Da and theoretical pI of 5.82. The encoded protein shares a $\tilde{9}6$ % identity with Leishmania oligopeptidases B-like, $\tilde{7}5$% identity with Trypanosoma oligopeptidases B-Like and $\tilde{2}2$% with Bacterial Oligopeptidases B. Phylogenetic analysis showed that oligopeptidase B-like is a new member of Oligopeptidase B. By sequence alignment, we determined a catalytic triad (Ser 629, Asp 717 and His 758) and S1 subsite (Glu 674 and Glu 676) of oligopeptidase B-Like. The oligopeptidase B-like gene is expressed in all cycle stages of *L. amazonensis*. Heterologous expression of recombinant oligopeptidase B-Like in E. coli produced only insoluble protein. Antibody anti-promastigote *L. amazonensis* lysate recognized recombinant oligopeptidase B-Like. Now, we are engaged in oligopeptidase B-Like refolding. This work is the first study to characterize Oligopeptidase B-Like.

BM032 - Cloning a phosphomevalonate kinase gene from *Trypanosoma cruzi*

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Chagas disease continues to be an important public health problem in Central and South America. The etiological agent of the disease is the hemoflagellate protozoan *Trypanosoma cruzi*, and the occurrence of the protozoan and the respective insect vectors encloses an area from the south of the United States until the north of Argentina. There are some drugs that are being used to treate this disease, like nitrofurans and nitroimidazoles, but they are active only against the acute phase. Them, it is essential importance the development of new effective drugs against this parasite, principally to chronic phase. We used AnEnπ, a computational tool that possibilities the detection of analogous enzymes, for the identification of possible cases of drug targets, based on the structural differences of enzymatic activities shared by humans and *T. cruzi*. One of these enzymes is that catalyzes an important step of mevalonate pathway. The objective of this study is the molecular and biochemical characterization of the phosphomevalonate kinase, a putative analogue enzyme that can lead us to develop new kinds of drug that can inhibit or decrease the catalytic activity of this enzyme of the protozoan, but do not do the same with the human form. In other words, they have similar functions, but with differences on the 3D structure. In this study we have cloned and sequenced the gene of phosphomevalonate kinase of *T. cruzi*. The amplicon with about 1,431 bp, was inserted in pBad-Thio-TOPO^R vector and cloned in Escherichia coli TOP10 strain. This polypeptide will be used for protein expression and for mouse polyclonal antibody production. This work is of considerable relevance for the study of the parasite metabolism and for the development of new strategies for drug design against this pathogen. Supported by: PDTIS-FIOCRUZ; CNPq, FAPERJ.

BM033 - CHARACTERIZATION, EXPRESSION ANALYSIS AND SUBCELLULAR LOCALIZATION OF THE MASP FAMILY OF TRYPANOSOMA CRUZI

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MASP is a large multigene family of surface proteins identified by the *T. cruzi* sequencing consortium (El-Sayed et al., 2005). MASP family contains 1377 members corresponding to $\tilde{6}\%$ of the T.cruzi diploid genome and is characterized by conserved N- and C-terminal domains and a central highly variable and repetitive region. To initiate the functional characterization of MASP, peptides derived from the family were selected for synthesis and generation of the corresponding antibodies. The anti-MASP affinity-purified antibodies have been used in immunofluoresence assays and in experiments of Western blot using extracts from epimastigote, tripomastigote and amastigote forms. Our results indicate that MASP is expressed on the trypomastigote surface. Northern blot, RT-PCR and screening of a trypomastigote cDNA library suggest a limited set of MASP group is expressed at a given time. To confirm experimentally in silico predictions that suggest that MASP is a GPI-anchored surface protein, we are performing Western blot experiments after the treatment of the parasites with Phosphatidylinositide-specific phospholipase C (PI-PLC). Financial Support: WHO, FAPEMIG.

BM034 - MOLECULAR ANALYSIS OF *Leishmania sp.* APYRASES

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Leishmania major has two mapped apyrase genes in its genome (putatives NTPDase and GDPase). Apyrase function, characterized as tri and di-nucleotide hydrolysis, was previously demonstrated in intact *L. amazonensis*, *L. braziliensis* and *L. major* cells. The very distinct ecto-nucleotidase capacity between *Leishmania* species suggests its involvement with virulence and control of host-immune responses. In order to evaluate if a molecular difference could explain these data, we analyzed both genes in *L. major*, *L. braziliensis* and *L. infantum* whole genome. Analysis of deduced proteins showed higher similarity between *L. major* and *L. infantum* isoforms (90%) and lowest similarities between *L. braziliensis* and the isoforms presented in the other species (70%). GDPases have an extended amino-terminal domain exclusive present in kinetoplastide apyrases. All putative proteins have only one transmembrane region localized in the amino terminal domain, but only putative GDPases possesses a peptide signal just after the predicted transmembrane region. These results suggest that GDPases could be secreted and NTPDases could be ecto-membrane proteins. Analysis of glycosilation sites revelead that NTPDases have more putative N-glycosilation sites than GDPases. Our results show that *Leishmania* apyrases have molecular differences that could explain the distinction in ecto-nucleotidase activities. Furthermore, respective genes from *L. amazonensis* were isolated by PCR, cloned and will be subjected to molecular analysis. This data and future comparison between biochemical characterizations of purified recombinant proteins will be the next steps in this work. Financial Support: FAPEMIG, UFV, UFOP, MEC

BM035 - Analysis of the *surf* gene family in *Plasmodium falciparum* field isolates

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During a major effort aiming to characterize the protective immune response, understood as non-symptomatic infection, we amplified and cloned a number of different *Plasmodium* genes which encode erythrocyte surface-exposed antigens. One of these antigens are encoded by the surf gene family which consists of 10 members in the *Plasmodium falciparum* strain 3D7 genome distributed in 13 annotated ORF in 5 chromosomes. No data about transcription mode or function of surf genes are currently available. Despite of being considered highly variant, we were able to PCR-amplifiy several surf genes in a number of field isolates and laboratory isolates, using oligonucleotides which anneal in the ecto-domain encoding region of the surf genes. Since the observed amplicons rarely vary in size, we expect that -in contrast to the var and rif gene families- the surf genes are fairly conserved despite of their subtelomeric localization and that their antigens are exposed at the red blood cell and also the merozoite. The sequencing of numerous clones is in progress and the data will be presented. Supported by FAPESP

BM036 - A survey of phosphatidylinositol kinase subtypes by mining the trypanosomatid genome

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Phosphatidylinositol 3-kinase (PI3K) is at the heart of one of the major pathways of intracellular signal transduction. The signals made by the enzyme influence a wide variety of cellular functions, including cell growth, differentiation and survival, glucose metabolism and cytoskeletal organisation. This enzyme, which consists of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110), catalyses the formation of a phosphoinositides family with phosphate at the D-3 position of the inositol ring. Wortmannin interacts with many biological targets, but binds *in vitro* most strongly to PI3K being therefore a potent antiproliferative agent. Lys-802, which is the target for wortmannin covalent attachment, resides in the ATP binding site of the p110 subunit and thus has a crucial role in the phosphotransfer reaction. PKB/c-Akt protein kinase has been proposed to depend on functional PI3K. Recent studies highlighting a role for host cell PI3Ks in the *T. cruzi* invasion process have revealed surprising new insights into the mechanism of host cell invasion by this pathogen. Moreover, the treatment of trypomastigotes with PI3K inhibitors prior to infection reduced parasite entry, indicating that PI3K and PKB/Akt activation in parasites, as in host cells induced by *T. cruzi*, is an early invasion signal required for successful trypomastigote internalisation. Herein we present the preliminary results of a survey made by bioinformatics searches of *T. cruzi*, *T. brucei* and *Leishmania* genomes available to date for PI3Ks and phosphatidylinositol-related kinases (PIK-related). PI3K and PIK-related were retrieved from GeneDB throught a combination of searches with Pfam domain, BLAST analysis, COGs examination, search for the presence of kinase (catalytic) domain structure and also for the recently described FAT and FATC motifs. In addition, each phosphatidylinositol kinase identified was analysed for the presence of additional domains by hidden Markov model analysis of the Pfam database. Experimental approaches exploring the noncovalent wortmannin interaction within the catalytic cleft of PI3K might lead to the design of subunit-specific inhibitors for PI3Ks and related enzymes. Unlike wortmannin, such inhibitors might be able to inhibit specific members of the PI3-kinase family mainly pursuing a goal in inhibiting *T. cruzi* invasion process by neutralising PI3K.

BM037 - Cloning and characterization of a Hexose Transporter gene in *Trypanosoma rangeli*

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Like other trypanosomatids, the metabolism of D-glucose is an essential source of energy to *Trypanosoma rangeli*, a kinetoplastid parasite found in several Latin American countries. The D-glucose transporter was formerly characterized for several other kinetoplastida species, including *T. rangeli.* In a previous study, our group characterized the D-glucose transporter in both epimastigotes and trypomastigotes as being a diffusion facilitated and high affinity transporter. Recently, during the *T. rangeli* Transcriptome Project cDNA fragments with high similarity with *T. cruzi* Hexose Transporter gene were clusterized in a $1,291pb$ sequence, representing 80% of the predicted gene. Thus, the aim of this work was to obtain and characterize the full sequence of the *T. rangeli* Hexose Transporter gene. For that, *T. rangeli* SC58 strain cDNA was used for PCR amplification using primers designed to the conserved gene region and to the spliced-leader. The obtained fragments of 563bp were cloned into pGEM-T-easy vectors and sequenced. After quality analysis (Phred ≥ 20), a new 1,724bp contig was obtained resulting in an ORF of 1,632pb, which represents the totality of the predicted gene to *T. cruzi.* Inter-specific comparisons with other Kinetoplastid species revealed 86% identity with T. cruzi and $\tilde{5}$7% with *T. brucei* and *Leishmania* sp. The resulting protein (544aa) contains the complete domain of "Transmembrane Transporter Glucose Multigene Family Hexose 2A HT1 1E"and 10 putative transmembrane segments. Previous analysis of paralogous genes in *T. rangeli* transcriptome has revealed at least duplication of this gene in the parasite genome; however, Southern Blot analysis is been performed to determine the gene copy number. Additional studies are in progress to determine the expression level and the intraspecific variability. Supported by CNPq and UFSC.

BM038 - Protein tyrosine phosphatase (TrPTP1): A novel differential marker for *Trypanosoma rangeli* KP1 strains

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Trypanosoma rangeli strains have been divided in two primary lineages based on the kDNA mini-circle sequences, named as KP1(+) and KP1(-), which have important epidemiological and evolutionary aspects for this taxon. Proteins tyrosine phosphatases (PTP) are described as having a key role on cell differentiation of trypanosomatids. Initially, our group has characterized the partial sequence of the *T. rangeli* PTP (TrPTP) gene, which allowed detecting some intraspecific variability. In this study, the complete TrPTP sequence was obtained for 10 distinct strains and a detailed comparative analysis was performed. For

that, PCR amplification was carried out from total RNA using a set of designed primers (conserved intergenic regions, spliced-leader, poliA tail) and, after cloning, three clones of each strain were sequenced in both strands. After quality analysis (Phred>20) the complete sequence, named TrPTP1, showed 987nt that predict a 328aa protein with a mass of approximately 36kDa, revealing a catalytic domain located between aminoacids 31-300. The 5' and 3' end UTRs are 48nt and 235nt in length, respectively. Alignment analyzes revealed eleven substitutions (Phred>60) in KP1(-) compared to KP1(+) strains sequences, among which, seven are located within the ORF and four at the 3'UTR. The majority of the differences observed within the TrPTP1 ORF are silent, changing a single aminoacid at position 321(A/S). Also, on the 3'UTR a variable poly-A region was observed, having 10 nucleotide for KP1(+) and fourteen for KP1(-) strains. Phylogenetic analysis using bootstrapped maximum parsimony and neighbor-joining methods showed strong support to distinguish KP1(+) and KP1(-) strains as observed for other markers. Due the variability observed within the 3'UTR, further studies are in progress to determine the PTP expression levels and the RNA secondary structure to assess possible functional and/or structural implications of the observed divergences between KP1(-) and KP1(+) strains. Supported by CNPq/UFSC.

BM039 - PCR as molecular tool in the detection of *Leishmania (Viannia)* DNA in different samples from naturally infected dogs.

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American tegumentary leishmaniasis (LTA) in dogs is frequently associated to a low or even negative immune response making unviable the use of serological conventional methods as a single tool in the diagnosis. The PCR technique has shown to be efficient in clinic-epidemiological studies and as an alternative and sensitive method in the LTA diagnosis. Conventional methods of diagnosis have not been able of detecting the parasite in different anatomic sites different from that of cutaneous lesions in naturally infected dogs. On the other hand, it has been reported that the PCR is able to detect the presence of parasite DNA in different anatomical sites in dogs with LTA. **Objective**: To utilize the PCR associated to the molecular hybridization with specific kDNA probe for detecting and typing of *Leishmania* DNA in fragments of cutaneous lesions, skin, spleen, liver and lymph nodes (popliteal, cervical and mesenteric) from dogs naturally infected by *L. (V.) braziliensis*. **Results**: Our results showed the detection of *Leishmania (Viannia)* DNA in biopsies of cutaneous lesions as well as in skin, lymph nodes (popliteal and cervical), spleen and liver from two dogs. Curiously was not possible to detect parasite DNA in mesenteric lymph nodes opening perspectives for further studies. All positive PCR products after hybridization revealing high copy number of homologous sequences with the kDNA of *L.(V) braziliensis* used as probe. In four other animals PCR positive reactions were only observed in cutaneous lesions. **Conclusion**: Our results reinforce the use of PCR associated to the hybridization as a diagnosis method in canine tegumentary leishmaniasis. Also suggest the possibility of the existence of *L.(V.) braziliensis* strains with distinct characteristics of virulence and tropism infecting dogs in Rio de Janeiro State. And contribute to corroborate the importance of dogs as domestic reservoirs in endemic areas of LTA. *Support: CNPq (PIBIC-Fiocruz), PAPES/Fiocruz.*

BM040 - Identification of potential MHC class I and II epitopes and cloning of the Nucleoside hydrolase of Leishmania donovani aiming the development of a synthetic vaccine against visceral leishmaniasis.

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The Nucleoside Hydrolase (NH36) of Leishmania donovani is the main antigen of the FML complex, inducing a TH1 immune response in DNA vaccination of mice against visceral leishmaniasis. To identify the NH36 main MHC class I and II linking epitopes, we cloned the sequences of its three fragments composed by the aminoacids 1-103 (F1), 104-198 (F2) and 199-314 (F3) in the Pet28A plasmid. Sequences were obtained by PCR amplification of the pMAL-NH36 plasmid with BamHI and EcoRI restriction site containing primers. The PCR products were cloned in the pMOS plasmid, sequenced, removed by BamHI and EcoRI digestion and cloned in the digested pET28a vector. E coli Bl21DE3 cells were transformed to express the peptides using 1 mM IPTG. The peptides were purified in a Ni-NTA column and monitored by Western Blot with anti-his antibody. Furthermore, the Sette algorithm of the Protean Pad program allowed the identification of six Balb/c CD4+ linking epitopes (29-E L L A I T T V V G N Q; 278-F R Y P R P K H C H T Q; 106-V Q L I I D L I M S H E; 298-K F W C L V I D A L K R I G; 217-F M L Q I L D F Y T K V Y E; 54-D V A G I V G V P V A A G C T) while the HLA peptide motif search and SYFPEITHI programs identified 3 H2 Ld CD8 linking epitopes (92 Y P P E F K T K L; 162 S P V A E F N V F and 172 D P E A A H I V F). The analysis of the reactivity of the peptides and epitopes against lymphocytes of NH36 vaccinated Balb/c mice is in progress.

BM041 - Applied PCR in different organs in seropositive dogs for visceral leishmaniasis

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Dogs are considered to be the main domestic reservoir of visceral leishmaniasis since they present intense cutaneous parasitism and are able to infect the insect vector. The successful diagnosis of CVL may be difficult since the clinical signs of the disease are varied and non-specific. It is estimated that around 50% of dogs with CVL show no clinical signs of the disease but constitute infection sources for the insect vector. Considering the high spectrum of clinical manifestations in CVL, the chronic aspect of the disease and its long incubation period may generate a delay or failure in clinical diagnosis. In the present work was evaluated the use of PCR to confirm the infection in seropositive dogs. A total of 40 dogs (20 asymptomatic, 20 symptomatic) seropositive by IFAT and ELISA were evaluated. The PCR was carried out in different organs: bone marrow, blood, skin, liver, spleen, and lymph node. The PCR was positive at least in three of six organs analyzed in all dogs. No significant correlations were observed when compared PCR in different tissues versus clinical group. However, the sensitive of PCR to diagnosis the infected dogs were variable according to analyzed organs and clinical status. In the asymptomatic animals, spleen and blood are the most sensitive followed by lymph node, skin, bone marrow and liver. By other hand, in symptomatic animals the PCR were more sensitive when used the spleen followed by blood, skin and lymph node, bone marrow and liver. The liver was the worse sample in both clinical groups. On basis of these results we can conclude that the PCR is an efficient tool for the confirmatory diagnosis of CVL. The use of blood can also be suggested as choice sample for serodiagnosis confirmation taking into account that these sample is easily obtained. Supported by:FAPEMIG,CNPq,PAPES IVB,FIOCRUZ

BM042 - Molecular and functional characterization of L-Asparaginase and Asparagine Synthetase: possible therapeutic targets in the amino acid metabolism of *Trypanosoma cruzi*

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Trypanosoma cruzi has a metabolism largely based on consumption of amino acid, which constitute carbon and energy sources. Some amino acids also participate in the differentiation process from non-infective to infective forms of the parasite. Asparagine is one of the essential amino acids and has a vital importance for correct protein synthesis and for post-transductional modification processes such as N-glycosylation. In other organisms, L-asparagine is known to be synthesized from aspartate in ATP-dependent amidation reaction catalyzed by asparagine synthetase. On the other hand, the L-asparagine hydrolyzes is made by L-Asparaginase to yield aspartate. Both enzymes, Asparagine Synthetase and L-Asparaginase have been characterized in bacteria and eukariotic organisms but have not been study in Trypanosomatids. In the present work we aim to characterize the genes involved on asparagine metabolism in *T. cruzi*. Our bioinformatic analyses from GENE DB and GENEBANK showed that the Asparagine Synthetase gene is present in *Leishmania*, *Trypanosoma cruzi*, *Trypanosoma brucei* and *Trypanosoma brucei congolensis* with 53 and 99% of identity between them while with the human gene it has only 21% of identity. The L-Asparaginase gene is present in species of *Leishmania* (51 to 94% of identity) but was not found in the *T. brucei* complex genome. Moreover, this gene has low similarity with its human countepart (3-27%). Southern Blot analysis indicated that L-Asparaginase could be present in only one copy in the genome of *T. cruzi* CL-Brener. We designed specific primers for Asparagine Synthetase e L-Asparaginase from *Trypanosoma cruzi* to clone the gene by PCR and subclone into pTEX expression vector to generate overexpressed transgenic parasites. Currently we are analyzing the phenotype of the overexpressed parasites and cloning these genes into the tetracycline inducible system to determine with accuracy the role of these genes in parasite surviving and aminoacid metabolism.

BM043 - PfATPase6 gene in Brasilian Amazon isolates: Identification of point mutations

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Resistance to antimalarials is the great challenger in malaria control. Drugs like chloroquine, sulfadoxine-pirimetamine and mefloquine, which are safe and easily administered, are no more effective against *P. falciparum* in different endemic regions. Artemisinin based combination therapy is now the recommended strategy to multidrug resistant parasites and recently was introduced as first line therapy in Brazilian Amazon region. The mechanism of action of this drug has not yet been precisely defined, but pfATP6 is proposed as target for artemisinin. Field studies of several hundreds isolates of *P. falciparum* from patients in French Guiana showed a reduced susceptibility to artemisinin derivatives, associated with a S769N mutation in pfATP6 gene. Monitoring mutations in target genes of circulating population parasites helps to understanding the molecular basis of drug

resistance. Limited data is available on allelic variation of this gene around the world. Trying to answer which polymorphisms are present in Brazilian Amazon isolates we have analyzed the pfATP6 gene of field samples from 10 different endemic regions in Amazon state. Our preliminary study did not detect the S769N mutation in 60 isolates analyzed but we observed different point mutations in pfATP6 gene.

BM044 - Lack of association between the CD28 +17 T/C gene polymorphism and the occurrence of human Chagas disease

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Human infection with *Tripanosoma cruzi* leads to Chagas disease, which can evolve to an asymptomatic (indeterminate) form or very severe forms that may cause death. Chronic chagasic cardiomyopathy, the main cause of disability and death in human Chagas disease, is characterized by an intense inflammatory response that ultimately leads to heart failure. The pathology associated to Chagas disease involves parasite factors, as well as the host immune response. We have previously shown that indeterminate and cardiac chagasic patients display high levels of activated T cells in their blood and that most T cells from these patients lack CD28 in their surface. Moreover, we have shown that *in vitro* exposure of T cells from chagasic patients to autologous monocytes infected with *T. cruzi* does not significantly alter the expression of CD28 by T cells. We hypothesized that the lower expression of CD28 by T cells from chagasic patients could be due to a gene polymorphism, as previously suggested in other diseases. To test this hypothesis, we evaluated the expression of the +17T/C CD28 gene polymorphism in a sample of 169 Brazilian individuals (22 individuals without Chagas disease, 54 indeterminate, 42 non-dilated cardiac and 51 dilated cardiac patients). Our results showed that polymorphism +17T/C on CD28 gene is not associated with Chagas disease outcome and that the frequency of the polymorphic allele is very low in the sample of brazilian individuals evaluated. These results suggest that *T. cruzi* infection rather that gene polymorphism is influencing CD28 expression in T cells from individuals with Chagas disease. We are currently evaluating others polymorphisms in genes involved with immunoregulation as an important strategy for identifying susceptibility patterns for develoment of Chagas disease cardiomyopathy. Financed by: WHO/TDR, NIH, CNPq and CAPES.

BM045 - A second form of TRAP (Thrombospondin Related Anonymous Protein) is present in tachyzoites of the Apicomplexan *Neospora caninum*

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The protozoan *Neospora caninum* is responsible for infecting a wide range of animals and in bovines it can induce abortions, which represents huge economical losses. *Neospora* is an Apicomplexan parasite, where the invasion step is crucial for survival. One important group of proteins secreted prior to invasion is the TRAP (Thrombospondin Related Anonymous Protein) family. Upon contact with host cells the TRAPs undergo exocytosis onto the apical surface of the parasite where they initiate tight binding. The adhesive complexes are translocated towards the posterior pole of the parasite via actin-myosin-based motility machinery, resulting in invasion of the host cell. In *Neospora caninum* one member, NcTRAP1 was previously described. A second possible member called NcTRAP2 was detected by non-annotated EST clustering. The aim of this work was the cloning of the full length NcTRAP2 by amplification of the 5‘ cDNA end through RNA ligase-mediated reaction (RLMRACE). Simultaneously, non annotated genomic sequence was released, and the analysis of the adjacent region of NcTRAP 2 contig enabled the amplification of the full-length gene. The predicted protein sequence has 39% of identity and 53% of similarity with its homologues of *Toxoplasma gondi*(TgMIC2); 39% and 53% with *Neospora caninum*(NcTRAP1); 39% and 52% with *Eimeria tenella*(EtMIC1).The TRAP homologues are characterized by a signal peptide and two adhesive domains: an integrin-like domain and one or more thrombospondin type I repeats, which presumably participate in host cell attachment. It has also a cytoplasmic domain responsible for movement during invasion and a transmembrane domain. In the transmembrane domain a cleavage site by rhomboid proteases is present between alanine and glycine. This site is conserved within *Toxoplasma*, *Babesia*, *Theileria* homologues and also in NcTRAP2. NcTRAP2 is currently being produced in recombinant form for localization within the parasite as well as its influence in the adhesion and cellular invasion process.

BM046 - Polymorphism at the Apical Membrane Antigen-1 domain I locus and population history of *Plasmodium vivax* in Brazil.

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The gene encoding AMA-1 is highly conserved among the *Plasmodium* species suggesting that this protein is essential for parasite‘s biology, although exhibits allelic diversity. This study characterized the genetic diversity of pvama-1 polymorphic domain in *P. vivax* populations isolate from patients coming from different endemic areas in the Brazilian Amazon. By examining polymorphism at this locus in Brazil and

comparing it to other populations throughout the world, we shed light on the demographic history of *P. vivax* in the New World, which in turn has implications for the epidemiology and control of this parasite in Brazil. One hundred and five sequences were generated and analyzed from samples from six different Brazilian states showing 28 nucleotide polymorphic sites, leading to 26 amino acids mutations. A phylogenetic tree of Brazilian and worldwide sequences showed no tendency toward geographic clustering of isolates. The numbers of synonymous nucleotide substitutions per synonymous site (dS) and the number of nonsynonymous substitutions per nonsynonymous site (dN) were estimated within and between states showing non-significant values. By contrast, in worldwide comparisons, dS and dN between Brazilian states were significantly lower than corresponding values between world regions. These results show that genetic diversity in PvAMA-1 in Brazil was reduced in comparison to that in Old World populations, implying evidence of a genetic bottleneck and of recent spread of *P. vivax* in Brazil, which is consistent with archaeological evidence that *P. vivax* was introduced to the New World after European colonization. Moreover, there was little evidence of genetic differentiation among the Brazilian states, consistent with a recent and relatively rapid spread of the parasite in Brazil, in contrast with what is seen in Southeast Asia, where there is evidence of marked geographic subdivision, indicative of a long history infecting human populations in Asia. Financial Support: FAPEMIG

BM047 - Characterization of TcTXNPm gene tryparedoxin peroxidase mitochondrial in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole.

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Tryparedoxin peroxidase catalyzes the reduction of hydrogen peroxide or small-chain organic hydroperoxides to water or alcohols, respectively. It can be found in the citosol or in the mitochondria of tripanosomatids. In this work, tryparedoxin peroxidase mitochondrial (TcTXNPm) was characterized in *T. cruzi* populations susceptible and resistant to Benznidazole (BZ). Bioinformatics analysis showed that this gene is present in four contigs in the *T. cruzi* genome. Artemis and "Clustal X"softwares were used for annotation and multiple sequence alignments for nucleotide and amino acid sequences, respectively. After manual annotation only two contigs (AAHK01005244 and AAHK01001562) presented correct complete ORF (Open Read Frame) for TcTXNPm protein. The amino acids sequences of the four contigs TcTXNPm have 97% of identity. *In silico* restriction analysis of *TcTXNPm* with enzymes *Hinf*I and *Xho*I showed that the gene can be organized in multiples copies in the genome this parasite. Northern blot analysis probed with the *TcTXNPm* gene showed a transcript of 0.76Kb to 17WTS/17LER populations and a transcript of 0.85Kb to other *T. cruzi* strains analyzed. In the 17LER resistant *T. cruzi* population, the levels of *TcTXNPm* mRNA were 2-fold higher than its drug-susceptible counterpart 17WTS. The copy number of *TcTXNPm* gene in *T. cruzi* samples was determined by Southern blot analysis of the parasite DNAs digested with two restriction endonucleases: *Hinf*I, which that cuts once inside the gene sequence and *Xho*I that does not. The results showed that *Hinf*I-digested DNA from 17WTS and 17LER populations contained bands of 0.3, 0.6 and 0.87Kb, whereas the others populations contained bands of 0.3, 0.56 and 0.77Kb. *Xho*I-digested DNA samples showed a unique band of 0.86Kb for all samples analyzed. Comparative densitometry analysis of the bands showed no amplification of the *TcTXNPm* gene between *T. cruzi* populations resistant or susceptible to BZ. Supported by CNPq, FAPEMIG and CPqRR/FIOCRUZ.

BM048 - Maxicircle NADH dehydrogenase subunit 7 gene deletion in *Trypanosoma cruzi* human isolates and mitochondrial respiration

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INTRODUCTION -Comparison of transcript profiles of T.cruzi isolates from asymptomatic (ASY) and cardiac patients (CCC) with a DNA microarray showed 30-fold upregulation in CCC strains of a probe corresponding to the maxicircle gene of NADH dehydrogenase subunit 7 (ND7) (Baptista et al., 2006). This result is the consequence of a deletion of 455 base pairs in the middle of the ASY strains ND7 sequence. GOALS 1-Evaluation of the prognostic value of ND7 structure for characterization of Chagas disease manifestation. 2-Assessment of mitochondrial respiration. RESULTS -The structure of ND7 was analyzed by PCR in 89 recent parasite isolates from chronic patients of Minas Gerais: 24 CCC and 65 ASY. The complete ND7 sequence was observed in 45.8% of the CCC strains (11/24), whereas 49,2% of the ASY strains (32/65) showed the ND7 deletion. Five strains (1CCC; 4ASY) showed both amplicons. CONCLUSION - ND7 deletion is not a molecular marker for clinical manifestation. Because the ND7 deletion produces a truncated product that could impair mitochondrial complex I function, we measured the oxygen consumption in digitonin-permeabilized epimastigotes of two isolates with ND7 deletion and two "normal"isolates, in the presence of NADH-linked mitochondrial substrates (malate+pyruvate) and $FADH_2$-generating succinate. All the substrates stimulated oxygen consumption. Addition of ADP to the preparations caused a transient increase in the respiration rate. The respiratory control ratio was calculated to be $\tilde{2}$ for the four isolates, suggesting no differences in mitochondrial res-

piration or oxidative phosphorylation. Several reports propose that the NADH-ubiquinone segment in the respiratory chain of trypanosomatids is inactive, and replaced by NADH-fumarate reductase, which reoxidizes mitochondrial NADH and generates succinate. Accordingly, work is in progress to evaluate the effect of this enzyme and complex II inhibitors on mitochondrial respiration of the two classes of isolates. Other mitochondrial parameters will also be determined. Support: FAPESP; MCT/CNPq/MS-SCTIE-DECIT.

BM049 - Does differential Mismatch Repair efficiency affect genetic variability in *Trypanosoma cruzi* ?

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T.cruzi presents extensive intraspecific variation which makes this organism an interesting model to study the mechanisms involved with the generation of genetic variability. The post-replicative DNA repair pathway MMR eliminates errors that scape from the proofreading activity of the DNA replication machinery and could be associated with high mutation rates. We have previously described that polymorphisms observed in the *Tcmsh2* gene among several strains of *T.cruzi* result in three different isoforms of the protein (TcMSH2a, TcMSH2b and TcMSH2c). We also proposed that strains could be grouped into three TcMSH2-based haplogroups, named A, B and C, which can be correlated to the division proposed by Momen (1999): haplogroup A corresponds to *T.cruzi* I, haplogroup C corresponds to *T.cruzi* II and the haplogroup B corresponds to hybrid strains and strains that were later classified as *T.cruzi* III. Moreover, our results suggest an increased efficiency of MMR in parasites expressing the TcMSH2a isoform, when compared to "b"and "c"isoforms. Several groups (including ours) have reported a decreased genetic variability in *T.cruzi* I lineages possibly as a consequence of decreased mutation rates. We propose to evaluate possible roles of polymorphisms in TcMSH2 protein in generating genetic variability in *T.cruzi.* We measured the levels of oxidative stress-induced DNA damage and cell viability in the presence of different genotoxic agents. Our analyses support the hypothesis that strains belonging to *T.cruzi* I lineage present a more efficient MMR. To further evaluate the role of TcMSH2 protein in *T.cruzi* MMR, we performed similar analyses using CL-Brener Tcmsh2 single knockouts. Attempts to knock-out the second allele were unsuccessful, suggesting that the *Tcmsh2* gene is essential, and may be involved with cellular functions other than MMR. To investigated these questions, studies with a CL-Brener lineage superexpressing the TcMSH2 are underway. Finantial support: CNPq, FAPEMIG and HHMI.

BM050 - Analysis of variability of repetitive amino acid motifs in Trypanosoma cruzi

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Trypanosoma cruzi expresses many repetitive antigens during its life cycle, most of them encoded by multi-copy genes. Our group has previously identified several antigens containing repetitive amino acids that react with sera from chagasic patients, such as TcRpL7a, TcAG48 and TcAG26. TcRpL7a antigen, a ribosomal protein, has an N-terminal repetitive domain containing several blocks of the amino acid motif AAKX. To compare the numbers of repetitive motifs among different *T. cruzi* strains, the corresponding region of TcRpL7a was amplified from the genome of various strains. Sequencing analyses of individual PCR products indicated that the numbers of repetitive motifs are lower in sequences derived from *T. cruzi* I strains (in which 14 to 15 repeats were found) when compared to *T. cruzi* II strains (which contain 15 to 18 repeats). Sequences from the hybrid CL Brener clone showed a pattern similar to *T. cruzi* II sequences. TcAG48, a putative RNA binding protein, also contains an N-terminal repetitive region similar to TcRpL7a. Sequence analysis of TcAG48 in various strains shows that variations in the number of the four amino acid repeat were only observed in strains belonging to the *T. cruzi* II lineage. Differently from TcRpL7a and TcAG48, TcRpL19, another ribosomal protein, contains various numbers of the Ala-Lys-Pro repeat domain in its C-terminal region. To identify all the *T. cruzi* proteins containing repetitive amino acid motifs, a whole genome scanning is being performed. To this end, a *T. cruzi* database of predicted protein sequences was generated after the exclusion of pseudo and partial genes. After data mining for the presence of repetitive amino acid sequences, using PERL scripts, the identified motifs will be classified based on the motif sequences and the functional categories of the corresponding proteins. Supported by Howard Hughes Medical Institute, CNPq and FAPEMIG.

BM051 - Characterization of *TcADH* gene encoding the alcohol dehydrogenase enzyme in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole

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Alcohol dehydrogenases (ADH) belong to a group of en-

zymes that catalyze the reversible ethanol acetaldehyde oxidation with consequent NAD reduction. In the present study, we have characterized the *TcADH* gene from 11 *T. cruzi* strains susceptible or resistant to benznidazole (BZ). The gene encoding ADH (*TcADH*) in *T. cruzi* was selected through microarray analyses due to its 4-fold lower transcription level in the *in vitro*-induced resistant *T. cruzi* population (17LER) when compared with its drug-susceptible counterpart (17WTS). TcADH encodes a protein of 393 amino acids representing a conserved domain of an iron-containing alcohol dehydrogenase. Analysis of the primary structure showed that TcADH in *T. cruzi* exhibited higher identity levels with prokaryote ADHs than with its orthologs identified in *Leishmania sp.* Western blot analyses evidenced that the anti-TcADH polyclonal antibody recognized a 41.7 kDa peptide in all *T. cruzi* strains tested. The level of expression of this polypeptide was approximately 2-fold lower in the 17LER *T. cruzi* population when compared with 17WTS. Enzymatic activity analysis of TcADH showed a correlation between protein expression and activity. Northern blot hybridization analyses showed that the *TcADH* probe was able to recognize a 1.9 Kb transcript with similar intensity in all *T. cruzi* samples analyzed, except for 17LER population that showed to have 2-fold lower intensity values. Immunolocalisation assays by confocal microscopy revealed that TcADH is located into the kinetoplast of parasite. In this work, we report, for the first time, the characterization of the *TcADH* gene and a correlation between its low expression levels with *in vitro*-induced BZ resistance in *T. cruzi.* Supported by: CAPES, CNPq, FAPEMIG, PAPES III- FIOCRUZ

BM052 - Survey of microsatellite loci in the *Trypanosoma cruzi* genome

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Although the first draft of the *T. cruzi* genome sequence has already been published, most aspects of its population structure and evolution remain to be elucidated. Previous studies using eight dinucleotide microsatellites loci have provided valuable information about the structure of *T. cruzi* populations. With availability of the *T. cruzi* genome, the development of a comprehensive panel of microsatellites is now feasible. To this end, we evaluated the abundance, composition and distribution of perfect microsatellite loci constituted by di, tri and tetranucleotide motifs in the *T. cruzi* genome. All the annotated contigs were submitted to the SSRIT algorithm. Approximately 1% of the *T. cruzi* genome corresponds to microsatellite loci. Of the 11,892 identified microsatellites, 47.7% are dinucleotides, 38.7% are trinucleotides and 13.7% are tetranucleotides. The most frequent di, tri and tetranucleotide microsatellites are AC, AAT and AAAT, respectively. Microsatellites composed by CG motifs are very rare and contain few repetitive units. The inter-CDS sequences showed approximately 12.6 more microsatellite loci than CDS regions, mainly constituted by dinucleotide motifs. Contrasting to the inter-CDS regions, the vast majority (88%) of the microsatellites associated with the CDS regions are trinucleotides and no perfect CG microsatellites were identified within the CDS regions. Moreover, preliminary analyses reveal that, CAA and CAG repetitions are the most abundant within the CDS regions, suggesting that the parasite proteome is probably glutamine rich. We are currently generating a panel of microsatellite markers derived from different CL Brener cromossomal bands which can be used in evolutionary and population genetic studies. Financial support: WHO, CAPES, CNPq and FAPEMIG.

BM053 - Expression analysis of six different proteins in *Leishmania amazonensis* and *Leishmania guyanensis* populations susceptible and resistant to antimoniates

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Recently, our group selected populations of *L. guyanensis* (M9945 strain) and *L. amazonensis* (PH8 and 10995 strains) with *in vitro* resistance to potassium antimonyl tartrate, an Sb(III)-containing drug. These populations were obtained by step-wise selection starting with drug concentrations corresponding to the 50% effective concentration (68-237μM) of each strain until we obtained the resistant populations SbR-1, SbR-3 and SbR-4, which were resistant to 900μM. In this work we investigated expression level of six different proteins in these populations and in the clone R-21 from *L. guyanensis* (M9945 strain), previously described as resistant to meglumine antimoniate (Glucantime) (Ferreira-Pinto *et al.*, 1996). Enzymes involved with antioxidant defense (iron superoxide dismutase, FeSOD-A and tryparedoxin peroxidase, TRYP), drug-stress (70 kDa heat shock protein, HSP-70) and metabolism (flavin oxidoreductase/NADH oxidase, dihydrolipoamide dehydrogenase, LPD and alcohol dehydrogenase, ADH) were analyzed. Protein expression was determined by western blot using polyclonal antibodies raised against these recombinant proteins from *T. cruzi.* Alignment analysis the amino acid sequences these proteins from *T. cruzi* showed 31 to 84% of identity with sequences from different species *Leishmania.* Antibodies anti-*T. cruzi* proteins also recognized *Leishmania* proteins. Differences in protein profiles were observed between *L. amazonensis* and *L. guyanensis.* The resistant populations SbR-1 and SbR-3 selected from M9945 and 10995 strains, respectively, presented levels of TRYP at least 1.5-fold higher than its wild-type populations. The resistant populations SbR-3 and SbR-4 selected from 10995 and PH8 strains, respectively, showed low expression levels of HSP-70. Clone R-21 showed overexpression of proteins: FeSOD-A, TRYP, flavin oxidoreductase/NADH oxidase and low expression of ADH compared to wild-type parasites. LPD was the only enzyme that showed the same

expression level among all samples analyzed, independent of drug-resistance phenotype. Further studies are under way to investigate the involvement of those proteins with drug-resistance phenotype in *Leishmania*. **Supported by: CNPq, CAPES, FAPEMIG**.

BM054 - PCR Isolation of Microsatellite Arrays (PIMA) and Analysis of Frequency and Density of Microsatellite repeats in *Trypanosoma rangeli* Coding Sequences

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Microsatellites, or simple sequence repeats (SSRs), consist of tandem repeats of a short nucleotide sequence (1-6bp) which evolve through mutational processes such as replication slippage, point mutation, and recombination. Also, SSRs show high levels of polymorphisms and Mendelian codominant inheritance which along their good stability under laboratory conditions and high detection sensitivity make SSRs a promising genetic marker for studies involving genetic variability and population genetics, genotype identification and discrimination. Regarding pathogenic trypanosomatids, SSRs have been identified in *T. brucei* and used in recognition and profiling of *T. cruzi* major phylogenetic lineages, but not in *T. rangeli*. In the present study we have used available *T. rangeli* CDS or EST/ORESTES sequences to study the frequency and density of various types of microsatellites. Also, PIMA methodology was used to identify novel microsatellite arrays from *T. rangeli* genomic DNA. All data were analyzed by TRF and TRAP softwares, limiting repeat units to 1-6bp. Around 1.6Mb of both coding and non-coding sequences from epimastigotes and trypomastigote forms of SC-58 and Choachi strains were analyzed, among which was possible to identify 207 distinct SSRs varying from 1 to 445 the total number of repeat loci. The SSRs frequency was of 1/1.44Kb and 1/1.19Kb for SC-58 and Choachi strains, respectively. Hexanucleotide repeats (about 30% for both strains) are the most abundant class of SSRs, following monomeric (21%), trimeric (19%) and dimeric/pentameric (9-11%) repeats with no significant variation between the strains. Furthermore, A-rich repeats are predominant in each of SSR type, whereas G-rich repeats are rare in the coding regions (except for trimeric repeats). After stability analysis, the ongoing genotyping of the herein identified microsatellites could be useful in addressing *T. rangeli* population genetics for better understanding of the parasite spreading pattern and co-evolution with *T. cruzi*. Supported by CNPq, CAPES and UFSC.

BM055 - ANALYSIS OF GENETIC POLYMORPHISM IN *Trypanosoma cruzi* ISOLATED FROM CHAGAS DISEASE CHRONIC PATIENTS AND TRIATOMINE FROM THE SEMI-ARID POTIGUAR, RN, BRAZIL

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Trypanosoma cruzi stocks from northeast of Brazil were among the first to be genetically characterized by means of isoenzymes and led to the description of three main zymodemes circulating in Brazil. However, little is known about the specific *T. cruzi* genotypes circulating in the state of Rio Grande do Norte and the population structure of the parasite in that area. In this work we evaluated genetic polymorphism of *T. cruzi* from the human and triatomines from the semi-arid region. Twelve *T. cruzi* isolates have been obtained from human through hemoculture and xenodiagnosis and 13 from triatomines that were captured in the intra and peridomicile, and in wild environments. DNA from these isolates has been analyzed by (i) Ribosomal 24Sα RNA gene amplification; (ii) typing by microsatelite and (iii) amplification of the region of spliced leader genes (SL-IR). Results showed that among 13 triatomine isolates, ten of them were *T. cruzi* III profile (DTU IIc) differentiated by SL-IR and three *T. cruzi* II (DTU IIb). Four stocks *T. cruzi* II or III isolated from triatomines presented the identical monoclonal homozygous or heterozygous profiles. Interestingly, seven isolates from humans and one from *T. brasiliensis* nynph instar were *T. cruzi* II and showed polyclonal profile by different microsatelite loci, suggesting that profile seems to be identical among them. These results can indicate a large distribution of *T. cruzi* populations in this area, and the multiclonal stocks isolated from the humans demonstrate genetic heterogeneity even after establishment of the chagasic infection. Supported by CAPES, CNPq/Edital Universal, MCT/CNPq/MS-SCTIE-DECIT, CNPq/Bolsista PV

BM056 - Genetic polymorphisms reveals *Trypanosoma cruzi* II infection in Colombian patients

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Introduction and objective: Recently, has been suggested that *T. cruzi* populations should be divided into three distinct major lineages, I, II and III, based on mitochondrial gene polymorphisms. Interestingly, carrying out the characterization by diverse molecular methods from isolates of patients, *T. cruzi* I, seems to be the only group causing chagasic cardiomyopathy in Colombia and other countries of northern part of South America and Central America. Also, this group (corresponding to Z1 zymodeme) is the only one isolated from hemoculture of Chagasic patients, and represents the 92% of isolates from vectors and reservoirs in Colombia, with occasional isolation of Z3 zymodeme. In the present work, we search the presence of *T. cruzi* II parasites directly in blood of Colombian infected patients. In order to do this, we characterize parasites detected in peripheral blood of 24 chagasic patients from endemic region of Santander, Colombia, by analyzing polymorphisms in mitochondrial gene Cytocrome Oxydase II and rRNA 24Sα. **Results and conclusion:** Results showed that *T. cruzi* II was detected in 7 Colombian patients, alone or mixed with *T. cruzi* parasites. Frequent isolation of *T. cruzi* I parasites instead *T. cruzi* II from Colombian patients, could indicate a clear selection of *T. cruzi* I in culture. The reason for this is even unknown; however, in other analysis we obtained evidences indicating the presence of *T. cruzi* II parasites in tissues of Colombian chagasic patients. Further studies are needed to establish the exact role of *T. cruzi* II parasites in the pathogenesis of Chagas disease and domestic transmission cycle in Colombia, and, to know the reason why *T. cruzi* II causes less cases of Chagas disease than *T. cruzi* I, contrary to that described in regions of Brazil, Chile and Argentina. **Financial Support:** Colciencias, grant 1102-05-17591 and Universidad Industrial de Santander.

BM057 - Synanthropic and sylvatic rodents found naturally infected by *Leishmania (Viannia)* in an endemic area American Tegumentary Leishmaniasis

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Introduction: The presence of natural infection by *Leishmania* in synanthropic and sylvatic rodents proceeding from the Municipality of Araçuaí, Minas Gerais has been investigated through molecular markers. The active transmission of human and canine tegumentary leishmaniasis as well as the existence insect vectors and naturally infected rodents (Oliveira et al., Vet. Parasitol., v 129, p. 219-227, 2005) has contributed for a better understanding of the transmission cycle associated to *Leishmania (Viannia) braziliensis*. **Objectives**: To detect and type *Leishmania* DNA directly in blood, skin and spleen samples from rodents using PCR and molecular hybridization. **Materials and Methods**: Samples were collected during an epidemiological study carried out in the Municipality of Araçuaí, Minas Gerais in 2001. The DNA extraction from blood and skin and spleen biopsies were carried out using the kits FTA Cards (GIBCO BRL) and GenomicPrep^{TM}Cells and Tissue DNA (Amersham Pharmacia), respectively. PCR analyses were processed using primers directed to the variable regions of kDNA minicircles which were specific for *L. braziliensis*, *L. mexicana* e *L. donovani* complexes. Southern blot hybridization experiments were carried out using specific radioactive kDNA probes. **Results**: Eight seven samples from 29 rodents were analysed. Spleen samples from two synanthropic rodents (*Rattus rattus*) and skin samples from two sylvatic rodents (*Galea spixii*) were PCR positive. The 750 bp diagnosis bands specific for species of the *L. braziliensis* complex were revealed after electrophoresis of the amplified products. Molecular hybridization showed high number of homologous sequences with the kDNA of *L. (V.) braziliensis* reference strain used as probe. **Conclusion**: Our results reinforce the importance of the rodent *Rattus rattus* in the domestic cycles of *L.(V.) braziliensis* transmission. The present work also contributes to alert the sanitary authorities for a proper surveillance in the control of this rodent species. *This work received financial support from FAPERJ and PAPES/Fiocruz.*

BM058 - Analysis of chimeric genes in the Trypanosoma cruzi genome

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The *Trypanosoma cruzi* genome contains a large number of repetitive sequences including large gene families and noncoding regions. The majority of the *T. cruzi* large gene families encodes for surface proteins which are clustered into the

parasite genome. Within these arrays of surface proteins, members of each family are not duplicated in tandem; instead the different families are alternated among each other in a not well defined fashion. Using the *T. cruzi* dataset generated by the genome project and sequences derived from a trypomastigote cDNA library, we have identified chimeric coding sequences containing conserved regions derived from different surface protein gene families. PCR analysis of selected chimeric genes in *T. cruzi* strains belonging to different lineages suggests that some of these mosaic sequences may have been originated before the *T. cruzi*I and *T. cruzi*II divergence while others were probably originated more recently during the *T. cruzi* evolution. In sharp contrast to the Tri-Tryp syntenic regions, intense rearrangements such as duplications, inversions and translocations were identified in the *T. cruzi* specific regions containing large clusters of surface proteins. We are currently scanning the *T. cruzi* large family of surface proteins to evaluate whether chimeric sequences derived from different gene families can be generated without the involvement of the N- and C- terminal regions. Financial Support: FAPEMIG, WHO, CNPq

BM059 - DNA research from Leishmania kinetoplast by Polymerase Chain Reaction in biological samples from patients with American Cutaneous Leishmaniasis

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American Cutaneous Leishmaniasis (ACL), as others Leishmaniasis, is a zoonosis presenting several clinical manifestations, which difficult a conclusive diagnostic. The use of Polymerase Chain Reaction (PCR) with different primers allows the identification of the etiological agent and helps in the ACL diagnosis. In this study it was compared the positivity of PCR reactions directed to conserved (150/152 primers) and variable (13Y/13Z primers) regions from Leishmania kDNAs minicircle in biological samples from suspected and confirmed ACL patients. Thirty-nine suspected ACL patients from South and Southwestern (16) and Rio Doce Valley region (23) of Minas Gerais State - Brazil were submitted to laboratorial exams (Montenegro Skin Test, parasitological and/or histopatological exams). The biopsies and Giemsa-stained imprint slides from patients samples were digested with Proteinase K (65C, 3 hours) and submitted to heating (70C, 15 minutes), respectively. The extracted DNA was used in PCR with primers 150/152 ($\tilde{1}$20bp expected amplicon) and 13Y/13Z ($\tilde{6}$50bp expected amplicon). Thirty-two patients were confirmed for ACL by the laboratorial methods. Among the 39 samples from suspected ACL patients, 13Y/13Z and 150/152 positivity index was 56.4%, and 76.9%, respectively. Moreover, among the 32 samples from confirmed ACL patients, the positivity with primers 150/152 (93.7%) was higher ($p < 0.05$) than 13Y/13Z (68.7%). The variable region from Leishmania kDNAs minicircle is a helpful target, because of the possibility of generation of different length and sequence PCRs products. The use of these primers is helpful in Leishmania identification in spite the positivity index with primers 13Y/13Z was smaller than 150/152. This is the first work to use the primers 13Y/13Z with extracted DNA from Giemsa-stained imprint slides from patients living in South, Southwestern and Rio Doce Valley regions of Minas Gerais State. Supported by Capes, CNPq, Fapemig, Finep and Unifal-MG.

BM060 - Characterization of antimonial-resistant *Leishmania* mutants

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The diseases caused by *Leishmania* parasites are responsible for significant morbidity and mortality worldwide. Despite extensive use of antimonial compounds in the treatment this disease, their mode of action remains uncertain. Resistance to antimonials is a major problem in treating leishmaniasis in India and has already been described for New World parasites. The aim of this work was to characterize the antimonial resistance elicited in *Leishmania* species for both *Leishmania* and *Viannia* subgenera.
Using a stepwise selection protocol, we isolated *L. (L.) major* and *L. (V.) braziliensis* cell lines that were resistant to different antimonial forms (SbIII and/or SbV). The accumulation of the trivalent form of the drug was quantified in wild type and resistant lineages using inductively coupled plasma mass spectrometry. SbV−resistant mutants accumulated twice as much SbIII when compared to SbIII−resistant lineages. When total thiol levels were analyzed in these mutants we observed a decrease of about 60% in intracellular thiol levels in the resistant lineages. Northern analysis revealed that resistant mutants presented a lower level of transcripts for genes involved in thiol biosynthesis and SbIII uptake, a profile that is in agreement with that of *L. donovani* resistant strains isolated from patients in eastern Nepal. Current work is focused in a detailed characterization of the expression pattern of genes possibly involved in the antimonial resistance phenotype observed in these selected mutants. Supported by FAPESP, CNPq and CAPES.

BM061 - eIF4E and PABP homologues from *Leishmania amazonensis* are represented by distinct isoforms which vary according to the parasite growth stage.

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The 5' and 3' ends of the majority of eukaryotic mRNAs are defined by the presence of a cap structure and a poly-A tail respectively. Both are very important to the recognition of mRNA and translation initiation. The cap structure is recognized by eIF4E, the cap binding protein, which is part of the heterotrimeric translation initiation complex eIF4F. At the poly-A tail binds the poly-A binding protein (PABP). Interactions between eIF4F and PABP allow the recruitment of the 40S ribosomal subunit and initiation of translation. Multiple eIF4E and PABP homologues have been identified in *Leishmania*. Here we have compared the expression pattern of selected homologues (EIF4E3-4 and PABP1) during different phases of a *L. amazonensis* promastigote growth curve. Our results show that all three proteins were found throughout the curve; however each is represented by two distinct isoforms, one of which is presumably phosphorylated. Immediately after feeding PABP1 and EIF4E4 are represented by mainly non-phosphorylated forms whilst EIF4E3 is found as its phosphorylated isoform. Soon after (less than 2 hours) phosphorylated forms of PABP1 and EIF4E4 appear, and predominate, whilst EIF4E3 is dephosphorylated. In exponentially grown cells (2-3 days after feeding) PABP1 is found as a non-phosphorylated protein whilst both EIF4E3-4 are represented by the two isoforms in roughly similar levels. In stationary phase cells (> 4 days after feeding) PABP1 and EIF4E4 are predominantly non-phosphorylated contrasting with EIF4E3 which is nearly all in its phosphorylated forms. Feeding the cells in the absence of foetal calf serum induces a premature phosphorylation of PABP1 and a hyperphosphorylation of EIF4E3, associated with a lack of cellular growth. Our results indicate that phosphorylation may play a significant role in regulating the activity of translation initiation factors in *Leishmania* and presumably protein synthesis as a whole.

BM062 - CHARACTERIZATION OF TcAPX GENE ENCODING THE ASCORBATE PEROXIDASE ENZYME IN *Trypanosoma cruzi* POPULATIONS SUSCEPTIBLE AND RESISTANT TO BENZNIDAZOLE

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One of the antioxidant enzymes involved in the defense of tripanosomatids is the Ascorbate peroxidase (APX). It is involved in the metabolism of hydrogen peroxide and it is not present in mammals. Therefore, it becomes as a potential target for chemotherapy of the Chagas disease. Here we have characterized the gene that codifies APX enzyme in a panel of *T. cruzi* (TcAPX) populations susceptible and resistant to benznidazole (BZ). Southern blot was used to determine the genomic organization of the TcAPX in the *T. cruzi* samples. Parasite DNAs were digested with the *Ava*I enzyme that cuts once inside the gene sequence, and *Xho*I that does not. The digestion profile with *Ava*I and *Xho*I hybridized with a specific P^{32}-TcAPX probe showed fragments of 0,85 Kb and 4,6 Kb, respectively. No differences in band intensities between samples were observed suggesting that the TcAPX gene is not amplified in *T. cruzi* populations analyzed. TcAPX gene is located on a chromosome of approximately 2,000 Kb in all samples. TcAPX gene expressed a recombinant protein of 62 kDa, corresponding to 33kDa from TcAPX fused with glutationa-S-tranferase (29kDa). Polyclonal antibodies anti-rTcAPX raised in rabbits showed a native protein of 33 kDa. The level of protein expression was the same for all samples except BZR (BZ resistance selected in vivo) that was two fold more intense than its susceptible pair (BZS). Currently, we are investigating the mRNA level of APX in *T. cruzi* populations susceptible and resistant to BZ.

BM063 - Isolation and characterization of Leishmania (L.) chagasi from patient infected with the human immunodeficiency virus in the Goiás state, Brazil.

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American Visceral Leishmaniasis (AVL) is endemic in 18 (66.7%) states of Brazil with clear spreading throughout the country. The occurrence of AVL in Brazil is associated with the precarious socio-economic conditions. We present here a Leishmania-HIV co-infection case in a woman, 41-year-old from Jataí-Goiás, a Center-West region of Brazil. The initial symptoms were pulmonary symptoms as pleural effusions, dry cough, dyspnea and fever. She was treating with clindomicin and ceftrioxone with remission of pulmonary symptoms. Later were observed bone marrow aplasia with pancytopenia. The diagnosis of AVL was being by mielograma with parasitic culture in 199 medium. Isolation of the promastigotes was assessing by direct visualization under a light microscope. The parasites were isolated and cultivated in 199 medium supplied with 20% of fetal bovine serum. The patient was treating with intravenous N-methyL glucamine antimoniate (Glucantime, 20 mg of Sb/Kg per day) for 30 days. Isolate was identified as *L.(L.)chagasi* by the analy-

sis of amplified DNA with specific primers. The biological characterization consisted on the growth curves evaluation in vitro (5x105 parasites/ml cultivated during 10 days). The curves of growth in vitro showed that the stationary phase was achieving between the 4th and 5th day. The stationary phase finished in the 8th day. During the phase logarithmic growth phase, parasites were stored in liquid nitrogen of Leishbank stock. Leishmaniasis/HIV co-infection is emerging as an extremely serious, new disease and it is increasingly frequent. There are important clinical, diagnostic, chemotherapeutic, epidemiological and economic implications of this trend. AIDS and AVL are locking in a vicious circle of mutual reinforcement. On the one hand, LV quickly accelerates the onset of AIDS and shortens the life expectancy of HIV-infected people. On the other hand, HIV spurs the spread of LV. AIDS increases the risk of VL by 100-1000 times in endemic areas.

BM064 - Phylogenetic reconstruction of *Eimeria* spp. of domestic rabbit and association with biological features

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Eleven species of the genus *Eimeria* infect the domestic rabbit causing an enteric disease known as rabbit coccidiosis. The most commonly colonized organ is the intestine, but *E. stiedai* also affects the biliar ducts. The different species also differ in the size and morphology of the oocysts, site of the intestinal lesions, severity of the disease, pre-patent period, etc. Since no molecular markers and phylogenetic studies have been reported so far for this group of parasites, we aimed at reconstructing the phylogeny of rabbit *Eimeria* using three distinct molecular markers: the fast-evolving ITS1 ribosomal sequence, and the18S rRNA and mitochondrial cytochome b genes. Based on previously described sequences of chicken *Eimeria*, we designed primers, amplified the targets and determined their corresponding nucleotide sequences. Phylogenetic reconstruction consisted in neighbor-joining and maximum likelihood analyses. The topology of the phylogenetic trees revealed a partial agreement. *E. exigua* and *E. irresidua* in a clade, and *E. flavescens* and *E. piriformis* in another one, were grouped together in a major clade. Another major group was composed by the remaining species. This clade was not well resolved, presenting either polytomies and/or low support values. Nevertheless, some relationships were observed, with *E. media* and *E. perforans* grouping together, closely to *E. magna*. From a biological standpoint, no correlation between the pathogenicity level of the species and the phylogenetic reconstruction was observed. Conversely, there was an excellent correlation of the topology of the tree, and the presence of the oocyst residuum. Some correlation was also observed with the parasite localization in the host tissues. This set of results suggest that a confident phylogenetic reconstruction will only be attained by using a larger set of molecular markers, in order to dilute possible differences on the evolutionary rate and history of single markers.

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BM065 - PHYLOGENY OF *LEISHMANIA* SPECIES BY ANALYSIS OF CODING SEQUENCES FOR TRYPANOTHIONE REDUCTASE

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Leishmania are protozoan parasites that cause a variety of diseases in human. It is estimated that 12 million people are infected worldwide, with 2 million new cases reported annually. Although a variety of approaches have been used in the identification of these parasites, few methods have provided comparative data suitable for investigating the evolutionary relationships among them. Over the last years contradictory hypothesis for either a Neotropical or a Paleartic origin of the genus *Leishmania* have been proposed. The formulation of a robust phylogenetic framework for these organisms is needed for the built up of a consensus picture based on extensive analyses of molecular sequences from several unlinked *loci*. In this work we determine the partial gene sequence encoding the enzyme trypanothione reductase (TR) from New World and Old World *Leishmania* species (*L. chagasi, L. infantum, L. major, L. major Friedlin, L. donovani,L. tropica, L. colombiensis,L. gymnodactyli,L. amazonensis,L. pifanoi, L. mexicana,L. lainsoni, L. panamensis, L. guyanensis, L. naiffi and L. braziliensis*). TR is a NADPH-dependent oxidoreductase flavoprotein, central to thiol metabolism in all the trypanosomatids. This molecule has a crucial role in the parasite‘s defense against the oxidative stress inside the macrophage phagocytic vacuole, and therefore in the survival of the parasites during the host immune response. The data obtained in this work with gene amplification and sequencing was used to infer a phylogenetic relationship between the studied species in order to contribute for the solution of the geographic origin and evolution of the genus *Leishmania*. This work was financially supported by PIBIC/ CNPq.

BM066 - Chromosomal size variation in *Trypanosoma cruzi* clones CL Brener and CL14 derived from the CL strain

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The examination of chromosomes by pulsed-field gel electrophoresis (PFGE) has revealed that length polymorphism is widespread in *T. cruzi*. The sizes and number of chro-

mosomal bands vary among strains and clones of this parasite. Most *T. cruzi* chromosome-length polymorphisms are currently uncharacterized with respect to content and origin. The objective of our project is to characterize the chromosome-length polymorphism and possible mechanisms of chromosome length change. The karyotypes of two cloned stocks, CL Brener and CL14 (derived from the CL strain) were studied by PFGE followed by ethidium bromide staining and hybridization with 30 different probes, 10 of which identified single chromosomes. Clones CL Brener and CL14 were isolated from the blood of mice infected with the parental CL strain and cloned on agar plates according to published protocols (Chiari & Camargo 1984, Genes and antigens of parasites, Fiocruz, pp. 95-109; Zingales *et al* 1997, 68:159). The ethidium bromide staining pattern of the gels revealed quantitative differences between the CL strain and its clones, and also either disappearence or appearence of several bands in the CL-strain derived clones. For instance, the parental strain and clone CL Brener exhibited a large chromosomal band of 2.6 Mbp which was not found in the clone CL14. In the CL14 this band underwent a large deletion resulting in a band of 2.0 Mbp. Due to the rearrangements of some chromosomes, we observed rearrangements in the chromosomal location of genes encoding immunodominat antigens (H49, CRA), calpain, α-tubulin, SL RNA, surface glycoproteins and repetitive DNA sequences. Our results suggest the existence of dispensable chromosomes and dispensable chromosome regions which could contribute to the variability of the *T. cruzi* karyotype. The range of karyotypes observed in *T. cruzi* indicates that many karyotypic changes may be genetically neutral, at least under some conditions. FAPESP, CNPq and CAPES.

BM067 - Genome size and molecular karyotype from isolates of *Trypanosoma cruzi* type I

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Molecular karyotype and genome size from different isolates (G and Trycc:1161 strains; clone DM28c) of *T. cruzi* type I were determined and compared to clone CL Brener (*T. cruzi* II). Total DNA content was estimated in hydroxyurea-arrested epimastigotes using an ultra-sensitive fluorescent nucleic acid stain for double-stranded DNA. The absolute DNA content of *T. cruzi* I epimastigotes was estimated at 0.08 to 0.10 pg/cell in the isolates of *T. cruzi* I and 0.12 pg/cell in CL Brener. Our data is agreement with previous report that suggested that the genome size of *T. cruzi* I isolates is smaller than that of *T. cruzi* II (Pedroso *et al.*, 2003). The isolates from *T. cruzi* I showed a variable number of chromosomal bands (17-20) comprised between 0.55 and 3.03 Mb. Many of the bands were present in different relative intensities suggesting that the number of individual chromosomes per cell could be considerably higher. Significant differences in molecular karyotype and in the chromosomal locations of the repetitive elements were found. We have determined the amount of repetitive sequences (minisatellite; E13; retrotransposons: C6, TcTREZO, L1Tc) and genes from transialidase superfamily. The relative abundance of these sequences was very similar among isolates from *T. cruzi I*. In agreement with previous report (Vargas *et al.*, 2004.), we confirmed that several repeated elements (minisatellite, TcTREZO, E13) were three- to four-fold more abundant in clone CL Brener. Despite the karyotype polymorphism, we have found that several the syntenic and linkage groups are conserved among the *T. cruzi* I isolates analyzed in this study. It has been suggested that the conservation of chromosome content is an important trait of the *T. cruzi* genome (Vargas *et al.*, 2004.). The range of karyotypes observed in *T. cruzi* suggests that many karyotypic changes may be genetically neutral. Supported by FAPESP, CNPq and CAPES

BM068 - Molecular analysis of the SSUrRNA coding sequence of kinetoplastid protozoan of the Furnas UHE reservoir (Alfenas, MG) indicated a possible new species of Neobodo.

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Free-living protozoan play important roles in the aquatic environment. At this milieu composed of a great nutritional diversity and considerably distributed all over the world, the protozoa can be used as bioindicators or in microbiological purification of water. The correct identification is a relevant problem for protozoan study and taxonomic classification. Nowadays molecular biology techniques have been used as a resource to surpass difficulty in the better identification and classification of these organisms. Using molecular approach, we started the characterization of two cultures of free-living flagellates protozoan isolated from Furnas UHE reservoir (MG). DNA of the organisms, from here named sp1 and sp2, provided template for PCR of the SSUrRNA gene using conserved primers that amplified the whole sequence. Products of about 2000bp for sp1 isolate and 1900bp for sp2 isolate were obtained, cloned and used for RFLP and sequencing analysis. Partial fragments of the 18S rRNA gene were sequenced for nucleotide alignment with literature kinetoplastid sequences. The RFLP analyses and the partial sequences of the 18S rRNA gene pointed to patterns similar but not identical to *Neobodo curvifilus* for isolate sp1 and *Neobodo designis* for isolate sp2. Nucleotide sequences of medRNA of both isolates showed to be similar to *Dimastigella* sp (Ribeiro, 2006); however, that organism displays the 5S rRNA coding sequence inside the medRNA

array, a fact that was not observed for the two isolates. Taking together, the results indicate that we probably have two new isolates of *Neobodo* or two new species of kinetoplastid species in the Furnas UHE reservoir, Minas Gerais, Brazil. Supported by: UNIFAL-MG, FAPEMIG, FAPESP, CNPq and FINEP.

BM069 - The proteasome inhibitor PSI blocks replication and development of *Trypanosoma cruzi*

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The 26S proteasome is a multicatalytic complex that plays important roles in the regulation of a broad cellular process including protein degradation and cell-cycle control from archaebacteria to mammalian. The identification of proteasome inhibitors has helped to define its role in various protozoan parasites processes. Thus, the objective of this work was to evaluate the effect of the proteasome inhibitor, PSI in the parasite growth *in vitro* and Chagas disease experimental development by *T. cruzi* Y strain. For analysis *in vitro*, $3x10^6$ parasites were incubated with different concentrations this inhibitor. The number of parasites was quantified in Newbauer chamber during ten days after the culture starting and showed 90% of parasite growth inhibition after 1μM of PSI addition in the LIT culture medium, suggesting that replication, probably requires proteasome activity. The experimental development in Chagas' disease observed 67,7% of parasitemia reduction detected in Swiss mice infected with $5x10^3$ blood trypomastigotes previously incubated with PSI. Histopathological analysis of heart, spleen, liver and skeletal muscle in the acute phase infection showed an increase of tissue parasitism in these organs, whereas the inflammatory process was reduced compared to the control group. Our results suggest that the proteasome activities are not essential for parasite cell invasion and transformation of tripomastigotes into amastigotes and amastigote replication, but the transformation into blood trypomastigotes and their releases into the blood circulation can be proteasome dependent. Taken together, our results and the fact that proteasome inhibitors are presently used as anti-cancer drugs forms a solid basis for further development and makes them potentially drugs also for Chagas‘ chemotherapy.

BM070 - Effects of Naphtoquinones upon proliferation of trypanosomatids and upon proteinases of *Trypanosoma cruzi*

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Chagas disease is a Latin America endemic infectious disease caused by the protozoon *Trypanosoma cruzi*. Chagas disease remains without an efficient treatment as all drugs presently used causes severe collateral effects on patients. Within this context, naphtoquinones appears as powerful chemotherapeutic agents for this illness, and are well known due to their action on biological activities of some microorganisms and against several lineages of cancer cells. These work main objectives are to evaluate the effects of naphtoquinones on epimastigotes forms of *Trypanosoma cruzi*, and to define one of its possible targets of action. Additionally, we evaluated these compounds interference on *Crithidia deanei* multiplication. Initially we carried out cytotoxicity tests in Vero cells with compounds in concentrations of $3,1\mu M$, $5,0\mu M$, $12,5\mu M$, $25\mu M$ and $50\mu M$, as well as tests to evaluate these compounds toxicity on *T. cruzi*. Additionally, assays were performed to evaluate a possible interference of these compounds on proteinase activity, located in the protein extract of epimastigotes forms of *T. cruzi* Dm 28*c* clone and *Y* strain. Results demonstrated that the orto-naphtoquinone β-lapachona shows powerful toxicity on Vero cells and high toxicity towards *T. cruzi*. However, Oxirano 10 did not showed cytotoxic effects on Vero cells, and presented high trypanomicidal activity, which was observed on *T.cruzi* and on *Crithidia deanei*. Data also indicated that β-lapachona showed inhibitory activity on cysteine proteinase from Dm 28*c* clone of *T. cruzi* in protein extract and Oxirano 10, showed inhibitory activity toward serine proteinase from Dm 28*c* clone and *Y* strain in protein extract. Finally, the set of data considered in our experimental drawing allows us to indicate that the β-lapachona and Oxirano 10 compounds can have effects on epimastigotes forms of *T. cruzi* multiplication after intervening with the proteinase activity of this parasite.

BM071 - Protein and mRNA associated to *T. cruzi* Dhh-1 containing granules.

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In trypanosomatids, regulation of gene expression occurs mainly at the post-transcriptional level. Accordingly, it is believed that mRNA stability and access to polysomes must be tightly regulated for allowing the rapid adaptations to different environmental conditions at which *T. cruzi* is exposed during its life cycle. Defined cytoplasmic foci containing non-translating mRNPs, known as P-bodies, have been reported in lower and higher eukaryotic cells. P-bodies are sites where mRNA can be decapped and degraded or stored for subsequent return to polysomes. Since there is evidence for the existence of non-translated mRNA in *T. cruzi* cytoplasm, we hypothesized that P-bodies must be present and functional in this parasite. The highly conserved DEAD box helicase Dhh1p is a marker protein of P-body functions. We have identified and cloned *T. cruzi* Dhh1 orthologue gene and showed that its expression is not regulated through the life cycle or under stress conditions. TcDhh1 is present as a free protein or in small to large polysome-independent complexes and localized to discrete cytoplasmic foci resembling P-bodies. These foci vary in number through the life cycle of the parasite, under nutritional stress conditions or puromycin/cycloheximide treatments. To evaluate the composition of these granules we have performed immunoprecipitation assays with polyclonal anti-TcDhh1 using epimastigotes lysates. The ribonucleoprotein complexes (mRNPs) were analyzed for protein content by mass spectrometry. Simultaneously, a ribonomic approach was used to identify the mRNAs associated with proteins within the complex. RNAs were purified from the immunoprecipitated mRNPs, amplified and hybridized against a *T. cruzi* oligonucleotide microarray. We are studying other putative components of P-bodies and defining the role of each one in the dynamics and function of these cytoplasmic foci. Altogether our data will contribute to get further insight into the mechanisms of gene expression regulation in *T. cruzi*. Financial support: CNPq, CAPES (Fiocruz).

BM072 - Binding analysis of complexes formed by TcPUF6 and its target mRNAs

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Unlike higher eukaryotic cells kinetoplastida regulate gene expression mainly post-transcriptionally. Control of mRNA stability, localization or translation is achieved through interaction of trans acting factors with sequences or structural elements present in the mRNAs 3'UTR. PUF proteins reduce target mRNA expression by repressing translation or inducing mRNA deadenylation and subsequent degradation. In Trypanosoma cruzi, the PUF protein family consists of ten proteins. In previous work we have characterized TcPUF6, a member of the Pumilio family in T. cruzi. TcPUF6 is expressed in the different life cycle forms of the parasite showing no clear stage specific regulation and it is localized to multiple discrete foci in the cytoplasm of epimastigotes. By affinity purification of TcPUF6 containing mRNPs and microarray hybridization with the purified mRNAs we identified the putative target transcripts of the TcPUF6 protein. To characterize the binding of TcPUF6 to its putative targets we subjected a recombinant protein and different RNA probes to electrophoretic mobility shift assays. A PGEX4T plasmid containing the recombinant GST-TcPUF6 protein was transformed in E. coli GroELS strain. Cells were stressed by addition of 3% ethanol prior to IPTG induction which allowed us to recover at least 10% of the recombinant protein in its soluble form. We tested the binding specificity of GST-TcPUF6 by using different oligoribonucleotides as probes. We tested probes with the core sequence of the PUF family binding element (UGUR) and in vitro transcribed probes with the sequence of the 3'UTRs of the putative targets. We analyzed the specificity and apparent dissociation constants of the ribonucleoprotein complexes formed in order to asses the target RNA sequence recognized by TcPUF6. We are currently identifying other putative TcPUF6 regulated mRNAs by pull-down assays followed by microarray hybridization.
Financial support: CNPq, FIOCRUZ, PRONEX/Fundação Araucaria. G.A.A. holds a fellowship from Fundação Araucaria

BM073 - Pre-SSU rRNA *trans*-splicing in trypanosomatids

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In kinetoplastids *trans*-splicing of a hypermethylated capped spliced-leader (SL) to polycistronic pre-mRNA precursors is an essential step in the generation of mature translatable mRNAs. However, preliminary experiments in our laboratory suggested that 5' ETS rRNA region of *Typanosoma cruzi* and *Leishmania amazonensis* could also include native 3' *trans*-splicing acceptor sites. We first identified and characterized this acceptor sites in *Trypanosoma brucei, Trypanosoma cruzi, Leishmania (Leishmania) amazonensis* and *Crithidia fasciculate* using a semi-nested RT-PCR strategy

(Mayer& Floeter Winter, 2006). Sequence analysis of RT-PCR products showed that generation of SL-5'ETS hybrid molecules follow the same laws as for pre-mRNA *trans*-splicing. To strengthen that SL-5'ETS molecules indeed correspond to native processed products we used two additional techniques for their detection in *L. (L.) amazonensis*. Using RNAse protection approach, we identified a transcript fragment with the expected molecular weight for SL-5'ETS hybrids as well as a fragment corresponding only to the 5'ETS molecule, although the proportion of SL-5' ETS/5'ETS molecules was very low. Primer extension experiments, using two oligonucleotides complementary to 5'ETS region, identified the same 5' end which correlates to an expected end point for the SL-5'ETS hybrid molecule. For the first time our results provide strong evidence that the RNA pol I transcribed pre-SSU rRNA of trypanosomatids is processed by SL-addition*trans*-splicing, suggesting an antique behavior of RNA molecules to join each other even after a partial specialization of RNA polymerases. We are currently performing real-time RT-PCR experiments to accurately quantify the proportion of SL-5'ETS/5'ETS molecules. The broadening of the process in relation to the total rRNA processing can give clues of a possible physiological role. Supported by FAPESP and CNPq

BM074 - TcYchF - A NOVEL GTP-BINDING PROTEIN ASSOCIATED WITH THE TRANSLATION MACHINERY OF THE PROTOZOAN Trypanosoma cruzi

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We describe the characterization of a gene encoding a GTP-binding protein, TcYchF, potentially involved in translation in *T. cruzi*. This gene has a coding region of 1,185 base pairs long and encodes a 44.3 kDa protein. BlastX searches showed that TcYchF is highly similar (45 to 86 %) to other putative GTP-binding proteins from eukaryotes, including some species of trypanosomatids *(Leishmania major and Trypanosoma brucei)*. A lower but significant level of similarity (38 to 43%) was also found between the predicted sequences of TcYchF and bacterial YyaF/YchF GTPases of the Obg family. Important features of the G domain of this family of GTPases are conserved in TcYchF. Although the function of YyaF/YchF is unknown, other members of the Obg family are known to be associated with ribosomal subunits. Immunoblots of the polysome fraction from sucrose gradients showed that TcYchF was associated with the ribosomal subunits and polysomes. Moreover the ablation of the *T. brucei* homolog of TcYchF by RNA interference inhibited the growth of the procyclic forms of the parasite. Taken together these data suggest an important role of this protein in the translational machinery of trypanosomes.

BM075 - THE RNA RECOGNITION MOTIF PROTEIN (TcRRM) IS DIFFERENTIALLY EXPRESSED DURING Trypanosoma cruzi LIFE CYCLE

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The identification and characterization of RNA binding proteins in *T. cruzi* is particularly relevant as they may play key roles in the regulatory mechanisms of gene expression. We have identified two coding genes for TcRRM1 and TcRRM2 which contain two RNA binding domains (RRM) each and are very similar to two *T. brucei* RNA binding proteins previously reported, Tbp34 and Tbp37, and to an annotated ORF in *Leishmania major* genome project. The *T. cruzi* RRM genes are organized in a tandem of at least 8 copies, alternating with copies of Tcp28, a gene of yet unknown function. However, TcRRM transcripts accumulation is higher in the spheromastigote stage, while for Tcp28 this accumulation is higher in the trypomastigote stage. Polyadenylation and trans-splicing sites were mapped for TcRRM and Tcp28 genes, and the untranslated regions are being analyzed in order to identify possible cis elements involved in this developmental regulation. Immunofluorescence assays using Tbp34/37 antibodies suggest that TcRRM have cytoplasm localization in the three different cell stages. Gel shift assays are being performed in order to verify the binding of TcRRM to specific oligoribonucleotides.

BM076 - A 62 BASE PAIR INDEL IN THE INTERGENIC REGION OF THE UNIVERSAL MINICIRCLE SEQUENCE BINDING PROTEIN (TcUMSBP) LOCUS AFFECT THE POLICISTRONIC RNA PROCESSING

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The kDNA is formed by minicircles and maxicircles con-

nected in a net-like structure. The replication of minicircles is not completely understood and most of this knowledge is due to studies in Crithidia fasciculata. Individual minicircles is known to contain a dodecamer highly conserved denominated Universal Minicircle Sequence (UMS). The UMS, which is the replication origin, binds to the Universal Minicircle Sequence Biding Protein. Our laboratory has characterized the Universal Minicircle Sequence Biding Protein of Trypanosoma cruzi (TcUMSBP). The protein contains five zinc-fingers motifs and presents 60% of identity with the corresponding protein of Crithidia fasciculata. The TcUMCBP gene locus presents two alleles in the T. cruzi CL Brener clone, which differ by the presence of the indels containing 62bp and 2,0 Kbp. We have mapped the polyadenylation and trans-splicing sites in the TcUMSBP gene locus. The 62bp indel is localized in the 5‘ intergenic sequence of the TcUMSBP gene and affects the mRNA processing generating two sites of polyadenylation for the upstream gene (the beta five proteasome subunit). We have investigated the sequences involved in the mRNA processing in this locus. We have cloned the 5‘ and 3‘ untranslated region (UTR) of the TcUMSBP and HSP70 genes in plasmids containing the Chloramphenicol acetyl transferase (CAT) reporter gene and transfected in Trypanosoma cruzi. The 62bp indel results in a differential accumulation of the polycistronic and the mature transcripts originated from each allele. The presence or absence of the 62bp in the TcUMSBP intergenic region also revealed different secondary structure, suggesting a role in the control of RNA processing.

BM077 - Structure of the A6 mRNA/gRNA pair involved in trypanosome mitochondrial RNA editing

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Sleeping sickness and nagana are diseases caused by *Trypanosoma brucei*, which is transmitted by the tsetse fly in Africa. In the vector, *T. brucei* survival relies on the expression of mitochondrial genes and most of these genes have their mRNAs edited to become translatable to functional proteins. RNA editing comprehends the insertion and deletion of uridylates (Us), a developmentally regulated process that is directed by guide RNAs (gRNAs) and catalyzed by a multicomplex of proteins determined editosome. It is uncertain how the editing complex is assembled onto specific RNAs. There are no conserved sequences but it is known that RNA structure affects editing. It has been suggested that different mRNA/gRNA pairs fold into a similar structure and we hypothesize that recognition of this common structure may be important for efficient editosome assembly. The secondary structure for the apocytochrome b (CYb) pair has been previously determined and corroborates with our model of a three helical structure. One helix is formed by the gRNA itself and very curiously is maintained throughout the first few editing events. In this work we used solution structure probing and crosslinking experiments in vitro to determine the structure of the ATPase subunit 6 mRNA with gA6-14 gRNA. Our results indicate that the A6 pair also folds into a three-helical structure like the CYb pair, supporting our hypothesis. We also observed that as editing proceeds through 3 editing sites, the gRNA U-tail does not seem to move from its preferential binding site on the mRNA and the gRNA helix is maintained. With these experiments we conclude that at least two different mRNA/gRNA pairs fold to form a three-helical configuration that persists after a few editing events and this core could potentially serve as the recognition target for the editosome.

BM078 - Conformational Stability of Oligopeptidase B from *Trypanosoma cruzi*

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Prolyl Oligopeptidases of *Trypanosoma cruzi* are potentials drug targets for Chagas disease chemotherapy. Oligopeptidase B of *Trypanosoma cruzi* (OligoBTc), a member of proly oligopeptidase family, seems to trigger the lysosomes recruitment that is crucial to parasite invasion into mammalian cell by a dependent-Ca2+ process. In this work, we analyzed the conformational stability of OligoBTc, under different conditions, in order to obtain structural data and to understand the kinetics of this enzyme. To this end, the OligoBTc gene was amplified by PCR and cloned into pET-19b. The recombinant OligoBTc was expressed in *E. coli* BL21(DE3) and purified by affinity in nickel-agarose resin. The fully active recombinant OligoBTc allowed us to study its unfolding process associating it with pH, thermal and chemical stability through circular dichroism and fluorescence emission experiments. The enzyme s secondary structure content was predicted using the spectra data obtained by circular dichroism. The physicochemical characterization of OligoBTc can help establish a better condition to perform biological assays, to understand its interactions with the environment and host cells and to facilitate its structural analysis. Supported by CNPq.

BM079 - Approaches to the identification of protein-protein interactions for the *T. cruzi* Pumilio TcPuf 6 protein.

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In trypanosomes regulation of gene expression occurs mainly at the post-transcriptional level. However the molecular bases of this process are still poorly understood and only

a few regulatory factors have been characterized. Pumilio proteins are RNA binding proteins that modulate mRNA expression in lower and higher eukaryotes. We have previously identified TcPuf6, a Pumilio protein, that is expressed throughout the *T. cruzi* life cycle and is localized to multiple discrete foci in the cytoplasm of epimastigotes. In addition we have demonstrated that TcPuf6 shares the Pumilio protein highly conserved characteristic of *in vitro* binding to the *Drosophila hunchback* NRE (*nanos* response element). With the aim of getting an insight into the TcPuf6 cellular functions, among other approaches, we have tried to identify its protein-protein interactions. In order to accomplish this goal, a pTEX vector which expresses a fusion of TcPUF6 to the TandemAffinityTag (TAP tag) sequence has been constructed. Transfectant *T. cruzi* epimastigotes have been selected and the correct expression of the fusion protein has been verified. Protein extracts of the transfected epimastigotes were submitted to recommended standard affinity purification steps followed by PAGE, Western-Blot and finally Maldi-TOF. In addition, we carried on an approach based on bioinformatics. Firstly we analyzed TcPuf6 within the Pumilio protein family of the Tritryp. Peculiarities on the primary, secondary and predictive tertiary structures (when available appropriate information) for the Tritryp Pumilio proteins have been observed. Finally, we studied the homologies to Pumilio proteins with reported cellular function. In spite of the increased number of Pumilio proteins in lower eukaryotes compared with the higher ones, we aimed to build an updated predictive protein-protein interaction map for the *T. cruzi* Pumilio proteins.

BM080 - Investigation of an alternative function for the Tryptophanyl tRNA Synthetase of *Leishmania major*

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Tryptophanyl tRNA Synthetase (TTRS) is the enzyme responsible for the activation of Tryptophan prior to protein synthesis. In humans, a shortened version of TTRS, generated by alternative splicing and induced by IFN$-\gamma$, inhibits vascular formation. A similar polypeptide, endowed with the same biological activity, can be generated by treating TTRS with leukocyte elastase.

The *Leishmania major* genome contains two TTRS genes, coding for the cytosolic and mitochondrial forms of the protein. The mitochondrial TTRS gene is located on the H region, a locus which is amplified in lineages selected under drug pressure. The H region also encodes the Pteridine Redutase 1 gene (PTR1), which is associated with the parasite‘s virulence. Considering that the parasite‘s TTRS expression within the vertebrate host could lead to the formation of its truncated version, we speculate if these proteins could play a relevant role in the host$-$parasite interaction.

In order to investigate an alternative function for the parasite TTRS, each gene was amplified from *L. major* genome by PCR and cloned into pET28a vector His-tag site. These clones were transformed into *Escherichia coli* BL21 Rosetta strain. The conditions for heterologous expression are currently being optimized. These genes were also cloned into the shuttle vector pXG1, which will allow the generation of overexpressing lineages of the parasite. The recombinant TTRS will be subjected to leukocyte elastase activity and the products generated will be tested as antagonists of an angiogenic factor such as VEGF (vascular endothelium growth factor). Supported by FAPESP and CNPq.

BM081 - The role of chromosome context in site-directed disruption of subtelomeric targets of *Leishmania major*

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Gene targeting strategies are invaluable tools for the study of gene function in the protozoan *Leishmania.* However, attempts to disrupt essential genes may lead to gene amplification and/or changes in the parasite‘s ploidy. We have previously shown that attempts to target a telomere-located essential gene did not elicit the typical phenomena of gene amplification, translocation and/or changes in ploidy. Instead, despite extensive rearrangements, the locus preserved its copy number and location. The aim of this work is to investigate the role of different subtelomeric regions in the genetic variability elicited by targeting genes encoded therein. We have targeted intergenic sequences across the subtelomeric region of chromosomes 6 and 20. To target chromosome 6 ends, a 1.7 Kb fragment containing the region between ORFs LmjF06.0020 and LmjF06.0030 was amplified by PCR and clonned in vector pGEM T-Easy. The fragment contains a single XhoI restriction site that was used to clone the selection cassette SAT (Streptotrycin Acetyl Transferase). This construction was linearized and transfected into *Leishmania major* for integration by homologous recombination. The heterozygous mutant will be subjected to loss of heterozygosis (LOH) protocols and the anatomy of the integrated locus will be investigated in selected cell lines. Supported by FAPESP and CNPq.

BM082 - *Trypanosoma cruzi* ecto-nucleotidase activity, infectivity and virulence relationship in mouse infection

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Recently, ecto-nucleotidases have been implicated in the control of many biological processes such as immune response, cellular adhesion, pathogen infectivity and virulence. In this context, the main goal of this work is to evaluate the effects of apyrase inhibitors in *T. cruzi* infectivity and virulence using the Swiss mouse as a model of infection. Blood trypomastigotes from Y strain were expanded by one passage in VERO cells culture, recovered and used to evaluate ecto-nucleotidase activities and in infection experiments after pre-treatment with or without apyrase inhibitors (Suramin, Gadolinium and ARL). *In vitro* trypomastigotes ecto-nucleotidase activities presented the following order of inhibition Suramin>Gadolinium>ARL. Suramin inhibited approximately 70% and 50% of ATPase and ADPase activities, respectively. In the same way, Gadolinium inhibitory effects achieved 30% and 60% and ARL showed 30% and 47% of inhibition. Groups of mice were infected with parasites pre-incubated with apyrase inhibitors (suramin and gadolinium) at concentrations that achieved maximum inhibitory effects. The results showed a significantly decrease in infectivity, parasitemia and mortality when gadolinium was used as apyrase inhibitor. The highest ADPase inhibitory activity of gadolinium could suggest that this enzyme may be involved in the establishment of the infection. Our results showed that the inhibition of *T. cruzi* ecto-nucleotidase activity could change parasite infection course and will became a new good target to interfere in this disease. Supported by: UFOP, UFV, CNPq, FAPEMIG

BM083 - Effect of Aureobasidin A on *Leishmania (Leishmania) amazonensis* promastigotes

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Aureobasidin A (AbA) is an antifungal antibiotic that inhibits the inositol phosphorylceramide (IPC) sintase, which is present in fungi, plants, and some protozoans such as *Trypanosomatids*. Recently we described that AbA decreased the IPC synthesis, and inhibited the growth of axenic cultures of *Leishmania (Leishmania) amazonensis* promastigote forms in about 90%. Promastigote growth curves returned to normal after AbA removal from culture medium, indicating that the AbA did not affect the parasite viability. Since mitogen-activated protein kinase (MAPK) pathways are conserved in eukaryotes and are often involved in cell growth and differentiation, in the present study we analyzed the presence of MAPK kinase kinase (MEK kinase) in AbA treated *Leishmania*. In a preliminary study, *L. (L.) amazonensis* promastigotes were grown in absence of serum for 24 hours, and after this period it was added complete medium containing or not AbA (10μM). Parasite proteins were analyzed by Western Blot using anti-phospho-MEK1/2 antibody. It was observed that the expression of pMEK1/2 is decreased in AbA treated *L. (L.) amazonensis* promastigotes, suggesting that MAP kinase pathway is probably affected by this drug. On the other hand, by flow cytometry assays using propidium iodide, it was observed no difference in the DNA content of AbA treated parasites in comparison to control, i.e., it was not observed any non-phase specific arrest of the cells. The elucidation of MAP kinase pathways, such as MEK kinase, affected by AbA treatment, and also the involvement of IPC and other products in the parasite growth may provide new insights about the mechanism of action of this antibiotic in *Leishmania*. Supported by FAPESP and CNPq.

BM084 - RNA aptamers as inhibitors of the glucose transport in *Trypanosoma cruzi*

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The protozoan parasite *trypanosoma cruzi* is the etiological agent for Chagas‘ disease. It presents a high rate of glucose consumption, a main energy and carbon source. Many enzymes involved in the glucose metabolism have been proposed as target for development of anti-trypanosome drugs. In this way the inhibition of the glucose transporter can constitute an efficient strategy to block glycolysis, allowing the control of the parasite infection. In this study we have obtained, by the first time to our knowledge, an aptamer which inhibits the *T. cruzi* glucose uptake. We determined the number of glucose binding sites for one epimastigote parasite cell, being 0.075 fmol. Ten rounds of *in vitro* selection using live epimastigotes as target and 30 mM glucose to elute RNA molecules bound to glucose transporters were performed. RNA pools from seventh and tenth round of *in vitro* selection were evaluated for inhibition of the glucose uptake into the parasites. The transport of glucose was determined as the incorporation of [^{14}C]-U-glucose by the parasite for 30 seconds in the presence or absence of selected RNA molecules. Glucose transport inhibition assays with the 7th round RNA pool showed a competitive inhibition profile, with an inhibition constant (K_i) of 1.2 - 2.0 μM. We are currently identifying individual aptamer clones by DNA sequencing. Individual identified RNA aptamers will be further characterized. Supported by FAPESP and CNPq.

BM085 - ANALYSES *In vitro* AND *In vivo* OF INFECTIVITY CAPACITY OF TRYPOMASTIGOTES TRANSFECTANTS FORMS OF *Trypanosoma cruzi* OVEREXPRESSING A TcPUF6, A PUMILIO-LIKE PROTEIN.

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Gene expression in *Trypanosoma cruzi*, a kinetoplastid parasite that causes Chagas‘ disease, is essentially regulated at the posttranscriptional level. This regulation involves specific interactions between regulatory transacting factors and conserved cis elements present in the 5‘ and 3‘ untranslated regions (UTR) of the transcripts. The PUF (Pumilio/FBF) protein family controls mRNA stability and translation in eukaryotes and several members of these posttranscriptional regulators have been identified in trypanosomatids. Recently, we showed that the TcPUF6, a member of the *T. cruzi.* PUF family, is expressed by various stages of the parasite life cycle and is not associated with the translation machinery. In this study, we analyzed the infectivity capacity of TcPUF6 overexpressing trypomastigotes transfectants using *in vitro* and *in vivo* systems. Groups of BALB/c mice were inoculated intraperitoneally with TcPUF6 and control (pTEX vector) metacyclic trypomastigotes obtained *in vitro* conditions. The number of circulating parasites was significantly higher in mice from TcPUF group than the control group and the parasitemia also persisted for a longer period. The mortality rate of the hosts was higher in control group. In *in vitro* assays, the TcPUF6-overexpressing parasites displayed a higher capacity for VERO cell infection and for survival and multiplication in the cell cytoplasm. But, the TcPUF6 parasite populations were slightly less able to infect purified peritoneal macrophages and far fewer intracellular amastigotes per cell were observed for these populations, when compared with control parasites. These results suggest a decrease in the capacity of macrophages to eliminate the transfected parasites, potentially resulting in the large number of circulating parasites observed in the *in vivo* assays. TcPUF6 overexpression seems to affect host mechanisms involved in parasite clearance and the higher parasitemia observed may result from this effect and from an increase in the ability of the transfected parasites to infect and replicate in nonphagocytic cells.

BM086 - BIOCHEMICAL AND BIOLOGICAL STUDIES WITH RECOMBINANT *TRYPANOSOMA CRUZI* APYRASE (NTPDASE I)

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An ecto-apyrase activity was characterized on the surface of *T. cruzi* and a 2282 bp cDNA encoding a full-length NTPDase was cloned (Fietto et al., 2004). Trypomastigotes were shown to have a 2:1 ATP/ADP hydrolysis ratio, while epimastigotes presented a 1:1 ratio, suggesting a possible role for the NTPDase in the parasite's virulence mechanism. To further characterize *T. cruzi* NTPDaseI we performed heterologous expression of active recombinant enzyme. *In silico* analyses of the sequence predicts a possible cleavage signal peptide at amino acid position 36, immediately following an amino-terminal predicted transmembrane segment suggesting that NTPDaseI could be produced as a soluble exported protein. Using this information we designed a strategy to express the soluble NTPDaseI. Full-length NTPDaseI cloned in pGEM vector was used as template to amplify a 1700 bp DNA fragment that was transferred to pET21b vector. This construction was used to transform *E. coli* BL21 cells. Recombinant protein was expressed after 1 hour of induction. Soluble and insoluble recombinant apyrases were purified from bacterial lysates using Ni-NTA-agarose and showed specific activity for ATP hydrolysis between 2-17 nmol.mg protein^{-1}.h^{-1}. Substrate specificity assays showed preference for tri-phosphate nucleotides. Activity was higher at pHs between 6.5 - 7.5 and in the presence of Mg^{2+} rather than Ca^{2+}. Apyrase inhibitors were tested and only Suramin inhibited NTPDAseI significantly. We concluded that rNTPDaseI was produced in an active form that should be suitable to start crystallization tests and to evaluate its potential as new target in specific drug design tests, such as the use of suramin.

BM087 - Studies of Anti-Silencing Factor 1 (ASF1) and its roles in Trypanosomatids

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ASF1 is a histone chaperone that contributes for the histone deposition during the nucleosomes assembly in newly replicated DNA. Moreover, it has been shown that activation of ASF1 is involved in the cell response to DNA damage. In our early studies, we have shown that *ASF1* knockdown in *T. brucei* leads to an arrest in the cell cycle progress of procyclic forms in axenic culture. Following these studies, DAPI-stained *T. brucei* procyclics were analyzed under fluorescence microscopy using the number of nucleus (N) and kinetoplasts (K) as markers of cell cycle position. We found that after RNAi induction the 1N1K population decreased from 88 to 63% with a concomitant marked increase of the 0N1K population (from 3 to 26%). These results suggest an inhibition of nuclear division following the kinetoplast segregation, resulting in anucleated daughter cells. Similar forms previously reported were taken as anticipated changes from a mitotic arrest. In addition, the alkylant agent Methyl Methanesulfonate affected the growth rate of *T. brucei ASF1*-depleted mutants. Preliminary results suggest that over production of ASF1 mRNA in *L. major* turn the parasite more resistant to this DNA damaging agent, as indicated by the analysis of karyotype integrity after an acute treatment (15 minutes) with MMS. These facts suggest that a balanced *ASF1* expression is important to the processes of DNA damage repair in trypanosomatids. We have found that *ASF1* overexpressors have a chromatin structure more resistant to MNase digestion than wild-type cells suggesting that the level of chromatin packaging of *Leishmania* is affected by *ASF1*overexpression. Therefore, this tight condensed state of chromatin could be an obstacle to the access of the DNA repair machinery. We may hypothesize that this effect would lead to a delay in the growth, and at the same time, would protect the chromosomes from the MMS action.

BM088 - CIS-REGULATORY AU-RICH ELEMENT MODULATES THE EXPRESSION OF ALPHA-TUBULIN mRNA IN *TRYPANOSOMA CRUZI*

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α/β-tubulin mRNAs expression in *T.cruzi* is under an autoregulatory control that affects these transcript half-lives during the parasite life cycle. Whereas epimastigotes present high levels of tubulin mRNAs, an excess of free tubulin monomers is correlated to a decrease in tubulin mRNA levels in amastigotes. This reduction is not a result of changes in transcription; instead, it is due to a reduction in α and β tubulin mRNA half-lives. By incubating epimastigotes with vinblastine (a microtubule depolymerization inducer) we have observed an inverse correlation between free tubulin and mRNA stabilization. Transient transfection assays have indicated that the 3UTR and the first four aminoacids of the α-tubulin might be involved in mRNA destabilization. Therefore, the aim of this work is to further investigate the role of 3UTR of the α-tubulin mRNA (and more precisely, the AU-rich element present within this region) on the control of mRNA abundance. Stably transfected epimastigotes with plasmids containing the wild-type or ARE-deleted α-tubulin 3UTR downstream the luciferase reporter gene were generated. Our results suggest that, similar to tubulin expression in amastigotes, luciferase mRNA containing the deleted element is less abundant and less stable in epimastigotes when compared to the mRNA containing the wild-type 3UTR. Sequences present in the α-tubulin 3UTR however, do not interfere with the destabilization of α-tubulin mRNA in response to vinblastine treatment. These findings indicate that differently from other AREs found in trypanosomatids, which cause degradation of mRNAs, the α-tubulin ARE is an stabilizing element responsible for maintaining high levels of α-tubulin mRNA in epimastigotes. We are currently investigating the role of α-tubulin 5UTR and coding sequences as well as possible interactions between regulatory trans-acting factors and ARE-binding domain on α-tubulin mRNA stability. Financial Support: CNPq, FAPEMIG and Howard Hughes Medical Institute.

BM089 - Post-transcriptional mechanisms involved in the control of expression of *Trypanosoma cruzi* GP82 gene: AU-rich elements in the 3'-UTR of GP82 mRNAs.

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Sequence elements rich in A and U nucleotides, called AU-rich elements (AREs) are able to target mRNAs for rapid degradation. These elements are found in the 3′-UTR of several mammalian mRNA transcripts. It has been shown that *T. cruzi* mucin mRNA steady-state level is developmentally regulated by post-transcriptional mechanisms, being stable in the epimastigote insect vector stage, but unstable in the trypomastigote infective stage of the parasite. Its turnover is controlled by an AU-rich element (ARE) localized in the 3′-untranslated region (3′-UTR) (Di Noia *et al.* 2000, JBC 275:10218).
Examination of the GP82 gene (GenBank L14824) reveals a 230 nucleotides 5′-UTR, a 1.55 kb protein coding region,

and a 0.34 kb 3′-UTR. The 3′-UTR contains one AUUUA (ARE) sequence which may bind cytosolic proteins and modulate mRNA stability. To further analyze the 3′-UTR of GP82 gene, we isolated cDNA clones from a metacyclic form library. Comparison of 3′-UTR sequences of eight GP82 clones revealed that there are three AU-rich regions, which sizes are 57, 99 and 153 nucleotides, respectively. Two AUUUA motifs are present in the 3′-UTR of GP82 mRNAs. This pentamer was reported to be essential and the minimal sequence motif of AREs. The AU_4A motif, present on mucin S group transcripts, also appeared twice in the 3′-UTR of GP82.
We tested if the AU-rich sequences found in the 3′-UTR of GP82 transcripts were functional by transfection of epimastigotes with pTEX vector carrying the coding region of Green Fluorescent Protein in fusion with the 3′-UTR of GP82. From the experiments described above we suggest that 3′-UTR AU-rich regions might be responsible for the control of GP82 levels, functioning as a destabilizing element in epimastigotes, but being protected in metacyclic trypomastigotes.

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BM090 - Finding putative regulatory motifs in the 5' and 3' Untranslated Regions of *Leishmania spp.* genes: an *in silico* approach

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The recent availability of the complete sequences of *L. braziliensis*, *L. infantum* and *L. major* genomes is a landmark and should accelerate understanding of these pathogens biology. Comparative analysis of multiple genomes is a powerful tool to expand the understanding of genetics and evolutionary bases of shared and distinct features of each species. Trypanosomatids protein-coding genes are grouped in long polycistronic units of unrelated genes and accumulating evidence indicates that control of gene expression happens by diverse mechanisms at the posttranscriptional level. Our goal is to conduct a computational analysis of sequence conservation in the 5' and 3' untranslated regions (UTR) of annotated protein coding genes in the three genomes to unravel regulatory elements. Those conserved elements will be further explored and validated. In our in silico approach, taking *L. braziliensis* as model organism, we developed a sequence analysis pipeline to find conserved intercoding sequences in the three genomes and to group them according to their neighboring CDS putative function using the Gene Ontology (GO) Family categories. Many conserved sequences were found and six putative regulatory motifs were selected to be statistically validated by another algorithm (MEME). These elements flank orthologous genes from five GO families: tetrahydrobiopterin biosynthesis (1), hydrogen peroxide metabolism (2), histone acetylation (1), histone deacetylation (1) and pathogenesis (1). We are currently exploring the role of these elements using Electrophoresis Mobility Shift Assay (EMSA) to investigate possible protein-DNA and/or protein-RNA interactions and northern blotting analysis to verify the target-mRNAs expression levels in different life stages of the parasite. Supported by FAPESP and CNPq.

BM091 - A 62 BASE PAIR INDEL IN THE INTERGENIC REGION OF THE UNIVERSAL MINICIRCLE SEQUENCE BINDING PROTEIN (TCUMSBP) LOCUS AFFECT THE POLICISTRONIC RNA PROCESSING

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The kDNA is formed by minicircles and maxicircles connected in a net-like structure. The replication of minicircles is not completely understood and most of this knowledge is due to studies in *Crithidia fasciculata*. Individual minicircles is known to contain a dodecamer highly conserved denominated Universal Minicircle Sequence (UMS). The UMS, which is the replication origin, binds to the Universal Minicircle Sequence Biding Protein. Our laboratory has characterized the Universal Minicircle Sequence Biding Protein of *Trypanosoma cruzi* (TcUMSBP). The protein contains five zinc-fingers motifs and presents 60% of identity with the corresponding protein of *Crithidia fasciculata*. The *TcUMCBP* gene locus presents two alleles in the *T. cruzi* CL Brener clone, which differ by the presence of the indels containing 62bp and 2,0 Kbp. We have mapped the polyadenylation and trans-splicing sites in the *TcUMCBP* gene locus. The 62bp indel is localized in the 5‘ intergenic sequence of the *TcUMCBP* gene and affects the mRNA processing generating two sites of polyadenylation for the upstream gene (the beta five proteasome subunit). We have investigated the sequences involved in the mRNA processing in this locus. We have cloned the 5‘ and 3‘ untranslated region (UTR) of the *TcUMCBP* and HSP70 genes in plasmids containing the Chloramphenicol acetyl transferase (CAT) reporter gene and transfected in *Trypanosoma cruzi* The 62bp indel results in a differential accumulation of the polycistronic and the mature transcripts originated from each allele. The presence or absence of the 62bp in the *TcUMCBP* intergenic region also revealed different secondary structure, suggesting a role in the control of RNA processing.

BM092 - POST-TRANSCRIPTIONAL REGULATION OF HSP70, HSP60 and HSP10 GENES IN *Trypanosoma cruzi*

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The understanding of the control of gene expression of members of the chaperone family will shed light on the mechanisms of post-transcriptional regulation through which trypanosomatids govern the fate of most of their mRNAs. The gene organization and gene expression pattern of HSP70, HSP60 and HSP10 genes of *Trypanosoma cruzi* have been previously characterized by our group. Our aim is to investigate the presence and function of heat shock-responsive elements in the mRNAs of these genes. Plasmids containing the chloranfenicol acetyltransferase (CAT) reporter gene under the control of the 18S rRNA promoter were constructed in which the CAT gene is flanked by of intergenic regions containing either the 5‘ or 3‘ UTR and their respective regulatory sequences of the HSP70 mRNA. Rab7 5‘ and 3‘ UTR-containing sequences were used as control plasmids. CAT assays of transiently transfected epimastigotes show that heat shock-responsive elements are present in both the 5‘ and 3‘ UTRs of HSP70 mRNA. We have constructed a new set of plasmids with deletions of 5‘ and 3‘ UTR to look for the responsive heat-shock element. A similar series of reporter plasmids are being constructed with HSP10 and HSP60 sequences. CAT mRNA levels of transfected cells are being determined to assess the contribution of mRNA stability and its translation in the induction of the CAT enzyme. We are also determining the half-life of the endogenous HSP10, HSP60 and HSP70 mRNA under stressing and non-stressing conditions. For HSP70, our preliminary results suggest a half-life of 40 minutes at 29^oC, increasing to 2 hours at 37^oC. The kinetics of this mRNA stabilization is currently being investigated. Supported by CNPq and FAPERJ.

BM093 - Study of the effect of chromosome location in the expression of a telomere-located essential gene of *Leishmania major*

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The protozoan parasite *Leishmania major* is mostly diploid and believed to have an asexual life-cycle. Attempts to inactive its essential genes invariably induce DNA amplification, translocation and/or changes in ploidy, revealing significant genome plasticity. We have generated a heterozygous mutant for an essential gene encoding the putative second-largest subunit of RNA Polymerase III *(RPC2)*, the nearest gene to one of the telomeres of the parasite‘s chromosome 20. The heterozygous mutant was subjected to drug pressure to induce loss of heterozygosis (LOH). However, we were unable to select a *RPC2* null mutant, even after the functional complementation of the gene in an episomal context. The selected mutants did not carry amplicons and maintained the number of *RPC2* copies. Here we investigated the organization of the *RPC2* locus in the mutants selected after the complementation and LOH protocols. The anatomy of the locus of different mutants was analyzed in Bal 31 assays followed by restriction and Southern analysis. The results revealed that despite the extensive rearrangement of the locus, the *RPC2* gene was maintained at the telomere end of chromosome 20. These findings underline the importance of chromosome location in the control of expression of the parasite genes. Current experiments are investigating the relevance of the genomic context in the expression of the *RPC2* gene of *L. major.* Supported by FAPESP and CNPq.

BM094 - (TG/CA)n ROLE ON GENE EXPRESSION IN T. cruzi.

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The kinetoplastid protozoan Trypanosoma cruzi is the causative agent of American trypanosomiasis or Chagas disease, which affects millions of people in Central and South America. Absence of canonical signals for transcription by RNA polymerase II is a distinction of trypanosomatids. Previously, our group suggested that the repeated sequence (TG/CA)n could constitute a signal in T. cruzi. In order to define the putative functionality of this repeat on expression, we constructed vectors bearing cat reporter gene under RNA pol II control and the (TG/CA)n elements were inserted in the 3' and 5' UTR regions. T. cruzi epimastigotes were transfected with these vectors and the insertions in the plasmids from the selected parasites were checked. CAT protein levels were analyzed by ELISA and normalized according to both the vector copy number obtained by real time PCR and total protein level. When (TG/CA)n are inserted in the UTRs a statistically significant increase of the reporter protein were observed (three fold increase for TG and ten to thirty for CA). These results could be suggesting different ways of action of the TG and CA repeats. In order to distinguish between functional role at transcriptional and/or translational levels mRNA stationary levels and stability were analyzed.

BM095 - Molecular characterization of UDP-GlcNAc:Thr polypeptide α-*N*-acetyl-D-glucosaminyltransferase-like (pp-αGlcNAcT) genes of *Trypanosoma cruzi* and heterologous expression in *Leishmania tarentolae*

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Mucin-like glycoproteins, the major surface components of the protozoan parasite *Trypanosoma cruzi*, appear to participate in parasite-host interactions, but genetic evidence is lacking. Their *O*-glycans are attached via α-*O*-*N*-acetylglucosamine linked to Thr, and the pp-αGlcNAcT responsible for addition of α-*O*-*N*-acetylglucosamine has been characterized biochemically. The social amoeba *Dictyostelium discoideum* also assembles *O*-linked glycans via α-*O*-*N*-acetylglucosamine, and the gene for the enzyme that catalyzes formation of this linkage (*gntB*) is known. A search for similar gene sequences in *T. cruzi* yielded 3 candidates (*TcOGNT1-3*). The biochemical function of these genes was tested by expression of various combinations of full-length and truncated fragments of TcOGNT1-3 in *Leishmania tarentolae.* Overexpression of full-length TcOGNT1 or TcOGNT2 in *L. tarentolae* approximately doubled apparent activity of microsomes relative to the parental control, using synthetic *Dictyostelium* peptide acceptors. Overexpression of the TcOGNT2 catalytic domain with a heterologous signal peptide quadrupled activity in the culture supernatant over controls, and also increased UDP-[^{3}H]GlcNAc hydrolysis, resulting in 10-fold higher activity in the culture supernatant from the catalytic domain strain. It was shown that [^{3}H] was incorporated as GlcNAc in direct linkage to an amino acid, based on chromatographic analysis of the products of acid hydrolysis and β-elimination. Both transferase and hydrolytic activities were demonstrated to directly involve the expressed TcOGNT2 polypeptide, detected by an anti-TcOGNT2-specific antiserum, based on co-chromatographic elution separate from the endogenous activity, and on the absence of activity of versions of *TcOGNT2* rendered inactive due to point mutations. These results will enable further studies of the function and mechanism of these genes in mucin-type *O*-glycosylation biosynthesis. Support: CNPQ, FAPERJ, OCAST.

BM096 - pROCK-DualLUC: a new transfection vector to study gene expression in *Trypanosoma cruzi*

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Trypanosomatids regulate the expression of their genes primarily at the post-transcriptional level, usually by mechanisms affecting mRNA stability. To better analyze the contribution of 5 and 3 untranslated sequences (UTR) on gene expression throughout the parasite life cycle, we generated a vector named pROCK-DualLUC, carrying the renilla and firefly luciferase reporter genes as well as the neomycin resistance (NeoR) gene. Flanking the renilla luciferase (RLUC), used as an internal control, we placed UTR plus intergenic sequences from constitutively expressed genes. Downstream from the firefly luciferase (FLUC) coding sequence, we inserted 3UTR+intergenic sequences derived from three differentially regulated genes (α-tubulin, amastin or MASP) or two constituvely expressed genes (TCR27 and gapdh). Transient transfections of epimastigotes with each construct showed that parasites carrying TCR27 and GAPDH 3UTRs controlling luciferase have higher FLUC activities, followed by alpha-tubulin 3UTR. In agreement with earlier reports showing the preferential expression of amastin and MASPs genes in amastigotes and trypomastigotes, respectively, sequences derived from these two genes showed the lowest level of FLUC expression in epimastigotes. PFGE analyses of stable cell lines containing these plasmids showed similar patterns of integration of the pROCK-DualLUC vector in the parasite genome, both in drug resistant transfected populations as well as in cloned transfected cell lines. Consistent with the transient assay results the amastin 3UTR showed 100x more FLUC expression in amastigotes than in the insect stage whereas the α-tubulin 3UTR conferred 10x more expression in epimastigotes compared to amastigotes. Analyses of luciferase expression in trypomastigotes are underway. Support: FAPEMIG and HHMI.

BM097 - Characterization of differences in the expression of epissomal genes in *Leishmania major* and *Leishmania braziliensis.*

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The Pteridine reductase 1 (PTR1) of *Leishmania major* was shown to play an important role in this parasite virulence by controlling the level of metacyclogenesis. The *PTR1* gene is encoded within the H region, a locus that is promptly amplified when this species of the *Leishmania* subgenus is cultivated in the presence of drugs, such as methotrexate (MTX), an inhibitor of dihydrofolate redutase-timilate syntase (DHFRTS). In resistant cell lines PTR1 can reduce folate and thus, compensate for the inhibition of DHFRTS. In this work we have investigated the *PTR1* gene from a *Viannia* subgenus parasite. The *Leishmania (Viannia) braziliensis* gene (*LbPTR1*) was isolated and cloned into the shuttle vector pELHYG. Surprisingly, transfection of *LbPTR1* into this species did not result in MTX resistance. However, *LbPTR1* mediated resistance to the DHFRTS inhibitor when transfected into *L. major.* The level of *PTR1* transcripts in *L. braziliensis* transfectants were not different from

that observed in wild type cells, despite the presence of the pELHYG-LbPTR1 episome. On the other hand, *L. major* transfectants carrying the same episome presented a substantial increase in *PTR1* transcript level when compared to wild type cells. We have recently demonstrated that the presence of amplicons is poorly tolerated in drug-resistant mutants of *L. braziliensis*. These findings, and the possible functionality of the iRNA machinery in this *Viannia* species, may explain the results described above. The pattern of expression of this gene in the different transfectants is currently being investigated. Supported by FAPESP, CAPES and CNPq.

BM098 - HETEROLOGOUS EXPRESSION AND PARTIAL CHARACTERIZATION OF A PUTATIVE GDPase FROM *Leishmania major* AS A GENUINE NTPDase

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L. major has two mapped apyrase genes in its genome (putatives NTPDase and GDPase). Apyrase function, characterized as tri and di-nucleotide hydrolysis, were previously demonstrated in intact *L. amazonensis*, *L. braziliensis* and *L. major* cells. The very distinct ecto-nucleotidase capacity between *Leishmania* species suggests its involvement with virulence and control of host-immune responses (Maioli et al., 2004). In order to evaluate the role of putative GDPase in *L. major* nucleotidase activity and to further characterize this protein we performed heterologous expression of active recombinant enzyme in *E. coli*-pET21 system. End primers were designed and used to amplify the full-length putative GDPase by PCR using genomic DNA as template. Full-length GDPase (2022 bp) was cloned in pET21b and this construction was used to transform *E. coli* BL21 strain. The recombinant protein was expressed after 3 hours of induction with IPTG. Insoluble recombinant protein was purified from bacterial lysates using Ni-NTA-agarose and showed specific activity for ATP hydrolysis around 35 nmol.mg protein $^{-1}$.h^{-1}. ATP/ADP hydrolysis was 1.05 characterizing this enzyme as a genuine apyrase. Full biochemical characterization , and search for potential inhibitors are included amongst the future goals of this work. Supported by: UFV, UFOP, FAPEMIG, CNPq, MEC

BM099 - On the origin of the putative indolepyruvate decarboxylase gene in *Phytomonas* spp.

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The plant trypanosomatid *Phytomonas* spp. is associated to devastating diseases in commercially important crops, whereas no apparent damage is observed in other plant species. The consensus sequence of one contig of ESTs of *P. serpens* (Pappas *et al.*, 2005) showed high similarity (BLAST X) with indolepyruvate decarboxylases (IPDCs) of phytobacteria, which in turn can be pathogenic and non-pathogenic to plants. IPDCs are involved in the pathway for the conversion of tryptophan to indole-3-acetic acid (IAA) phytohormone. The similarity of *P. serpens* IPDC with those of phytobacteria suggests that the gene could have been acquired by horizontal gene transfer from microorganisms that co-exist in plants. The goal of the present study was to investigate *IPDC* origin in *Phytomonas* through phylogenetic analyses. Sequence similarity searches were performed with the BLAST X program. High similarity with IPDCs and pyruvate decarboxylases (PDCs) were obtained, both of which are Thiamine pyrophosphate (ThDP)-dependent enzymes, along with putative pyruvate/indole-pyruvate carboxylases of *L. major*, *L. braziliensis* and *L. infantum*. The annotation of the *Leishmania* enzymes was generated automatically in the genome projects and, most probably, it is mistaken, because we found that these proteins have ThDP domains, whereas the coenzyme of carboxylases is Biotin. Interestingly, no orthologs were observed in the *T. cruzi* and *T. brucei* genomes. Phylogenetic analyses with several ThDP-dependent enzymes (protein sequences) from bacteria, fungi, plants and *Leishmania* were performed by several methods. The analyses show a clear separation between the various ThDP-dependent enzymes. We observed that *Phytomonas* IPDC is monophyletic with the *Leishmania*'s putative proteins, and that this clade is included in the IPDCs clade of phytobacteria. Work is in progress to characterize IPDC activity in *Phytomonas* and *Leishmania* organisms. Support: FAPESP; CNPq.

BM100 - New insights on the interaction between *Crithidia deanei* and fibroblasts: influence of the endosymbiont

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Crithidia deanei is a monoxenous trypanosomatid that harbors a bacterial symbiont in its cytoplasm. A previous study from our group showed that *C. deanei* was able to interact with mammalian fibroblasts. In an effort to identify some surface molecules responsible for this cellular interaction, we described the presence of homologues of gp63

on the cell surface of *C. deanei* as well as in a released form. We also demonstrated that the gp63-like molecule was more expressed in the wild type than in the aposymbiot strain.Moreover, this molecule is relevant for the flagellate adhesion to the insect gut as well as to the fibroblasts. Here, we have showed that both wild and aposymbiont strains recognized the same receptor on the fibroblast cells. In this context, living biotinylated parasites were able to bind to two distinct polypeptides expressed by the fibroblasts. In addition, we observed a 3-fold increase in the adhesion index after 24 to 96 h of parasite-fibroblast interaction when compared to the interaction process performed for 1 to 4 h, irrespective to the presence of the endosymbiont. Both parasite strains were capable in inducing fibroblast lysis. Interestingly, the wild strain led to a 2-fold increase in fibroblasts death in comparison to the aposymbiotic strain. We also looked for the detection of gp63-like molecules during the interaction. In this sense, the spent culture media, obtained after cellular interaction for 24-96 h, were analyzed by gelatin-SDS-PAGE and by western blotting using an anti-gp63 polyclonal antibody. Our results identified a peptidase of 66 kDa and a 62 kDa polypeptide, which were revealed by the anti-gp63 antibody, in all systems analyzed. Taken together, these results corroborated that both endosymbiont and gp63-like molecules are essential factors during the interaction of *C. deanei* with mammalian fibroblasts. Financial support: CNPq, CEPG-UFRJ, FUJB and FAPERJ.

BM101 - Real time PCR quantification of metacyclic forms in a *Leishmania (L.) amazonensis* promastigote culture.

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The *in vivo* differentiation of procyclic promatigotes into the infective metacyclic form occurs at the insect midgut. In an *in vitro* culture, the differentiation occurs at the final log/stationary phase, but the distinction between the two forms is not an easy task, neither is the estimative of their numeric relation. The estimative of infective form however is a very important parameter to find out the efficiency of mutant strains in establishing the infection. Particular genes, exclusively expressed during metacyclogenesis, can be used as stage markers. We used META-1 regulated gene sequence (Uliana et al., 1999) to establish a protocol to quantify metacyclic forms of *L. (L.) amazonensis* present along the different phases of a promastigotes growing curve. The protocol is based in real time PCR, using as template total RNA, to determine the number of mRNA molecules of the target gene. To carry on the standardization, sequences of arginase and G6PD coding genes, which are not regulated (da Silva et al, 2002; Castilho et al., in prep.) were used for total molecule calculation. Oligonucleotides for all target sequences were design to produce similar length fragments of nearly 100 bp. The products were cloned and the plasmid DNA containing each target sequence was used to construct a standard curve, with known number of molecules. After that, total RNA, extracted from different points along a promastigote growing curve, was used as template to obtain cDNA by reverse transcription. The cDNA was then used in the real time PCR protocol to determine the number of molecules for each target. The percentage of metacyclic in each point of the curve could then be determined by the relation between the numbers of molecules of META1 and arginase and/or META1 and G6PD. Supported by FAPESP and CNPq.

BM102 - Correlation between tissue and blood parasitism levels during the long-term experimental infection of *Beagle* dogs by *Trypanosoma cruzi*

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The positivity of parasitological tests is related to parasitemia levels, and some times the parasitemia levels are associated with tissue parasitism. However the evaluation of these two parameters is not possible to be performed in humans. In the present work, we evaluated the correlation between blood parasitism $freshbloodtestFB, hemocultureandPCR$ and cardiac parasitism *in situ*PCR in *Beagle* dogs infected with *Trypanosoma cruzi* strains that show different pathogenicity and virulence. Twelve *Beagle* dogs were inoculated with 4.0 x 103 blood trypomastigotes of Be78, Y or ABC *T. cruzi* strains. FB was always positive during the acute phase. However, the frequency of positive tests, the minimum number of tests necessary for detection of the parasite and the period of the infection in which the parasite was detected during acute phase were different among animals infected with these strains. All dogs showed positive blood culture during the acute phase. In the other hand, during the chronic phase, all dogs inoculated with Be78 or ABC showed at least one out of four positive tests performed, while only one dog with Y strain was positive. The perceptual of positivity of hemoculture was similar between animals inoculated with Be78 75% and ABC 62.5% strains and higher than hemoculture observed in dogs inoculated with Y 6.2% strain. The PCR positivity was higher than being 100%, 93.7% and 56.3% positive in dogs infected with Be78, ABC and Y strains, respectively. During the chronic phase, the kDNA presence verified by PCR in cardiac fragments was similar among animals inoculated with the three strains. The results showed that parasitism level in peripheral blood and cardiac tissue is dependent of the *T. cruzi* strain and no correlation between them was verified.