Biologia Molecular - Molecular Biology

BM001 - COMPARISON OF TWO METHODS AND PARASITOLOGICAL TESTING PRIMERS FOR THE DIFFERENTIATION BETWEEN TAENIA SOLIUM AND TAENIA SAGINATA

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The **Taeniasis** and **Cysticercosis** caused by **Taenia solium** are serious public health problems that are prevalent in urban and rural areas from Latin America, associated with poor sanitation and hygiene, poverty and education.
A further objective of this work has been studying the presence of eggs ***Taenia sp***. and then use a specific method of PCR-REA that allows us to differentiate ***Taenia solium*** of ***Taenia saginata***. We analise 1096 university students from Cochabamba-Bolivia for detection ***Taenia sp***. We used the inform consent and all the participants who agreed to participate in the study. In one year of our study we had 76 individuals carrying the parasite (***Taenia sp.).*** We used two coproparasitologic methods: the direct and flotation method. In the direct method we get 21 individuals with ***Taenia sp*** , with the flotation method we showed 76 individuals in a total of 1096 students, demonstrated a good results with flotation method.
Of the 76 students infected with ***Taenia sp*** through the PCR technique-REA was parasitized 11 students with ***Taenia solium***, and 65 students were parasite with ***Taenia saginata.*** We get new tools for do a adequate treatment in our patients.

Support: Agencia Sueca para el Desarrollo Internacional (ASDI)

BM002 - CLONING AND EXPRESSION OF *T. rangeli* UBIQUITIN FOR A POTENTIAL USE IN A DIFFERENTIAL DIAGNOSIS OF CHAGAS DISEASE

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Human and *T.cruzi* ubiquitin differ by only three amino acids (14, 19 and 57), with all changes being conservative except for position 19 were a proline in human ubiquitin is substituted for a serine in *T. cruzi* ubiquitin. In a previous report we showed that despite this high homology, the ubiquitin antibodies present in chagasic patients do not recognize human ubiquitin. The species-specificity of this response is further sustained by results where chagasic sera did not recognize purified *Leishmania.sp* ubiquitin where amino acid 19 and 57 are unchanged in relation to the human counterpart. Due to the cross reactivity in standard serological kits between *T..rangeli* and *Leishmania sp* and based on this previous results we isolated and cloned the ubiquitin DNA of *T.rangeli* and *Leishmania Brasilensis* in pET100 directional TOPO, and further sequenced them to evaluate the difference in the secuence of the ubiquitin gene between both parasites and compare it with that of *T.cruzi* and human ubiquitin to determine the potential use of recombinant ubiquitin for a differential diagnosis among these parasites. Analysis of sequences indicated that between *Leishmania brasilensis* and *T. rangeli* there is only one residue changed (19 from P to A). On the other hand if we compared the sequence of *T. rangeli* with *T. cruzi* ubiquitin, we found difference in the residues 19, 52, 57, and with human ubiquitin a difference in aminoacids 19, 57 and 52 . Additionally we studied the theorical antigenicity of ubiquitin through the programs antheprot2000 and CEP (Conformational Epitope Server). The analysis of human uquititin by CEP showed that the protein does not have sequential epitopes, but predict four conformational epitopes including 19 and 57 residues. We are currently expressing the ubiquitin of *T. rangeli* to see the antigenicity against chagasic and leishmanian sera.

BM003 - Tc-CRP GENE AMPLIFICATION: A PUTATIVE NOVEL TECHNIQUE FOR TYPING TRYPANOSOMATIDES STRAINS

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Trypanosoma cruzi is a protozoan parasite represented by a group of strains with high biological and genetic heterogeneity. Nowadays, this parasite strains are divided in two main groups, with a third controvertible group. Considering this variability and the presence of other trypanosomatides such as *T. rangeli* and others, which co-exists with *T. cruzi* in some endemic areas, it's highly necessary the development of a single step method capable of discriminate these species. The *Trypanosoma cruzi* complement regulatory protein (Tc-CRP) protects the parasite against complement mediated lysis, by binding to C3b factor and inhibiting MAC formation. This antigen was tested in chronic chagasic patients' sera and the results demonstrated optimal sensitivity and specificity for the diagnosis, besides a high ability to detect therapeutic efficacy in ELISA tests. However, all studies involving the Tc-CRP protein were performed with parasites from the Y strain. Recently, our group verified the Tc- CRP coding gene presence in 26 strains, using two specific primers: CRP-1 and CRP-2. The aim of this work was to evaluate the pair of primers CRP-2 as a molecular marker for differentiation among the *T. cruzi* phylogenetic lineages, and other trypanosomatides. The amplification product using the primer CRP-2 resulted in a fragment of 1569pb corresponding to the N-terminal portion of the gene. The analysis of the electrophoretic profile generated by this PCR presented a differential pattern of non-specific bands correlated to each groups of strains evaluated. These data evidenced the high usefulness of this single step PCR in the differentiation among strains of the lineages *T. cruzi* I, II and hybrid, besides the discrimination from *T. cruzi*-like and *T. rangeli*. Moreover, the present work suggests a possible new molecular marker for trypanosomatides typing that will increase the number of tool options for this research line. Financial support: CAPES, CNPq, FAPEMIG, UFTM.

BM004 - ANTISERUM AGAINST RECOMBINANT NCTRAP-2 DECREASES THE IN-VITRO INVASION PROCESS OF *NEOSPORA CANINUM*

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Neospora caninum is responsible for infecting a wide range of animals, inducing abortions in bovines and, as every Apicomplexan protozoan the invasion step is crucial for its survival. One important group of proteins secreted during invasion is the Thrombospondin Related Anonymous Protein (TRAP) family. *Neospora caninum* NcTRAP-1 was previously described and a second possible member called NcTRAP-2 was detected by our group on ESTs. The aim of the present work was the cloning of the NcTRAP-2 full-length sequence, production of recombinant antisera, localization of native form in 2D western blot and *in vitro* invasion inhibition assay. The full-length gene was obtained with a predicted protein sequence with 39% of identity and 53% of similarity with its homologues of *Toxoplasma gondi* (TgMIC-2); 39% and 53% with *Neospora caninum* (NcTRAP-1). The TRAP homologues have a signal peptide, two adhesive domains (an integrin-like domain and one or more thrombospondin type I repeats) and a transmembrane domain. Two recombinant fragments (fragments 1 and 2, both without signal peptide and transmembrane region) of NcTRAP-2 were generated (pET28 vector), MW of 50 and 78 kDa (fragment 2 is 163 aa longer towards the C-terminal end). Antisera against the recombinant forms were obtained and monitored by ELISA. The antiserum against recombinant fragment 1 recognized the native NcTRAP-2 (80 KDa, acidic PI) and putatively two isoforms (50 KDa, neutral PI) in 2D western blot. *In vitro* assays were performed to test inhibition of the invasion with the sera against recombinants 1 and 2 which were estimated both by: microscopic counting (10 fields/well in Giemsa stain) on 8 well plates and Real Time PCR on 24 well plates. The sera against recombinants forms of NcTRAP-2 (fragments 1 and 2) decreased the *in-vitro* invasion process in 46% and 27% respectively.

Keywords: *Neospora caninum*, Apicomplexa, thrombospondin related anonymous protein

BM005 - Down regulation of the selenocysteine synthesis pathway by RNA interference on *Trypanosoma brucei* cells: Is this a novel target?

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The biosynthetic pathway of the 21º amino acid (selenocysteine - Sec - U), whose incorporation in selenoproteins occurs at an UGA-stop codon and by a selenocysteine insertion sequence (SECIS) on the mRNA, represents the main biological form of selenium. The selenoproteins are present in a wide range of organisms and are mostly involved in oxidative pathways. Recently the selenocysteine biosynthetic pathway was identified in Kinetoplastida and all the homologue genes for Sec synthesis are present in this order. We have tested *Trypanosoma brucei brucei* and *Leishmania major* cells with auranofin, a compound that specifically inhibit selenoproteins. This compound caused parasite death with an LD50 of 1,73µM and 0,79µM for *T. brucei* and *L. major* cells, respectively, suggesting that the parasite form tested is dependent on the selenocysteine biosynthetic pathway. This prompted the possibility of the selenocysteine synthesis pathway proteins representing a potential target for drug design. In order to validate this pathway and to provide new insights on the relationship between the selenoproteins and their biological role, we employed the RNA interference (RNAi) technique in the SELD (selenophosphate synthase) protein. SELD is responsible for the synthesis of selenophosphate, a substrate for the selenocysteine synthesis and therefore is of central importance in this pathway. A 1076bp fragment of selD was cloned into the p2T7 vector for transfection into procyclic and bloodstream forms of *T. brucei* cells. Preliminary results of the RNAi knock-down assay indicate that in the procyclic forms a decrease in cellular SELD causes significant growth inhibition. Experiments on the blood stream form have been done aiming at comparing the gene expression variation and the role of this pathway on the parasite life cycle stages. To better understand the function of SELD, we are currently working to characterize the cellular phenotype caused by gene silencing using markers for specific subcellular structures and Immunofluorescence microscopy. Supported by FAPESP, CNPq and PET.

BM006 - USE OF FLUORESCENT *LEISHMANIA* (GFPs) FOR THE DEVELOPMENT OF DRUG SCREENING PROCEDURES

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Leishmaniasis is a neglected disease considered of high importance according to the World Health Organization (WHO). They are caused by protozoan parasites from the genus *Leishmania*. Increasing in the incidence of the disease is mainly due: environment modifications, difficulties in controlling vectors and reservoirs, the absence of a vaccine and lack of efficient drugs. Actually, control of Leishmaniasis is primarily based on the treatment of the human cases. However, chemotherapy of leishmaniasis still faces some difficulties such as: toxicity of the available drugs, route of administration and natural resistance of some strains of the parasite. The technology of the gene reporter allowed the construction of vectors expressing the fluorescent green protein (GFP). In this project, this protein was transfected into *Leishmania* parasites including the Brazilian species of higher medical importance: *L. chagasi*, *L. braziliensis*, *L. amazonensis* and *L. guyanensis*. Parasites were transfected with the vector pIR1Phleo-GFP+(a)(sense). Fluorescent *Leishmania* were checked in the Flow Cytometry (FACS) and in the laser confocal microscopy (LCM). All four species were successfully transfected and became fluorescent. Further, murine macrophages will be infected with those strains prior to in vitro drug screening tests. These genetically modified organisms will enable an improvement in the technique of screening of drugs, since the method routinely used is extremely laborious, subject to errors and individual experience. Through the direct measure of the fluorescence in the FACS, it will be possible to test a great number of molecules aiming a future identification of new drugs against Leishmaniasis.

Supported by CPqRR/Fiocruz.
Key words: *Leishmania*, Green fluorescent protein, Screening-drug

BM007 - GENERATION OF *LEISHMANIA* MUTANTS TO BE USED IN LARGE-SCALE DRUG SCREENING

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Search for novel druggable compounds against leishmaniasis are hampered by the lack of *Leishmania* mutants fitted for high-throughput screenings. The objective of this work is the generation of *Leishmania* mutants to overexpress constitutively a reporter gene inserted in the rDNA locus. They must lack a drug resistance gene to avoid potential interference with tested compounds. Different strategies were envisioned to obtain such mutants including removal of resistance gene from available vectors (A. Misslitz et al, MBP, 2000) using various approaches for recovery of transfectants based on the fluorescence of GFP+ cells and co-transfection of the targeting fragment with a circular plasmid bearing *neo*, with no success. The next strategy was to generate constructs carrying a positive (hygromycin phosphotransferase - HYG) and a negative (HSV-1 Thymidine Kinase - TK) selectable marker to be used in two consecutive rounds of transfection to recover parasites with a reporter gene (secreted placental alkaline phosphatase - SEAP) and no selectable marker. Strains of *L. major, L. braziliensis* and *L. donovani* were used. In the first transfection a cassette containing HYG, TK and SEAP genes flanked by fragments of the 18S (pSSU_HYG_TK_SEAP_SSU) was used and selection of transfectants was made with hygromycin B (HYG+ mutants). Another cassette containing SEAP and the rDNA fragments (pSSU_SEAP_SSU) was transferred to each HYG+ *Leishmania* in a second round of transfection; counter-selection was made with gancyclovir and the expression and secretion of SEAP was confirmed by luminometry. Phenotypic characterization of mutants will be presented. Besides its potential application for drug screening, the reported HYG+ *Leishmania* mutants are useful tools to facilitate the stable over-expression of any gene of interest, with no interference of drug resistance genes.

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BM008 - Cloning of the Nucleoside hydrolase of Leishmania donovani aiming the development of a synthetic vaccine against visceral leishmaniasis.

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Introduction: the Nucleoside Hydrolase (NH36) is the main antigen of the FML complex, inducing a TH1 immune response in DNA mice vaccination against visceral leishmaniasis. Objectives: 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 pET28b plasmid. Sequences were obtained by PCR amplification of the pMAL-NH36 plasmid with NcoI and XhoI restriction site containing primers. PCR products were cloned in the pMOS plasmid, sequenced, removed by NcoI and XHoI digestion and cloned in the digested pET28b 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. Balb/c females were vaccinated with 3 sc doses of NH36 recombinant protein obtained from the pMAL expression system (100ug/mice) in formulation with Riedel de Haen saponin (100ug/mice). Results: one week after the last vaccination dose, the DTH response against *L. chagasi* lysate was increased (76%, $p<0.005$) in vaccinated mice. The FACS analysis of *Leishmania*-specific splenocytes after *in vitro* incubation with the recombinant peptides disclosed significant differences ($p=0.048$) in CD4+ lymphocytes proportions, indicating that the F2 as the most immunogenic fragment. The CD8+ lymphocyte proportions were increased in all vaccinees (mean average 28. 93) over the untreated controls (mean 20. 94; IC 95% 15.2-26.28). IFN gamma was only secreted by vaccinees ($p<0.017$). Significant differences were detected in the induction of IFN gamma secretion by the NH36 fragments ($p=0.041$). Only F2 induced a 4.8 fold increase in IFN gamma secretion by splenocytes of vaccinees (88pg/ml) over controls (18.33pg/ml) Discussion: our results

suggest that relevant epitopes recognized by T cells producing IFN gamma are located within the F2 fragment peptide sequence.
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BM009 - A COMPARISON OF GENOMES YIELDS KEY DETERMINANTS FOR ANTHROPONOTIC CRYPTOSPORIDIUM.

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Our comparison of the newly sequenced genomes of *Cryptosporidium parvum* and *Cryptosporidium* hominis yielded over twenty apparently species specific coding sequences. Validation of these sequences on a range of clinical isolates has revealed remarkable polymorphisms which correlate not only with species but with isolate virulence and epidemiology. In particular we describe the discovery of a gene product, secreted by sporozoites and apparently central to the parasite-gut interface, which may have a role in host range and thus serve as the primary species determinant for *C. parvum.*

BM010 - CHARACTERIZATION OF GENES OF UNKNOWN FUNCTION WITH A HIGHER EXPRESSION IN *TRYPANOSOMA CRUZI* METACYCLIC TRYPOMASTIGOTES

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Our group has previously produced a DNA microarray containing a representation of the *Trypanosoma cruzi* genome which has been used for selection of stage-increased genes. The present project aims to characterize genes coding for hypotetical proteins which have a significant higher expression in metacyclic trypomastigotes compared to other life cycle forms. In general little is known about these genes function. The genes were cloned and expressed in *E. coli* using the Gateway platform (Invitrogen). The purified proteins were used to produce polyclonal antibodies in mice, which are been used to evaluate the protein expression patterns during the parasite life cycle by Western Blots and immunofluorescence. Among the selected targets, one codes for a 43 kDa protein that possess domains that characterize the Hsp40 group I co-chaperone (DnaJ I). We performed a bioinformatics analysis aiming to identify *T.cruzi* proteins containing a PFAM Hsp40 domain (e-value threshold of 10). After redundancy elimination, we have remained with 83 proteins, including 8 group I HSP40. Due to the high conservation of the domain, the protein was also expressed without the conserved N-terminal J-domain, to produce a more specific serum. Future work using expression vectors containing reporter genes or purification tags will bring more insights about the function of these genes.

Financial support: CNPq, Fiocruz, Fundação Araucária, NIH.

BM011 - PYROSEQUENCING OF *Trypanosoma cruzi* Dm28c STRAIN TRANSCRIPTOME: INSIGHTS INTO mRNA STRUCTURE AND INTER-STRAIN DIVERSITY

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Recent advances in the sequencing technology have enabled researchers to produce genome and transcriptome datasets in an unprecedented speed, allowing the identification of broader and deeper patterns of biological information. We have applied one of these next-generation sequencing technologies (pyrosequencing, 454/Roche FLX System) to analyze the transcriptome profile of the Dm28c strain of *Trypanosoma cruzi*. Briefly, total mRNA was extracted from the four main life cycle stages (epimastigote, amastigote, bloodstream and metacyclic trypomastigotes), transformed by a

SMART-oligo-dT approach, normalized by double strand normalization (DSN) and submitted to two sequencing runs (half plate for each stage). In the end, we were able to generate 616,611 distinct reads considered of adequate quality, averaging 212 nucleotides in length (range from 23 to 640). Using the GS *De Novo* Assembler, we have generated a total of 25,728 contigs from the four stage libraries (ALL), being 14,026 contigs for epimastigote, 14,331 for amastigote, 8,182 for metacyclic and 12,123 for trypomastigote. A total of 71,028 reads remained as singletons. Comparing the ALL set against the CL Brener sequenced genome, we were able to map the transcript data to almost all predicted genes, indicating both the success of the normalization process and the broad transcriptional profile obtained using the four stages total mRNA. There was a trend of increased transcript coverage towards the 3' end (26.2% 3'UTR, 48.0% CDS-3'UTR, 17.9% CDS, 2.4% 5'-CDS, 4.2% whole transcript), reinforcing the need of a different sequencing approach to increase transcript coverage. We have observed 43 contigs stage-specific and 413 contigs discriminating two stages from the others (selection criteria, positive has more than ten reads and negative has no reads). We are currently comparing this dataset to the CL Brener genome sequence, aiming to identify protein-coding and UTR structures differences between these two strains, as well as evidences of alternative SL and poly-A addition, and cis-splicing.

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BM012 - CHARACTERIZATION OF GENES DIFFERENTIALLY EXPRESSED DURING TRYPANOSOMA CRUZI METACYCLOGENESIS

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The process of cell differentiation from epimastigotes to metacyclic trypomastigotes forms of *Trypanosoma cruzi* is called metacyclogenesis. Functional and morphological changes of the parasite throughout this process are related to the pattern of differential expression of many genes. Together with the genome sequence of CL Brener strain of *T. cruzi*, data obtained by our team using DNA microarray experiments confirmed that during this event there are several genes whose expression is modulated. Also, we observed that nearly fifty-five percent of these genes codes for hypothetical proteins. From these results, we selected ten differentially expressed genes coding for hypothetical proteins that possibly have an important role in metacyclogenesis. In order to characterize these genes, we have been using a vectors system that allows their easy propagation and expression, the Gateway plataform. So far, all selected genes were cloned in pDEST17 and eight of them generated recombinant proteins produced in *Escherichia coli* BL21 pLysS strain. The expressed proteins were purified and inoculated in mice to obtain policlonal antibodies. The sera from mice inoculated with the recombinant proteins were taken by cardiac puncture and purified. Antibodies generated specifically against the recombinant proteins will be used on immunolocalization assays in *T. cruzi* and western blots to compare with data previously observed by microarray. Additionally, different vectors will be used for the complementar characterization of the selected genes.

Financial support: CAPES, CNPQ, Fiocruz, Fundação Araucária, NIH.

BM013 -- A HIGH-THROUGHPUT GENE CHARACTERIZATION SYSTEM FOR *Trypanosoma cruzi*

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The availability of the *Trypanosoma cruzi* genome is only a small piece of the puzzle of this parasite's biology. A high percentage of hypothetical proteins, few genes characterized and limitations on heterologous protein expression are the present scenario for this biological model. Thus, the need for development of a high-throughput gene characterization platform becomes evident. Ideally, such platform would allow efficient cloning and compatibility with different interests. In this context,

the goal of this study was to use the Gateway technology (Invitrogen) to construct plasmid vectors suitable to different uses. Our constructs contained 35.1 intergenic regions, *T. cruzi* Dm28c 18S ribosome or the T7 bacteriophage promoters, antibiotic resistance genes, and several tags for multiple purposes. Besides *T. cruzi* vectors, a *Crithidia fasciculata* heterologous protein expression system was created based on pNUS-H1 and modified for use of the bacteriophage T7 RNA polymerase. The advantages of the *C. fasciculata* system are that it is related to *T. cruzi* but it is not pathogenic for humans. Three *T. cruzi* genes were used to validate our strategy. Antibodies against the products of these genes were used to detect the presence of the recombinant proteins in *T. cruzi* and *C. fasciculata* extracts, and to compare the localization of the native proteins with that of the recombinant proteins tagged with *c-myc* epitope or fluorescent proteins GFP, CFP and YFP in *T. cruzi*. The results obtained with these antibodies corroborated the results obtained with our system. TAP-tag protein complex purification was also used for strategy validation. Our results show that this platform is a fast and efficient cloning system that allows the characterization of *Trypanosoma cruzi* genes with different biological approaches. The development of this high-throughput platform is a step closer to large scale applications such as the *T. cruzi* ORFeome.

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BM014 - GENE EXPRESSION PROFILE OF *Trypanosoma cruzi* INFECTED CARDIOMYOCYTES.

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Host-*Trypanosoma cruzi* interactions are dynamic and complex processes, where the parasite exploits strategies to survive and establish the infection. Therefore, a more comprehensive knowledge of the interplay between the host cell and *T. cruzi* regarding genome expression profiles is central to the understanding of the pathogenesis of Chagas' disease. In the present study, we have employed an oligonucleotide microarray to analyze the transcriptional response of cardiomyocytes to *T. cruzi* infection. Previous analysis using a Genechip® microarray (covering 12,422 genes) provided a starting point for the description of the transcriptome response to infection. In order to gain further insights into the dynamics of gene regulation during *T. cruzi* infection, we performed a temporal analysis of gene expression in cardiomyocytes infected by *T. cruzi*, clone Dm28c. A detailed time course at early stages (1, 2, 3, 4, 5, 6 hours) and one later stage (24 hours) of infection was performed using a dense oligonucleotide microarray that covers a large portion of the mouse genome (39,000 probes corresponding to 34,944 genes). Most of the genes up and down regulated were related to cell signaling, apoptosis, cytoskeleton, extra cellular matrix and immune response. We also found a great increase in differential gene expression at 6 and 24 hours after infection. More importantly, we identified a small set of genes including genes related to TNF-α and TGF-β that show changes in gene expression, and their metabolic pathways are involved in disease progression and fibrosis leading to heart failure. These findings will provide new insights into molecular mechanisms by which intracellular *T. cruzi* influences the host cell transcriptome and lead to pathogenicity development.

BM015 - GENETIC BACKGROUND AND BASIC GENE EXPRESSION LEVEL OF MURINE PERITONEAL MACROPHAGES INFLUENCE THE RESPONSE TO *LEISHMANIA AMAZONENSIS* INFECTION

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CBA/J mice are highly susceptible to *L. amazonensis* and C57BL/6 mice once infected with

L. amazonensis develop a chronicle disease. In addition, CBA/J peritoneal macrophages present twice the percentage of *L. amazonensis* infection compared to C57BL/6 macrophages. We hypothesized that the mouse genotype and basic expression level of either CBA/J or C57BL/6 macrophages influence their response to *L. amazonensis* infection. DNA microarray experiments were used to determine CBA/J or C57BL/6 macrophages differences in gene expression before and after *Leishmania* infection. Non-infected C57BL/6 macrophages differentially expressed 3 times more genes compared to CBA/J cells. After infection, C57BL/6 macrophages modulated 6 times more genes compared to CBA/J cells in response to *L. amazonensis*. One important finding was a higher stability in gene expression of C57BL/6 macrophages when compared to CBA/J cells. These data could explain the differences in the number of modulated genes in response to infection and justify the limit number of modulated genes in the CBA/J model and also that the CBA/J genotype represents a more complex model than C57BL/6. In addition, despite the differences observed between these two mouse models, we were able to identify slight differences in gene expression by these macrophages in response to the same *Leishmania* specie. Differentially expressed genes were confirmed by qRT-PCR. In conclusion, *Leishmania* infection produces subtle alterations in gene expression and the caos level of gene expression by macrophages can influence our ability to identify modulations induced by *Leishmania* infection. Financial support: CNPq & FAPESB.

BM016 - HIGH-THROUGHPUT CHARACTERIZATION OF mRNA DEGRADATION PROFILE ON *Trypanosoma cruzi*: MICROARRAY ANALYSIS OF EPIMASTIGOTES TREATED WITH ACTINOMYCIN

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Trypanosoma cruzi, as others trypanosomatids, controls its gene expression mainly post-transcriptionally. Theoretically, this control can be exerted at different steps, but research has been focused in mRNA degradation and translational control, with the identification of several examples. We have started a project aiming to identify the final outcome of this post-transcriptional network at a high throughput level, by performing a microarray analysis of actinomycin-treated epimastigotes of *T. cruzi* Dm28c strain. Briefly, $1x10^7$ cells were incubated for 15, 30, 45, 60, 90, 120, 150, 180 and 240 minutes with 10 µg and 50 µg of actinomycin-D and each experimental unit (two drug doses and 10 data points) were replicated, totaling 40 samples. These samples were hybridized in a full-loop design for each experimental unit to a whole genome oligonucleotide microarray (10,816 probes), obtaining two independent hybridization for each sample. A time-series statistical analysis was performed using the SAM software, including data from all four experimental units, as well as for each experimental unit independently. Setting two distinct thresholds (5% false discovery rate, 2-fold change) and comparing the extreme data points, we have observed 2000-3000 differentially expressed genes for each experimental unit. We have observed a statistically significant enrichment of retrotransposon hot spot protein (RHS) and metabolism processes, among the most stable mRNAs, and DNA binding among the most unstable mRNAs. This dataset represents the first mRNA half-life analysis in a protozoan and provides degradation profiles of an organism that regulates its gene expression mainly post-transcriptionally and is a rich repository for data mining. These profiles are being integrated with distinct datasets obtained in our *T. cruzi* Functional Genomics Project, aiming to identify gene expression regulatory networks in this organism.

Financial support: CNPq, FIOCRUZ, NIH, Fundação Araucária.

BM017 - ANALYSIS OF PERFECT AND DEGENERATED REPETITIVE SEQUENCES IN THE PROTEOME OF PROTOZOAN PARASITES

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Although the precise function of repeat segments in eukaryotic proteome remains obscure, several studies have shown that repeat-containing proteins in protozoan parasites are highly immunogenic, involved in immune evasion, cytoadherence and protein-protein interactions. Here we have performed an *in silico* analysis searching for perfect and degenerated repeats in the predicted proteome of *Trypanosoma cruzi*, *Leishmania major*, *Plasmodium vivax*, *Plasmodium falciparum*, *Toxoplasma gondii* and *Trypanosoma brucei* using the algorithm AA-RepeatFinder developed by our group. The *P. falciparum* proteome has the largest percentage of proteins containing both perfect (42.2%) and degenerated (58.1%) repetitive segments while *T. brucei* has the smallest percentage of repeats: 7.1% and 25.9% of the parasite proteome contains perfect and degenerated repeat segments, respectively. Although there is a large difference in the amino acid composition of the repetitive motifs in each species, the amino acids over-represented in the repeats are those known to be involved in O- and N-glycosylation. Threonine is the most frequent amino acid in *T. cruzi* repetitions encompassing around 23% of the all amino acid derived from repeats; serine is the most frequent amino acid in *L. major* repeats (28.2%) and asparagine is the most frequent amino acid of *P. falciparum* repetitive segments, totalizing 49.7%. On the other hand, there is no strong bias in the amino acid composition of *T. brucei* repetitive sequences. Analysis of the *T. cruzi* surface proteins have shown that the largest content of perfect and degenerated repetitive segments is derived from TcMUC mucin and MASP superfamilies, respectively. Also, the amino acid composition of the repeats in each *T. cruzi* superfamily is also very different, suggesting different functions for these repetitive motifs.

BM018 - PROTEOMIC IDENTIFICATION OF MOLECULES INVOLVED ON ESTABLISHMENT AND CONTROL OF CUTANEOUS LEISHMANIASIS

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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. Using microarray approach we showed that *L. amazonensis*- and *L. major*-infected macrophages express different sets of genes related to early cell-Leishmania interaction and immune-inflammatory response. Although microarray technology is useful to identify molecules at transcriptional level, it is necessary to associate expressed genes to theirs respective proteins. Besides that, several important modulations occurring during infection are only seen at proteome level. Proteomic experiments using inflammatory macrophages demonstrated that only 62 proteins were exclusive of *Leishmania* infection and 162 proteins were modulated on macrophages infected by *L. amazonensis* in comparison to *L. major*-infected cells. In order to better evaluate the macrophage response to Leishmania infection, we decided to use bone marrow derived macrophages (BMMφ), since these macrophages are not pre-activated by thioglycollate. Besides that, BMMφ cultures are synchronized and homogeneous. First, we performed kinetic studies and compared the capacity of *L. amazonensis* and *L. major* to infect BMMφ. We evaluated percentage of infection and parasite burden 1.5, 3, 6, 12 and 24h after infection and no difference was observed for 3h between the two groups. In contrast, after 6h we observed that infection was higher in *L. amazonensis*- than in *L. major*-infected cells. In this study, using proteomic approach, we compared 6 and 24h of *L. major* and *L.*

amazonensis infection on CBA/J BMMφ. Protein extracts were obtained to characterize peptides by LC-MS/MS in a MudPIT approach. The results from 5 independent experiments are being analyzed by bioinformatics. The identification of molecules involved on the establishment and control of cutaneous leishmaniasis could help the design of new therapeutic drugs against this disease.

Financial support: CNPq & CAPES

BM019 - A combined in silico and in vitro strategy to analyze hipothetical proteins in *Giardia intestinalis*

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Giardia intestinalis causes giardiasis, which is a notorious health problem throughout the world. Giardia is a member of the diplomonads, often described as an ancient protist group, whose primitive nature is suggested by the lack of typical eukaryotic organelles. In 2003 Tovar et al, described an IscU and IscS proteins that show a presence of mitochondrial remnant organelles, called Mitosomes. Now with the completed Giardia intestinalis genome, we are interested in looking for new mitossomal proteins that could provide a target to develop new antigiardiasis drugs.

For Mitosomas we seek markers analyzing 3553 hypothetical proteins obtained from giardiadb.org/giardiabd databases using a combined strategy with mitochondrial prediction online software (Target P, signal P, Mitopred) and developing an algorithm able to cluster protein expected values (E-values), additionally we find an inherent hierarchical structure in data (based on the Baire metric, Murthag F. et al, 2008).
10 genes targetp +, Signal p – mitopred + were chosen from 128 putatives genes with a high mitosomal probability to be characterized. The genes are being cloned in fusion with an haemaglutinin epitope tag in a pPac giardia vector to create over-expressed transgenic parasites. Cellular localization and function of these genes will be analyzed (in progress).

BM020 - THE *Trypanosoma cruzi* PHOSPHOPROTEOME

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Phosphorylation is a reversible post-translation modification widespread across the proteome, regulating several signal transduction pathways and different biological processes. Thus, the identification of phosphorylated sites is a valuable tool to address cell signaling control at the molecular level. Protein phosphorylation is proposed to be of even greater importance for the protozoan parasites of the order Kinetoplastida (e.g. *Trypanosoma cruzi*) due to the high concentration and different domain organization of both kinase and phosphatase genes on these organisms, which present a complex life cycle with different hosts. To characterize the phosphoproteome of *T. cruzi*, protein samples from cells at different stages of metacyclogenesis were enriched for phosphopeptides using TiO_2 chromatography and analyzed using an LTQ-Orbitrap mass spectrometer. All together, we identified 412 phosphoproteins with a total of 1135 phosphorylation sites, with a phosphosite distribution of 961 (84.67%) serine, 165 (14.45%) threonine and 10 (0.88%) tyrosine residues. Surprisingly, the phosphorylation distribution was similar to results previously reported for other organisms with a different kinase, phosphatases and phosphodomains proportion. To our knowledge, this is the first work to describe site-specific phosphorylation events in the Kinetoplastida order.

Financial supported by Fiocruz, Cnpq, CAPES and NIH.

BM021 - *LEISHMANIA (LEISHMANIA) CHAGASI* FROM NORTHWESTERN AND SOUTHEASTERN REGIONS OF SÃO PAULO HAVE DIFFERENT ANCESTRAL BASED ON MICROSATELLITE MARKERS

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Recent findings have shown great dispersion of *Leishmania (L.) chagasi* through noncontiguous areas of São Paulo (SP) State. Noteworthy is the absence of its natural vector in some of these areas, pointing to possible atypical ways of transmitting. To understand the epidemiology of the disease, we have initially characterized six dinucleotide microsatellite markers, which were selected based on their allele numbers described elsewhere. This panel was used to analyze 22 specimens collected at eight cities from the SP Northwestern (SPNW) region and two from the SP Southeastern (SPSE) region. Two additional samples were collected at Minas Gerais (MG) State and used as outgroup. Our preliminary results have shown no variation for four markers, despite two of these have 13 and 15 alleles described. The other two markers were polymorphic and present three and four alleles on these populations. The observed frequencies were 81.25%, 16.67% and 2.08% for Li71-7 and 39.13%, 34.78%, 15.22% and 10.87% for Lm4TA. Unfortunately, no useful information for epidemiology studies can be obtained from Li71-7 marker because alleles are present on both SP populations. On the other hand, all but one sample collected at SPSE region present identical allelic pattern which was not found among samples from SPNW region. Interestingly, allelic pattern of MG samples was identical to SPSE region. Therefore, *Leishmania* from SPSE and SPNW regions may have been originated from different ancestral and the former probably share the same ancestral to MG protozoans. This is in accordance with the hypothesis that leishmaniasis reaches SPNW region following the construction of natural gas pipes while no one knows how it reaches SPSE region, maybe via MG touristic cities which are common destination of many people. To test this hypothesis, we will extend our microsatellite panel and include samples from other Brazilian regions. Financial support: Genoa Biotecnologia SA.

BM022 - THE DIFFICULTY IN VALIDATING THE TAXONOMIC STATUS OF *LEISHMANIA (VIANNIA)* SPECIES

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Among the 14 *Leishmania* species circulating in Brazil 6 belong to the *L. (Viannia)* subgenus. The genetic distance observed among these species can be higher than the observed distance between some species in this subgenus. In fact, there is no appropriate concept to define species in the genus *Leishmania;* some of them were described only considering eco-epidemiologic features, but not all are supported by biochemical and/or molecular characteristics. The high homology among *L. (Viannia)* species, the sympatric occurrence and the observation of natural hybrids between some *L. (Viannia)* indicate that genetic flow might be occurring in this group. Several markers have been employed to characterize *Leishmania* species. Isoenzymes and PCR-RFLP of hsp70 are good markers to discriminate *Leishmania* species. In this study it was selected 18 *Leishmania* strains representing the 5 *L. (Viannia)* described species associated with human CL in Brazil. All of them were typed by isoenzymes and PCR-RFLP hsp70. The samples were classified in 12 zymodemes and they were clustered according to their specific classification. When the reference strains of each species were analyzed by the PCR-RFLP produced after the digestion with *Hae*III and *Bst*UI it was also possible to discriminate among all the species. However, when the other isolates representing each species were included in the study, only *L. braziliensis* classified in the same zymodeme as the reference strain showed a profile compatible to this species. The others *L. braziliensis* strains were similar to *L. naiffi*. It was observed distinct patterns among the *L. guyanensis* strains and some were identical to the reference strain of *L. guyanensis* for one restriction enzyme. The other enzyme showed a similar pattern to *L. naiffi*/*L. braziliensis*. So far our results indicate the difficulty of defining criteria to name *Leishmania* species and the appropriated method to characterize the described species.

Supported by IOC-Fiocruz, CNPq, FAPERJ (Cientista do Nosso Estado), European Community (FP6)

BM023 - PHYLOGENETIC ANALYSIS OF *Trypanosoma cruzi* Dm28c GENES DIFFERENTIALLY EXPRESSED DURING METACYCLOGENESIS

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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 were to produce folding information aiming to attribute function by structure comparison. We have initially selected 192 proteins, whose coding regions were amplified from the Dm28c strain genome and cloned in the Gateway platform (Invitrogen). There are two main lineages in *T. cruzi*, represented by the strain used in this project and CL Brener. 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 and evolutive divergence. 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 successfully sequenced the complete CDS for 178 genes in Dm28c, searched for orthologs of CL Brener, *T. vivax*, *T. brucei, Leishmania major, L. infantum* and *L. braziliensis* by a BLAST reciprocal best hit approach, and aligned these genes using the ClustalW software. We are presently analyzing multiple sequence alignment with several evolutionary models and methods, such as distance methods, likelihood, bayesian inference, codon bias index and d_N/d_S ratio. Correlating the phylogenetic distribution of genes with their expression pattern in *T. cruzi* (microarray data), several distinct functional groups can be assigned, particularly focused in the phenotypic and genotypic intra-specific diversity of *T. cruzi*. Furthermore, due to the great number of genes analyzed, the concatenated results can improve the knowledge about the evolutionary relationship between the trypanosomatids species analyzed.

Keywords: Structural proteomics; *Trypanosoma cruzi*; evolutionary models; lineages.

Financial support: CNPq, CAPES, NIH, Fundação Araucária and FIOCRUZ

BM024 - COMPARATIVE ANALYSIS OF *Trypanosoma rangeli* HISTONE H2A GENE INTERGENIC REGION WITH DISTINCT INTRA-SPECIFIC LINEAGE MARKERS

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Considering the biological and epidemiological interest, research on *T. rangeli* genotypic characterization is of major importance and has been carried out using distinct markers and methods. In this study, sequences of the intergenic region of the *T. rangeli* histone H2A gene (H2A IR) were comparatively analyzed in order to examine the relationship between KP1(+) and KP1(-) sub-populations with their respective hosts and/or geographic origins. Also, a comparative analysis of the H2A IR with the rDNA and mini-exon gene PCR profiles was carried out in order to point out the usefulness of the H2A IR for strains characterization. Detailed H2A IR sequence analysis revealed a discrete size polymorphism among *T. rangeli* strains and the presence of SNPs and mini-satellite repeats, allowing an inter-specific differentiation from *T. cruzi* strains representing the main parasite lineages, but not allowing intra-specific differentiation. Differently from the H2A IR, UPMGA analysis of the 24S□ rDNA and the mini-exon genes profiles clearly separated *T. rangeli* KP1(-) and KP1(+)strains, clearly clustering the Brazilian and Colombian KP1(-) strains on separate branches. Our results point out that H2A IR is a good marker for inter-specific characterization of the genus *Trypanosoma*. Supported by COLCIENCIAS, CNPq, PUJ and UFSC.

BM025 - *Trypanosoma cruzi* GENOTYPES CIRCULATING IN SEMI-ARID REGION OF RIO GRANDE DO NORTE STATE, BRAZIL

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Little is known about the specific *Trypanosoma cruzi* genotypes circulating in the state of Rio Grande do Norte and the population structure of the parasite in that area. Twenty five *T. cruzi* stocks from the semi-arid region isolated from 13 triatomine bugs and 12 from humans were analyzed by a panel of PCR-based genetic markers (rDNA 24Sα, mitochondrial cytochrome oxidase subunit II, intergenic region of spliced-leader DNA genes and microsatellite *loci*) to identify and characterize the parasite lineages. The presence of the phylogenetic subdivisions *T. cruzi* I, II and III were recorded. The exclusive presence of the *T. cruzi* III, or subgroup DTU IIc, circulating at silvatic environment was observed by the presence this lineage in all stocks isolates from *Panstrongylus lutzi* and in 50% from *Triatoma brasiliensis*. The genotype *T. cruzi* II (DTU IIb) was found in humans and vectors and *T. cruzi* I (DTU I) was observed only in one human stock. Polymorphism analysis of these isolates by six microsatellite loci revealed an extraordinary predominance of identical *T. cruzi* populations supposedly polyclonal in 58.3% human isolates and in one from triatomine bugs. The high proportion of these multiallelic isolates was identified by the presence of three peaks for the SCLE10, SCLE11, TcTAT20 and TcAAAT6 microsatellite *loci*. Our next goal is to dissect the population structure of these potentially polyclonal stocks employing microsatellite loci analyses in a full nested PCR approach using single cells sorted by a FACS Vantage apparatus.

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BM026 - STUDY OF CHAGAS DISEASE CONGENITAL CASES FROM TWO DIFFERENT ENDEMIC AREAS: MINAS GERAIS (MG), BRAZIL AND BUENOS AYRES, ARGENTINA

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After the successful control of vector-borne Chagas disease in Brazil, the importance of other forms of infection such as congenital transmission has increased. Congenital transmission depends on the established infection in pregnant women and on the risk of vertical transmission which varies according to the region. The exact causes of this variation are still unknown but could be related to hosts' capacity to stop parasite proliferation or due to parasite genetic polymorphism. We analyzed paired mother-child cases from Minas Gerais (MG) and Buenos Aires. The microsatellite profiles showed a perfect genotypic identity between the mother-offspring pairs analyzed, confirming the congenital infection. The rDNA 24Sα, COII and mini-exon profiles demonstrated that in MG the parasite isolates of all congenital cases belong to *T. cruzi* II major lineage (DTU IIb), whereas the isolates parasites from congenital cases from Argentina belong to hybrid strains (DTU IId). Once the prevalence of congenital cases of Chagas disease seems to be much higher in Argentina than in Brazil, we hypothesized that the high frequency of congenital cases of Chagas disease in an endemic area is associated with the higher predominance of *T. cruzi* hybrid populations circulating. To test this idea we are initially

analyzing morphological and biological characteristics of two strains belonging to *T. cruzi* II lineage from MG: one from a confirmed congenital case and another not transmitted congenitally. Preliminary results demonstrated that the congenital strain presents smaller trypomastigote forms and a higher number of these forms liberated from the infected LCC-MK2 cells when compared to the non-congenital strain. These results can indicate that parasite characteristics may also influence the rates of congenital transmission. Our next goal is to investigate the infection profile of these strains on placental cells and also to compare these parameters employing *T. cruzi* hybrid populations.

Financial support: FAPEMIG, CNPq, CAPES.

BM027 - POPULATION GENETIC ANALYSIS OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *L. (V.) GUYANENSIS* NATURAL POPULATION CIRCULATING IN BRAZIL

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Leishmania (Viannia) braziliensis and *L. (V.) guyanensis* are the most important etiologic agent of human cutaneous leishmaniasis in Brazil. Although the reported genetic distance between these two species is not high, the epidemiology of the disease caused by each species is quite different and it is known that the SbV treatment outcome in patients with CL can vary according to the species causing the disease. Therefore, parasite identification is of utmost clinical and epidemiological importance. Besides taxonomic identification, population genetics analysis contributes to epidemiological studies, for example, determining the relationship between species and/or strains and making correlation with eco-epidemiological features. In this study several *L. braziliensis* (Lb) and *L. guyanensis* (Lg) strains, isolated from different CL endemic region in Brazil, were identified by multilocus enzyme electrophoresis (MLEE), the gold standard for taxonomy and strain typing of *Leishmania*. The strains were also typed by 15 polymorphic microsatellite markers (MLMT), specific for the *L. (Viannia)* subgenus. Fragment analyses were conducted using automated sequencers and were analyzed using different population genetics/phylogenetics programs (*GeneMapper, MSAnalyser, Populations, Mega 3.*1, *Structure*). Population structures were inferred by combination of different methodologies and the results show that the Lg group was more homogeneous than the Lb. Lb and Lg are genetically distinct population. However, one Lg strain grouped together with Lb. In the Lb group it was observed a cluster corresponding to the isolates classified in the same zymodeme, IOC/Z27, which is the most prevalent and widespread in the American continent. The Lb strains typed in other zymodemes formed a distinct group inside the Lb group or grouped together with Lg. The results suggest that MLMT provides a powerful tool for taxonomy, population genetic and epidemiological studies of important *Leishmania* species.

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BM028 - MOLECULAR MARKERS BASED IN THE *prp1* GENE, THAT CODIFIED A GLYCOPROTEIN RELATED WITH PENTAMIDINE RESISTANCE, IS CAPABLE TO DIFFERENTIATE FOUR LEISHMANIA SPECIES.

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The classic diagnosis for leishmaniasis is the demonstration of the parasite through its isolation in cultures and/or identification in microscopic analysis. Nevertheless, the gold standard diagnostic method for visceral leishmaniasis is conventional serology, as IFI or ELISA based in total extracts of the parasite, besides the low sensitivity presented by these methods. With the current knowledge of the genome of different species of *Leishmania*, molecular techniques, as Polymerase Chain Reaction (PCR), have been improved for leishmaniasis diagnosis, now demonstrating the presence of the parasite with higher sensitivity and specificity than serological or classical parasitological methods. In such a way, our main goal here was to evaluate new molecular markers for PCR diagnosis of canine leishmaniasis, based in structural differences demonstrated by our group in the *prp1* gene (Pentamidine Resistance Protein-1). PRP1 is a P-glycoprotein member of the ABC transporter superfamily, which mediates Pentamidine

resistance following overexpression in *Leishmania*. First, we verified that PCR reactions with primers directed to specific regions of PRP1 render different patterns of amplification when sampled with DNA from promastigotes cultures of *Leishmania amazonensis*, *L. braziliensis*, *L. chagasi and L. major*. Then, PCR preliminary reports using these primers in a field sample of dogs serologically positive for leishmaniasis collected at the city of Embu das Artes (on the São Paulo boundary), shown amplification patterns compatible with *L. chagasi*, in accordance with the clinical symptoms observed. We expected these proposed molecular markers could confirm the power to differentiate *Leishmania* species after PCR analysis with new samples of Embu das Artes contaminated dogs, as well as human samples from other cities of São Paulo state, where patients with cutaneous leishmaniasis were reported. We also expected that PCR analysis with these specific primers allow their use as a new molecular tool for the diagnosis of canine and human leishmaniasis.

Supported by: CNPq, FAPESP and LIM-48 from FM-USP.

BM029 - STUDY OF ALTERNATIVE VECTORS FOR *LEISHMANIA (LEISHMANIA) CHAGASI*

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American visceral leishmaniasis is becoming a serious problem of public health in the last years for different regions of Sao Paulo State (SP). In some of these areas, it has been observed high incidence of canine visceral leishmaniasis (CVL), despite the low or no occurrence of its natural vector *Lutzomia longipalpis.* Interestingly, dogs from these regions are highly infested with ticks (*Rhipicephalus sanguineus*) and/or fleas (*Ctenocephalides felis felis*) that might be acting as vector. To test this hypothesis, we designed molecular protocols to detect *L. (L.) chagasi* from ticks and fleas collected from 31 CVL dogs with positive diagnosis confirmed by PCR and/or ELISA laboratorial tests. Ticks and fleas from nine healthy dogs were also tested to the presence of protozoan. We were able to successfully extract DNA from all samples as confirmed by PCR to ectoparasite genes (18S). Considering only positive CVL dogs, we were able to detect protozoan DNA from ectoparasites collect from 19 of 31 dogs, (sensibility of 61.3%). Ectoparasites from other 12 positive dogs were negative as well as the nine collect from healthy dogs (100% specificity). These preliminary data show the presence of viable *L. (L.) chagasi* inside ticks and fleas, suggesting a possible importance of dog ectoparasites in visceral leishmaniasis dissemination.

BM030 - BLOODFEEDING- AND INFECTION-INDUCED DIFFERENTIAL GENE EXPRESSION IN THE BRAZILIAN MALARIA VECTORS *A. AQUASALIS*.

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Malaria still affects 300 million people worldwide every year, causing 1.5-2.7 million deaths. In Brazil there are 450.000 cases per year, 99.7% of which occur in the Amazon. Molecules that take place in the interaction between mosquito vectors and plasmodia, which might be involved with the parasite's developmental cycle within the vector, have been the focus of studies aiming at novel malaria control strategies. However, almost all such studies are based on Old World anopheline species. Thus, our main goal is to analyze the effects of blood-feeding and infection with *Plasmodium vivax* on gene expression induction in the Brazilian malaria vector, ***Anopheles aquasalis*.** The following experimental procedures were employed in order to address the main points proposed in this project: (1) evaluation of the differential gene expression: subtractive suppressive hybridization (SSH) and RT-PCR; (2) characterization of molecules of interest: cloning, sequencing, and function prediction through GenBank Blast searches and phylogenetic comparisons with other insect groups. After the subtraction analysis, important differences in gene expression were detected in ***A. aquasalis*** after feeding and infection for the two established times

(two and 24 hours). For example, expression of fibrinogen cDNA was observed only in the infected insects, whereas digestive enzymes cDNAs\prime (chymotrypsin, serine proteases, carboxypeptidases) were detected solely in the blood-fed insects. The expression of some of these differentially expressed genes was confirmed by RT-PCR. These results indicate that genes are differentially expressed during ***A. aquasalis*** feeding and infection with *P. vivax,* generating important information on molecules possibly involved on the interaction between Brazilian malaria vectors and their parasites, and thus contribute to the development of new malaria transmission-blocking strategies.

BM031 - Trypanosoma cruzi ecto-nucleotidase inhibition, infectivity and virulence relationship in mouse infection

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Chagas disease is one of the main diseases with high economical and social impacts in Latin America. The current drugs used to treat this disease lead to low cure, especially in the chronic phase and present high toxic non-desired side effects. Because of these there are necessity to develop new drugs and strategies for the control and treatment of Chagas disease. From this point of view biochemical pathways related with surviving capacity and virulence of *Trypanosoma cruzi* could represent good targets. In this work we evaluate the roles of *T. cruzi* ecto-nucleotidases in the infection and virulence procedures in mouse, using parasites pre-treated with the known E-NTPDase inhibitors (Suramin, Gadolinium and ARL67156). The pre-treatment with all inhibitors leaded to significantly decreases in parasitemia and mortality. This influences were dose-dependent and the betters concentrations of inhibitors were: 300µm, 300µm and 1mM, to $GdCl_3$, ARL and Suramin, respectively. The pre-treatments lead to high levels of INF-γ expression in the heart and serum production, in the earlier stages of infection. These data suggest that ecto-nucleotidase inhibitions could be responsible to increments in extra-cellular ATP concentration that could stimulate hosts inflammatory mechanisms against parasites, evidencing the participation of these ecto-enzymes in the modulation of host immune system. Our data showed that the pre-treatment no change IL-10 heart expression and serum production too. In addition, when we evaluate the inflammation intensity in animal hearts, we observed that $GdCl_3$ was able to reduce the number of inflammatory cells, both in the 8^{th} an 15^{th} days of infection. Concluding, together our data suggest an effective participation of parasite E-NTPDases in the infection process and in the modulation of host immune system. We believe that the inhibition of these pathways emerge as new good targets to block T*. cruzi* infection.
Supported by: UFOP, UFV, CNPq, FAPEMIG

BM032 - MOLECULAR CHARACTERIZATION OF *TRYPANOSOMA CRUZI* SINGLE CELLS ISOLATED BY FACS CELL SORTER FROM NATURAL MULTICLONAL STRAINS

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The development of a technique suitable to analyze individualized parasites became indispensable after the identification of *T. cruzi* multiclonal strains. Techniques such as micromanipulation and limiting dilution have been already used in the attempting of isolating parasite clones from *T. cruzi* multiclonal strains. However, these techniques are laborious, time consuming and yield very few clones suitable for molecular analyses. To avoid these drawbacks, we described herein an optimized methodology to characterize *T. cruzi* single cells isolated by FACS Vantage. This apparatus allow sorting of a large number of single cells in a relatively short time presenting high purity and accuracy in the number of cell set per well in a microplate. During to the methodology optimization step we used fluorescent beads and artificial mixture of two *T. cruzi* monoclonal strains. Our results demonstrated excellent sorting index by FACS and high sensitivity of the single cells nested PCR strategy, which allowed us to identify each subpopulation present in the artificial mixture. Using this strategy, we analyzed also a naturally occurring multiclonal strain, A316A R7, recently

isolated from a *Triatoma sordida* vector in Paraná, Brazil. The 24Sα rDNA, COII haplotypes and microsatellite analyses performed in A316A R7 derived single cells showed that this strain is composed by two subpopulations: one belonging to *T. cruzi* II lineage (24Sα rDNA type 1 and COII C haplotype) and another belonging to *T. cruzi* I lineage (atypical 24Sα rDNA of 117bp and COII A haplotype). Furthermore, these two subpopulations displayed distinct microsatellite profiles. The *T. cruzi* II subpopulation was more frequent in the original strain (approximately 90%). The major goal of this kind of analysis is to evaluate the populacional structure of several natural multiclonal *T. cruzi* strains and to determine the occurrence of recombination events within these populations.

Financial support: FAPEMIG, CAPES, CNPq, WHO.

BM033 - PROBING THE DYNAMICS POPULATIONAL OF THE *TRYPANOSOMA CRUZI* DURING DISEASE PROGRESSION IN THE CHRONIC PHASE

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The structure of the *Trypanosoma cruzi* population is still not completely understood. In this work we analyzed the genetic profile of 102 *T. cruzi* isolates from 44 Chagas disease chronic patients from different regions of Minas Gerais (MG) and Goiás (GO) states. At least two isolates were obtained from each patient at different times aiming to understand the parasite population dynamics during disease progression in the chronic phase. All *T. cruzi* DNA were characterized by different PCR assays: rDNA 24Sα; COII mitochondrial gene and SL-IR. Seventy-seven isolates were further analyzed for nine microsatellites loci. Results from rDNA24Sα and the COII gene characterization show a strong correlation of these, since most of the isolates presented rDNA24Sα 1 and COII haplogroup C, corresponding to the *T. cruzi* II major lineage. On the other hand isolates from one patient from MG and one from GO, showed rDNA 2 and 1/2 suggestive at the first sight of *T. cruzi* I and hybrid strains, respectively. However the further COII and SL-IR genes typing confirmed that the last belonged to the hybrid group, but the former was a *T. cruzi* III. These results strengthened the risk for misclassifying *T. cruzi* isolates on basis in a single molecular marker analysis. The microsatellites profiles have shown that in general different isolates obtained from the same patients were genetically identical and monoclonal. Exceptions were observed for *T. cruzi* isolates from two patients that presented differences in the allele sizes detected for the SCLE11 locus and also for other two patients that showed the amplification of three peaks for one single microsatellite locus (TcAAAT6). These findings suggest that at least two populations of parasites were present in these patients. Being thus, which would be the importance of the existence of multiclonality in the population structure of the *T. cruzi*? We demonstrated for the first time the necessity of study the population structure of samples of the *T. cruzi* present in patientes chagasics chronics.

Financial support: PRONEX/CNPq/FAPEMIG and CNPq fellowships

BM034 - CD14 -260 T/C GENE POLYMORPHISM AND THE OCCURRENCE OF CHRONIC CHAGASIC CARDIOMYOPAHTY

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Chronic Chagasic Cardiomyopathy (CChC), the main cause of disability and death in human

Chagas' disease, is characterized by an intense inflammatory response that ultimately leads to heart failure. Inate immune response is essential for mounting primary response against pathogens and orchestrating adaptative immunity. CD14 is a receptor constitutively expressed on macrophages and monocytes, best known as LPS receptor. It can also associate with other receptors of the inate immunity, such as toll like receptors, acting as adjuvant, enhancing signal transduction. It has been recently shown that TLR2 participates in recognition of GPI-anchored mucins from trypomastigotes, triggering expression of inflammatory cytokines. Although our previous work did not show association between TLR2 functional gene polymorphism with CChC, a distinct expression pattern of CD14 could lead to differential macrophage responses to *T. cruzi*. The occurrence of CD14 -260T/C gene polymorphism is associated with high transcriptional activity, leading to high expression of CD14. In fact, our group showed higher expression levels of CD14 by monocytes of healthy individuals with CC genotype, *versus* TT. Here, we aimed to search for association between the CD14 -260T/C gene polymorphism and the occurrence of CChC. DNA was extracted from oral swabs of 149 chagasic patients and genotypic analysis was performed by RFLP. Chagasic patients were further subdivided into indeterminate (n=57) and cardiac (dilated n=54; non-dilated n=38). We did not observe any association between genotypic nor allelic frequency with clinical forms. However, the homozygous occurrence of the polymorphism (T-), was associated with dilated cardiomiopathy when compared with indeterminate disease (p=0.04; OR: 1.02; CI: 1.03-5.52), suggesting that -260T/C polymorphism may influence cardiomiopathy outcome. Investigating other genetic variants of key players in immunopathogenesis of CChC could be helpful for identifying markers of susceptibility to severe disease.

Financed by: WHO/TDR, NIH, CNPq and CAPES.

BM035 - IDENTIFICATION AND COMPARISON OF TRYPANOSOMA CRUZI - COMPLEMENT REGULATORY PROTEIN (TC-CRP) CODING GENE IN STRAINS FROM GROUPS I, II AND 1/2 (HYBRID)

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Chagas disease still represents a major public health problem in the Americas, affecting nearly 15 million people. During its chronic phase, the diagnosis is often performed by conventional serology, based on antibodies search that remain circulating for a long period, leading to a high frequency of false-positive results post-treatment. In this context, Tc-CRP, a tripomastigote-specific membrane glycoprotein has been evaluated as a molecular marker in ELISA assays, showing optimal sensitivity and specificity. However, these data were achieved in studies using exclusively Y strain, prompting the necessity of identifying and differentiating the Tc-CRP encoding gene in other strains. Our aims consisted in identify and comparatively characterize the Tc-CRP–coding gene from *T. cruzi* strains belonged to groups I, II and 1/2 (hybrid). A total of 26 strains were selected, their DNA were extracted and used for PCR assays, with primers CRP-1 and CRP-2, which were specific for Tc-CRP gene. The amplicons obtained were fractionated by PAGE and then digested with the endonuclease *Hae* III for posterior characterization by RFLP. The results demonstrated that the Tc-CRP gene is present in all evaluated strains. The amplification profiles generated by primer CRP-2 demonstrated a differential pattern able to discriminate the two major strain groups, opening the perspective of its use as molecular markers in this differentiation. This data was, subsequently, corroborated by the RFLP analysis. As concluding remarks, we can mention that Tc-CRP–coding gene is present in all evaluated strains, which reinforces the great usefulness of this glycoprotein in ELISA tests for diagnosis and post-treatment therapeutic success evaluation. The polymorphisms identified on the studied samples suggest differences between the Tc-CRP genes, which, meantime, do not interfere in the efficacy of this antigen for detection of anti-*T. cruzi* lytic antibodies induced by any other strains.

Financial support: CAPES, CNPq, FAPEMIG, UFTM.

BM036 - ASSESSING THE GENETIC POPULATION STRUCTURE OF *TRYPANOSOMA RANGELI* BY MICROSATELLITE GENOTYPING

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Microsatellite repeats (MS) occurs in a variety of genomes and are due mutational processes such as replication slippage, point mutations and/or recombination events. Presenting a co-dominant Mendelian inheritance, their size and structure are highly informative for genetic analyzes. Despite the importance of *T. rangeli* on *T. cruzi* diagnosis and epidemiology, MS-based studies have been exclusively carried out on *T. cruzi*. Using a *T. cruzi* MS locus (MCLE-01), we have performed genotyping and analysis of the population structure of 21 *T. rangeli* strains (9 KP1+ and 11 KP1-) from distinct geographical regions. After checking the identity of the locus on the *T. rangeli* genome by sequencing, contigs ranging from 470 to 475bp containing "A" repeats were observed. PCR products of all strains generated with 5´- labeled primers were analyzed using a MegaBace™ 1000 Genotyping System. A total of seven alleles were observed for the MCLE-01 locus in all *T. rangeli* strains. The presence of one or two peaks for MCLE-01 in distinct *T. rangeli* SC-58 strain clones suggests a diploid genome, as observed for *T. cruzi*. A disequilibrium on the Hardy-Weinberg proportion indicated that KP1- strains showed higher heterozygosity than KP1+ strains, despite their geographical origin. Such observation can be due genetic drift, mutation, gene flow and/or natural selection, but further analyses using other loci are underway to address such hypothesis. Phylogenetic analysis based on the results obtained for the MCLE-01 locus are in agreement with kDNA-based classification of *T. rangeli* lineages. Supported by CNPq, CAPES and UFSC.

BM037 - ANALYSIS OF ECTOPIC RECOMBINATION OF VAR GENES IN PLASMODIUM FALCIPARUM ISOLATES.

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The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is encoded by the multi-copy *var* gene family and is an important virulence factor. The ability of P. *falciparum* parasites to generate new *var* genes contributes to a long term persistent infection through immune escape. Different from other regions where falciparum malaria is present, the *var* DBLalpha repertoire of Brazilian parasites is limited, implicating that a large number of *var* tags is shared among distinct isolates. In this study, we have investigated the generation of genetic diversity by ectopic telomeric recombination during meiosis and mitosis. Using pulsed-field gel electrophoresis we examined var tags redistribution in Brazilian *P. falciparum* isolates and 3D7 clones grown for 180 generations. We have analyzed 12 isolates and 15 clones from the 3D7 strain obtained using limited dilution and Southern blot analyses at high stringency showed that the same *var* tag is found in most of the isolates. In general cases, the chromosomal position is not altered, while in other isolates duplication or redistribution occurred. These findings suggest that ectopic recombination is functional during meiosis events in natural transmission. We did not observe any alterations among the 3D7 clones, indicating that ectopic telomeric recombination during mitosis is a rare or absent event. We are now testing more *var* tags in order to estimate the frequency of individual *var* tag redistribution in field samples.

BM038 - DIFFERENTIAL EXPRESSION OF GLUTAMATE DEHYDROGENASE IN BENZNIDAZOLE-SUSCEPTIBLE AND RESISTANT POPULATIONS OF *Trypanosoma cruzi*

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Glutamate dehydrogenases (TcGluDH) are essential enzymes for the metabolism of amino nitrogen and catalyze the NAD and/or NADP-dependent reversible deamination of L-glutamate to form alpha-ketoglutarate. In the human parasite *Trypanosoma cruzi* the *TcGluDH* gene was identified through microarray analysis as having reduced transcription in an *in vitro*-induced benznidazole (BZ) resistant population. In the present study, we have characterized *TcGluDH* gene from 13 *T. cruzi* strains susceptible and

resistant to BZ. Northen blot showed a 2.2 and 3.8Kb transcripts with a similar levels of *TcGluDH* mRNA in parasite populations, except for the 17LER, Colombiana, Noel and VL-10 resistant strains, where transcripts levels were 2-fold lower. We determined whether the increase in mRNA levels was due to gene amplification using Southern blot analysis of the *T. cruzi* strains. *TcGluDH* gene is organized in multicopy array and no amplification was observed in the parasite genome. The cromossomal location of the *TcGluDH* gene was variable, but was not associated with the zymodeme or with de drug-resistance phenotype. In Western Blot analysis, anti-TcGluDH antisera recognised a 49kDa protein in all strains tested. The level of expression of this polypeptide was approximately 2-fold lower in 17LER and Noel resistant strain than in the susceptible strains. Our findings show a reduced expression of the TcGluDH enzyme in the *T. cruzi* BZ-resistant strain and suggested that amino acid metabolism may be provide multiple as yet unexplored targets for the therapeutic drugs in Chagas disease. Supported by: CNPq; FAPEMIG and CPqRR/FIOCRUZ.

BM039 - CHARACTERIZATION OF A GENE ENCODING ALDO/KETO REDUCTASE IN BENZNIDAZOLE-SUSCEPTIBLE AND RESISTANT POPULATIONS OF *Trypanosoma cruzi*

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Aldo-keto reductases (AKRs) are a superfamily of enzymes that reduce aldehydes and ketones, and have a broad range of substrates. Moreover, in *Trypanosoma cruzi*, the causative agent of Chagas disease, TcAKR is over-expressed in strains that are resistant to benznidazole (BZ), a drug currently used in chemotherapy. TcAKR has thus been indicated as a potential target for the development of new chemotherapeutic agents. In this work, we have characterized *TcAKR* gene from 11 *Trypanosoma cruzi* populations susceptible, naturally resistant or with *in vitro*-induced (17 LER) or *in vivo*-selected resistance to benznidazole (BZR). Northen blot showed a 1.02 and 2.05Kb transcripts with a similar levels of *TcAKR* mRNA in parasite populations, except for the BZR and Sc-28 resistant strains, where transcripts levels were 3-fold higher than in drug-susceptible strain. We determined whether the increase in mRNA levels was due to gene amplification using Southern blot analysis of the *T. cruzi* populations. We found that the number of gene copies was similar for all samples tested and TcAKR gene is organized in a tandem multicopy array. The cromossomal location of the *TcAKR* gene was variable, but was not associated with the zymodeme or with de drug-resistance phenotype. Our findings show an increased of the *TcAKR* mRNA in the *T. cruzi* population with resistance to BZ. Further studies will be performed, in order to investigate possible TcAKR protein overexpression in the resistant populations, to confirm our hypothesis that TcAKR is involved in *T. cruzi* resistance to BZ. Supported by: CNPq, Fapemig and CPqRR/FIOCRUZ.

BM040 - *Trypanosoma cruzi*: characterization of the gene encoding Ascorbate peroxidase (TcAPX) in benznidazole-resistant and susceptible populations

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Ascorbate peroxidase (APX) is one of enzymes involved in the antioxidant defense of trypanosomatids. It is responsible for reduction of hydrogen peroxide to water in the endoplasmic reticulum of the *T. cruzi*. The absence of the APX in mammals makes it an important target for antimicrobial chemotherapy. Here we have characterized a gene encoding ascorbate peroxidase in *T. cruzi* (TcAPX) populations susceptible and resistant to benznidazole (BZ). The TcAPX gene complete sequence was compared against the local compiled database for contigs presenting similarities against the query sequences. From the 4 contigs initially identified by means of manual annotation in the Artemis software, we were able to select 2 contigs (AAHK01002212 and AAHK01001145). The data suggest that TcAPX gene present two full copies dispersed throughout the parasite genome. Alignment of these TcAPX contigs by “Clustal X” indicated 99% amino acid sequence identity. Southern blot analysis of DNA digested with two restriction enzymes (AvaI and XhoI) hybridized with a specific P^{32}-TcAPX probe showed fragments of 0.85 and 4.6 Kb, respectively. Comparative densitometry analysis of the fragments intensity

showed no amplification of the TcAPX gene between *T. cruzi* populations resistant or susceptible to BZ. The *in silico* restriction map analysis performed with AvaI endonuclease confirmed the southern blot results. Furthermore, chromosomes from 10 *T. cruzi* strains were separated by pulsed field gel electrophoresis and hybridized with a TcAPX gene-specific probe. The results revealed that TcAPX gene is located on a chromosome of approximately 2.0 Mb in all *T. cruzi* samples independent of the phenotype resistance or susceptible to BZ. Studies are underway to evaluate the TcAPX mRNA level in the *T. cruzi* strains susceptible and resistant to BZ.

Supported by CNPq, FAPEMIG, CPqRR/ FIOCRUZ

BM041 - PROTEOMIC ANALYSIS OF *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES SUSCEPTIBLE AND RESISTANT TO BENZNIDAZOLE.

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There are 10 million people infected with the Chagas' disease causative agent, *Trypanosoma cruzi*, in Central and South America. The drug currently used in the treatment of Chagas' disease in Brazil, benznidazol (Bz), presents a reasonable efficacy in the acute phase, very low activity in the long-term chronic phase, undesirable side-effects and variable efficacy according to the parasite strain infecting the patient. A better understanding of *T. cruzi* drug resistance mechanisms, may lead to the identification of new drug targets and new chemotherapeutic agents. Proteome comparative analysis seems to be a particularly important tool because it assesses directly the proteins. It overcomes the post transcriptional events that frequently happen in the gene expression regulation in *T. cruzi*. The aim of this work is to identify trypomastigote proteins potentially involved in the BZ resistance mechanisms. Trypomastigote forms from two *T.cruzi* populations previously selected *in vivo*, BZ resistant (BZR) and BZ susceptible (BZS), were maintained in Vero cell monolayers, collected and purified. We developed an appropriated drug test *in vitro* to evaluate the drug susceptibility using the vital dye Alamar Blue®. The difference in BZ susceptibility between the tissue culture BZR (LC_{50}=95.1μM) and BZS (LC_{50}= 12.0μM) trypomastigotes was approximately eight-fold. Bidimensional gel electrophoresis (2-DE) followed by Mass Spectrometry (MS) were used to separate and identify differentially expressed proteins between BZR and BZS trypomastigotes. In a pH 3-10NL 2-DE separation range we found 238 spots for BZR and 241 for BZS. Further, in a pH 4-7 2-DE separation range we found 211 spots for BZR and 251 for BZS. Seven out of nine differentially expressed proteins were identified: tyrosine aminotransferase, cofilin/actin depolymerizing factor, L-threonine 3-dehydrogenase, eukaryotic initiation factor 5α, malic enzyme, elongation factor 1α, alanine aminotransferase and ATPase β subunit. The first three were previously identified as differentially expressed between BZR and BZS epimastigotes.

Financial support: CNPq, FAPEMIG, PAPES e PDTIS – FIOCRUZ.

BM042 - Comparative analysis of nucleotide and peptide sequences and expression of five proteins from populations susceptible and resistant to potassium antimonyl tartrate in four L*eishmania* species.

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In the present study we have selected four populations of L*eishmania* species with *in vitro* resistance developed to potassium antimonyl tartrate (SbIII): *L.amazonensis* (PH8 strain), *L.braziliensis* (M2904 strain), *L.chagasi* (PP75 strain) and *L.guyanensis* (M9945 strain). We have analyzed the nucleotide sequences and the expression level of five proteins involved with: antioxidant defense (iron superoxide dismutase-Fe-SODA and tryparedoxin peroxidase -TXNP); drug-stress (70 kDa heat shock protein - HSP-70) and metabolism (NAD(P)H flavin oxidoreductase - OYE and dihydrolipoamide dehydrogenase - LipDH). Nucleotide and peptide sequences of those proteins from *L.braziliensis*, *L.infantum*, and *L.major* were compared to each other, to homologue sequences from *T.cruzi* and used for

primer design for gene amplification through the Polimerase Chain Reaction (PCR). Protein expression was determined by Western blot, using rabbit polyclonal antibodies raised against these recombinant proteins from *T.cruzi*. Nucleotide sequence polymorphisms were observed in the genes encoding Fe-SODA, TXNP and LipDH in the different *Leishmania* species. Polymorphisms were also observed in the band profile recognized by Western blot analysis in the proteins Fe-SODA, OYE and LipDH. The analysis of the level of protein expression showed that Fe-SODA is 1.7-fold more expressed in the resistant population of *L.guyanensis* and 2.0-fold in the resistant population of *L.braziliensis*, compared with their respective wild-type populations. The level of expression of OYE from *L.braziliensis* resistant population was 3.0-fold less expressed than its wild-type population. Further studies are under way to investigate the involvement of those proteins with drug-resistance phenotype in *Leishmania*.

Supported by: CNPq, CAPES, FAPEMIG.

BM043 - Characterization of mechanisms of antimonial resistance in *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) major* mutants

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Clinical resistance to pentavalent antimonial compounds may constitute a major problem in the treatment of leishmaniasis. Understanding of resistance mechanisms is essential to define treatment strategies and to monitor the emergence and spreading of resistance. In this study we selected *Leishmania braziliensis* and *Leishmania major* (SbIII) and (SbV)-resistant cell line and analyzed: (i) (SbIII) accumulation, (ii) total thiol levels and (iii) patterns of expression of relevant genes. (*ACR2*) antimoniate reductase, (*TDR1*) thiol-dependent reductase 1, (*GCS*) γ-glutamylcysteine synthetase, (*ODC*) ornithine decarboxylase, (*MRPA*) multidrug resistance protein A and (*AQP1*) aquaglycerporin 1 were the genes investigated in this study. *L. braziliensis and L. major* (SbIII)-resistant mutants presented a decrease in (SbIII) accumulation, possibly related to a lower level of *AQP1* expression and increased drug extrusion mediated by increased *MRPA* gene expression. A different resistance profile was observed in *L. braziliensis* (SbV)-resistant mutants. These cell lines showed a higher level of SbV activation, possibly due to a higher level of *ACR2* expression and active drug extrusion via a higher expression of *MRPA*. Moreover, *L. major* (SbV)-resistant mutants presented a similar phenotype when compared to *L. donovani* (SbV)-natural resistant isolates. These mutants had an altered thiol metabolism, possibly resulting in inhibition of (SbV) activation. Current work is focused in 2D electrophoresis protein analysis of different resistant mutants to establish a correlation between the expression profile and resistance phenotype observed in these cell lines.

Supported by FAPESP, CNPq and CAPES

BM044 - CHARACTERIZATION OF THE DIVISION PROTEINS OF THE ENDOSYMBIOTIC BACTERIUM OF THE TRYPANOSOMATID *Crithidia deanei*

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Some monoxenous protozoa of the *Trypanosomatidae* family such as *Blastocrithidia culicis*, *Crithidia deanei*, *Crithidia desouzai* and *Herpetomonas roitmani* harbor endosymbiotic bacteria in their cytoplasm. The relationship between the endosymbiont and the trypanosomatid provides an intersting model to understanding the emergence of eukaryotic organelles mitochondria and choroplasts. It is noteworthy to note that in the case of trypanosomatids there is only one symbiotic bacterium per cell, which implies the existence of a perfect control of the endosymbiont division. The factors that perform and control this process as well as their mechanisms remain unknown. In this regard, we characterized some key proteins involved in the processes of assembling and

regulation of the Z ring, which are important steps in the division site formation in the mid-cell. Among these proteins, there are FtsZ, FtsK, ZipA and the complex MinCDE. The genes encoding these proteins were identified in the genome of the endosymbiont of *C. deanei* that has been sequenced in the Carlos Chagas Institute. All the studied genes were amplified by PCR from the genomic DNA of the endosymbiont purified from *C. deanei.* The analysis of the deduced amino acid sequences from these genes show that they share greater similarity with the orthologues from the *Bordetella* genus *(*subdivision of β Proteobacteria). We showed that the expression of the genes *ftsZe, zipAe and minDe* in *E. coli* induce filamentation, indicating that these recombinant proteins were functional and could destabilize the division machinery in *E. coli*. The functionality of the proteins FtsKe and MinDe was also showed by their hability to hydrolise ATP. Regarding the FtsZe, we show that it was able to self-polimerize, an important feature for the Z ring assembly. Taken together, these data suggest that proteins of the endosymbiont divisome have maintained their functionality during the evolution of the symbiotic relationship.
Financial support from CAPES, Fiocruz.

BM045 - TRANCRIPTIONAL PROFILE OF RIF GENES IN *Plasmodium falciparum*

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The *P. falciparum* rif gene family is composed of ca. 150 members per haploid genome and not much is known about its function, transcriptional modus and the importance in immune escape. In order to investigate the global transcriptional profile of rif genes we designed oligonucleotides for 150 copies using primer 3 and e-pcr. *P. falciparum* 3D7 wild types and Selectin-adhesive phenotypes were cultured, synchronized and recovered at trophozoite stage (18-24h). RNAs were reverse transcribed and relative mRNA expression to Seryl t-RNA synthetase (PF070073) was analyzed using Real-Time PCR. Four genes PFI0055c, PF100397, Mal13P1500 and PF130006 are upregulated in selectin-cytoadherence selected parasites. In unselected parasites, PFD0070c is up-regulated while this gene is down-regulated in selectin parasites. Further experiments are underway using other adhesive phenotypes as well as testing parasites after a couple of reinvasions in order to elucidate if there is transcriptional switching or not in 3D7 *P. falciparum*.

Financial support: FAPESP

BM046 - THE EXPRESSION OF *Leishmania major* TAGGED GENES IS DEPENDENT ON GENOME CONTEXT.

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We have previously shown that transposon technology can be used as a reliable tool for gene expression studies in *Leishmania*. *In vitro* transposition events can also be the source of fragments for genome integration by homologous recombination, which allows the characterization of a fusion protein expressed from a chromosomal context. We have used shuttle mutagenesis to integrate a reporter fusion between a hypothetical gene (LmjF23.0310; orf4) and the neomycin phosphotransferase II gene (NEO). Northern analysis of the selected heterozygous cell line (HZorf4) and a cell line expressing the fusion on an episome revealed the presence of transcripts for both wild-type and quimeric loci (orf4::NEO). Despite the significant level of transcripts, western analysis detected the fusion protein exclusively in the cell line expressing it from an episome. Moreover, amplification of the locus, generated during attempts to select a homozygous cell line, resulted in the expression of the fusion from the extrachromosomal molecule. Altogether, these data suggest not only a strict expression control of genes encoded within chromosomes, but also that it occurs at post-transcriptional level.
Supported by: FAPESP and CNPq.

BM047 - Targeting subtelomeric sequences of *Leishmania major*.

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Chromosome ends can play an important role in virulence and survival of protozoan parasites. In *Trypanosoma brucei*, for instance, antigenic variation is mediated by the genes expressed from telomeric loci. During attempts to disrupt *RPC2*, a telomere-located essential gene of *L. major*, we did not observe alterations in ploidy or translocation events. These phenomena are normally elicited when essential genes of the parasite are targeted. However, we observed the preservation of gene copy number and location.

We set out to investigate if the observed phenotype was related to either the disruption of an essential gene or to the telomeric nature of the targeted locus. Intergenic sequences within subtelomeric regions were targeted for the integration of a selectable marker. For the construction of integration reagents, the Streptotrycin Acetyl Transferase (SAT) cassette was cloned into amplified fragments from chromosomes 06 and 20 of *L. major*. The integration fragments were purified and transfected into the parasite. A heterozygous mutant bearing SAT at 10 Kb from the telomere of chromosome 06 was selected and characterized. The pattern of gene plasticity of this mutant is currently being investigated.

Supported by FAPESP and CNPq.

BM048 - ECTOPIC EXPRESSION OF THE GLYCOSOMAL PHOSPHOGLYCERATE KINASE IN *LEISHMANIA* IS MODULATED BY BOTH 5' AND 3' UNTRANSLATED REGIONS

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The protozoan parasite *Leishmania* expresses two isoforms of phosphoglycerate kinase (PGK): PGKB is found in the cytosol and PGKC in the glycosome. PGKB and PGKC open reading frames are 99.5% identical, diverging with respect to the signal peptide present at the 3' end in *PGKC.* The cytosolic isoenzyme is approximately 4 times more abundant than its glycosomal counterpart, throughout the parasite life cycle. In this study we used ectopic overexpression of *PGKB* or *PGKC* in *Leishmania major* to study the role of 5' and 3' untranslated regions (UTRs) of these genes in the control of the levels of expression of each gene. We observed that the presence of both 5' and 3' UTRs of PGKC affected the control of replication of the plasmid; PGKC overepressors bear approximately $1/10^{th}$ of plasmid copy number than control and PGKB transfectants. In addition, in PGKC overexpressors the level of episomal RNAs is ~4 times lower than in PGKB and vector transfectants. Nevertheless, PGKC transcripts have a longer half life; it is twice as long as the one observed for PGKB overexpressors and it is affected by the absence of either 5' or 3' UTRs of *PGKC*. As a result of the mRNA stability we observed that the glycosomal protein accumulates in the organelle at higher levels than the cytosolic isoform, despite a faster kinetics of degradation of the glycosomal protein.

Supported by FAPESP and CNPq

BM049 - STUDIES OF NON-CODING RNA FROM *LEISHMANIA MAJOR*.

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The presence of various forms of non-coding RNAs (ncRNAs) involved in gene expression regulation in other organisms and the fact that in trypanosomatids gene expression is controlled at the posttranscriptional level supported this work. We selected some non-coding RNA candidates from a cDNA library to investigate their potential role in the control of gene expression in *Leishmania*. Here we report the study of one of the candidates, named *ODD3.* The phenotype more than four independent *Leishmania major* transfectants overexpressing *ODD3* was analyzed. Promastigotes grow poorly in axenic culture, present a noticeable altered morphology and some

of them gained an extra copy of chromosome 5 suggesting a deleterious effect of *ODD3*. Putative mRNA targets of *ODD3* were identified by RT-PCR and computational analysis. We identified a region close to an ORF of chromosome 18 (LmjF18.0730, a putative dynein light chain), a hypothetical protein from chromosome 21 (LmjF21.0725, with a conserved domain of Leucil / Fenilalanil-tRNA-transferase) and a region between two ORFs for hypothetical proteins (LmjF36.2060 and LmjF36.2070). The level of transcript for the putative L/F t-RNA transferase is lower in *L. major ODD3* transfectants. To further investigate the correlation between *ODD3* and LmjF21.0725 we generated co-transfectants for both genes. *L. major* co-transfectants present a tendency to revert the dramatic phenotype of *ODD3* mutants. In addition, Northern analysis indicates that LmjF21.0725 expression is under the control of *ODD3.*

Supported by FAPESP

BM050 - INVESTIGATION OF A POSSIBLE TELOMERE-POSITION EFFECT IN THE EXPRESSION OF *LEISHMANIA* GENES

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We have previously generated a *Leishmania major* heterozygous mutant for the *RPC2* gene, in which a selectable marker (SAT) was integrated at one of the telomeres of chromosome 20. Unsuccessful efforts to generate a RPC2 null mutant did not lead to amplification of the locus suggesting that maintenance of copy number and expression of this telomere-located gene is under strict control. The expression pattern of SAT in this cell line was notably different from that observed when the selectable marker was integrated internally into chromosome 23. Unlike the internal marker, the expression level of telomere-located SAT was dependent on the presence of the selective drug in the culture medium. Moreover, the expression of Anti-Silent Factor 1 (ASF-1) was sufficient to suppress the observed effect. This result suggested a possible telomere-position effect (TPE) on *Leishmania* gene expression. TPE is widely described in *Saccharomyces cerevisae* and is mediated by epigenetic control of chromatin packing and telomere lenght. We are currently studying the expression of the LmSir2 protein and its involvement in the observed phenotype. The effect of nuclear position in the expression of SAT is also being investigated in these cell lines.

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BM051 - STUDY OF A NON CODING RNA IN *LEISHMANIA BRAZILIENSIS*

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Non coding RNAs (ncRNAs) execute their role in the cell as RNAs and are not intermediates of protein codification. The ncRNAs are involved in a variety of cellular processes, such as replication of DNA and chromosomal maintenance, regulation of transcription, RNA processing, RNA stability, translation and it regulates the stability and translocation of proteins. Previous work of our group characterized a possible ncRNA called *ODD3. Leishmania major* transfected with the *ODD3* gene has evident genotypic and phenotypic changes; axenic promastigotes present marked growth impairment, the ultra-structural analysis revealed an altered morphology and ploidy changes are also observed. The goals of this project are to extend the studies with *ODD3* to *Leishmania braziliensis* and to investigate the effect of ODD3 direction in the vector on the transfectants' phenotype. Therefore, the same recombinant transfected in *L. major* was expressed in *L. braziliensis* and a recombinant containing the *ODD3* gene positioned in the same direction as of the neomycin phosphotransferase was generated and transfected in *L. major* and *L. braziliensis.* We are currently analyzing phenotypic changes of the *L. major* and *L.braziliensis* transfectants. We observed that the presence of the ectopic copy of *ODD3* is deleterious to *L. braziliensis* and that the inversion of ODD3 direction within the plasmid leads to different phenotypic changes. We will present growth curve profiles, molecular karyotype (PFGE) and morphological changes in addition to a comparative analysis of levels of expression of plasmidial genes and the ODD3 target gene.

Supported by FAPESP and CNPq

BM052 - A 62BP INDEL IN THE INTERGENIC REGION AFFECTS THE RNA PROCESSING AND STABILIZATION

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1.Introduction

A 62bp indel localized in the intergenic region (62bp indel-IR) of the *TcUMSBP* gene affects the mRNA processing generating differential RNA accumulation and two sites of polyadenylation. This was shown by RT-PCR and transient transfection assays using the 62bp indel-IR cloned upstream to the CAT (Chloramphenicol acetyl transferase) gene (Coelho et al, 2006, Biochem Bioph Res Com, 341:382).

2. Proposition

To investigate the role of the 62bp indel-IR downstream to the CAT gene in transient and permanent transfection in *Trypanosoma cruzi* CLBrener clone.

3. Methods and Results

Transient transfections were done using the plasmid pKS containing the CAT gene flanked by the HSP70 intergenic region at 5' and the 62bp indel-IR at 3'. Purified plasmids (100μg) were electroporated in epimastigotes cells. After 48 hours, the cells were collected and the protein extract were obtained. CAT assays were performed using Amersham kit protocol. CAT activity was higher in cells transfected with the 62bp insertion. This suggests that the presence of 62bp stabilizes the CAT mRNA. To confirm these results, total RNA was also obtained for future quantification. To further analyse the regulation of the RNA stabilization and avoid problems concerning the amount of transfected DNA, we are preparing new plasmids for permanent transfections. The plasmids contain the entire GADPH intergenic region upstream to the CAT gene and the 62bp indel-IR; the GADPH intergenic region also regulates the neomycin resistance gene. Similar plasmids that substitute the GAPDH at 5' for the 62bp indel-IR are being prepared to better comprehend the RNA processing steps. We are current investigating the half-lives of the mRNA beta-proteasome in order to determine the role of 62bp insertion at the 3'UTR.

Supported by: FAPERJ and CNPq

Key words: TcUMSBP, polymorphic alleles, kDNA, minicircle, RNA processing.

BM053 - *En masse* CHARACTERIZATION OF mRNAS ASSOCIATED WITH RNA BINDING PROTEINS OF THE RRM FAMILY IN *Trypanosoma cruzi*

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Trypanosoma cruzi can be considered as a model organism for studying the posttranscriptional mechanisms that regulate gene expression in eukaryotes. These mechanisms are mediated and facilitated by RNA binding proteins (RBPs) and regulatory elements present in the untranslated regions (UTR) of the mRNAs which associate in ribonucleoprotein complexes (RNPs). In these RNPs, functionally related mRNAs and RBPs associate in a combinatorial way to define regulatory networks known as RNA regulons. Characterizing the proteins present in the different RNPs and their target mRNAs may contribute to the comprehension of posttranscriptional mechanisms in eukaryotes. The ribonomic approach consists in the purification of RNPs or a single RBP with their associated transcripts followed by the isolation of the mRNAs and microarray hybridization to define their identity. We focused in the RBP protein family with the RNA Recognition Motif (RRM). We have selected several proteins that are expressed in the epimastigote forms of *T. cruzi* and show some degree of regulation during differentiation. His-tagged recombinant proteins were obtained in their soluble form in order to use them in pull-down assays with total RNA from epimastigote forms. Target mRNAs are currently being identified by allowing them to form complexes with the recombinant proteins *in vitro*. These complexes are isolated by affinity chromatography in niquel-agarose columns, and the resulting RNAs are amplified and analyzed in the *T. cruzi* oligonucleotide microarray. Bioinformatics analysis are being performed in order to identify the recognition elements in the 3'UTRs and to construct the regulatory gene networks of which these proteins and mRNAs are part.

Financial support: NIH, PRONEX/Fundação Araucária, CNPq, CAPES.

BM054 - ANALYSING THE ROLE OF AU-RICH SEQUENCES IN THE ALPHA-TUBULIN MRNA OF TRYPANOSOMA CRUZI

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Several studies have shown that sequences in untranslated regions (mainly in 3'UTR) of trypanosomatid transcripts are involved in gene regulation. One of these sequences is named AU-rich elements (AREs), which can be involved in mRNA regulation, as shown for the SMUG mRNA of *Trypanosoma cruzi*. In this work, our goal was to evaluate the role of an AU-rich sequence present in the alpha-tubulin 3' UTR of *T. cruzi* using a transfection vector, pROCKDualLUC that contains 2 reporter genes: the fire-fly (FLuc) and *Renilla* (RLuc) luciferases. After generating stable transfectants with the complete or an ARE-deleted 3' UTR of alpha-tubulin mRNA downstream from the FLuc gene, luciferase mRNA quantifications and protein activity assays were carried out. Parasites transfected with pROCKDualLUC containing the complete alpha-tubulin 3' UTR showed higher luciferase mRNA levels and activity than parasites transfected with ARE-deleted 3' UTR. However, when the AU-rich sequence was inserted within the TCR27 and amastin 3' UTR, we observed no differences in luciferase activity compared to the wild type 3' UTRs in transiently transfected parasites. These findings suggest that (1) either more than one ARE sequence motif is necessary to confer regulation to a heterologous gene, or (2) a different poly-adenylation may occurs due to an alternative poly-pyrimidine tract, so that the 3' UTR sequences of the transfected genes are different from the endogenous mRNA. To test the second hypothesis, sequencing analysis of cDNA amplification products of mRNA derived from stable transfected parasites using primers specific for FLuc and alpha-tubulin sequences is currently underway.SUPPORT: CNPq, FAPEMIG, HHMI

BM055 - CHARACTERIZATION OF THE *Trypanosoma rangeli* TYPE II DNA TOPOISOMERASES DOMAINS

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Type II topoisomerases (topoII) catalyze DNA changes by introducing transient double-strand cuts. Eukaryotic type II topoisomerases are important due their essential role in maintenance of chromosomes structure and segregation. Along with their key role on nuclear DNA processes, these enzymes are also involved in replication and organization of the kinetoplast DNA in trypanosomatids. In addition, DNA topoisomerases have emerged as targets for the development of antitumoral, antibacterial and antiparasitic drugs. We have cloned and sequenced two genes encoding topoII in *T. rangeli* (*TrTOP2mt* and *TrTOP2α*), both showing high similarity with topoII genes from other trypanosomatid species. While amino acid sequences of the topoII N-terminal and central domains are conserved, the C-terminal domain is quite divergent and, thus, species-specific. Recombinant expression of the C-terminus of both *TrTOP2mt* and *TrTOP2α* in bacteria resulted in non-soluble proteins. Polyclonal sera raised against both proteins in mice exclusively recognized *T. rangeli* cells in Western blots. A total of ten distinct fragments of *TrTOP2mt* and *TrTOP2α* genes, including the ATPase, the clevage and the carboxy domains, were PCR amplified, cloned in pET14b and expressed. Since amino acid sequences of these domains shows high inter-specific conservation, recombinant proteins were purified (NI-NTA) and used for antiserum production in mice to assess their expression levels and sub cellular distribution. Supported by CNPq, CAPES, Fiocruz and UFSC.

BM056 - CHARACTERIZATION OF THE DNA TOPOISOMERASE IIIα FROM *TRYPANOSOMA CRUZI*

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DNA topoisomerases are ubiquitous enzymes involved in many cellular processes such as DNA replication, transcription and recombination. Topoisomerases are divided in two classes (type I and type II) according to their mechanism of action. Type I enzymes act by introducing a transitory cut in one DNA strand, whereas type II enzymes cut both DNA strands.

Type I topoisomerases are further classified in two subfamilies (IA and IB). The type IA enzymes, as exemplified by bacterial DNA topoisomerases I and III, and eukaryotic DNA topoisomerase III, form a tyrosyl linkage with a 5' phosphate. The type IB enzymes form a tyrosyl linkage with a 3' phosphate and include the eukaryotic DNA topoisomerase I, the poxvirus DNA topoisomerase and the bacterial topo V.

The role of topoisomerase III (a type IA enzyme) is still unclear and might be related to processes that control the genome stability. To further get insight into the role of topo III for *T. cruzi* we have analyzed the genome of this parasite for the presence of the gene encoding this enzyme. Blast analysis showed that the genome of *T. cruzi* encodes two distinct topo III (α and □). We have successfully produced the functional Tctopo III□ enzyme using a baculovirus expression system. The recombinant Tctopo IIIα (102 kDa) was purified by Ni-NTA chromatography and assayed for plasmid relaxation activity. Our data showed that the Tctopo IIIα is able to relax a supercoiled plasmid in a fashion similar to other eukaryotic topo III enzymes. The activity of Tctopo IIIα was tested at 28 °C and 37 °C and no significant difference was observed in the ability of the enzyme to relax the plasmid. Antiserum will be produced to immunolocalize the enzyme and analyze the expression pattern during the life cycle of *T. cruzi*.

Financial support: CNPq, Fundação Araucária-PR.

BM057 - DNA Polymerase Rev1 from *Trypanosoma cruzi* presents catalytic properties not encountered in its described orthologs

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Trypanosoma cruzi, the causative agent of Chagas disease, is an unicellular protozoan that presents high genetic variability. The generation of this variability can be associated to the action of low-fidelity DNA polymerases during the process of DNA replication. This group of DNA polymerases shares some common properties: lack of associated exonuclease 3' to 5' proofreading activity; very limited processivity; and more permissive catalytic site. DNA polymerase Rev1 belongs to this group and is high specialized in the incorporation of C opposite template G, using a mechanism of DNA synthesis which employs a protein template. As previously described in different organisms, Rev1 is involved in UV light-induced mutagenesis and performs the bypass of N^2-adducted G and abasic sites. In this work, we studied the gene *REV1* from *T. cruzi* (*TcREV1*). In contrast to its orthologs, *TcREV1* deduced peptide sequence presents only the catalytic domain, lacking protein-protein interaction motifs. In the catalytic core, almost all the key aminoacids related to the high-specialized incorporation of C opposite template G are present, including the arginine that pairs with incoming dCTP. Curiously, the protein TcRev1 incorporates correct dNTPs opposite A, C, T and G templates. *In vivo* experiments will be needed to investigate the role of this polymerase in *T. cruzi.*

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BM058 - *TRYPANOSOMA CRUZI* MOLECULAR CHARACTERIZATION: OPTIMIZATION OF PCR-RFLP TECHNIQUE FOR CYTOCHROME OXIDASE SUBUNIT II (COII) GENE ANALYSIS

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The molecular characterization of *Trypanosoma cruzi* major lineages throughout gene regions contained in the maxicircle of the parasite's KDNA has been largely used in the last years. Techniques such as PCR-RFLP are frequently used for this characterization, more specifically for the COII gene of *T. cruzi*. Previous studies based on COII-RFLP profiles were able to discriminate *T. cruzi* strains into three major haplotypes. However some difficulties in the amplification of this gene in strains belonging to *T. cruzi* I lineage have been observed. To solve these problems DNA sequences of COII gene from *T. cruzi* I strains available on GenBank were aligned by the MultiAlin software and revealed the existence of SNP variation in the in 3´-end of primer annealing sites (TcMit10 and TcMit21). Thus, the objective of this present work was to design and test a new primer pair (TcMit17-Forward and TcMit17-Reverse) to improve the sensitivity of the PCR amplification for COII gene using strains belonging to different *T. cruzi* lineages and the full-nested-PCR protocol. After these sensitivity assays it was possible to verify that the new primer pair was more sensitive than the previous primer pair for *T. cruzi* I strains. Furthermore, the new RFLP-patterns employing the *Alu* I restriction endonuclease facilitated the differentiation of the strains belonging to *T.cruzi* II lineage from the other *T. cruzi* lineages due to larger differences observed among the banding patterns. The analyses of the results employing full-nested PCR allowed us to detect as little as 200 fentograms of DNA, corresponding to the DNA content of a single parasite cell. This sensitivity level allow the use of the new primers by the full nested-PCR protocol in assays containing *T. cruzi* DNA obtained from tissues of chronically infected patients and single cells sorted by FACS Cell Sorter.

Financial support: FAPEMIG, CNPq and CAPES

BM059 - ENDOSYMBIOSIS IN TRYPANOSOMATIDS: THE FINDING OF A PUTATIVE PORIN SUGGESTS THE PROKARYOTIC ORIGIN OF THE OUTER MEMBRANE IN THE SYMBIOTIC BACTERIUM

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Some protozoa of the Trypanosomatidae family, as *Crithidia deanei*, present an obligatory intracellular bacterium, which maintains a mutualistic relationship with the host. This association constitutes an excellent model to study the origin of organelles and cellular evolution. The endosymbiont is surrounded by two unit membranes, but lacks a typical cell wall of Gram-negative bacteria. Regarding the origin of the outer membrane, some authors suggest that it is derived from the host protozoan, but other data indicate its prokaryotic nature. The presence of porins, which are typical outer membrane proteins, in the endosymbiont envelope would confirm its Gram-negative ancestral, since molecular analysis classified this bacterium in β subdivision of Proteobacteria, close to *Bordetella* genus. In this study, we used biochemical and molecular methods to identify porins in the *C. deanei* endosymbiont envelope. Search on the endosymbiont genome annotation database identified a sequence that shares homology with porin genes of Gram-negative bacteria. From then, primers were designed and a PCR amplification produced a 1,2 Kb DNA fragment that was cloned in the pET21dHis-Tev vector to overexpress the recombinant protein. We purified the recombinant porin using Ni^{2+} column and ASB-14 was used for the protein solubilization. To check its localization by immunocytochemical techniques, antiserum against this recombinant porin was produced in mouse. Our data showed that the recombinant protein presents 64% (262/408) of positive identity with a *B. pertussis* porin, suggesting the prokaryotic origin of the outer membrane in the endosymbiont. Support: CNPq and CAPES.

BM060 - IDENTIFICATION OF MAJOR SURFACE PROTEASE (MSP) MEMBERS IN *TRYPANOSOMA RANGELI*

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Key words: *Trypanosoma rangeli*, Major Surface Protease, bioinformatic.

Introduction and objectives: Major Surface Proteases (MSPs), also known as gp63 or leishmanolysin, play a major role in trypanosomatids survival and infection. In *Trypanosoma rangeli*, a non-pathogenic trypanosome which is able to infect humans, members of this gene family have not been described yet. This study aimed the characterization of recombinant clones isolated from a *T. rangeli* genomic library which present significant sequence identity with MSPs described in other trypanosomatids. **Results:** A partial *T. rangeli* genome library was constructed by cloning *Bam*HI/*Bgl*II restriction fragments of the P07 strain of the parasite in the *Bam*HI site of the vector pUC18. BLASTP searches allowed the isolation of fourteen recombinant clones from a *T. rangeli* genome library for their significant identity with MSP sequences described in *Leishmania* spp., *Trypanosoma brucei* and *Trypanosoma cruzi* available in GenBank. Multiple sequence alignments with MSPs from several trypanosomatid species showed that *T. rangeli* MSPs presented the highest percentage of identity with *T. cruzi* sequences (38% to 69%) and the lowest percentage of identity with *Leishmania infantum* (27% to 45%). A detailed analysis of *T. rangeli* MSP protein sequences allowed the identification of the region HEIAHTGF which contains a histidine residue essential for the enzyme catalytic activity. **Conclusion:** These results show the presence of members of the MSP gene family in *T. rangeli*. The presence of a conserved region previously associated with the catalytic site of the enzyme strongly suggests that MSPs present in *T. rangeli* are active. Further studies on the genomic organization of these genes and their expression will open new perspectives on MSP function in a non-pathogenic trypanosome. This work supported by FAPEMIG.

BM061 - IDENTIFICATION AND CHARACTERIZATION OF PROTEINS INVOLVED IN mRNA EXPORT FROM NUCLEI TO CYTOPLASM IN T. cruzi

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Regulation of gene expression in eukaryotes involves transcriptional and post-transcriptional mechanisms. In Trypanosomatids, transcriptional mechanisms appear to play a minor role and there are several examples of changes in gene expression that are controlled post-transcriptionally. These changes are controlled principally by RNA binding proteins that are part of ribonucleoprotein (mRNP) complexes. The nuclear export of mRNAs is a post-transcriptional event that involves the shuttling of mRNP from nuclei to citoplasm. This mechanism of regulation may be important in Trypanosomatids but it is still little understood. In other eukaryotes, it is also a major point for controlling protein expression during cell development and differentiation. The goal of this work is the identification of the molecular factors involved into nuclear export of mRNAs to characterize their role in the regulation of gene expression during the life cycle of these parasites. Bioinformatic analyses allowed the identification of five proteins genes from *Trypanosoma cruzi* and *Trypanosoma brucei* orthologous to proteins from *Saccharomyces cerevisiae* that are involved with mRNA transport from nucleus to cytoplasm. We present phylogenies that illuminate the evolutionary relationships of each of these proteins. We performed molecular evolution analyses of each of these genes to evaluate the role of positive Darwinian selection in the evolution of these proteins as well. In addition, we used *in silico* three-dimensional homology modeling techniques

to reconstruct structural changes. Inferences derived from these computational analyses will be validated by functional analyses. The combination of computational and biochemical data generated in this work will consolidate the identification of protein factors and pave the way for a better understanding of the mechanisms of nuclear export of mRNA in Trypanosomes and its relevance in the regulation of gene expression.

Financial Support : CNPq, Fiocruz

BM062 - STUDIES OF RECOMBINATION IN *TRYPANOSOMA CRUZI:* GENERATION OF TCRAD51 SINGLE KNOCKOUT CELLS AND IMMUNOLOCALIZATION

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Key words: Recombination, Rad51, *Trypanosoma cruzi*, DNA Repair e Gamma radiation.

Trypanosoma cruzi is the etiologic agent in Chagas' disease. Our group has recently characterized a gene encoding one of the key proteins involved in *T. cruzi* homologous recombination, TcRad51. Previous studies have demonstrated that this gene overexpression increases the parasite resistance to double strand breaks agents such as gamma radiation. Besides this, the chromosomal reconstitution kinetics after exposure to these agents becomes faster in these cultures. In order to better understanding these processes we performed TcRad51 immunolocalization experiments during the *T. cruzi* recovery process after gamma radiation (0, 4 and 24 hours after the exposure). Before the treatment, the protein was localized diffusely in the epimastigote form. Immediately after the damage, TcRad51 accumulates in the nucleus and the protein intensity increases up to 24 hour. These results indicated that TcRad51 is an important protein in the double strand breaks recovery. In addition to studying the recombination process in *T. cruzi*, we also generated TcRad51 single knockouts cells for each allele in the CL Brener strain. To confirm the knockouts, we carried out western blot assays and showed a decrease in TcRad51 protein expression in the transfected cells. We then submitted these cultures to different genotoxic agents. When exposed to ultraviolet light, the TcRad51 single knockouts cells showed a resistance similar to that observed for the wild type cells. However, these cultures revealed a diminished resistance when exposed to agents that cause double strand DNA breaks. These results indicate that TcRad51 has a role in the parasite genomic stability, and participates in the process of double strand break repair after genotoxic agents exposure. Further analysis using these cells and double knockout cells that are already in progress for this gene will allow us to better understand the role of the TcRad51 protein in *T. cruzi* DNA metabolism.

Financial support: CNPq, FAPEMIG and Howard Hughes Medical Institute

***BM063 - TRYPANOSOMA CRUZI* MSH2: INVESTIGATING ITS ADDITIONAL ROLE IN THE OXIDATIVE STRESS RESPONSE AND ITS INFLUENCE IN THE PARASITE INTRASPECIFIC VARIATION**

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T. cruzi has an heterogeneous population with strains showing distinct characteristics including virulence, sensitivity to drugs, antigenic profile and tissue tropism. In spite of its genetic diversity, three major lineages of the parasite, named *T. cruzi* I, II and III have been identified, with *T. cruzi* II strains being preferentially associated with the human infection. Several studies have also indicated that *T. cruzi* II strains have higher levels of genomic sequence variability compared to *T. cruzi* I strains. By studying the molecular mechanisms affecting

the *T. cruzi* genetic diversity, we identified three distinct isoforms of the TcMSH2, a protein that plays a major role in DNA mismatch repair pathway (MMR): TcMSH2a, TcMSH2b and TcMSH2c, which are specific for *T. cruzi* I, III and II strains, respectively. We are thus investigating whether *Tcmsh2* gene polymorphisms may have a role in generating such differences in genetic variability amongst *T. cruzi* strains. The levels of oxidative stress-induced DNA damage (by HPLC analysis and *in situ* studies using a specific FITC-Avidin conjugate) as well as the response of cell cultures to different genotoxic agents were determined. Our analyses support the hypothesis that strains belonging to *T. cruzi* I strains (which express the TcMSH2a isoform) present a more efficient MMR. When we tried to perform similar analyses in *Tcmsh2* knockouts we found evidences indicating that this gene could be multifunctional and, consequently, essential. Together with the studies performed in *T. brucei* knockouts, our results indicate that, in addition to its role in MMR, TcMSH2 may be also directly involved with the response to oxidative stress. We are further investigating this hypothesis, by generating parasites overexpressing the *Tcmsh2* gene, as well as by performing immunolocalization of the protein.
Support: CNPq, FAPEMIG and HHMI.

BM064 - DEVELOPMENT OF A METHODOLOGY TO ANALYZE MUTATION RATE IN *TRYPANOSOMA CRUZI*

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DNA mutations are the major source of genetic diversity in asexual organisms and are caused by many distinct factors, such as replication errors, spontaneous deamination of bases and by the action of mutagens. Usually, mutation rates are kept at lower levels by different DNA repair pathways. However, at stressful conditions a mutator phenotype – caused by a defective DNA repair – can provide a selective advantage to a population since the increase of its genetic variability enhances its adaptability to the environment. Our research group predicted that this phenomenon could be the source of the high genetic variability and biological diversity encountered in *Trypanosoma cruzi*, the causative agent of Chagas disease. In order to test this hypothesis we developed a methodology to analyze and measure the mutation rate in *T. cruzi*. The assay is performed in semisolid medium plates and is based on the selection of clones that spontaneously reverts the neomycin-sensitive phenotype caused by a single base substitution in the neomycin resistance gene (*neo)* inserted into the parasite genome. Our preliminary results indicate that this experimental approach is functional since (*i*) clones that grew in neomycin containing plates have the revertant mutation confirmed by DNA sequence analysis and their resistance phenotype was confirmed by survival curves; (*ii*) a proportionally higher number of mutant clones are observed after long-time culture in liquid medium (*iii*) the number of revertant clones is higher when cells are cultivated in the presence of a mutagenic agent. Once the methodology has been completely implemented in our laboratory, we will be able to quantify the mutation rate in distinct *T. cruzi* strains, as well as analyzing the effect of mutagens to the mutation levels. FINANCIAL SUPPORT: CNPq, CAPES, FAPEMIG, Howard Hughes Medical Institute

BM065 - DNA POLYMERASE KAPPA FROM *Trypanosoma cruzi* CAN ACT AGAINST OXIDATIVE STRESS AND MAY BE INVOLVED IN RECOMBINATION

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DNA Polymerase Kappa (Polκ) is a low-fidelity polymerase that has the ability to perform DNA synthesis across several damaged bases. It belongs to the DinB subfamily of Y-family DNA polymerases and for years its biological role remained unclear. Here we studied the DNA Polymerase Kappa from the protozoan *Trypanosoma cruzi* (TcPol□), encoded by *TcPOLK* gene. The role of TcPol□ in this parasite was investigated by analyzing its subcellular localization, studying its activities *in vitro* and also by performing experiments with parasites overexpressing this polymerase. *TcPOLK* sequence is a hybrid between eukaryotic and prokaryotic DinB members since its N-terminal extension is related to eukaryotic orthologs, but its

C-terminal region is more related to prokaryotic and archaeal counterparts. Our results indicate that, in contrast to its previously described orthologs, this polymerase has mitochondrial localization. The overexpression of *TcPOLK* increases *T. cruzi* resistance to H_2O_2 and *in vitro* polymerization assays showed that purified TcPol efficiently bypasses 8-oxoguanine lesions. Remarkably, this work also provides evidences – *in vivo* and *in vitro* – that the DinB subfamily polymerases can act in homologous recombination. TcPol increases *T. cruzi* resistance to agents that cause severe double-strand breaks – gamma irradiation and zeocin – and is capable to perform DNA synthesis in a recombination intermediate. This is the first evidence – to our knowledge – that this subfamily of DNA polymerases could play a role in homologous recombination as well as we also provide new experimental data supporting the existence of mitochondrial recombination.

Financial support: CAPES, FAPEMIG, Howard Hughes Medical Institute

BM066 - GENOME-WIDE SURVEY AND LOCALIZATION OF PHOSPHATIDYLINOSITOL-3 AND RELATED- KINASES CLASSES AND SUBTYPES IN *TRYPANOSOMA CRUZI*

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Phosphatidylinositol 3-kinase (PI3K) is at the core key of intracellular signal transduction pathways. PKB/c-Akt protein kinase depends on functional PI3K. Trypomastigote treatment with PI3K inhibitors reduced parasite entry indicating that PI3K and PKB/Akt activities are essential and may be potential drug targets. Herein we present the results of a survey made by similarities searches against *Trypanosoma cruzi* genome available to date for phosphatidylinositol-3 (PI3K) and -related kinases (PIK-related). In addition, we examined the *T. cruzi* genome localization of two PI3K members. PI3Ks and PIK-related sequences were retrieved from GeneDB (http://www.genedb.org) with Pfam domains, BLAST analysis and COGs together with the search for kinase (catalytic) domains. PI3K and PIK-related were classified according to five models (1, 2, 3, 4 and 5) which were designed on the basis of the following domains: Phosphatidylinositol 3- and 4-kinase catalytic domain (PF00454); kA, accessory PIK domain (PF00613); RBD, ras-binding domain (PF08771) and FAT / FATC (PF02260). PI3K genes were cloned and used as molecular markers to map *T. cruzi* chromosomes. Chromosomes of CL Brener and G strain were separated by pulsed-field gel electrophoresis (PFGE) and hybridized with radiolabeled probes. *Trypanosoma cruzi* displays twelve phosphatidylinositol kinase genes divided into five PKs models. A PI3K gene corresponding to Model 1 displays a FYVE domain located at the N-terminus. FYVE-containing gene hybridization located in two chromosomal bands in CL Brener and in a single band in G strain, whereas a model 2 PI3K showed a distinct hybridization pattern. These results suggest that PI3K members are widespread in *T. cruzi* chromosomes and show chromosomal polymorphism between different parasite stocks, possibly due to the hybrid origin of the CL Brener genome. Antibodies to these proteins will allow us to examine their cellular localization and to study their possible role on host cell invasion. Support: FAPESP, CAPES, CNPq.

BM067 - IDENTIFICATION OF LmHUS1-INTERACTING PROTEINS THROUGHOUT STRESS BY DNA DAMAGE.

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The overexpression of *LmHUS1* in *Leishmania major* confers resistance to drugs such as phleomycin, a radiomimetic drug, and hydroxyurea, a replication halting drug, suggesting that LmHus1 is involved in DNA repair events. It has been well documented that Hus1 forms a trimeric complex with proteins Rad1 and Rad9 that associates with Rad17-RFC and telomeres. Hus1 has also been shown to interact with RPA, a DNA single-stranded binding protein that interacts with other proteins involved in DNA replication, recombination and repair. In order to determine the role of LmHus1 in these processes, we have generated and expressed tagged versions of LmHus1 in the parasite. GFP::Hus1 and Hus1::Myc fusions were

used in subcellular localization experiments. Such reagents were also used to co-immunopreciptate other proteins that interact with LmHus1. In yeast, activation of Hus1 in response to DNA damage involves its phosphorilation. To determine if such modulation takes place in L. major DNA repair mechanisms, we investigated the phosphorilation pattern of Hus1 and RPA throughout DNA damage stress. Altogether, our results suggest the involvement of LmHus1 in genome maintenance and plasticity.
Supported by: FAPESP and CNPq.

BM068 - *LEISHMANIA MAJOR* NUCLEAR PROTEIN LMHUS1 PROTECTS DNA FROM DAMAGE

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The 45 Kb H locus of *Leishmania major* is found amplified in resistant lineages of the parasite after selection with unrelated drugs. The 1 Kb gene *LmHUS1* is among the genes encoded within the H region of different species of *Leishmania.* The *HUS1* gene is widely conserved and, in yeast and mammalian cells, the Hus1 protein interacts with Rad9 and Rad1 proteins forming the 9-1-1 complex. This trimeric complex has been implicated in DNA repair and events that involves mechanisms such as homologous recombination which results in the maintenance of genome integrity. The *L. major* gene was cloned into pXG shuttle vector and transfected into the parasite. *LmHUS1* transfectants were resistant to genotoxic drugs such as phleomycin. Resistance was mediated by protection against DNA damage as seen in TUNEL assays. A N-and C-terminus tagged versions of LmHUS1 allowed its subcellular localization. Respectively, GFP::HUS1 and HUS1::Myc fusions protein were localized to the parasite nucleus. The localization was unaffected when different fusions protein was analyzed. We also investigated the involvement of LmHUS1 in cell cycle progression. Wild type and LmHUS1 transfectant cells were synchronized and treated with phleomycin. These cell lines were monitored by flow cytometry showing that increased LmHUS1 expression alters the pattern of progression through the cell cycle. Considering the participation of this protein in DNA repair and replication control, current work is focused on the investigation of the involvement of LmHUS1 in the parasite genome plasticity. Supported by: FAPESP, CAPES and CNPq.

BM069 - INVOLVEMENT OF THE LEISHMANIA MAJOR BRCA2 HOMOLOG IN DOUBLE STRAND BREAK REPAIR

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Introduction and objectives: In several organisms, the *Breast Cancer Susceptibility 2* (BRCA2) gene is involved in mechanisms of DNA repair by homologous recombination by the formation of a protein complex with the recombinase Rad51. The BRCA2 homolog in *Leishmania major* (LmBRCA2) has been previously identified; however, its function in the parasite remains unclear. The aim of this work is to investigate the involvement of the *LmBRCA2* gene product in the mechanisms of DNA repair in *L. major*. **Results**: A 7.1kb *Stu*I fragment obtained by the digestion of a cosmid containing the LmBRCA2 gene was gel-purified and cloned in the plasmid pXG1. Plasmids pXG1 (control) and pXG1LmBRCA2 were transfected in the *L. major* CC1 clonal lineage and the transfectants were selected in M199 supplemented with 2.4ug/mL of G418. After isolation, tranfectants were maintained at 1.2ug/mL of G418. The radiomimetic drug methyl methanesulfonate (MMS) was added to the *L. major* culture medium in order to generated double-strand breaks (DSB) in the parasite DNA. The IC50 for MMS was calculated for CC1 promastigote forms cultivated in M199 and in M199 supplemented with increasing concentrations of the drug (6,25ng/mL to 200ng/mL) and was determined in 7.4ng/mL. Transfected and non-transfected cell lines were cultured in increasing concentrations of MMS (0 to 40ng/mL) and it was observed that in the highest concentrations (20 and 40ng/mL), transfectants bearing the plasmid pXG1LmBRCA2 reached cell densities higher than the control cells ($p<0.05$). **Conclusions**: These results suggest that the LmBRCA2 gene product might participate in the mechanisms of DSB repair in *L. major*. Further experiments will determine the

LmBRCA2 transcript and protein levels in non-transfected and transfected cell lines.

This work is supported by FAPESP (06/50323-7) and PESL-J receives a fellowship from FAPEMIG

BM070 - MOLECULAR CHARACTERIZATION OF A LEUCINE RICH PROTEIN IN *LEISHMANIA BRAZILIENSIS*

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Leucine-rich repeats (LRRs) are versatile binding motifs found in a variety of proteins involved in protein-protein interactions. The *La*LRR17 gene, identified initially in the *L. amazonensis* genome, encodes a protein with 6 "LRRs" in its central region that presented similarity with the human NOD3 protein. NODs are members of a recently identified family of cytosolic proteins that have been implicated in the intracellular recognition of pathogens. Many of these proteins contain a leucine rich repeat (LRR) region, a nucleotide-binding domain known as NOD (nucleotide-binding oligomerization domain) and a third domain involved in a variety of functions including apoptosis regulation, activation of the nuclear factor κB and regulation of MHC class II expression. It was shown by immunohystochemistry and immune electron microscopy that the *La*LRR17 protein of approximately 70 kDa is secreted to the cytoplasm of *L. amazonensis*-infected macrophages (Franco et al. Proceedings of the XXIII, SBPZ, Annual Meeting, 2007:73), suggesting that the *La*LRR17 protein can interact with macrophage proteins and modulate the immune response. *L. major, L. infantum and L. braziliensis* genomes, show a high degree of synteny and conservation in the coding sequences. In *L. braziliensis* we identified an orthologue of LaLRR17 in the chromosome 17 (*Lbr*M17_V2.0920). This ORF was PCR amplified and cloned into the histidine tagged expression vector pAE. The recombinant protein was purified and used to raise specific antibodies. A more detailed study of this genomic region identified a truncated second copy of this gene, which encodes the first 225 amino acids of the protein *Lbr*M17_V2.0920. The characterization of this gene and of the encoded protein is under way. The study of those gene products will produce the necessary tools to investigate the participation of the two proteins in the macrophage-parasite interaction and in the host cell invasion. Financial support: FAPESP.

BM071 - Heterologous Expression and Partial Characterization of putative *Leishmania major* guanosine diphosphatase as a genuine apyrase from E-NTPDase family

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L. major has two mapped E-NTPDase genes in its genome (putative nucleoside diphosphatase and putative guanosine diphosphatase). 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 capacities demonstrated in *Leishmania* species suggest their involvement with virulence and control of host-immune responses (Maioli et al., 2004). In order to evaluate the role of putative guanosine diphosphatase in *L major* ecto-nucleotidase activity and to prove its action as a genuine E-NTPDase, we performed heterologous expression of recombinant enzyme in *E. coli* system. End primers were designed and used to amplify the full-length putative GDPase using genomic DNA as template. Full-length GDPase (2022 pb) was cloned in bacterial expression vector and this construction was used to transform *E. coli* BL21 strain. The recombinant protein was expressed after IPTG induction and purified by Nickel affinity chromatography using soluble and insoluble fractions. The purified recombinant protein showed specific activity for ATP hydrolysis around 35 nmol.mg protein^{-1}.h^{-}1. ATP/ADP ratio hydrolysis was 1.05 characterizing this enzyme as a genuine apyrase from E-NTPDase family. Full biochemical characterizations, polyclonal specific antiserum and search for potential inhibitors are included amongst the future goals of this work.

Supported by: FAPEMIG, UFV, UFOP, MEC.

BM072 - INVESTIGATION OF AN EXTRA-RIBOSOMAL FUNCTION FOR THE RIBOSOMAL PROTEIN L19 IN LEISHMANIA MAJOR

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In the course of studying one region of *Leishmania major* genome we observed that the transcript for the ribosomal protein L19 (RPL19) is more abundant in the amastigote stage. Given that several reports show different examples of extra-ribosomal functions for ribosomal proteins, we decided to investigate a possible role of RPL19 in the control of gene expression in *Leishmania*. In order to identify possible targets of control of this ribosomal protein we chose to evaluate modifications on the parasite proteome induced by overexpression of RPL19. Therefore, we constructed a vector to express RPL19 (pNeo_RPL19) exogenously in *Leishmania*, which was transfected in *L. major* (LV39) and *L. braziliensis* (Lb2904). Transfectant parasites and wild type strains were submitted to comparative proteomic analysis and differentially expressed proteins detected in 2D gels will be identified by MS. In addition, to evaluate levels of RPL19 throughout the parasite life cycle and its subcellular localization we raised polyclonal antibodies against *L. major* RPL19 in rabbits. We used the vector pET28a for the protein expression in *E. coli* and the expected gene fusion with a His tag was confirmed. The recombinant protein was kept in inclusion bodies and solubilization was achieved in the presence of Guanidine-HCl 6M, following refolding and purification. Rabbits were immunized and antiserum specificity was analysed by western blot. Generated antibodies recognized RPL19 isoforms of *L. major* in cellular extracts. Preliminary immunofluorescence experiments revealed the widespread distribution of RPL19 in granules in the cytoplasm. In addition, both immunofluorescence and western blotting experiments confirmed the decrease of RPL19 levels as the promastigote culture progresses.

Supported by FAPESP.

BM073 - MOLECULAR CLONING OF TWO PUTATIVE E-NTPDASES FROM *Leishmania (Leishmania) infantum/chagasi*

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L. infantum/chagasi is the etiological agent of visceral leishmaniasis in the New World. This parasite (reference strain JPCM5) has two mapped E-NTPDase genes in its genome: putative guanosine diphosphatase (GDPase) and putative nucleoside diphosphatase (NTPDase). E-NTPDase function characterized as tri and di-nucleotide hydrolysis, were previously demonstrated in intact *L. amazonensis*, *L. braziliensis* and *L. major* cells. A very distinct ecto-nucleotidase capacity between *Leishmania* species suggests its involvement with virulence and control of host-immune responses. The goals of this work are elucidate the biochemical properties of these putative proteins. To achieve these we amplified and cloned the coding region of GDPase and NTPDase into pJET vector using pJET system (Fermentas). These constructions were used to transform *E. coli* DH5α. The successes of cloning were evaluated by colony PCR using specific primers and restrict digestion analysis. The partial sequencing of cloned amplicons showed 100% identities with reference sequences. These genes are now transferred to bacterial and yeast expression vectors. Future heterologous expression and comparisons between biochemical characterizations of purified recombinant proteins will be the next steps in this work.

Supported by: FAPEMIG, UFV, UFOP

BM074 - MOLECULAR ANALYSIS OF *Leishmania sp.* E-NTPDases

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L. major, *L. infantum* and *L. braziliensis* have two mapped E-NTPDase genes in their genomes: putatives nucleoside diphosphatase (NTPDase) and guanosine diphosphatase (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 E-NTPDase 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 only present in kinetoplastidae E-NTPDases. 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 transmembrana region. These results suggest that GDPases could be secreted and NTPDases could be ecto-membrane proteins. Analyses of glycosilation show that NTPDases have more putative N-glycosilation sites than GDPases. We can conclude that *Leishmania* apyrases have significant molecular differences that could explain the distinction in ecto-nucleotidase activities. Furthermore, respective genes from *L. amazonensis* were isolated by PCR, cloned and predicted coding region of NTPDase was completely sequenced, showing high identity with *L. major* reference sequence. Confirmation of predicted sub cellular localizations and future comparisons between biochemical characterizations of purified recombinant proteins will be the next steps in this work.

Financial Support: FAPEMIG, UFV, UFOP.

BM075 - Heterologous expression and purification of Tryptophanyl tRNA Synthetases of *Leishmania major*

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Aminoacyl tRNA Synthetases (aaTRS) catalyzes the acylation of aminoacids and subsequent ligation of this aminoacyl acid to its respective tRNA. Practically each enzyme is specific to one single aminoacid. aaTRS are divided into two classes according to its three dimensional structure and acylation mechanism.

Besides its role in aminoacid activation, human Tryptophanyl tRNA Synthetase (TTRS) has been characterized as an inhibitor of angiogenesis after proteolysis by leukocyte elastase. The *Leishmania major* genome encodes two different TTRSs, a citosolic (TTRS1) and a mitochondrial (TTRS2) forms. *In silico* analysis of parasite's *TTRS* genes has shown that the encoded proteins are equivalent to the product generated by elastase cleavage of human TTRS. We set out to investigate if *L. major* heterologous TTRSs present the same angiostatic behavior observed in the processed human protein. We have cloned TTRS1 and TTRS2 genes into pET28a vector and induced their heterologous expression. The recombinant proteins were expressed in BL21 Rosetta strains as inclusion bodies. Polypeptides were solubilized in 2% sarkosyl/25mM triethanolamine/2mM EDTA and extensively dialyzed for sarkosyl/EDTA removal. Ni sepharose affinity chromatography was performed after determination of purification parameters. Structural analyses are currently being performed through Circular Dichroism and enzymatic activity assay.

Supported by FAPESP and CNPq.

BM076 - IDENTIFICATION OF A PUTATIVE AMINOPHOSPHOLIPID TRANSLOCASE IN PROMASTIGOTES OF *LEISHMANIA AMAZONENSIS*

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The exposure of phosphatidylserine (PS) on the cell surface is a conserved feature of apoptosis and plays an important role in the infectivity of *Leishmania (L.) amazonensis*. However the molecular mechanism that drives PS exposure in the parasite remains elusive. Recent studies on *Caenorhabditis elegans* propose the action of an aminophospholipid translocase (TAT-1) as responsible for the possible PS outsourcing (Züllig et al., Current Biology, 2007). The objective of this work is to identify and characterize a similar molecule in promastigotes forms of *Leishmania (L.) amazonensis.* From the alignment and analysis of the sequences obtained from the TAT gene of *Leishmania (L.) major*, *Leishmania (L.) donovani*, *Leishmania (L.) infantum*, *Leishmania (L.)*

braziliensis and *Leishmania (L.) mexicana* species, deposited in GeneDB, we desined 12 primers which were used to screen the TAT gene in *Leishmania (L.) amazonensis*. Until now we have sequenced 3.100bp of the gene TAT in *Leishmania (L.) amazonensis*. The expected size of the gene range from 3.470pb and 3.480bp. BLAST sequence comparisons of the gene obtained from *Leishmania (L.) amazonensis* with their orthologous counterparts revealed 91% of identity with TAT gene of *Leishmania (L.) major*, 93% with *Leishmania (L.) donovani* and *Leishmania (L.) infantum* and 99% with *Leishmania (L.) mexicana*. We are currently characterizing if - similarly to what happens in *C. elegans* - the TAT molecule displays an aminophospholipid translocase activity in *Leishmania* spp. Support: CNPq and FAPERJ.

BM077 - CLONING AND CHARACTERIZATION OF THE ERGOSTEROL BIOSYNTHESIS GENES OF *LEISHMANIA MAJOR*

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The therapeutic arsenal against *Leishmania* infection is limited. Antimony-containing compounds are the mainstream form of anti-leishmanial therapy. Potentially exploitable chemotherapeutic targets of *Leishmania* can be the enzymes of the ergosterol biosynthesis pathway. In this study we investigated the participation of *ERG1*, *ERG7*, *ERG10* and *ERG11*, the *Leishmania major* genes encoding squalene epoxidase, lanosterol synthase, 3-ketoacyl-CoA thiolase and lanosterol 14□ demethylase, respectively, on the resistance/susceptibility to terbinafine and amphotericin B. Initially, the genes amplified by PCR were cloned into vector pGEM-T easy (Promega) and after were sub-cloned into shuttle-vector pXG1. These constructions were transfected into the wild-type cell LT252 of *L. major*. Presence of the transfected genes was confirmed by short-run PFGE and Southern analysis. Overexpression of *ERG1, ERG7* and *ERG10* genes, revealed in Northern analysis, was correlated to increased resistance to terbinafine. Resistance in these mutants was up to 2 times higher than that of wild-type cells. Increased expression of *ERG11* gene resulted in an increased susceptibility to this drug. On the other hand, *ERG1* and *ERG10* transfectants presented a higher susceptibility to amphotericin B. The characterization of ERG genes transfectants may shed light on the mechanisms of ergosterol biosynthesis regulation and may improve our ability to design better anti-leishmanial therapeutic strategies. Supported by CAPES and FAPESP.

BM078 - IDENTIFICATION OF TWO DIFFERENT PROTEINS RELATED WITH TUBERCIDIN RESISTANCE IN LEISHMANIA

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Leishmaniasis is an emergent parasitic disease caused by protozoa of the genus *Leishmania*. Treatment is based in chemotherapy and most of the treatment fail is associated with parasite drug resistance. Gene identification involved drug resistance has contributed to a better understanding of the mechanisms of antiparasite compounds action. Using transfection and overexpression selection strategy, we isolated two *loci* of *L. major* capable to confer resistance to tubercidin (TUB), a toxic purine analogue with a potent antiparasitic action. Nucleotide sequence analysis of the first TUB resistant *locus* of *L. major* shown a 77% identity with the *TOR* gene (**TO**xic nucleoside **R**esistance) described in *L. amazonensis*. Although transcription of most genes does not appear to be controlled in *Leishmania*, *TOR* presents high identity with *Oct-6*, a mammalian transcription factor. This homology suggests that TOR could act as a repressor of the genes related with purine transport. The second and new TUB resistant *locus* of *L. major* (*Tub2*), codified an open reading frame of 606 amino acids with unknown function, accordingly the genome database. *Tub2* was transfected into wild type cells alone and in the presence of a 10kb upstream region. After overexpression induction, cells were tested in the presence of increasing concentrations of TUB. Functional analysis shown that overexpression of *Tub2* alone presents a value of TUB resistance smaller than the value obtained when *Tub2* were over-expressed with the

upstream region. Like the repression hypothesis described above for the *TOR* gene, these data suggest that another component could also be implicated in the role of *Tub2* in the TUB resistance. New functional analysis must be done to better characterize this hypothesis. Looking for new anti-leishmania targets, we expected that a better description of the *Tub2* role in the TUB resistance can contribute to better understanding of the components of purine biosynthetic pathway in *Leishmania*.

Supported by: CAPES, CNPq, FAPESP and LIM48-FMUSP.

BM079 - YIP1 of *Leishmania major:* Characterization and possible role in terbinafine resistance

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Yip1p is a Rab-interacting integral protein which is the founder member of the YIP1 protein family. Orthologs and paralogs of YIP1 are found in all eukaryotic genomes. In budding yeast, Yip1p is essential for transport from ER to Golgi stack during the early secretory pathway. Membrane transport and ER vesicle budding are severely compromised in Yip1p defective mutants (Yang et al 1998, EMBO *Journal* 17: 4954-4963). Three putatives YIPs are annotated. In the *Leishmania major* genome. One of them, named LmHTBF, confers resistance to terbinafine, an inhibitor of Esqualene epoxidase (Marchini et al 2003; *MBP* 131: 77-81). Our hypothesis is that LmHTBF is involved in the formation and/or redirection of vesicles, improving mechanisms of drug extrusion or membrane repair. Here we report the identification and characterization of *Leishmania* YIP1. Sequence analysis revealed that leishmania YIP1 (LmYIP1), like most of the other YIP1 protein family members, contains multiple transmembrane segments. Double immunofluorescence study revealed co-distribution of LmYIP1 with Golgi markers. To delineate the function of LmYIP1, we generated the reagents for yeast complementation assays. Both *LmYIP1* gene and the Gal1-10 promoter were cloned into pRS415vector. The construct was transformed into yeast strain RCY1610 – *MAT*α *ura3-52 leu2-3,112 YIP1*Δ*KAN*^{R} [YCP50 (pRC1245) *YIP1*] (Calero et al 2003; *Mol Bio Cell* 14: 1852-1867) and plated in media contained 5-fluorootic acid (5-FOA) and galactose. After three days, cells expressing *LmYIP1* were found in the plates. Our results suggest not only that HTBF is a YIP1 of *L. major*, but also that the terbinafine resistance observed in HTBF overexpressors involve the vesicle trafficking of the parasite. Financial support: FAPESP, Capes and CNPq.

BM080 - The CCCH zinc finger protein TcZFP2: characterization and expression analyses during the metacyclogenesis of *Trypanoaoma cruzi*

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The kinetoplastida exhibit an almost total dependence on post-transcriptional mechanisms for their gene expression regulation. This control can be influenced by the basal mRNA decay machinery, regulatory factors that respond to various stimuli and sequence-specific decay components.

In recent years, research has focused on the identification of the trans-acting factors that interact with sequence motifs in mRNA and modulate translational efficiency or mRNA stability in this order.

The complete genome sequencing of *T. cruzi*, *T. brucei* and *Leishmania major* has identified a large number of genes encoding putative CCCH zinc finger proteins. Such proteins have an established role in RNA binding, localization and stability in a wide variety of organisms.

Here, we report the characterization of a small CCCH zinc finger protein of *T. cruzi,* named TcZFP2. TcZFP2 is a cytoplasmic protein with differential expression pattern during metaciclogenesis and is associated with polysomes. This association was corroborated by proteomic studies from the immunocomplexes of TcZFP2, where some specific factors from the translational machinery were identified.

The TcZFP2-associated mRNAs were identified by RNA pull down and microarray analyses. Gel shift

assay showed that TcZFP2 is able to recognize an A-rich sequence element in the 3'UTR of its target transcripts. TcZFP2 could be therefore a regulator of developmental events in *Trypanosoma cruzi* that acts at the level of the post-transcriptional control of gene expression

Financial support: CNPq, Fiocruz, Pronex (Fundação Araucária).

BM081 - THE eIF4F COMPLEX IN *Leishmania major*: PRELIMINARY MAPPING INTERACTIONS BY PULL DOWN ASSAYS

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THE TRYPANOSOMATIDS ARE PATHOGENIC PROTOZOAN RESPONSIBLE FOR RELEVANT HUMAN ILLNESSES SUCH AS CHAGAS' DISEASE, SLEEPING SICKNESS AND LEISHMANIASIS. THEY ARE ALSO CHARACTERIZED BY UNIQUE MOLECULAR MECHANISMS INCLUDING THE CONTROL OF THEIR GENE EXPRESSION AT THE POST-TRANSCRIPTIONAL LEVEL. THE INITIATION STAGE OF PROTEIN SYNTHESIS, OR TRANSLATION, IS A POSSIBLE TARGET FOR THIS CONTROL BUT VERY LITTLE IS KNOWN ABOUT HOW IT HAPPENS IN THESE PATHOGENS. IN PLANTS, YEAST AND ANIMALS, TRANSLATION INITIATION STARTS WITH THE BINDING OF THE TRANSLATION INITIATION COMPLEX EIF4F (FORMED BY THE SUBUNITS EIF4A, EIF4E AND EIF4G) TO THE MONOMETHYLATED CAP PRESENT ON THE 5'END OF THE MRNAS. THE EIF4F ACTIVITY IS ENHANCED BY THE POLY(A) BINDING PROTEIN (PABP), WHICH ACTS THROUGH A DIRECT INTERACTION WITH EIF4G. SEVERAL HOMOLOGUES TO THE EIF4F SUBUNITS AND PABP HAVE BEEN PREVIOUSLY IDENTIFIED IN *LEISHMANIA MAJOR*. THEIR GENES HAVE BEEN CLONED AND EXPRESSED AS RADIOACTIVE LABELLED PROTEINS OR RECOMBINANT GST-FUSIONS. HERE WE HAVE INVESTIGATED POSSIBLE INTERACTIONS BETWEEN SEVERAL OF THESE HOMOLOGUES THROUGH PULL-DOWN ASSAYS. SO FAR, WE HAVE CONFIRMED DIRECT INTERACTIONS BETWEEN TWO EIF4G HOMOLOGUES (*LM*EIF4G3-4) AND HOMOLOGUES OF THE REMAINING SUBUNITS OF THE EIF4F COMPLEX, AS WELL AS PABP. THUS, *LM*EIF4G3 BINDS TO *LM*EIF4AI, *LM*EIF4E4 AND *LM*PABP1 WHILST *LM*EIF4G4 BINDS TO *LM*EIF4AI AND *LM*EIF4E3. THE CENTRAL *LM*EIF4G3'S HEAT DOMAIN IS SUFFICIENT FOR ITS BINDING TO *LM*EIF4AI WHILST BOTH THE HEAT DOMAIN PLUS THE PROTEIN'S C-TERMINUS IS REQUIRED FOR *LM*EIF4G4 TO INTERACT WITH THIS EIF4A HOMOLOGUE. FOR THE BINDING TO *LM*EIF4E3 ONLY THE SHORT N-TERMINAL DOMAIN OF *LM*EIF4G4 IS REQUIRED. SITE DIRECTED MUTAGENESIS OF SELECTED RESIDUES IN *LM*EIF4G3 REDUCED DRASTICALLY IT'S INTERACTION WITH AN EIF4A HOMOLOGUE AS IDENTIFIED IN OTHERS EUKARYOTES. THESE INTERACTIONS CONFIRM THE EXISTENCE OF AT LEAST ONE EIF4F COMPLEX IN *L. MAJOR* AND INDICATE BOTH NOVEL AND CONSERVED INTERACTIONS AND INTERACTION-MOTIFS WHEN COMPARED WITH OTHERS EUKARYOTES.

Supported by: CAPES, UFPE, FIOCRUZ, FACEPE.

BM082 - SUPEREXPRESSION OF A GLYCOSOMAL AND A CYTOSOLIC ARGINASE IN *LEISHMANIA (LEISHMANIA) AMAZONENSIS.*

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In the mammal host, *Leishmania* survive inside the macrophages by escaping from their microbicidal mechanisms such as production of nitric oxide (NO) and super oxide radicals (Bogdan et al., 1996). The production of NO by inducible nitric oxide synthase (iNOS) requires L-arginine as substrate, the same amino acid required by arginase to generate ornithine and urea, so arginase may play a dual role in parasite survival, competing with the iNOS reducing the NO generation and/or providing ornithine. Although a mutant with one copy of arginase coding gene knocked out had shown to be less infective, its instability in culture lead us, in a new approach, to construct mutants that superexpress the enzyme to evaluate how the mutants behave, mainly in respect to infection. Previously, we showed that arginase of *L. (L.) amazonensis* is compartmentalized in glycosome (da Silva et al., 2008), by immunolocalizing the enzyme in a mutant expressing enhanced green fluorescent protein (EGFP) targeted to the glycosome. Adding compartment signaling to the superexpression, we would also be able to evaluate the importance of the glycosomal location for the parasite. With this aim in mind, we constructed plasmids with the arginase gene followed or not by the targeting signal for glycosome (SKL). The selected mutants were used to prepare protein extracts to evaluate arginase expression and activity. The Western blot experiments revealed that the mutants expressing

the glycosomal arginase produced more protein than the mutants expressing the cytosolic one. Besides, the enzymatic activity experiment showed a greater arginase activity with the protein extract of mutants expressing glycosomal arginase. Now, we are performing infections with the different mutants obtained to evaluate their infection capacity.

Supported by FAPESP and CNPq.

BM083 - PABP IN TRYPANOSOMATIDS: DIFFERENT FUNCTIONAL PROPERTIES OF ITS HOMOLOGUES

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The poly(A) binding protein (PABP) is the major cytoplasmic mRNA binding protein. It binds to the 3'end poly-A tail of eukaryotic mRNAs and participates in an extensive range of cellular functions. PABP facilitates the formation of a "closed loop'' structure of the mRNA that is crucial for some of its activities, such as translation initiation and termination and recycling of ribosomes. Aside from translation, PABP participates in several steps of mRNA biogenesis, processing and degradation. Furthermore this protein may be involved in mRNA transport from the nucleus to the cytoplasm. Here we describe work focusing on the PABPs homologues previously identified from *Leishmania major* (*Lm*PABP1-3) and *Trypanosoma brucei* (*Tb*PABP1-2). First we have analyzed the expression pattern of the *Lm*PABP1 homologue, known to be regulated through phosphorylation, upon transcription inhibition with actinomycin D. Soon after inhibition (less then 30 min) a premature phosphorylation was induced when compared to the control (without actinomycin D). Cell death occurs within 24 hours, where only the non- phosphorylated isoform appear. Since transcriptional inhibition has influenced the expression pattern of this protein we have decided to investigate if it can also interfere in the sub-cellular localization. The sub-cellular localization experiment was performed using indirect immunofluorescence assays with cells exponentially grown and where *Lm*PABP1-3 normally localizes throughout the cytoplasm. In the presence of actinomycin D the localization of *Lm*PABP2-3 changes dramatically and they can be detected mainly in the nucleus whilst *Lm*PABP1 remains mostly cytoplasmic. Complementary RNAi analyses of *T. brucei* prociclic forms with the *Lm*PABP1-2 orthologues (*Tb*PABP1-2) indicate that both proteins are required to cellular viability. Additional studies must be done to understand how the PABP homologues differ functionally and what their roles in protein synthesis and mRNA metabolism are.

Financial support: CNPq, UFPE, FACEPE

BM084 - FUNCTIONAL COMPLEMENTATION OF *LAG1* IN YEAST BY THE ACYL-COA DEPENDENT CERAMIDE SYNTHASE FROM *TRYPANOSOMA CRUZI*.

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Ceramide plays a crucial role as a basic building block of sphingolipids, but also as a signaling molecule mediating the fate of the cell. Ceramide consists of a sphingoid long chain base to which a fatty acid is attached via an amide bond. This reaction is catalyzed by an acyl-CoA-dependent and Fumonisin B_1-sensitive ceramide synthase (CerS) which requires in yeast the gene products encoded by *LAG1* or *LAC1*. Several orthologs have now been identified in most species and these include a family of six mammalian paralogs, originally named Lass (longevity assurance) genes. In *S. cerevisiae*, the concomitant deletion of *LAG1* and *LAC1* causes a significant growth defect in the genetic background of W303 cells and the same double deletion does not yield viable cells in the YPK9 background, which was used for the present study. The lethality of YPK9 *lag1Δlac1Δ* was overcome by expression of a CerS gene ortholog from *T. cruzi* (*TcCerS*), a gene that shows 22% identity to yeast *LAG1*. Southern blot analysis suggests that *T. cruzi* has more than one copy of *TcCerS* in the genome, and northern blot analysis shows two mRNA species of 1.8 Kb and 3.0 Kb in epimastigotes. Also, a *TcCerS* 5`trans-splicing acceptor site was mapped by RT-PCR. The TcCerS activity, detected in epimastigotes' crude microsomal membranes, exhibited a preference for fatty acyl-CoA rather than free fatty acid as donor

substrate and was blocked by Fumonisin B_1, a known inhibitor of CerS in fungi, plants and mammals. Using the cell-free assay system described here, the TcCerS activity was characterized using different chain length fatty acyl-CoAs, protein concentrations and co-factors. The results suggest that TcCerS is an authentic acyl-CoA dependent ceramide synthase from *T. cruzi*. Support: CAPES, CNPq, FAPERJ, IFS.

BM085 - REGULATORY ELEMENTS IN HSP70 mRNA MODULATE STABILITY AND TRANSLATION DURING HEAT SHOCK IN *Trypanosoma cruzi.*

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The understanding of 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. Our aim is to investigate the presence and the function of heat shock-responsive elements in the mRNAs of HSP70, HSP60 and HSP10 genes, and the respective factors that bind to them as well. Plasmids containing the CAT reporter gene, flanked by intergenic regions containing either the 5' or 3' UTR and their respective regulatory sequences of the HSP70 mRNA were constructed. CAT assays of transfected epimastigotes show that when CAT gene is flanked by both 5` and 3` UTRs of HSP70 mRNA, the CAT enzyme levels increase 3 times upon heat shock. However, when CAT gene is flanked by either 5` or 3`UTRs, the CAT enzyme levels increase 1,3 and 1,7 times, respectively. CAT mRNA levels of transfected cells when both 5` and 3`UTRs of HSP70 mRNA are present increase two-fold at 37ºC, while with either 5` or 3`UTRs this stabilization at 37ºC is lost. The data indicate that both UTRs need to be present to increase HSP70 mRNA stability, being responsible for part of the protein induction at 37ºC. Nonetheless, each UTR contributes individually and in combination to increase translational efficiency at 37ºC. The determination of the half-life of the endogenous HSP70 mRNA under stressing and non-stressing conditions suggests a half-life of 60 minutes at 29ºC, increasing to 2 hours at 37ºC. The kinetics of this mRNA stabilization is being investigated. In addition, an *in vitro* RNA degradation assay is being developed to investigate a putative ARE sequence in the 3' UTR in mRNA stabilization upon heat shock.

Supported by CNPq and FAPERJ.

BM086 - MOLECULAR CHARACTERIZATION OF TCRPL7A, AN IMUNOGENIC RIBOSOMAL PROTEIN FROM *TRYPANOSOMA CRUZI* CONTAINING REPETITIVE AMINO ACID SEQUENCES

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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 a protein named TcRpL7a that shares homology to the eukaryotic L7a ribosomal protein wich carries a large repetitive amino acid motif. After searching the *T. cruzi* genome database, we also identified a total of seven orthologs of eukaryotic ribosomal proteins that contain amino acid repeats. To compare the number of repetitive motifs of TcRpL7a 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 number of repetitive motifs is lower in sequences derived from *T. cruzi* I strains when compared to *T. cruzi* II strains. Sequences from the hybrid CL Brener clone showed a pattern similar to *T. cruzi* II sequences. Western blots using recombinant protein, including truncated forms containing only the repetitive and the non-repetitive domains, showed that the humoral chagasic response is directed to the repetitive region. ELISA assays showed that 73% of chagasic patients sera (n=59) reacted with the TcRpL7a repetitive domain. 43% of sera reacted with a peptide harboring the repetitive region. TcRpL7a was localized in parasites transiently transfected with the cDNA cloned in fusion with GFP. GFP::TcRpL7a

accumulates in the nucleus where the biogenesis of the ribosome takes place. Antibodies raised against the recombinant antigen identified equivalent amounts of the native protein in cell extracts from all three stages of the parasite life cycle. Western blot analyses of subcellular fractions also indicated that TcRpL7a co-fractionates with polysomes. Ongoing experiments will evaluate whether the antigen may be used as vaccine candidate and the influence of the repetitive region in the capacity of generating a protective immune response against *T. cruzi* infection. SUPPORT: CNPq, FAPEMIG and *HHMI*.

BM087 - TcYchF – A NOVEL OBG-LIKE ATPASE ASSOCIATED WITH THE TRANSLATION MACHINERY OF THE PROTOZOAN *Trypanosoma cruzi*

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Many cellular processes, including protein synthesis, intracellular transport, cell signaling and differentiation, involve a group of proteins from the P-loop GTPase superfamily. This group contains many ubiquitous sub-families, some of them with unknown functions, such as the YchF sub-family. YchF belongs to the Obg family and is ubiquitous in bacteria and eukaryotes presenting a high level of sequence conservation. We characterized an YchF-related protein, TcYchF, potentially associated with the protein translation machinery of *T. cruzi*. TcYchF is a 44.3 kDa protein and is very similar (45 to 86 %) to putative GTP-binding proteins from eukaryotes. A lower but significant level of similarity (38 to 43%) was also found between bacterial orthologs. Important features of the G domain, which is involved in the recognition and hydrolysis of the GTP, are conserved in TcYchF. However, we found that TcYchF preferentially hydrolyzed ATP rather than GTP. The function of YchF is unknown. Some of them were found associated with the ribosome, suggesting that some of these proteins are translation factors. Immunoblots of the polysome fraction from sucrose gradients showed that TcYchF was associated with ribosomal subunits and polysomes, suggesting that TcYchF is involved with the translation machinery of *T. cruzi*. Proteomic studies from the TcYchF immunocomplexes identified ribosomal proteins, translation factors and the protein RPN7 from the regulatory subunit of the *T. cruzi* proteasome. These results were corroborated by immunoprecipitation assays that showed that the TcYchF coimmunoprecipitates with proteins from ribosomal subunits and proteins from the regulatory subunit of the *T. cruzi* proteasome. Furthermore, the silencing of the ortholog of *tcychf* in *T. brucei* by RNAi inhibited the growth of procyclic forms. These data suggest that this protein may potentially play an important role in the molecular link between the translation apparatus and the proteasome for the degradation of proteins damaged during translation.

BM088 - Molecular Characterization of *Tc-OGNT2* that Encodes an UDP-GlcNAc:Polypeptide O-α-*N*-acetyl-D-glucosaminyltransferase (pp-αGlcNAcT) Involved in the First Step of the Biosynthesis of *O*-glycans in *Trypanosoma cruzi*

Koeller, C.M.[1], van der Wel, H.[2], Abreu, F.[3], Previato, J.O.[1], Mendonça-Previato, L.[1], Todeschini, A.R.[1], Souto-Padrón, T.[3], West, C.M.[2], and Heise, N.[1]

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In all stages of *T. cruzi*, the synthesis of *O*-glycans present in cell-surface mucin-like glycoproteins initiate through the addition of α-GlcNAc to threonine, reaction catalysed by a Golgi pp-αGlcNAcT. Previously, we have identified three genes in the parasite genome (*Tc-OGNT1*, *Tc-OGNT2* and *Tc-OGNTL*) that encode for putative pp-αGlcNAcT. Overexpression of full-length TcOGNT1 or TcOGNT2 in *L. tarentolae* approximately doubled apparent activity of microsomes relative to the parental control, using synthetic *Dictyostelium* mucin-peptide acceptors. Overexpression of the TcOGNT2 stem region plus catalytic domain (TcOGNT2cat) 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. Here we show that both *Tc-OGNT1* and *Tc-OGNT2* are transcribed by epimastigotes and their 5`-*trans*-splicing acceptor-sites were mapped by RT-PCR. TcOGNT2cat was amplified by PCR, cloned into pET15-TEVi and the recombinant (rTcOGNT2cat) overexpressed in *E. coli* BL21-DE3-Rosetta using 0.75 mM IPTG at 18^{o}C for 18h, and purified by IMAC after solubilization of inclusion bodies with 8M urea/0.5M NaCl. The purified rTcOGNT2cat was precipitated with cold acetone, mixed with Complete Freund's Adjuvant (FA) and used to immunize Balb/c mice with two boosts done in 15 days interval (one with Complete and the last with Incomplete FA). Three days after the last immunization, the serum was obtained and titrated against the purified rTcOGNT2cat by ELISA. Unlike the pre-immune, the immune serum (1:1000 dilution) was able to recognize: **(i)** the rTcOGNT2cat produced by *E. coli* and by *L. tarentolae* through western-blot using anti-mouse-IgG coupled to AP and, **(ii)** mainly the Golgi in formaldehyde fixed and permeabilized epimastigotes by immunofluorescence using anti-mouse-IgG coupled to Alexa-Fluor 546. Together, the results show the production of polyclonal antibodies that efficiently recognize the TcOGNT2 of *T. cruzi*.

Support by: CNPq, FAPERJ and OCAST.

BM089 - HETEROLOGOUS EXPRESSION AND CHARACTERIZATION OF RECOMBINANT *TRYPANOSOMA CRUZI* APYRASE (NTPDASE I)

Souto, X.M.[1], Cunha, L. C. S.[1], Bastos, M.S.[1], Cardoso, F.G.[1], Santos, R.F.[1], Almeida, M.R.[1], Fietto, J.L.R.[1]

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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+}. In addition the presence of galactose 5mM showed significant enzyme activation. The use of apyrase inhibitors (Suramin, Gadolinium and ARL67156) showed a partial ATPase and ADPase inhibitions. Suramin presented higher inhibition action in lower drug concentrations than other apyrase inhibitors. The same effects were observed in live trypomastigotes. We concluded that rNTPDaseI was produced in an active form that should be suitable to biological assays, start crystallization tests and to evaluate its potential as new target in specific drug design assays.

Supported by: CNPq, FAPEMIG, UFV, UFOP.

BM090 - PRELIMINARY MOLECULAR CHARACTERIZATION OF THE STRESS INDUCIBLE PROTEIN 1 FROM *Trypanosoma cruzi* IN DIFFERENT DEVELOPMENTAL FORMS OF THE PARASITE

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The life cycle of *Trypanosoma cruzi* includes at least three morphologically distinct stages and exposure to several environmental stresses at the invertebrate and vertebrate hostess. Stress inducible protein 1 (STI1) is a co-chaperone whose expression is induced by heat shock and other stress conditions. This protein mediates the formation of a complex between Hsp70 and Hsp90 that seems to play a key role in signal transduction networks, cell cycle control, protein degradation, and genomic silencing. Since nutritional stress is an essential event in differentiation of epimastigotes into metacyclic trypomastigotes at the mid-gut of the insect host, STI1 protein might play a role in metacyclogenesis process. Cell localization and protein level of TcSTI1 protein were investigated during metacyclogenesis. The translated sequence of TcSTI1 gene exhibited significant similarity to other STI1 proteins already described in *Leishmania major* (64%), human (40%), mouse (39%), soybean (37%) and yeast (37%). The cloned gene was expressed in *E. coli* and the purified protein was used to produce polyclonal antibodies to perform Western blot and cellular localization analysis. Expression of TcSTI1 was detected in protein extracts of epimastigotes and of metacyclic trypomastigotes. We observed that the protein was more abundant in epimastigotes in late growth phase and in epimastigotes under nutritional stress. Indirect immune fluorescence analysis showed that TcSTI1 protein was dispersed in the cytoplasm. Immunoprecipitation assays have been performed to investigate TcSTI1 interaction with other proteins. These data indicate that STI1 levels are increased by nutritional stress and suggest that the protein might play a role in differentiation process.

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BM091 - HETEROLOGOUS EXPRESSION OF *Trypanosoma cruzi* APYRASE (NTPDase-I) WITH AND WITHOUT SIGNAL PEPTIDE

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An ecto-NTP diphosphohydrolase (NTPDase-I) was previously characterized on the surface of live *T. cruzi* parasites and a 2282 bp cDNA encoding a full-length NTPDase-I (~70.8 KDa) was cloned. In order to further characterize *T. cruzi* NTPDase-I we performed the heterologous expression. *In silico* analysis using Signal-P program predicts a possible cleavage signal peptide at amino acid position 36, immediately following an amino-terminal predicted transmembrane segment, thus suggesting that NTPDase-I could be produced as a soluble exported protein (~66.6 kDa). Using this information we design strategies to express full NTPDase-I and putative secreted protein. Full NTPDase-I was used as template to amplify DNA fragments that were transferred to pET21b bacterial expression vector. These constructions (full-NTPDaseI-pET21b and partial-NTPDase-I-pET21b) were used to transform *E. coli* BL21 cells. Recombinant proteins were expressed after 4 hours of 0.1mM IPTG induction. Soluble and insoluble recombinant proteins were purified using Ni or Co-agarose affinity chromatography. Our results showed that recombinant proteins with and without signal peptide were eluted with 80mM and 160mM of imidazol, respectively. Western blot analysis showed that anti-NTPDase-I polyclonal antiserum was able to recognize recombinant proteins and evidenced that signal peptide was recognized by the bacterial system. We intend to evaluate biochemical and structural properties of these proteins and to perform the expression in eukaryote system (*Saccharomyces cerevisiae*) to evaluate the influence of pos-translational modifications in recombinant purified proteins.

Supported by: FAPEMIG, CNPq, UFV, UFOP.

BM092 - COMPETITIVE INHIBITION OF THE HEXOSE TRANSPORTER OF *TRYPANOSOMA CRUZI* BY RNA APTAMERS

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The chemotherapy of Chagas' disease is still particularly inefficient in the chronic phase. Moreover most of tripanocidal drugs present undesired side effects. Therefore, the development of new strategies to obtain novel and efficient chemotherapic drugs is necessary. In spite of the fact that *Trypanosoma cruzi* undergoes at least

part of its life cycle in glucose-deficient environments, the parasite uses this metabolite as a main energy and carbon source. Thus the inhibition of the glucose transporter could constitute an efficient strategy to control the parasite infection. We have obtained, by the first time to our knowledge, RNA aptamers inhibiting glucose uptake by *T. cruzi*, which are now being characterized. Seven rounds of *in vitro* selection using live epimastigotes as target were performed for enrichment of RNA molecules with binding affinity to the glucose transporter in the previous random RNA pool. Several clones from 7th round and 4th round SELEX were sequenced and searched for conserved structural motifs. These consensus regions were analyzed *in silico* for RNA secondary structure prediction. Individual RNA clones were analyzed for affinity and inhibition of glucose uptake activity into the parasites. A clone was identified with enhanced binding affinity (Kd = 1006 ± 379 nM) and glucose transport inhibition. The effect of glucose transport inhibition on the epimastigote viability was assayed by MTT.
Supported by FAPESP and CNPq.

BM093 - THE STRUCTURE OF HSP100 OF *TRYPANOSOMA CRUZI*

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Heat shock protein 100 (HSP100) are a group of chaperones crucial to maintaining the native protein conformation and preventing nonspecific protein aggregation. Being a heat-inducible protein, HSP100 is a good model for studying gene regulation. We aim to study the HSP100 gene structure, expression and regulation in *Trypanosoma cruzi*. The predicted amino acid 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 2607 bp was identified, and gives rise to a protein of 869 aa and a pI of 10.4. 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 gene being present in just one copy in the genome. The complete coding region was cloned into pGEX-4T1 vector to obtain the recombinant protein which will be used to generate HSP100-specific polyclonal antibodies. The antibodies will be used to characterize the subcellular localization of the protein. Since previous attempts to detect the HSP100 mRNA by northern blots using total RNA were unsuccessful, probably due to low abundance, we are now using poly(A)$^+$ RNA. RNA processing sites are also being mapped by RT-PCR. Finally, we are currently investigating HSP100 protein induction and isoforms distribution by 2D gel electrophoresis.
Key-words: Trypanosoma cruzi; Clp/HSP100; gene expression.
Supported by CNPq and FAPERJ.

BM094 - FUNCTIONAL CHARACTERIZATION OF A RNA BINDING PROTEIN DIFFERENTIALLY EXPRESSED DURING *Trypanosoma cruzi* METACYCLOGENESIS

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We are characterizing hypothetical proteins differentially expressed during metacyclogenesis. Here we describe the functional characterization of a 25 kDa putative RNA binding protein, containing a domain probably related to nucleic acid binding activity, named ALBA. In addition, this protein has several RGG-related motifs at its carboxyl end, generally also involved in RNA binding. *T.cruzi* has four distinct genes coding for ALBA containing proteins, as contiguous pairs in two different genome regions. The proteins coded by these pairs differ in size (~13kDa and ~25kDa). The larger proteins are very similar to each other, differing only at the carboxyl end, where RGG motifs are located. All *Trypanosoma* sp. have four genes, but both pairs have only one member in *Leishmania* sp. We have cloned the larger ALBA CDSs into the Gateway platform (Invitrogen), followed by protein expression in *E. coli* using pDEST17 vector. After purification of the recombinant protein, we produced polyclonal antibody, used for protein expression analysis by western blot and immunolocalization during *T.*

cruzi metacyclogenesis, immunoprecipitation for protein-protein interaction identification, by LC-MS/MS, and protein-RNA interaction identification, by microarray. Semi-quantitative transcriptome and proteome analyses showed a profile of down-regulation during metacyclogenesis. One interesting finding is that it is easily found in descriptive proteomics analyses, suggesting it is highly expressed. It is a cytoplasmic protein, showing a dispersed granular pattern. This result, taken together with the domains function, suggests it is a RNA binding protein. Reinforcing this idea, we have identified 60 probably interacting proteins, being 60% ribosomal and 8.3% RNA-binding proteins. Protein-RNA complexes were obtained and are currently being evaluated by microarray. In summary, aiming to characterize a hypothetical protein differentially expressed during metacyclogenesis, we have described a highly expressed cytoplasmic RNA-binding protein, whose expression decreases during metacyclogenesis, that possibly interacts with the translational apparatus.

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BM095 - THE INTERACTION MAP OF *TRYPANOSOMA CRUZI* RIBOSOMAL P PROTEIN COMPLEX

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The ribosomal P proteins are located on the stalk of the ribosomal large subunit and play a critical role during the elongation step of protein synthesis through interaction with elongation factor 2 (EF2). The stalk, in *Trypanosoma cruzi*, is composed by four proteins of about 11 KDa, TcP1α, TcP1β, TcP2α, TcP2β and a fifth TcP0 of about 30 KDa. In order to study the ribosomal P protein complex assembly, a yeast two-hybrid interaction map was generated that indicated a central role for TcP0. Using Surface Plasmon Resonance the kinetics of each interaction between the members of this protein family and with the EF2 were analyzed. The assembly of ternary complexes was also assessed and the interaction domains of TcP0 and TcP2β were mapped using truncated proteins and synthetic peptides, respectively. Results showed that TcP0 and TcP2βproteins were able to form homo dimers and also interacted with both P1 and both P2 proteins. All ribosomal P proteins interact with EF2, but the small P proteins showed a stronger affinity than TcP0. The C-terminal region of TcP2β (peptide R15) seems to be involved in the interaction with EF2, since R15 was able to inhibit the second step of the association phase. Other regions of the protein may also be involved because R15 had no effect on the first step of the association phase. Compared to other species, *T. cruzi* displays a specific pattern of ribosomal P protein interactions that could be targeted by selective therapeutic agents. This work was supported by ECOS-SECyT, France-Argentine and FONCyT BID 1728/OC-AR 01–14389, Argentine.

BM096 - *TRYPANOSOMA CRUZI*: INSIGHTS IN THE FUNCTIONAL CHARACTERIZATION OF TcRBP19, AN RNA BINDING PROTEIN

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The protozoan pathogen *Trypanosoma cruzi* is the causative agent of Chagas disease. Its life cycle is developed between two hosts (insect and mammalian) and involves at least four stages. Consequently, several proteins have to be tightly regulated to allow the rapid adaptation that is essential for the parasite survival. Regulation of gene expression in trypanosomatids is not yet well understood presenting several deviations from standard eukaryotic paradigms. Genes are organized in long polycistronic transcriptional units separated by intergenic regions that contain the signaling information mRNA processing. There are no canonical promoters identified yet and there is no evidence for specific or regulated transcription initiation of protein coding genes by RNA polymerase II. Given these peculiarities, the regulation of gene expression occurs predominantly at the post-transcriptional level. Undoubtedly, RNA binding proteins (RBP) play a major role in many aspects of RNA processing,

function, and degradation of mRNA. We have previously described the characterization of TcRBP19, a 17 kDa RNA-binding protein containing an RRM in *T. cruzi*. Orthologous genes are present only in the TriTryp genomes, suggesting unique function in those parasites. TcRBP19 is a low expression protein only barely detected at the amastigote stage localizing in a diffuse pattern in the cytoplasm. In this work, we focused in the identification of mRNAs associated with TcRBP19, we performed GST-pull down assays and we found that TcRBP19 also binds its own mRNA suggesting autorregulation. Finally, epimastigotes overexpressing TcRBP19 showed no phenotypic alterations but, interestingly, those parasites have diminished their capacity of develop metacyclogenesis. Moreover, lower infection rates were observed in VERO cells with those transfectant parasites.

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BM097 - PROTEASOME β SUBUNIT: GENE RESEQUENCING AND PROTEASOME ACTIVITY IN STRAINS OF *TRYPANOSOMA CRUZI*

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The proteasome is a multisubunit cellular organelle that functions as a nonlysosomal threonine protease. Proteasomes play a critical role in the degradation of proteins, regulating a variety of cellular processes, and they are also the target for antitrypanosoma proteasome inhibitors. Each protease is formed by an activated beta subunit, beta5/X, beta1/Y, or beta2/Z, that exhibits chymotrypsin-like, caspase-peptide hydrolyzing, or trypsin-like activity, respectively**.** Little is known about the relative contribution of specific beta subunits in the degradation of endogenous protein substrates in Trypanosma cruzi, as well as the impact of genetic variation in proteasome subunits could influence both proteasome function and response to drug therapy**.** We resequenced genes encoding the three active proteasome β subunits using DNA samples from six strain of T. cruzi (ABC, CL-Brenner, Berenice-62, Berenice-78, Colombian and Y strains). Resequencing was followed by functional studies of polymorphisms identified in the coding region Tcbeta5. Resequencing identified a series of novel strain-specific polymorphisms that are not represented in public databases. The proteasome activity was monitored using fluorogenic peptides as substrates. Levels of ubiquitin and ubiquitin conjugates were determined by Western blotting. These results showns that Berenice 78 has seven fold more proteasome associated to chymotrypsin-like than Berenice 62. Also, the Colombian strains show similar levels of proteasome activity than Y and ABC. Future studies might focus on confirm the genotype-phenotype correlations between single nucleotide polymorphisms (SNP) in Tcbeta5 and mRNA expression and the significant effects on proteasome activity. Supported by FAPEMIG,CNPq,UFOP.

BM098 - Molecular Characterization and RNAi studies of the gene SECp43 of *Trypanosoma brucei*

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The inspiration of this work is an important biochemical pathway in the parasite *Trypanosoma brucei* as a genetic code translation of the UGA-stop codon in the 21th amino acid selenocisteine. These represent the major organic form of selenium and their synthesis and incorporation into selenoproteins co-translational requires complex molecular machinery. Trypanosomatids are a major cause of mortality in tropical regions of the world and are affected by the lack of effective treatments. Several intriguing molecular pathways are found in these parasites also, rendering them particularly attractive for biochemical investigation. In kinetoplastida, Secp43 appears to be a key enzyme in the $tRNA^{Sec}$ modification and presentation to the translation machinery. In eukaryotes the full repertoire of genes involved in this route of synthesis has recently been uncovered, but still several answers remain unchecked. We have cloned and characterized the *Trypanosoma brucei* SECp43 homologue. This gene is intriguing due to its architecture as resulting from a gene duplication and divergent evolution event. The counterparts of SECp43 of various organisms are preserved with each other, and have just a single module. To provide new insights on the relationship between the selenoprotein synthesis pathway and their

biological role in *T. brucei*, we used the RNA interference technique to knock down the gene expression. The use of p2T7 vector allowed the direct transfection and RNAi induction in both procyclic and bloodstream forms of *T. brucei*. The gene was also cloned and an expression and purification protocol established. Mouse polyclonal antibodies were obtained and revealed the presence of the translated Secp43 protein in *T. brucei* extracts. A detailed biochemical characterization of this protein and its interaction with $tRNA^{Sec}$ is under way. Supported by FAPESP, CNPq

BM099 - Functional analysis of adenylate cyclases in *T. brucei* reveals their involvement in cell cycle progression

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We investigated the role played by the adenylyl cyclases (AC) in cellular differentiation from long slender to short stumpy forms of the African trypanosome, in particular ESAG4, whose gene is located in VSG expression sites and is the only known stage-specific AC isoform specifically expressed in bloodstream forms. Several complementary reverse genetics strategies have been applied in bloodstream stages of both monomorphic and pleomorphic strains: (1) expression of several different catalytically dead dominant negative (DN) mutants of ESAG4, (2) RNAi mediated repression in various ESAG4 dsRNA expressing cell lines (p2T7-ribo, hairpin RNAi), (3) a targeted knock-out (KO) of ESAG4 in the active expression site. Inducible expression of an ESAG4 DN mutant in a monomorphic strain presented a transitory growth phenotype with cytokinesis defect, whereas no growth phenotype was detected upon constitutive expression of an ESAG4 DN mutant in a pleomorphic strain. ESAG4 knock down (KD) in a monomorphic strain induced a lethal phenotype linked to cytokinesis blockade. Moreover, while KD of ESAG4 and ESAG4 DN mutants decreased global adenylyl cyclase activity of trypanosomes, KO of ESAG4 alone did not affect this activity. These results suggest that the dominant-negative ESAG4 versions affected not only ESAG4 itself but also other members of the GRESAG4 family. A preferential flagellar localization observed with dominant-negative GFP tagged ESAG4 mutants confirmed our published EM results showing that ESAG4 is primarily located on the flagellar membrane of the parasite. This supports the view that the flagellum serves sensory functions in kinetoplastids. Further analysis of the phenotypes of our ESAG4 mutant collection upon differentiation processes is underway.

BM100 - Study of the Trypanosoma brucei chloride channels, and their involvement in lysis by apolipoprotein L-I

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African trypanosomes (the prototype of which is Trypanosoma brucei brucei) are protozoan parasites that infect a wide range of mammals. Human blood, unlike the blood of other mammals, has efficient trypanolytic activity. Studies showed the importance of chloride ions in this activity and there are 3 putative chloride channel (CLC) genes in the T. brucei genome. The CLC family is essentially ubiquitous and all characterized members transport chloride either in a voltage-regulated process or as antiports coupled to H^+.
In silico analysis showed that 2 of these genes present an antiport consensus sequence. The third, TbCLC-b, presents a channel consensus. TbCLCs also show a greater similarity to the hCLC-6 part of the human CLC family by northern blot we found no differences between the levels of expression of TbCLCs in bloodstream or procyclic form. Complementation experiments in S.Cerevisiae deleted for GEF1 showed no rescue of the phenotype by TbCLCs. When knocked down by RNAi, TbCLC-b is the only CLC inducing a delay in the kinetics of trypanosome lysis by human serum.
Therefore, we think that TbCLC-b is involved in the mechanism of trypanolysis and requires further study.

BM101 - FUNCTIONAL CHARACTERIZATION OF AN ESSENTIAL FKBP12 IN TRYPANOSOMA BRUCEI.

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FKBPs (FK506 Binding Proteins) belong to the family of peptidyl prolyl cis-trans isomerases (PPIase) which catalyze the cis-trans isomerisation of cis-prolyl bonds. They are conserved from bacteria to mammals and help in the folding of proteins. They are also called immunophilins as they are able to complex immunosuppressive drugs (forming a ligand-receptor pair) like the two macrolactones, FK506 and rapamycin, thereby inhibiting their PPIase activity. In mammals, the immunosupressive activity of the drugs is unrelated to their inhibition of the PPIase activity but is instead due to inhibition of the proteine kinase TOR (rapamycin-FKBP) or the proteine phosphatase calcineurin (FK506-FKBP12). This inhibition is subsequent to the formation of a tripartite complex with the ligand-receptor pair. FKBPs are involved in a wide variety of biological processes affecting the function and structure of target proteins. Some of them might play a role in parasite virulence (as TcMIP in *T. cruzi*) and could therefore be ideal targets of anti-parasitic drugs.

We investigated the role played by the FKBPs in the African trypanosome (*Trypanosoma brucei*) responsible for sleeping sickness in humans.

The genome of *T. brucei* contains four different FKBP genes (FKBP12 (archetype of the family), FKBP11, FKBP21 and FKBP47) which are all expressed at the different stages of the parasitic cycle. Systematic knock-down by RNA interference of the *FKBP* genes of the trypanosome leads to the conclusion that only FKBP12 is essential for the parasite survival in both bloodstream and procyclic forms. This lethal growth phenotype is related to a cytokinesis deficiency. Further analysis of the cell growth phenotype and confirmation of the phenotype by knock-out gene ablation are underway.

BM102 - Identification of *Trypanosoma brucei* pre-replication machinery components

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The assembly of pre-replication complexes on eukaryotes begins with the binding of an origin recognition complex (Orc_{1-6}) to chromatin. Chromatin-bound Orc recruits Cdc6 and Cdt1. Cdc6 stabilizes the binding of Orc and allows Cdt1 to load the hexameric Mcm complex, whose helicase activity is essential for replication initiation. Although the pre-replication machinery has been extensively studied in several eukaryotes, nothing is known how it is formed in *Trypanosoma*. Differently from other eukaryotes and based on genome predictions trypanosomes do not contain sequences in their genome that could codify for Orcs, Cdc6 or Cdt1. Instead, these parasites contain only one open reading frame homologous to both Orc_1 and Cdc6. Here we cloned the Orc/Cdc6 encoding gene and the recombinant protein was used to raise specific antibodies. Western and immunofluorescence assays showed that Orc/Cdc6 is expressed in *T. brucei* and remains associated to chromatin during the entire cell cycle. Extraction of soluble proteins followed by extraction of DNA bound proteins by DNAse digestion showed that a small fraction of Orc/Cdc6 is associated to nuclear matrix. Immunoprecipitation of *T. brucei* extract with anti-Orc/Cdc6 revealed coprecipitation of a 70 kDa protein. This protein is not recognized by anti-Orc/Cdc6 in immunoblots, showing that this 70 kDa molecule might be associated with Orc/Cdc6. The identity of this protein, probably a component of *T. brucei* pre-replication machinery, is under investigation.

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BM103 - Expression of recombinant SURFIN proteins of Plasmodium falciparum isolates from the Brazilian Amazon and humoral immune response analysis

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The effective humoral response against *P. falciparum* is directed against merozoites and parasite encoded proteins at the infected red blood cell surface. The discrimination between "useful" and mere "collateral" targets is essential for the formulation of an effective malaria vaccine.

The *surf* genes, a small gene family of 10 members, locate close to or within the subtelomeric region of chromosomes 1, 4, 8, 13 and 14 from *P. falciparum*. Their gene products are apparently presented at the surface of the infected erythrocyte and as a sheat around the apical complex of merozoites. We have cloned and expressed a number of SURFINs from *Plasmodium falciparum* field isolates of the Brazilian Amazon. Partial sequencing of exon 1 from 10 genes show a high level of similarity between field isolates, FCR3 and 3D7 strains, however, not all genes were amplifiable in all isolates, suggesting the absence of certain genes or significant polymorphisms at the primer site. We produced recombinant GST-fusion peptides from a portion of exon1 of PFA0625w, PFA0650w and PFA0725w *surf* genes and analyzed their recognition in Western Blots. Pooled sera from symptomatic and asymptomatic patients living in a seasonal malaria endemic area of Rondônia were utilized as primary antibodies. Preliminary data show a mixed recognition of rSURFINs by symptomatic individuals and asymptomatic individuals while the control antigen rMSP3, a vaccine candidate, was recognized stronger by asymptomatic individuals.

Elisa and western analysis of recombinant peptides from the others 7 *surf* genes are in progress and the results will be presented. Supported by FAPESP.

BM104 - Functional characterization of mRNA-proteins complexes (mRNPs) in Trypanosoma cruzi

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Gene expression mechanisms in trypanosomatids are quite unusual when compared to other eukaryotes. The polycistronic transcription and the lack of characteristic promoters indicate that gene expression regulation occurs mainly at the post-transcriptional level. It is believed that mRNA stability and access to polysomes must be tightly regulated, allowing rapid adaptations to different environmental conditions at which *T. cruzi* is exposed during its life cycle. Post-transcriptional regulation requires the association between mRNAs and certain proteins to form mRNPs complexes. In order to elucidate the composition of mRNPs in the polysomal and polysomal free fractions in *T. cruzi* the mRNA-protein complexes were captured using magnetic beads containing a poly-T tail. The mRNP components from *T. cruzi* epimastigotes in exponential growth phase and epimastigotes under nutritional stress isolated were characterized by microarray analysis and by mass spectrometry in tandem (LC-MS/MS). To determine the core proteins that compose the mRNPs and also the weaker protein-protein interactions high and low salt conditions were used respectively. The results allowed the identification of 107 (high and low salt) proteins in the polysomes of epimastigotes from at least two replicas. In the polysomal free fraction of epimastigotes 19 (high salt) and 119 (low salt) proteins were identified. For the stressed parasites we identified 46 (high salt) and 236 (low salt) proteins in the polysomes and 54 (high salt) and 428 (low salt) proteins in the polysomal free fraction. Based on these results some proteins were selected for antisera production in order to define the composition of specific mRNPs and determine their role in *T. cruzi* the gene expression regulation.

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