# Bioquímica e Biologia Molecular - Biochemistry and Molecular Biology

## BM001 - Identification of expressed proteins during *Trypanosoma cruzi* metacyclogenesis by High-throughput proteome analyses

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The study of *T.cruzi* metacyclogenesis is of great interest since it comprises the morphogenetic transformation of a non-pathogenic form (epimastigote) to a pathogenic form (metacyclic trypomastigote). Metacyclogenesis can be mimicked in vitro by incubating epimastigotes in a chemically defined medium e (TAU3AAG medium), rendering possible obtaining parasites at various time points of the differentiation process. The fact that most if not all trypanosomatid mRNAs result from processing of polycistronic transcripts suggests that most genes must have their expression regulated at the post-transcriptional level. Whole-organism, shotgun proteome LC-MS/MS analysis have been carried out in order to gain further insight into the relative abundance of proteins of *T. cruzi* during metacyclogenesis. The proteomic approach is an important tool for studying global protein expression and is being applied for obtaining protozoan parasite protein maps. In this work, proteomic maps of epimastigotes, metacyclic trypomastigotes and four intermediate forms during the parasite differentiation were performed to identify proteins and to understand the dynamics of protein synthesis during this differentiation process. Approximately 1,000 proteins were identified after searching databases with approximately 12,000 peptides mass fingerprints obtained by LC- MS/MS. The accuracy of peptide identifications made by usual database was provided by Peptide Prophet. These proteins were clustered according to each stage analyzed. These proteins categories might be key factors to understand the parasite biology as well as an important toll to confirm the hypothetical genes. Financial support from CNPq (Prosul and Pronex), Fiocruz, NIH

## BM002 - Preparation os a DNA microarray slide for the analysis of gene expression in *Trypanosoma cruzi* strains with different susceptibility to benznidazole

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Since the beginning of the 1970s Benznidazole (BZ) and Nifurtimox have been used for treatment of Chagas disease. One of the factors explaining the variability of the success of treatment is the occurrence of *T. cruzi* strains exhibiting different levels of drug susceptibility. Decreased drug uptake, increase in drug export and modification of the gene encoding the drug target have been implicated in resistance to chemotherapy in parasitic protozoa. To approach the identification of putative genes involved in BZ resistance in *T. cruzi*, we are using DNA microarrays to compare gene expression in three strains susceptible and three strains resistant to BZ. EST clustering was performed with the CAP3 program on 1,233 ESTs of amastigotes of the CL Brener strain (kindly provided by Dr. A. Gonzalez, Instituto de Parasitología y Biomedicina, Granada). We observed 585 clusters, of which 300 contained only one EST (singletons). The functional identity of the consensus sequences was inferred from similarity searches using the BLASTX program against NCBI nr database and KOG database. CL Brener amastigotes ESTs were also clustered with 44 ESTs of Tulahuén amastigotes (kindly provided by Dr. S. Teixeira, UFMG) and 723 ESTs of CL Brener epimastigotes (kindly provided by Dr. W. Degrave and Dr. A. Brandão, FIOCRUZ). Data obtained with CAP3 program indicated a total of 1,153 clusters of which 814 clusters were singletons. For amplification and immobilization on the microarray slide we selected 288 amastigote singletons, 782 representatives of clusters containing CL Brener and Tulahuén ESTs, 37 Orestes of CL Brener, 32 genes from different *T. cruzi* strains and 10 control DNA probes. The quality of the microarray slide was verified by two independent hybridizations with DNA of CL Brener or VL10 strains labeled with Cy3 and Cy5. Hybridization with cDNAs of *T. cruzi* strains is in progress. FAPESP; CNPq, CAPES

## BM003 - Contribution of YAC based physical maps to the final assembly of the *Trypanosoma cruzi* genome

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Repetitive sequences represent the major challenge for assembly of sequences generated in *Trypanosoma cruzi* genome project [whole genome shotgun (WGS) sequences]. They may confound shotgun approaches to complete genome sequencing. Thus the complete assembly sequence of megabase *T. cruzi* chromosomes are not yet available. In this work we integrate genome sequencing data into the physical map of megabase chromosomes XX, XVI and XVII of clone CL Brener. Physical maps of chromosomes XVI, XVII (Santos *et al.* 1999, *Genome Res* 9, 1268) and XX (Porcile *et al.* 2003, *Gene* 308, 53) have been constructed using YAC clones and chromosome-specific markers. We confirmed that large fragments of *T. cruzi* DNA are stable as YACs in *S. cerevisiae* and some regions of the megabase chromosomes are well covered by YACs. We have used chromosome-specific markers from chromosomal bands XVI, XVII and XX to find out homologous sequences in contigs of T. cruzi database (www.genedb.org/genedb/tcruzi/). Sequence contigs containing these markers can be anchored in both YAC clones

and chromosomes generating an integrated map of megabase regions of the genome. Fifty sequence contigs containing markers from chromosome XX and 19 sequence contigs from chromosomes XVI and XVII were identified. This strategy involved the selection of seed clones for mapping along the length of the chromosomes, and extending outwards from those to develop large sequence contigs. Manual editing improved the sequencing assembly by identifying potential joins between contigs, and performing merges, increases the overall contiguity of the resource. With this approach we generated sequence contigs covering megabase regions of the chromosomes XX, XVII and XVI of the clone CL Brener. Supported by FAPESP, CAPES, CNPq.

## BM004 - Microarray profiling of gene expression in *Trypanosoma cruzi* strains from asymptomatic and cardiad patients

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The majority of the individuals with positive serology for Chagas disease are asymptomatic. After several years, 10-40% of these patients develop lesions of the heart or digestive system. The variability of symptoms has been mainly attributed to the genetic diversity of *T. cruzi* strains. One possibility to explain the distinct pathogenicity of the strains is the differential expression of particular genes. We have reported that DNA microarrays bearing predominantly ESTs of CL Brener are a valid tool for comparative genomics and analysis of gene expression in parasite strains (Baptista et al. MBP 18, 183-194, 2004). In the present study, DNA microarrays were used for comparative gene profiling in isolates from individuals presenting the indeterminate and cardiac forms of Chagas disease (three strains of each group from the same endemic area; typed as *T. cruzi II*). To identify DNA sequences differentially expressed we used as premise to select the genes up regulated or down regulated in the three cardiac strains when compared with the three asymptomatic strains. For statistical analysis of the data, we developed a method called BayBoots, a non-parametric Kernel and Bayesian Bootstrap. We concluded that 10 probes were up regulated in the cardiac strains and 4 probes, up regulated in the asymptomatic strains. Fifty percent of the sequences had no similarity in databases. The differential transcription of the genes was investigated by Northern blot. One probe encoding the sequence of one component of the mitochondrial electron-transport chain showed cardiac/asymptomatic microarray hybridization ratios of 17-30. Southern blots indicate alterations in the organization of maxicircle kDNA between the two groups of strains. Studies are in progress to elucidate this aspect. It is anticipated that the genetic markers differentially expressed may be of potential use in diagnostic/prognostic tests and could assist the understanding of the pathogenesis of Chagas disease. FAPESP, CNPq.

## BM005 - The repetitive element TcTREZ0, a species-specific sequence, is not randomly distributed in the genome of *Trypanosoma cruzi*

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The repetitive element TcTREZ0 (*Trypanosoma cruzi* Tandem Repetitive Element Z0) accounts for 4% of the nuclear genome of clone CL Brener. Here we analyzed 38 contigs from the *T. cruzi* database (www.genedb.org) carrying sequences homologous to TcTREZ0. The element was found in tandem arrays of 1 to 5 copies per contig, and several copies were found at the end of the contig. Detailed analysis of the contigs showed the presence of a consensus sequence pattern upstream of the TcTREZ0, indicating the existence of an insertion site for this element. The size of the upstream sequence (68 nt) flanking TcTREZ0 is very conserved. The presence of a consensus sequence pattern downstream of TcTREZ0 was also confirmed by the analysis of the contigs. The size of downstream sequences can vary between 300 to 500 bp. These results indicate that TcTREZ0 is not randomly distributed in the genome of *T. cruzi*. This is in agreement with previous data obtained in our laboratory by two-dimensional PFGE hybridization, fiber FISH and YAC-based physical mapping. TcTREZ0 is located in large regions containing genes encoding surface antigens, satellite DNA and retrotransposons (L1Tc, VIPER). In *T. cruzi*, regions of synteny with other trypanosomatids (*T. brucei*, *Leishmania major*) are interrupted by genes belonging to these sequences (El Sayed et al. 2005, Science 309, 409), suggesting that these regions could be genetically instable. We demonstrated by hybridization and sequence analysis that TcTREZ0 was not present in other trypanosomatids (*Trypanosoma rangeli*, *Trypanosoma brucei* and *Leishmania L. amazonensis*). Further characterization of structural and functional features of TcTREZ0 repeated elements is underway in our laboratory. Supported by FAPESP, CNPq, CAPES.

## BM006 - *Leishmania (Viannia) braziliensis* Sequence Project Update and Comparative Genomic Analysis with other *Leishmania* species.

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The recent availability of the Tritryp *Leishmania major*, *Trypanosoma brucei and Trypanosoma cruzi* genomes is a marker for the knowledge of the biology of these three pathogens. Whereas analysis of a single genome provides remarkable biological insights on any particular organism, comparative analysis of multiple genomes provides

considerably more information. It expands our ability to understand the genetic and evolutionary bases of the shared and distinct parasitic modes and lifestyles and better assign putative function to predicted coding sequences. *Leishmania (Viannia) braziliensis* is one of the causative agents of cutaneous leishmaniasis (CL) in Brazil and its infection leads to a broad spectrum of clinical, histopathological, and immunological manifestations ranging from self-healing cutaneous lesions to severe destructive nasal/oral mucous membrane lesions. In this study we are using the sequencing information of *L. braziliensis* (MHOM/BR/75/M2904) project, together with the data from *L.major* and *L.infantum* in a broad comparative genomics analysis. Preliminary comparative results regarding gene content, genome architecture and the conservation of synteny (gene order & organization ) among these three organisms will be presented. Differences in the gene content between *L.braziliensis* and the other species have been identified as well as species-specific genes that may contribute to the individual pathologies presented by each *Leishmania species*.

## BM007 - Functional genomic characterization of *Trypanosoma cruzi* trypomastigotes

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Metacyclic and cell derived trypomastigotes are both infective forms of *Trypanosoma cruzi*. In order to improve our understanding about these two forms, a functional genomic analysis was performed using a microarray with approximately 6,000 probes. RNA samples, extracted from *T. cruzi* epimastigotes (Epi), amastigotes (Ama), metacyclic trypomastigotes (Meta) and cell derived trypomastigotes (Trypo), were extracted and amplified *in vitro*. There were 4 samples for each form (2 from total RNA, 2 from polysomal RNA), generating 4 groups of analysis. Differentially expressed probes (DEPs) were detected by the significance analysis of microarray (SAM). The comparison between Meta with Trypo showed that 543 DEPs were detected in at least 3 of the groups of analysis. 189 probes were shown to be specifically expressed when total RNA samples were compared and 129 probes were considered specifically expressed when polysomal RNA populations were compared. The comparison of genes equally expressed between Meta and Trypo, but differentially expressed when compared to Epi and Ama, allowed detecting 279 DEPs raised in Epi/Ama and 205 DEPs raised in Meta/Trypo. In addition, 342 DEPs were detected when total RNAs from Epi/Ama and Meta/Trypo were compared whereas the comparison of polysomal RNAs resulted in 47 DEPs. The results indicate that for some genes changes in the total RNA profiling are accompanied by a corresponding change in the profile of RNAs associated to the polysomes. However, there are several cases where great differences are observed in the total and polysomal RNA populations, corroborating the relevance of controlling mRNA access to polysomes as an active posttranscriptional gene regulation mechanism in *T. cruzi*. Financial support from CNPq, Pronex (CNPq-Fundação Araucaria), Fiocruz, NIH.

## BM008 - OPEN READING FRAME EXPRESSED SEQUENCE TAGS (ORESTES) OF *TRYPANOSOMA CRUZI*

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About 11,000 ESTs of epimastigotes and 2,800 ESTs of trypomastigotes and amastigotes of CL Brener are available. ORESTES is an alternative methodology for characterization of transcribed sequences, in which the central region of mRNAs is preferentially amplified (Dias Neto et al., 2000). ORESTES methodology has not been applied to human parasitic protozoa. Aiming the characterization of transcripts in the intracellular stages of T. cruzi strains, we standardized the ORESTES technique in CL Brener epimastigotes. The mRNA fraction was purified with mMACS kit (Miltenyi Biotec), DNAse treated and cDNA templates were generated by RT-PCR using five arbitrarily selected primers under low-stringency conditions. The same primer was used to synthesize cDNA second strand. The products (200-400 bp and $> 400pb$) corresponding to each primer were selected, purified and ligated in pGEM TEasy. We sequenced 584 ORESTES of epimastigotes. After analysis of the chromatograms, 540 sequences were accepted and used for similarity searches with the BLASTN and BLASTX programs against NCBI nr database. We found that 260 sequences (48.1%) had no matches and that rRNA sequences varied from 5 to 8% depending on the primer used for amplification. BLASTX analyses indicated the tendency of a specific primer to amplify particular classes of transcripts. Similarity to *T. cruzi*, *T. brucei* and *L. major* sequences was found, respectively, in 47.5%, 35.5% and 15.9% of the sequences. The data of ORESTES clustering and the functional identity of the consensus sequences will be presented. ORESTES were obtained from the VL10 human strain amastigotes and trypomastigotes obtained from LLC-MK2 cell monolayers. Until now, 192 ORESTES were obtained, of which 175 showed good quality. The percentage of sequences showing no similarity against public databases was 69.1%, a figure higher than that obtained for epimastigote ORESTES (48.1%). This suggests the usefulness of ORESTES methodology in gene discovery. FAPESP, CNPq.

### BM009 - Exploring the *Trypanosoma rangeli* transcriptome: Generation and analysis of Expressed Sequence Tags (ESTs) and ORESTES (ORF-ESTs)

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*Trypanosoma rangeli* is a hemoflagellate parasite occurring in sympatry with *T. cruzi* in a wide geographical area in Central and South America. Despite the overlaping distribution, the antigenic similarity and the occurrence single and/or mixed infections with *T. cruzi* in reservoirs and vectors, the molecular biology of *T. rangeli* is little studied. Thus, through the generation of GSS, EST and ORESTES, we aim to explore the *T. rangeli* genome and transcriptome using intra and inter-specific comparisons. In this work we present the updated results of the *T. rangeli* Transcriptome Project (http://www.biowebdb.org/). Up to now, sequencing of 11 cDNA libraries (3 ESTs/8 ORESTES), derived from epimastigote and trypomastigote forms of *T. rangeli* Choachi and SC-58 strains, produced a total of 3,168 sequences (1.9Mb). Sequence analysis was performed by the GARSA system (http://www.biowebdb.org/garsa). The 1,644 (51.9%) high quality sequences were distributed in 615 singlets and 1,029 clusters, totalizing 862 non-redundant sequences. The mean size of the sequences for all cDNA libraries was of 342bp, and the mean C+G content was of 52.0%. Around 49.0% of the sequences presented similarity to previously identified trypanosomatid sequences, among which, 5.05% were similar to *T. rangeli* sequences, while 48.4% presented no significant matches with the public databases used, and thus, may represent *T. rangeli* specific genes. Comparative analysis allowed the grouping of the sequences according to their putative biological functions, being cell surface molecules, such as sialidases, the largest category so far. Along with the continuing ESTs and ORESTES generation, the project is sequencing GSS libraries of the same strains. The information revealed by this project will be of major importance to bring up new markers for specific diagnosis, to discover, understand and/or compare some biological processes, revealing a genomic panorama of the parasite. Supported by CNPq, Fapesc and UFSC.

### BM010 - Setting Up a Reference Map For the Alkaline Proteome of *Trypanosoma cruzi*

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The particular *T. cruzi* post-transcriptional gene regulation mechanisms that allow transcription of many mRNAs whose information is not translated into polypeptides, makes unfeasible to extrapolate mRNA measurements into protein levels. Thus, the analysis of protein expression in the parasite is a necessary step into the elucidation of biochemical differences responsible for the particular biological traits of each developmental form of the protozoa. We have published before proteome maps of three life stages of the parasite using acid pH gradients (Paba et al., 2004. Proteomics 4, 1052-1059). Although there was analysed the expression of several hundreds of proteins, it represents only a small portion of the potential number of genes expressed by the parasite. In order to get a wider view of the parasite protein expression we are actually expanding covering capabilities using narrow range and alkaline IPGs in two-dimensional gel electrophoresis (2-DE) experiments. In the present work we tested and optimized conditions for constructing *T. cruzi* alkalyne 2-DE maps using epimastigote forms. Several experimental alternatives were assessed regarding sample application (in gel rehydration, cup loading, paper bridge loading), type and concentration of reducing reagents, total kVh in isoelectrofocusing (IEF), sample loads and addition of organic solvents to solubilization buffer. Loading samples in the anodic side of the IPG using the paper bridge method, coupled to optimized reducing conditions and the addition of ten percent isopropanol to the sample before IEF, provided the best 2-DE maps. The optimized protocol allowed the construction of proteome maps containing around two hundred spots, and displaying higher resolution and reproducibility. This protocol is now being applied to trypomastigote and amastigote forms of the parasite. This research is sponsored by TDR-WHO, FAPDF

### BM011 - Microarray analysis of *Trypanosoma cruzi* metacyclogenesis: newer and deeper insights in mRNA level regulation and biological functions involved

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*Trypanosoma cruzi* displays some unusual and interesting biological characteristics. Studying the biology of this parasite is of utmost importance, as it is the causative agent of Chagas disease. A key event during its life-cycle is the metacyclogenesis process, which occurs in the insect vector, consisting in the differentiation from a replicating, non-infective form (epimastigote) to a non-replicating, infective form (metacyclic trypomastigote). We have been studying the biology of *T.cruzi* through functional genomics and our previous microarray analysis has been recently expanded and corroborated by using a microarray with 6,200 probes in triplicate. We have measured the mRNA level at six different time points of the metacyclogenesis process comparing total and polysomal mRNA. An excellent level of reproducibility has been obtained, both at the technical and at the biolog-

ical level, mainly by using RNA *in vitro* transcription and three distinct metacyclogenesis experiments. After ninety-four hybridizations, the SAM method was used to achieve rigorous statistical differential expression. More than 1,500 probes were differentially expressed (criteria: fold change 75%, FDR 1%, at least two biological replicates), referring to nearly 1,000 genes, covering a wide variety of biological process, providing very interesting subjects for future research. Our results reinforce and expand the importance of the mRNA association to the translational machinery as a major mechanism of gene expression regulation in *T.cruzi*. We are presently expressing 300 genes differentially expressed during *T.cruzi* metacyclogenesis, which will be used in functional and confirmatory assays.
Financial support from CNPq, Pronex (CNPq-Fundação Araucaria), Fiocruz, NIH

## BM012 - Characterization of multiple cap binding protein (eIF4E) homologues from *Trypanosoma brucei*.

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The protozoans belonging to the Trypanosomatidae family are known for their complex biology and unusual molecular mechanisms. Regulation of gene expression is accomplished mainly by posttranscriptional mechanisms such as control of mRNA stability and translation. In order to study translation initiation in these parasites and its role in regulating gene expression we proposed to characterize the subunits of the translation initiation complex eIF4F. In eukaryotes, eIF4F is composed by the subunits eIF4E, the cap binding protein; eIF4A, a RNA helicase; and eIF4G, a large scaffolding protein. Here we describe work aimed at the characterization of four eIF4E homologues previously identified in *Trypanosoma brucei*. The four genes (*Tb*EIF4E1-4 ) were amplified, cloned, expressed in E. coli and the recombinant proteins used to produce polyclonal sera in rabbits. Northern and Western blots were then performed using RNA and protein extracts from both the insect procyclic and human bloodstream stages of the parasite lifecycle. mRNAs coding for *Tb*EIF4E1, 3 and 4 were found to be expressed in both stages at constant levels. In contrast, whilst the *Tb*EIF4E1 protein was detected at similar moderate levels in procyclic and bloodstream forms, *Tb*EIF4E3 and *Tb*EIF4E4 were found to be abundant in procyclic but absent from the bloodstream form. *Tb*EIF4E2 was not detected at all. In functional analysis *Tb*EIF4E1, *Tb*EIF4E2 and *Tb*EIF4E4 were able to bind the vertebrate cap. Through in vivo approaches, all four proteins were found to localize in the cytoplasm of the parasite, whilst only *Tb*EIF4E3 was found to be strictly required for cellular viability after RNAi using procyclic forms. Our results are consistent with the various homologues having distinct functions during the parasite lifecycle: whist *Tb*EIF4E3 seems to be essential at the procyclic stage (*Tb*EIF4E4 being complementary), *Tb*EIF4E1 and/or *Tb*EIF4E2 may be essential at the bloodstream stage despite their low intracellular concentration.

## BM013 - Overexpression of the META 2 gene of *Leishmania amazonensis*: the effect on parasite virulence

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The META cluster of *Leishmania amazonensis* contains both the META 1 and META 2 genes, which are upregulated in metacyclic promastigotes and encode proteins containing the META domain. These genes are conserved in other *Leishmania* species and in *Trypanosoma brucei*. META 1 overexpressing mutants show increased virulence in vivo. Previous studies defined META 2 as a 48 kDa protein localized in vacuoles in the cytoplasm and in the flagellum of the parasite. One of our approaches to characterize META 2 protein function is the study of *L. amazonensis* overexpressing mutants. As described previously, mutant lines obtained by the integration of META 2, META 2/GFP or GFP coding sequences into the rDNA locus were shown to induce increased size lesions in experimentally infected mice, as compared with the wild type parasite. The unexpected increase in virulence shown by the GFP overexpressor line led us to hypothesize that an altered phenotype could have been determined by the integration events. A second strategy was then used to obtain META 2 overexpressor mutants. Transfectants were obtained with constructs based on the episomal vector pTEX (Kelly et al., *Nucleic Acids Res*, 1992). Higher levels of the META 2 protein in M2/pTEX transfectants were detected by immunoblotting. The evolution of lesions was compared in BALB/c mice infected with lines transfected with the empty vector, with the M2/pTEX construct or without DNA. Disease progression in mice infected with the vector alone was again more severe than that observed with the wild type parasite. On the other hand, M2 overexpressor parasites were able to induce lesions even bigger than the ones determined by the line transfected with the empty vector. The results obtained with the control transfected parasites were unexpected and suggest that the insertion of foreign DNA in *Leishmania* may cause changes in phenotype.

## BM014 - ALTERATIONS IN TRAFFIC AND PRO-CRUZIPAIN MATURATION IN *Trypanosoma cruzi* OVEREXPRESSING CHAGASIN

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Chagasin, a protein recently characterized in *Trypanosoma cruzi*, is the prototype of a new family of inhibitors of papain-like cysteine proteases. These inhibitors were also identified

in other parasitic protozoa, as well as in *Pseudomonas aeruginosa*. In *T.cruzi*, we have recently shown that chagasin is able to interact with cruzipain, the major cysteine protease of this parasite, forming tight binding complexes and regulating the activity of the enzyme. *T.cruzi* epimastigotes engineered to express 2-4 fold higher levels of chagasin presented a significant reduction on the soluble cruzipain activity but not on enzyme turn-over. In addition, these parasites displayed lower capability to differentiate into trypomastigotes and increased resistance to a trypanocidal synthetic cysteine protease inhibitor, as compared to the wild-type. Furthermore, tissue culture trypomastigotes were less infective than wild type in vitro, due to lower membrane-associated cysteine protease activity. Here, we demonstrate that increased chagasin expression interferes with the intracellular traffic and maturation of cruzipain zymogens (pro-cruzipain), which we show to be present at the Golgi apparatus of epimastigotes. The reduction on the cruzipain activity in these parasites leads to an accumulation of pro-cruzipain and in increased secretion of these precursors to the flagellar pocket. The uptake of transferrin-FITC and the subsequent recycling of the fluorofore, but not the internalization of BSA-FITC, were compromised in chagasin overexpressing epimastigotes, suggesting an alteration of receptor-mediated endocytosis. In agreement with that, sub-cellular fractionation followed by biochemical and ultra-structural characterization of the endolysosomal compartments indicated alterations in the density of the microssomes of the transfected parasites, as well as, an accumulation of chagasin in the sub-cellular fraction containing Golgi apparatus. Taken together, these results indicate that chagasin contributes to the control of pro-cruzipain activation and sorting, ultimately influencing the traffic in the secretory compartments.

## BM015 - Molecular characterization of free-living kinetoplastid protozoan from the Furnas reservoir (MG, Brasil).

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Free-living protozoan species have a large distribution and play an important role in aquatic microecosystems as they take part in a complex food web including all trofic levels, thus collaborating for nutrient recycling. The Furnas reservoir is located in the south of Minas Gerais, Brasil and plays a very significant role as a font of economical interest for agriculture, human recreation and health. Water samples were collected using plankton nets in different points of the lake. Large numbers of isolated organisms were cultivated using 997 media (ATCC) supplemented with bacteria from the water samples. Two of the isolates (sp1 and sp2) were used for total DNA extraction. Those DNA when digested with appropriated restriction enzymes generated a kDNA-like pattern, compatible with kinetoplastids. In fact a culture of *Bodo* sp, whose identity was made using transmission electron microscopy (Attias et al., 1996) was included in the study as a control sample. DNA of the organisms provided template for PCR SSUrRNA and the medRNA using conserved primers specific for both genes. MedRNA derived primers resulted in a major product of $\tilde{5}$00bp and dimers of $\tilde{1}$000bp for the three species. The SSUrRNA derived primers produced $\tilde{1}$900bp fragments for *Bodo* sp e sp1 and about 1700bp for sp2. A RFLP analysis for the SSU product pointed to patterns compatibles with *Bodo designis* and *Bodo saliens*. Thus, the molecular analysis showed the presence of two different free-living kinetoplastid species isolated in the Furnas reservoir, most probably from the genus *Bodo*. These data also confirmed the *Bodo* identity of the cultivated kinetoplastid, however for the species level classification new approaches should be assigned as the ITS1-5,8S-ITS2 regions, or medRNA/SSU rRNA sequence determination. Supported by: Efoa/Ceufe, FAPEMIG, FINEP.

## BM016 - ELECTROCARDIOGRAPHIC ALTERATIONS IN MUTATED CHICKEN WITH kDNA OF TRYPANOSOMA CRUZI

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Horizontal sequence transfer of mitochondrial kinetoplast DNA (kDNA) of *Trypanosoma cruzi* to chagasic man, rabbits and birds has been demonstrated in the Chagas Disease Multidisciplinary Research Laboratory of the University of Brasilia. The inoculation of trypomastigote forms of protozoan in fertile eggs of hens (1 day) resulted in the transference of the genetic material of the parasite to vertebrate host. Ten days after inoculation, the trypomastigote had been destroyed by the innate immunity. Although its shortness permanence, it was possible to detect the parasite kDNA in the genome of the young chickens born of inoculated eggs. The birds with the kDNA mutation (FO) were crossed to obtain (FI) and this generated descendants (F2). All chicken were kDNA-positive. The mutations profile suggested DNA transference, this was proved by the examination of germinative cells of the kDNA positive birds. The birds FO, F1 and F2 were monthly examinated and submitted the electrocardiographic examinations. The average recorded on electrocardiogram eletric axis variation, suggestive of heart increase, was evaluate by comparing with controls, kDNA-negative birds. The electrocardiogram examination consisted of standards recording of frontal plain derivations. The animals in dorsal decubitus, with pectoral restriction, were examinated without anesthesia. The recordings of cardiac frequency and arrhythmias were analysed. It was observed that the mutated chicken, presented consistent shift of the electric axis to top and right (third and fourth) quadrants. The statistical analyses will be presented and argued. In summary, the kDNA mutated chickens have shown progressive shift of the eletric axis to top-right posetions, consistent with increasing heart size (cardiomegaly). This finding has been confirmed

in necropsies of birds that died with cardiac insufficiency.

### BM017 - Cloning and characterization of DNA Topoisomerases genes from the endosymbiont of *Crithidia deanei*

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DNA topoisomerases are enzymes that participate not only at the replication and transcription processes but are also involved in recombination and chromosome segregation. The replication and transcription are processes where the enzymatic machinery generates both positive and negative super coiling of DNA by the unwinding of the two strands at the replication and transcription fork. Topoisomerases are therefore required to rapidly relax the accumulated positive and negative super coils. These enzymes compose a heterogeneous class of molecules that can be classified in type I and type II. For type I enzymes, the DNA strands are transiently broken one at a time; for type II enzymes, a pair of strands in a DNA double helix are transiently broken. Some trypanosomatids present an obligate symbiotic bacterium in their cytoplasm, which divides synchronously with the host cell. The mechanism of co-evolution of the symbiotic bacteria within the host protozoon is of great interest because of its relationship with the origin of organelles as mitochondria and chloroplasts. In the present study, we investigated the presence of topoisomerases in the endosymbiont of *Crithidia deanei*. For this purpose, the genomic DNA used in molecular assays was obtained from isolated symbionts after cell fractioning. This DNA was used to construct a shotgun library and through the sequencing of this library the gene sequences of DNA gyrase subunit A, DNA gyrase subunit B and Topoisomerase III were obtained. These sequences show high similarities with those described in bacteria from *Bordetella* genus. Based on the obtained sequences, primers were designed to amplify the targets through PCR. The amplified targets were cloned and expressed in *E.coli* and the recombinant products were subsequently purified to produce polyclonal antibodies and to carry on functional assays in vitro. The genes coding for ParC and ParE subunits from Topoisomerase IV are also being investigated.

### BM018 - An expanded family of adenylate kinases in the protozoan parasite *Trypanosoma cruzi*.

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Adenylate kinases supply energy routes in cellular energetic homeostasis. In this work we identified and characterized the adenylate kinase activity in extracts from the flagellated parasite *Trypanosoma cruzi*. Adenylate kinase specific activity increases continuously during the epimastigote growth curve and is down regulated when other phosphotransferase, arginine kinase, is overexpressed. Two adenylate kinase isoforms named TzAdK1 and TzAdK2 were cloned and functionally expressed in *E. coli*. Based on the localization data from *T. brucei* orthologs, TzAdK1 and TzAdK2 are flagellar and mitochondrial isoforms, respectively. We have also cloned and partially characterized four additional adenylate kinase isoforms with a predicted subcellular localization in flagellum, mitochondria, glycosome and cytosol. Surprisingly, an unusual adenlate kinase sequence similar to the nuclear AdK6 (human) was detected in the *T. cruzi* genome. This work reports the presence of a large number of adenylate kinase isoforms suggesting a coordinated regulation of phosphotransferase-mediated ATP regenerating pathways in the unicellular parasite *Trypanosoma cruzi*.

### BM019 - SSU processome proteins are co-regulated during metacyclogenesis of *Trypanosoma cruzi*.

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*T. cruzi* , the causative agent of Chagas disease, presents peculiar mechanisms of gene expression control when comparing to other organisms. Among them, differential rRNA processing appears to be a mechanism of gene expression regulation. During metacyclogenesis the nucleolus disappears accompanied by $\tilde{1}0$ fold decay of the RNA polymerase I activity and a drastic reduction in the levels of gene expression in the metacyclic trypomastigotes forms. We have previously reported that the expression of the TcImp4, a protein from the 18S rRNA processing (SSU complex) is considerably reduced in these forms. Another SSU complex protein, TcSof1, also presents a great reduction in its expression level in metacyclic tripomastigotes forms. These data suggest a stage-specific regulation of the rRNA processing pathway. In yeast the SSU complex involves about 40 proteins, where only seven are considered specific to this complex (including the Imp4 and Sof1). Orthologues of the other five SSU specific proteins could be identified in the data base of *T. cruzi* , and were cloned in expression vectors for production of specific antibodies and analysis of their expression during the life cycle of the parasite. In addition we have started the analysis for identification of possible common targets of regulation in the untranslated regions (UTRs) of these 7 genes, since elements present in the 3' -UTR might be involved in gene expression regulation. Accordingly, four shared patterns were selected and analyzed by electrophoretic mobility shift assay (EMSA). We observed the formation of stage-specific complexes in epimastigotes and metacyclic trypomastigotes

forms. We are currently analysing the specificity and kinetic constant of the observed complexes. Cross-linking assays are being used to identify the proteins that participate from these complexes. Financial support: CNPq, PROSUL, PRONEX, Fundação Araucária, FIOCRUZ.

### BM020 - Lm *YPT* overexpression abolishes HTBF-mediated terbinafine resistance in *Leishmania major.*

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The *HTBF* gene of *Leishmania major* is involved in the acquisition of resistance to terbinafine, an inhibitor of Esqualene epoxidase (Marchini et al., 2003. MBP, 131: 77-81). *HTBF* is coded within the H region, a genomic locus that undergoes amplification when the parasite is subjected to drug pressure. The predicted aminoacid sequence of HTBF showed significant homology to the YIP1 protein of Saccharomyces cerevisae. In the yeast, YIP1 participates in vesicle trafficking by interacting with YPT, a Rab/GTPase. One of our hypotheses for the role of HTBF in resistance is based in its possible involvement in the formation and/or redirection of vesicles, improving mechanisms of drug extrusion or membrane repair. In order to investigate the possible functional interaction between HTBF and YPT, the L. major *YPT* gene was amplified by PCR and cloned into vector pXG1 (Ha et al, 1996. MBP, 77: 57-64). After transfection into the parasite, cell lines overexpressing Lm *YPT* showed a higher sensitivity to terbinafine when compared to wild type L. major. The sensitivity to the drug in this cell line showed an inverse correlation to the level of transcripts for LmYPT. Co-transfection of *HTBF* and Lm *YPT*, or transfection of Lm *YPT* in cell lines that overexpress *HTBF* led to the abolition of HTBF-mediated terbinafine resistance. Moreover, co-transfectant cell lines regained its terbinafine resistance phenotype after the loss of the pXG-YPT plasmid. Altogether, these results not only confirmed the participation of HTBF in terbinafine resistance, but also indicated the possible interaction between HTBF and YPT in the parasite cell. Current work is focused in the characterization of the subcellular localization of both proteins and the possible physical interaction between them. Supported by FAPESP, CAPES, CNPq and FAEPA

### BM021 - Identification, molecular cloning, functional characterization and immunolocalization of an octaprenyl pyrophosphate synthase in intraerythrocytic stages of Plasmodium falciparum

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This report is part of the studies on the biosynthesis pathway of isoprenic compounds in the intraerythrocytic stages of Plasmodium falciparum which is a target for the development of antimalarials. Herein, we identified and characterized another probable antimalarial target structure the enzyme prenyltransferase, involved in the elongation of the isoprenic chains to the benzoquinone ring of Coenzima Q. First, the PlasmoDB-predicted gene PfB0130w localized on chromosome 2 with apparent similarity to trans-preniltransferases was amplified by PCR, and afterwards cloned and sequenced. The transcription as well as the presence of the mature polypeptide was confirmed in different intraerythrocytic stages. After production of the recombinant enzyme, we characterized the enzymatic activity of both versions of the enzyme: the recombinant as well as the enzyme from semi-purified Plasmodium falciparum extracts. For both, the products of isoprenoid synthesis reactions were analysed by different means such as RP-TLC, RP-HPLC and mass spectrometry. Additionally, assays using the recombinant form of the enzyme with the terpene nerolidol revealed that the enzyme could be competitively inhibited. Finally, immunolocalization assays were performed. Supported by FAPESP

### BM022 - Comparative analysis of satellite DNA sequences in *Trypanosoma cruzi* putative hybrid strains

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Approximately 10% of the Trypanosoma cruzi genome is formed by a satellite DNA of 195-bp repeats. Recently, we described that satellite DNA is organized in $30 \pm 10$ kb clusters in some, but not all chromosomes of three *T. cruzi* strains (Elias et al., MBP. 129, 1-9, 2003). Later, the satellite DNA of six representative strains was sequenced and used for phylogenetic inference. The results show that CL Brener contains satellite repeats from *T. cruzi* I and *T. cruzi* II strains (Elias et al., MBP 140, 221-227, 2005). The presence of type I and type II sequences extends previous propositions that genetic exchange between the two major *T. cruzi* lineages has occurred in CL Brener. These studies also demonstrated the usefulness of satellite DNA for the analysis of the generation *T. cruzi* diversity. In the present study, we determined the nucleotide sequence of the satellite DNA of NR cl3, B147 and 115 human strains, which were typed as belonging to the rDNA group 1/2 (Souto et al., MBP 83, 141-152, 1996). For this purpose, genomic DNA was digested with SacI, the 195-bp fragments were cloned in pBluescript at SacI site and their sequences determined. These sequences were used as input for phylogenetic inference using Cluster analysis (DNASTAR). In this analysis we included 15 satellite sequences each of CL Brener (hybrid), Silvio X10 cl1 (*T. cruzi* I), Y (*T. cruzi* II) and Esmeraldo cl1 (*T. cruzi*

II) strains. The dendrograms showed the distribution of the 195-bp sequences of the rDNA group 1/2 strains in *T. cruzi* I and *T. cruzi* II clusters, confirming their hybrid nature and suggesting that one of the events that originated the present genome of the strains included genetic exchange between the two *T. cruzi* major groups. Additional analyses for phylogenetic inference will be performed. **FAPESP, CNPq and HHMI/USA.**

### BM023 - *Toxoplasma gondii* secreted proteins: Capacity to stimulate the immune response

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Many people in the world are infected with *T. gondii*. In Brazil, the incidence of toxoplasmosis is high. Toxoplasmosis is an asymptomatic infection in most of the cases. However, it presents high mortality and morbidity in congenital infection and in immunocompromised patients. When the parasites enter the host cells, different mechanisms can occur as a response to the parasite cells. The proteins formed in tachyzoites are responsible for many parasite-host interactions, such as immune system evasion and cell invasion. Despite this knowledge, much remains to be studied. Here we analyzed the immunogenic capacity from *T. gondii* proteins released in the infected cell culture supernatant. Tachyzoites from RH strains were used to infect VERO cells with DMEM medium without fetal calf bovine serum. The infected supernatants were removed, filtered, concentrated, dialyzed against PBS, and the proteins concentration was determined. SDS-PAGE analysis showed the presence of a 70 kDa protein. The protein probably was released when parasites penetrated the cell, because it was detected only until 4 hours after infection. ELISA and Immunoblot methods showed that this protein was able to recognize sera from infected but not from normal individuals. Antibodies from immunized mice specifically recognized crude tachyzoites extracts. These preliminary data suggest that the protein could perform an important role in host cell penetration. As the protein can be recognized by the immune system, it could be studied in immunization and diagnosis assays. However, other studies should be carried out so as to provide better knowledge about these points.

### BM024 - Molecular and biochemical characterization of the yeast longevity-assurance (*LAG1*) gene homologs in *Trypanosoma cruzi*

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Sphingolipids are important structural components of eukaryotic membranes and their precursor molecules are major participants in the regulation of cell proliferation and function. The initial bioactive signaling lipids formed during *de novo* sphingolipid biosynthesis includes the long-chain bases (LCBs) dihydrosphingosine (DHS) and sphingosine. LCBs are the substrates required for ceramide synthase to produce ceramide. In the present work, we assayed epimastigotes microsomal membranes with [$^3$H]-DHS, acyl-CoA derivatives, free fatty acids, or an *in situ* acyl-CoA generation system in the absence or presence of free-fatty acid BSA or detergents to identify and characterize the ceramide synthase activity of *Trypanosoma cruzi*. The radiolabelled products were extracted, separated on TLC ($CHCl_3$:MeOH:2N $NH_4OH$;40:10:1,v/v) and visualized after autoradiography. *T. cruzi* ceramide synthase can use acyl-CoA derivatives as substrates, but not free fatty acids. The activity was blocked by Fumonisin B1 (FB1), a known inhibitor of acyl-CoA-dependent ceramide synthase from fungi, plants and mammals. However, contrary to previous observations with yeasts and mammals, FB1 was not toxic to the parasite. In *Saccharomyces cerevisiae*, the essential acyl-CoA-dependent ceramide synthase reaction requires the presence of one of a homologous pair of genes, *LAG1* and *LAC1*. *lag1*Δ*lac1*Δ mutants cannot produce ceramide and exhibit a striking synthetic growth defect. To obtain further molecular evidences about the existence of the ceramide synthase in the parasites, the *T. cruzi* genomic database was searched for putative *LAG1* homologues using BLAST and the yeast Lag1p-motif. Two highly homologous sequences (GeneDB No. Tc00.1047053507395.10 and Tc00.1047053510087.30) were found and the full length of one of the genes was amplified by PCR using Dm28c genomic DNA as template. The amplified 1.2kb fragment was cloned into pCR4-TOPO and the obtained sequence presented all the expected features of *LAG1* homologues from other organisms. The heterologous expression of the full length gene is underway. Support: CNPq, FAPERJ, TWAS, IFS.

### BM025 - Genotypic characterization of *T. gondii* strains isolated from Brazilian patients with cerebral toxoplasmosis and AIDS

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Toxoplasmosis occurs worldwide, and it is one of the most common infections in humans. The primary infection is usually subclinical and the majority of the infected human remains asymptomatic or some patients present mild symptoms. The infection can cause significant morbidity and mortality when the reactivation infection in immunocompromised patients occurs, causing especially encephalitis. Cerebral toxoplasmosis is one of the most common opportunistic neurological infections in AIDS, and it is observed in the later stages of the HIV infection. In Brazil, it is the most common cerebral focal lesion in AIDS patients, and it is the third most frequent AIDS-defining condition. Early studies showed that

the parasite virulence and dissemination observed in mice were correlated with the clonal lineages designated types I, II and III. In Brazil, the genetic diversity of *T. gondii* is almost unknown. The aim of this study was to determine the lineages from DNA samples of patients with AIDS and cerebral toxoplasmosis from Instituto Infectologia Emilio Ribas. *T. gondii* infection was confirmed as polimerase chain reaction (PCR) of the conserved gene B1. The lineage type was determined by restriction fragment of the amplified *SAG2* gene of *T. gondii* using two nested PCRs amplifying the 5"and 3' ends of the gene. Restriction digestion of 5'-end-amplified products with *Sau3*AI distinguished the type III from type I and II strains. Digestion of the 3'-end-amplified fragments with *Hha*I differentiated type I and III strains. Controls were prepared from RH (tipe I), ME 49 (type II) and VEG (type III) strains. PCR products and the restriction fragments were analyzed by 2% agarose gel eletrophoresis. Our preliminary data showed that most of the samples were genotyped as type I (62%). These data can contribute to molecular epidemiological studies on *T. gondii*.

## BM026 - Cysteine peptidases in the tomato parasite *Phytomonas serpens*: similarities with the major cysteine peptidase of *Trypanosoma cruzi*

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The peptidase profile of *Phytomonas serpens*, a tomato trypanosomatid, was analyzed as well as how different growth conditions influenced its expression by gelatin SDS PAGE and the use of specific proteolytic inhibitors. In this sense, *P. serpens* cells were cultivated in four different media supplemented with fetal bovine serum: brain heart infusion, liver infusion trypticase, yeast extract and Warren. The cells were collected at the exponential and stationary phases of growth. The results showed that cellular associated proteolytic zymograms were very similar when these media were used. However, a clear distinction was observed in the peptidase expression with respect to growth phase. In this context, two major peptidases of 40 and 38 kDa were observed in the exponential phase in all media. Conversely, a markedly peptidase modulation was observed in parasites obtained on the stationary phase, in which several proteolytic activities were detected in the range of 20 to 50 kDa. All these peptidases are cysteine peptidases optimally active at pH 5.5. As previously reported *P. serpens* shares common antigens with the human pathogen *Trypanosoma cruzi*. The peptidases observed in *P. serpens* possessed similar biochemical characteristics with cruzipain, the major cysteine peptidase of *T. cruzi*. In this sense, we have shown through western blotting analysis that anticruzipain polyclonal antibodies recognized two major polypeptides with apparent molecular masses of 40 and 38 kDa in cellular extracts of *P. serpens*. Flow cytometric analysis provided measurements for the relative levels of *P. serpens* cruzipain like molecules in non permeabilized and Triton X 100 permeabilized cells. In addition, *P. serpens* promastigote forms were immunocytochemically labeled with antibodies associated with colloidal gold and analyzed by transmission electron microscopy. The results showed an intense labeling of the cell and flagellar membranes. Gold particles could be also observed in the cytoplasm. Financial support: FUJB, CNPq and FAPERJ.

## BM027 - CHARACTERIZATION OF ISOPRENOID MOIETIES ATTACHED TO THE *PLASMODIUM FALCIPARUM* PROTEINS

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Recently, our group has demonstrated the existence of prenylated proteins in *Plasmodium falciparum* using metabolic labeling with [1-(n)-3H] farnesyl pyrophosphate ([3H]FPP) and [1-(n)-3H] geranylgeranyl pyrophosphate ([3H]GGPP). [3H]GGPP-labeled proteins with molecular mass of approximately 8-10 KDa, 21-24 kDa and 200 kDa appeared in ring, trophozoite and schizont stages. When parasites were incubated with [3H]FPP, a similar pattern was detected but an additional 50 kDa protein was labeled in all intraerythrocytic stages. In order to elucidate the identity of the isoprenyl side chain of the different prenylated proteins, parasites were labeled with [3H]GGPP and [3H]FPP and proteins were separated in SDS-PAGE, excised from the gel and isoprenyl groups attached to these proteins were extracted and characterized using GC-MS and ESI-MS. Our results reveal a farnesyl group attached to proteins with molecular weight of approximately 200, 50 and 21-24 kDa in ring stage. In the proteins of 8-10 kDa a geranylgeranyl group was detected. In trophozoites, a farnesyl group was detected in the 50 kDa proteins and farnesyl and geranylgeranyl groups were detected in the 21-24 kDa and 8-10 kDa proteins. In schizonts, farnesyl groups were detected in the 200 and 50 kDa proteins. In the 21-24 kDa proteins a geranylgeranyl group was detected and in the 8-10 kDa proteins a farnesyl and geranylgeranyl groups were detected. We also detected the presence of an unidentified group with the same fragmentation spectrum of isoprenoid molecules attached to a 50 kDa proteins in the trophozoite stage and in the 21-24 kDa proteins in ring stages. In the 21-24 kDa proteins of trophozoite and schizont a dolichyl group of 11 isoprene units was identified. This is the first direct characterization of a dolichyl group attached to proteins and the first evidence of differences in prenylated proteins between the tree intraerythrocytic stages of *P. falciparum*. Support: FAPESP.

## BM028 - Repression of Connective Tissue Growth Factor by a Soluble Trypansoma cruzi Factor

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*Trypanosoma cruzi* infection of human foreskin fibroblasts results in specific repression of a group of related genes, including the fibrogenic cytokine Connective Tissue Growth Factor (CTGF). CTGF plays a central role in regulating extracellular matrix protein expression and has been identified as a promising therapeutic target for the treatment of fibrotic disorders. We have shown by quantitative real time PCR that *T. cruzi* causes a 3 to 5 fold reduction in the basal level of CTGF mRNA where the repression is both rapid (less than 2h post-infection) and sustained (greater than 48h post-infection). Interestingly, similar repression of CTGF is seen when fibroblasts are treated with parasite-conditioned medium (PCM), which contains a complex mixture of secreted/shed trypomastigote molecules. In addition to its ability to repress basal CTGF levels, PCM antagonizes several CTGF inducing agents that use disparate signaling pathways including TGF-beta, LPA, and endothelin-1. We have determined that the factor responsible for CTGF repression has a molecular mass of greater than 250 kDa by size exclusion chromatography. The factor is heat labile and trypsinization shifts the apparent mass of the activity to between 30 and 50 kDa, demonstrating that there is a protein component. We have explored the physical characteristics of the factor and have produced a highly enriched fraction by combining size exclusion and anion exchange chromatography. Currently, we are identifying proteins in the active fraction by mass spectrometry. Preliminary evidence suggests that the active factor does not affect CTGF mRNA stability. We are examining transcription initiation and transcription factor binding to the CTGF promoter. Overall, our results reveal a novel high molecular weight activity released by *T. cruzi* trypomastigotes capable of targeting mammalian CTGF expression.

## BM029 - Molecular characterization of *Rhodnius prolixus ultraspiracle* (*USP*): differential expression induced by terpenoids and retinoids

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Many of highly prevalent diseases in the world such as malaria, dengue fever and Chagas disease are transmited by hematophagous insects. One of the main strategies developed to reduce the spread of these diseases is based on the control of vector population through the use of insecticides. Several juvenile hormone (JH) analogs have already been synthesized aiming their use as insecticides since JH regulates many events in insects such as embryogenesis, moult and immune response. These effects are mediated by a class of nuclear receptors named retinoic acid receptors, which ultimately control the expression of target genes. In insects, homologues of retinoic acid receptors are known as ultraspiracle (USP) and have been already cloned and characterized in some insects such as *Drosophila, Locusta* and *Aedes*. Preliminary data from our group indicate that putative USP ligands, such as JH and 9-cis retinoic acid, caused an increase in immune response and altered morphology upon moult in *Rhodnius prolixus*. Attempting to characterize *R. prolixus* USP, we previously cloned the DNA binding domain (DBD) of USP. RT-PCR experiments reveled the presence of USP in salivary glands, fat body and ovaries and a semi-quantitative RT-PCR indicated that this gene is more expressed in ovaries than in fat body and salivary glands. Moreover, expression of USP in fat bodies increased during blood digestion. Injection of 120 pmoles of 9-cis retinoic acid and JH in the hemocoel of *R. prolixus* females led to an increase in USP expression. These results indicate that an authentic USP is coded in *R. prolixus* and that it may be mediating the physiological changes of retinoids and terpenoids injection in this insect. Financial support: CNPq, FUJB, Faperj, TWAS.

## BM030 - Effects of retinoids and terpenois in insect *Rhodnius prolixus*

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Hematophagous insects are important in public health, as they are vectors of highly prevalent diseases. One of the main strategies developed to reduce the cases of these diseases is based on the control of vector population. In insects, many physiological events are regulated by the hormones ecdysone and juvenile hormone (JH). JH levels are well regulated and the pharmacological intervention, through administration of several JH analogs, have already been used as insecticide strategies. These effects are mediated by a nuclear receptor known as *ultraspiracle* (*USP*), which regulates gene expression. Retinoids are molecules derived from retinol, which are similar to JH, and their functions are partially modulated through interaction to their nuclear receptors. Therefore, the main objective of this work is to investigate the effect of retinoids and JH on several physiological parameters in the Chagas disease vector *Rhodnius prolixus*. Injection of 120 pmols of all-trans retinoic acid, 9cis retinoic acid (9cis RA) or all-trans retinol in the hemocoel of adult females of *R. prolixus* caused no changes on oogenesis and embryogenesis. However, injection of 9cis RA in 4th instar nymphs led to remarkable changes in the external morphology upon moult, suggesting that 9cis RA may be acting as a morphogen. Injection of retinoids increased hemolymphatic phenoloxidase activity in adult insects. The *R. prolixus USP* was recently cloned by our group and may be involved in the retinoids/terpenoid physiological changes

since its expression is up-regulated 3h after injection of both 9cis RA or JH. Therefore, putative *USP* ligands, such as retinoids/terpenoids, seems to regulate morphogenetic events and also immune response in this insect. Financial support: CNPq, FUJB, Faperj, TWAS.

## BM031 - Are there integrins in *Trypanosoma cruzi* ?

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Integrins comprise a large family of highly conserved metazoan cell surface receptors, involved in cell-cell and cell-matrix interactions. These glycoproteins are also present in fungi (*Candida*) and protozoa (*Entamoeba*) although its presence in other protozoa, such as *Trypanosoma cruzi*, has never been demonstrated. Here, we report the identification of a 10kb ORF in *T. cruzi* genome database with significant similarity to a human $\beta$ integrin, using BLAST. Previous work has reported that this sequence, initially described as DGF-1 gene, is present in numerous copies in the parasite genome, but its function remains unknown. In the present work we have cloned and sequenced a copy of DGF-1 gene from *T. cruzi* strains G, Y and CL Brener. A small segment of the *T. cruzi* G DGF-1 cloned sequence, corresponding to a N-terminal region from the predicted protein, was expressed in *E. coli*, purified and used to produce mouse antisera. Analysis of Western blots showed that these antisera recognize a peptide of approximately 150kDa in *T. cruzi* Y trypomastigote cell extracts. FACS analysis using live trypomastigotes from *T. cruzi* G and CL demonstrated that this protein is expressed on the parasite surface. Also, immunofluorescence assays suggested that these integrin-like proteins be uniformly distributed on the parasite. Future work will focus on functional assays in order to confirm whether DGF-1 bears *bona fide* integrin binding capabilities.

## BM032 - *Plasmodium falciparum var* gene silencing/activation does not depend on the absolute promoter/intron sequence

Uta Gölnitz *(ICB II)*; Gerhard Wunderlich *(ICB II)*

The *Plasmodium falciparum* genome contains ca. 50 subtelomeric and centromeric *var* genes which encode variant antigens responsible for the pathology of this organism. *Var* gene transcription is controlled by an allelic exclusion mechanism and only one (or a few) *var* gene is transcribed in trophozoites. Previous data indicate that promoter from active or silenced *var* genes constitutively drive expression of episome-based reporter genes suggesting that all *var* promoters are competent for transcription but that the majority of them are silenced, which depends on the presence of the *var* intron. Since all *var* genes contain an intron, it remains puzzling what activates the transcription of a determined *var* gene. We selected parasites for transcription of a centromeric *var* gene (PFD1000c) and constructed plasmids containing the luciferase reporter gene under control of the PFD1000c-*var* promoter with and without the PFD1000c-*var* intron. As expected, the luciferase is expressed in unselected parasites transfected with the plasmid containing only the *var* promoter whereas no luciferase activity is observed in parasites transfected with a construct containing the PFD1000c-*var* promoter and the PFD1000c-*var* intron together. When plasmids were transfected in a selected parasite culture expressing the PFD1000c-*var* gene under the control of the promoter/intron also present in the transfected plasmid, the same transcription pattern was observed, indicating that the endogenous *var* gene is active whereas the episomal construction remained silenced. This indicates that i) mechanisms leading to the epigenetic modification of *var* transcription units to "silent"are independent on the sequence or eventual small RNAs of the promoter/intron site and ii) more factors than the promoter and intron sequences are necessary for the activation of *var* genes. The construction of other expression vectors containing novel elements is under way and data will be presented. Supported by FAPESP

## BM033 - GENERATION AND DETOXIFICATION OF HYDROGEN PEROXIDE IN THE MIDGUT OF *Aedes aegypti*

Oliveira, J.H. *(UFRJ)*; Alvarenga, P.H. *(UFRJ)*; Lara, F.A. *(UFRJ)*; Gandara, A.C.P. *(UFRJ)*; Oliveira, P.L. *(UFRJ)*

*Aedes aegypti* females ingest large amounts of vertebrate blood in a single meal. The hydrolysis of hemoglobin in the midgut lumen releases free heme, a well-known pro-oxidant molecule, which is able to increase the generation of reactive oxygen species. To counteract this situation hematophagous insects posses antioxidant mechanism that permit its survivor. One of these mechanisms is catalase that detoxifies hydrogen peroxide into water and oxygen preventing its interaction with iron, in the so-called Fenton reaction, avoiding the formation of hydroxyl radical, which is highly toxic to cells. Our aims are characterize catalase activity in the midgut during the blood digestion process in the mosquito and measure the amount of hydrogen peroxide present in this tissue in response to blood meal. In order to characterize catalase activity mosquitoes were fed with blood or plasma and dissected different hours after blood meal (ABM). Our results indicate that catalase activity in the midgut epithelium is similar when the mosquitoes are fed with blood or plasma. This activity peaks at 24 hours after the meal and than declines to the basal levels with 48 hours, which corresponds to the end of the digestive process. Concerning the peritrophic matrix we observed that catalase activity present in this structure until 24 hours ABM comes with ingested vertebrate blood. This idea is reinforced by the fact that the in vitro inhibition profile of catalase present in the epithelium is different from that profile present in the peritrophic matrix,

suggesting that they are different enzymes. After characterize catalase activity we determined the amount of hydrogen peroxide present in the midgut epithelium of mosquitoes that fed on blood or sugar. Preliminary data indicated that the mosquitoes that ingested a blood meal produce less hydrogen peroxide than those that fed on sugar.

## BM034 - Characterization of NOD-like genes in Leishmania

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Many of the proteins in the NOD family have been implicated in the intracellular recognition of pathogens and regulation of immune response. In several instances, the function of NOD proteins is dependent on the binding and cleavage of nucleosides through domains known as Walker A and B (Nuñez *et al.*, Annu. Rev. Biochem. 74:355-83, 2005). In the process of characterizing the genomic region of the *Leishmania amazonensis meta 1* gene (FEMS Microbiol Lett, 238(1):213-9, 2004), we identified a 2,03 Kb ORF that presented similarity with the human NOD3 protein. Northern blots indicated that this gene is upregulated in amastigotes of *L. amazonensis*. The complete nucleotide sequence of the *L. amazonensis* ORF was obtained. The predicted translated sequence of 672 amino acids was used to identify orthologous *L. major* and *L. infantum* proteins (*LmjF17.0900* and *LinJ17.0750*, www.genedb.org). The alignment of these sequences revealed high degrees of similarity. The 73-kDa recombinant NOD-like protein was expressed and used to raise specific antibodies that recognized a 45 kDa protein in total extracts of *L. amazonensis* amastigotes but did not react with extracts of *L. major* promastigotes or amastigotes. The search for *LmjF17.0900* orthologues on *T. brucei* and *T. cruzi* translated genome databanks identified predicted proteins of 478 (*Tb05.3C6.260*) and 470 amino acids (*Tc00.1047053511755.40*), respectively, with high degrees of similarity with the *L. amazonensis* protein. The sequence alignment of the 3 peptides showed that *T. brucei* and *T. cruzi* proteins lack the C-terminal region of the NOD-like protein found in *Leishmania* species. A sequence search in the unfinished *L. major* Friedlin genome project data allowed the identification of 5 further possible NOD-related genes. One of these genes (*LmjF 11.0890*) encodes a protein containing Walker A and B domains. The characterization of this gene and of the encoded protein is under way. Supported by CNPq.

## BM035 - Phospholipid biosynthesis in endosymbiot-bearing trypanosomatids: new clues on organelle origin.

Azevedo-Martins AC *(IBCCF)*; Frossard ML *(IBCCF)*; De Souza W *(IBCCF)*; Einicker-Lamas M *(IBCCF)*; Motta MCM *(IBCCF)*

In some trypanosomatids an endosymbiotic bacteria has coevolved with the host protozoan through a mutualist relationship. This is a valuable model to understand the origin of organelles, such as the mitochondrion and the chloroplast. Sterols are not commonly present in bacteria, the major phospholipid being phosphatidylethanolamine (PE), followed by phosphatidylglicerol (PG) and cardiolipin (CL). On the other hand, phosphatidylcholine (PC) is the major phospholipid in eukaryotes, being an essential structural component of cell membranes. There are two alternative pathways for PC biosynthesis; in the Kennedy pathway, free choline is converted to PC via the intermediates cholinephosphate and CDP-choline. This is the predominant pathway in mammalian cells. In the Greenberg pathway, PC is formed by three successive methylations of PE via specific N-methyltransferase. Only the methylation pathway of PC biosynthesis was thought to occur in prokaryotes, however, a novel pathway has been described, involving the enzyme phosphatidylcholine synthase, which condenses choline with CDP-diacylglyceride directly. Recent lipid analyses of purified endosymbionts indicated a complete absence of sterols and a phospholipid composition different from that of mitochondria or whole protozoan, but the lipid composition is similar to that described for eubacteria closely associated with eukaryotes. The goal of this study was to investigate the phospholipid biosynthesis in *Crithidia deanei*, an endosymbiont-bearing trypanosomatid grown in culture medium containing 32P. Our results showed that after 10 h of growth, the normal strain display a two fold higher content of phospholipids, as compared to the aposymbiotic strain. The major phospholipid was PC, followed by PE and PI. Cellular fractioning assays in *C. deanei* indicated that PC is the main phospholipid incorporating 32P. Interestingly, the 32P-PC was 2 fold higher in mitochondrial fractions, when compared to isolated endosymbionts. Ongoing studies address the characterization of lipid metabolic pathways involved in this co-evolutionary process. Supported by CNPq and FAPERJ.

## BM036 - Zymographic analysis of peptidases in non-infected and *Trypanosoma cruzi* -infected mouse embryo hepatocyte cell lineage using different proteinaceous substrates

Nogueira de Melo, AC *(UFRJ)*; d'Avila-Levy, C M *(UFRJ)*; Souto-Padrón, T *(UFRJ)*; Santos, ALS *(UFRJ)*; Branquinha, MH *(UFRJ)*; Meirelles, MNL *(FIOCRUZ)*; Vermelho, AB *(UFRJ)*

*Trypanosoma cruzi*, the causative agent of the American Trypanosomiasis (or Chagas' disease), exploit important cellular processes, for invasion and intracellular survival, including peptidase-dependent extracellular matrix (ECM) remodeling. In the mammalian host, *T. cruzi* is predominantly found infecting myocardial cells, macrophages and cells of the autonomic nervous system. However, previous studies demonstrated that primary cultures of mouse embryo hepatocytes allow not only the invasion by the *T. cruzi* but also

the development of a complete life cycle of the parasite. In this study, cellular and extracellular peptidases from normal and *T. cruzi* infected hepatocyte cell cultures were characterized through the incorporation of different protein substrates (gelatin, casein and hemoglobin) into dodecyl sulfate sodium-polyacrylamide gel electrophoresis (SDS-PAGE). A 100-kDa metallopeptidase activity was detected in the cells and in the culture supernatant fluids of both systems, with ability to degrade exclusively gelatin. Non-infected hepatocytes produced an additional extracellular metallopeptidase of 85-kDa. In the non-infected and in the infected hepatocyte cells, cysteine-peptidase activity migrating in gelatin-SDS-PAGE at 60-kDa presented the broadest specificity, since it was also able to degrade casein and hemoglobin. However, no difference in substrate specificity was observed in the normal and *T. cruzi*-infected hepatocytes. Western blotting analysis using rabbit anti-MMP-9 antibody recognized the 100 and 85 kDa protein bands, indicating that hepatocyte metallopeptidases correspod to the latent and active forms of the matrix metallopeptidase-9, an ECM-degrading enzyme. Notwithstanding, the 100-kDa component was predominantly expressed in non-infected hepatocytes, while the 60-kDa cysteine peptidase activity was augmented in *T. cruzi* infected cells, probably due to the cysteine peptidase synthesized by the parasites, as corroborated by the western-blotting analysis against the cruzipain enzyme. Collectively, these results suggest that peptidases may be involved in the interaction process between *T. cruzi* and hepatocytes in vitro. Supported by: MCT/CNPq, CEPG/UFRJ, FUJB, FAPERJ and FIOCRUZ

## BM037 - Identification and characterization of matrix metallopeptidase-9 and gp63-like activities in *Trypanosoma cruzi*

Nogueira de Melo, AC *(UFRJ)*; d'Avila-Levy, CM *(UFRJ)*; Santos, ALS *(UFRJ)*; Souto-Padrón, T *(UFRJ)*; Branquinha, MH *(UFRJ)*; Meirelles, MNL *(FIOCRUZ)*; Vermelho, AB *(UFRJ)*

Peptidases have been extensively studied to elucidate their roles during the penetration of *T. cruzi* trypomastigotes into host cells, in the nutrition of the parasite at the expense of the host, and in the escape mechanisms of the parasite from the immune system of the host. Moreover, cruzipain, the major cysteine peptidase from *T. cruzi*, has been regarded as promising targets for anti-parasite chemotherapy. Recently, gp63 homologues have also been described in *T. cruzi*, probably play a role in host cell interaction. In this work, the cell-associated and extracellularly released peptidases by *T. cruzi* epimastigotes and amastigotes were analyzed through the incorporation of distinct proteinaceous substrates into SDS-PAGE. The zymogram gels showed that the peptidases extracellularly released by *T. cruzi* epimastigotes displayed two distinct proteolytic classes: cysteine (80 kDa) and metallopeptidase (95 kDa) activities when casein was used as the protein substrate, suggesting that this protein substrate was the most appropriate for extracellular peptidase detection in this evolutive form of the parasite. The analysis of the cell-associated peptidases from *T. cruzi* epimastigotes and amastigotes showed only a 53 kDa cysteine peptidase capable of degrading solely gelatin. The proteolytic profile detected in the amastigote cells were identical with either casein or hemoglobin incorporated into the gels, comprising cysteine peptidases with molecular masses of 150 and 30 kDa, besides the proteolytic activity of 53 kDa. Additionally, some of the *T. cruzi* peptidases reacted with polyclonal antibodies raised against gp63, the major surface metallopeptidase of *Leishmania spp.*, and matrix metallopeptidase-9, an ECM-degrading enzyme for normal matrix remodeling or pathological tissue destruction. This approach could be potentially important in the characterization of distinct proteolytic enzymes, since alterations in methods of detection may help in the search for known enzymes as well as until yet unknown ones. Supported by: MCT/CNPq, CEPG/UFRJ, FUJB, FAPERJ and FIOCRUZ

## BM038 - Differential growth of *T. cruzi* strains in cardiac explants obtained from mice congenic for the H2 complex.

Freitas, JM *(UFMG)*; Lima, R *(CPqGM)*; Soares, M *(CPqGM)*; Santos, RR *(CPqGM)*; Pena, SDJ *(UFMG)*; Chiari, E *(UFMG)*; Macedo, AM *(UFMG)*

The causes of the variability of clinical presentation seen in Chagas disease are not understood, although both parasite and host genetic traits are thought to be involved. We have previously demonstrated that the host background is one of the determinants in the tissue distribution of parasites after artificial double infection with Col1.7G2 clone and JG monoclonal strain in four lineages of mice: three inbred (BALB/c, DBA-2 and C57Blk/6) and one outbreed (Swiss). BALB/c and DBA-2 mice, which have the same MHC haplotype ($h2^d$), also show the same pattern of parasite distribution, while C57Blk/6 mice present a different H2 haplotype ($h2^b$) and also a different tissue distribution of parasites. In order to investigate further a possible influence of H2 haplotypes in tissue distribution of specific types of *T. cruzi*, we infected cardiac explants obtained from mice diverse H2 haplotypes in different genetic backgrounds: C57Blk/sJ ($h2^d$) - C57Blk/6 ($h2^b$) versus DBA-2 ($h2^d$) - CB10H2 ($h2^b$). Hearts were aseptically removed from mice and cut in 0.5 mm slices. Two or three slices totalizing approximately a 10-$mm^2$ area were infected with 5 x $10^5$ trypomastigotes of Col1.7G2 and JG. Following 24 hours of incubation slices were washed and fresh media added to eliminate parasites that had not been internalized. After 24, 96 and 120 hours tissues slices were rinsed and analyzed by two PCR assays for detection of parasites. We observed a clear predominance of col1.7G2 clone in both mice lineages with $h2^b$ haplotype and a equilibrium between the two parasites strains for mice with $h2^d$ haplotype. Thus, our results indicate that the relative amount of each parasite strain is strongly linked to H2 haplotype regardless of the genetic background. These results have important implications for the pathogenesis of Chagas disease in humans.

Financial support: CNPq, FAPEMIG and WHO.

### BM039 - Glycoconjugates from *Blastocrithidia culicis* and *Crithidia deanei*: influence of the endosymbiont and implication on the insect gut interaction

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*Blastocrithidia culicis* and *Crithidia deanei* are parasitic trypanosomatid protozoa of insects that normally contain intracellular symbiotic bacteria. The protozoa can be rid of their endosymbionts by antibiotic, producing a cured cell line. The glycoconjugate profiles of endosymbiont-harboring and cured strains of *B. culicis* and *C. deanei* were analyzed by Western blotting and flow cytometry analyses using lectins, which recognize specifically sialic acid and mannose-like residues in glycoconjugates. All lectins showed a sugar-inhibited recognition of the parasite extracts. A marked difference on the glycoconjugate composition between the endosymbiont-containing and cured strains of *C. deanei* and *B. culicis* was identified in the present study: the absence of the endosymbiont increased the intensity of the lectins binding on both trypanosomatids. In addition, wild and cured strain-specific glycoconjugates were identified. The role of these surface saccharide residues on the interaction with the insect gut was assessed. The interaction of *B. culicis* (wild strain) with explanted guts from *Aedes aegypti* was inhibited in the presence of mucin (56%), fetuin (62%), sialyllactose (64%) and methyl-D-mannoside (80%); while in *C. deanei* (wild strain) the inhibition was 53, 56, 79 and 34%, respectively. In addition, the pre-treatment of the parasites with sialidase reduced the interaction of *B. culicis* and *C. deanei* in 93 and 90%, respectively. Collectively, these results suggest a possible involvement of sialomolecules and mannose-rich glycoconjugates in the interaction between insect trypanosomatids and the invertebrate host. SUPPORTED BY: MCT/CNPq, CEPG/UFRJ, FAPERJ and FUJB.

### BM040 - Gp63 Metallopeptidase-like Activity in *Phytomonas françai*, *Crithidia deanei* and *C. guilhermei*

Marinho, FA *(UFRJ)*; Santos, LO *(UFRJ)*; Dias, FA *(UFRJ)*; Lopes, AH *(UFRJ)*; Vermelho, AB *(UFRJ)*; Santos, ALS *(UFRJ)*; Branquinha, MH *(UFRJ)* d'Avila-Levy, CM *(UFRJ)*

In previous works, we have purified and characterized a metallopeptidase released by the trypanosomatids *P. françai*, *C. deanei* and *C. guilhermei*. The purified enzymes presented similar biochemical properties to the gp63 metallopeptidase from *Leishmania* spp., which is a well-known virulence factor expressed by these digenetic parasites. The present study aims to determine the relationship of the released metallopeptidases to gp63 and to infer a possible biological function in the vector midgut. Fluorescence microscopy and flow cytometry using anti-gp63 antibodies, raised against gp63 from *L. amazonensis*, demonstrated the presence of gp63-like molecules in the cell-surface of the parasites. Moreover, western blotting analysis using the anti-gp63 antibodies showed the presence of a reactive polypeptide of 63 kDa in the cellular extract, in the spent culture media from the parasites and in the purified enzymes, which demonstrates immunological similarities between these distinct trypanosomatids and indicates that the enzymes previously purified and characterized by our research group are in fact related to the gp63 from *Leishmania*. Binding assays with explanted guts of *Aedes aegypti* previously treated with the purified gp63 and anti-gp63-treated parasites demonstrated that the gp63-like molecules are relevant to the trypanosomatid-vector interaction. In addition, we have demonstrated for the first time that a 40-kDa protein in the insect gut is responsible for specific recognition of the gp63. Collectively, these results indicate that the gp63-like molecules are ubiquitously expressed in trypanosomatids. The gp63-like protein in the studied flagellates has a surface location, is actively secreted by the parasites and somehow improves the adhesive process of these trypanosomatids to the insect gut wall. SUPPORTED BY: MCT/CNPq, CEPG/UFRJ and FAPERJ.

### BM041 - The Inhibition of Magnesium-Dependent Ecto-ATPase Activity of *Trypanosoma brucei brucei* By Ferrous Iron and Heme: Effects on Lipid Peroxidation

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*Trypanosoma brucei brucei* is the causative agent of livestock disease nagana that is related to African sleeping sickness in man. We have previously characterized an $Mg^2 + -$dependent ecto-ATPase activity of *T. brucei brucei* procyclic forms. Ecto-ATPases are integral membrane proteins that catalyze the hydrolysis of extracellular nucleosides di- and tri-phosphate and can be modulated by $Ca^2+$ and/or $Mg^2+$. This enzyme activity can be measured using intact cells. Procyclic vector forms reside mainly in the midgut of the tsetse fly, which feeds exclusively on the blood. Hemoglobin digestion occurs in the midgut resulting in an intense release of free heme. Free heme is a powerful generator of reactive oxygen species that can interact with many biomolecules. Heme also associates with phospholipid bilayers of plasma membrane. Here we showed that 100 $\mu$M ferrous iron or heme inhibited more than 50% of $Mg^2 + -$ dependent ecto-ATPase activity. The inhibition of ecto-ATPase activity with 100 $\mu$M ferrous iron, but not with 100 $\mu$M heme, was prevented by pre-incubation of cells with

catalase. We also demonstrated that another surface protein of this parasite, a magnesium-dependent ecto-phosphatase that was characterized in our laboratory, was inhibited by both ferrous iron 100 $\mu$M (72.0%) and heme 100 $\mu$M (91.5%) and the pre-incubation with catalase partially protected from those effects. Lipid peroxidation was increased by exposing cells to ferrous iron. In contrast, incubation with heme did not enhance lipid peroxidation. Although both $Fe^2+$ and heme have oxidant properties, their effects on ecto-ATPase activity of *Trypanosoma brucei brucei* procyclic forms, may involve distinct mechanisms, but this possibility are now under investigation. Supported by: CNPq and FAPERJ

## BM042 - CHAGAS DISEASE: STUDY OF FIVE CASES OF CONGENITAL TRANSMISSION USING kDNA SIGNATURE, MICROSATELLITES, MITOCHONDRIAL COII TYPING AND rDNA PROFILES

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After the successful control of vector-borne Chagas disease in Brazil, the importance of other modes of infection, such as by congenital transmission, has grown. Although several studies have been done in an attempt to determine the mechanisms involved in *T. cruzi* transmission from mother to offspring, little knowledge has been acquired. We analyzed five paired mother-child cases from two distinct endemic areas: four cases from Minas Gerais and one case from Buenos Aires, Argentina. The DNA obtained from parasites isolated from all individuals were submitted to PCR for the study of kDNA signatures by LSSP-PCR and typing of 24S$\alpha$ rDNA, mitochondrial COII and five microsatellite loci. In all Brazilian cases the *T. cruzi* proved to belong to rDNA group 1 and COII haplotype C, thus corresponding to the *T. cruzi* II major phylogenetic line. The microsatellite profiles demonstrated that the parasite populations isolated from the mother-offspring pairs were genetically identical, confirming the congenital infection. In the Argentinean case, the parasites were analyzed directly in the blood of the mother and her twin children. The parasite populations identified in the daughter and son presented rDNA 1/2 and mitochondrial haplotype B, belonging to a putative *T. cruzi* III major line. Preliminary results obtained one microsatellite locus suggested that the *T. cruzi* population isolated from the mother and her children are policlonal. These results are in agreement with the histotropical-clonal model of Chagas disease, according to which infected humans can harbor several clones of the parasite presenting distinct characteristics as the virulence and capability of colonizing specific tissues. Supported by: FAPEMIG

## BM043 - Comparative studies of two polysome isolation techniques in *Trypanosoma cruzi*.

Mansur, F. C. B. *(IBMP)*; Correa, A. D. *(IBMP)*; Holetz, F. B. *(IBMP)*; Probst, C. M. *(IBMP)*; Pavoni, D. P. *(IBMP)*; Passos, T. *(IBMP)*; Krieger, M. A. *(IBMP - FIOCRUZ)*; Goldenberg, S. *(IBMP - FIOCRUZ)*

Genes are differentially expressed in the several developmental stages of *Trypanosoma cruzi*. We are comparing genes differentially expressed in the course of the metacyclogenesis process using microarray analyses. The differences between stages are much more pronounced when comparing polysomal than total mRNA populations. Brems *et al.* (2005) have compared quantitatively and qualitatively different stages of *T. brucei* by genomic microarray analyses using total and polysomal RNA also. However, it was shown that there were not significant differences between the two populations of RNA when comparing distinct developmental stages. These divergent observations might be due to either differences in protocols used for obtaining polysomes or microarray manufacturing, more than to differences existing between these two close related species. The objective of this work was to compare ours and Brem's protocols for *T. cruzi* polysome isolation. The comparisons were done by polysome sedimentation profiling in sucrose gradients and microarray analyses of the extracted mRNAs. Epimastigotes were processed according to each protocol to obtain polysomes. Comparison of polysomal profiles using sucrose gradient showed more similarities than differences between the procedures. However, some extra peaks were observed when using Brems's protocol in the non-polysomal portion of the profile. Polysomal mRNAs obtained by both protocols are being used in competitive microarray hybridization versus total RNA as reference. Preliminary results indicate that despite the differences between polysomal RNAs obtained from both protocols, they could not explain the divergent results obtained by the two laboratories. Accordingly, polysomal RNAs obtained using Brems's protocol are substantially different from the total RNA population when the comparison is done using our *T. cruzi* microarray, hence suggesting that the major cause of the divergence in the results is due to differences in the microarray manufacturing. Financial support, CNPq, PIBIC-Fiocruz, Fundação Araucária (Pronex).

## BM044 - Characterization of *Leishmania major* genes of unknown function amplified in drug resistant cell lines.

Augusto MJ *(FMRP-USP)*; Tosi LRO *(FMRP-USP)*

The H locus of *Leishmania major* contains at least three genes involved in drug resistance. PGPA (P glycoprotein A) is as ATP-binding cassette that renders the parasite resistant to antimonials possibly by sequestration of metals into vesicles that could be exocytosed through the flagellar pocket.

HTBF (H region associated terbinafine resistance gene) confers resistance through a still unclear mechanism. HTBF has a significant homology to the yeast protein YIP1, involved in vesicle docking and trafficking. The possible synergistic action of PGPA and HTBF is currently being investigated in our laboratory. Also, PTR1 (pteridine reductase 1) confers resistance to methotrexate and has been shown to limit pathogenesis within the mammalian host. This 45 Kb region also codes for at least 6 genes of unknown function. The aim of our work was to study the function of two unknown genes coded within the H region of *L. major* and investigate their involvement in drug resistance. In order to inactivate LmORF4, which is located 8 Kbs downstream of PTR1, the parasite was transfected with a DNA fragment containing the ORF4 interrupted by the insertion of a specialized transposable element (/NEO*SAT). This gene disruption reagent was easily generated by the mos1 mariner *in vitro* transposition system(Augusto et al. 2004. Exp.Parasitol., 108: 109-113). The disruption of the first ORF4 allele was confirmed by Southern analysis of digested genomic DNA and PFGE-separated chromosomes.The ORF4+/- cell line is currently being used to generate the null mutant for this locus.The heterozygous cell line was also subjected to protocols that elicit H region amplification and is currently being tested for resistance to various drugs, such as, methotrexate, antimonials and terbinafine. Supported by FAPESP and CNPq.

## BM045 - Embryonic cuticle of *Rhodnius prolixus*: morphological and biochemical characterization

Ferreira, PSS *(UFRJ)*; Bouts, DMD *(UFRJ)*; Ramos, IB *(UFRJ)*; Attias, M *(UFRJ)*; Moreira, MF *(UFRJ)*; Atella, GC *(UFRJ)*; Masuda, H *(UFRJ)*

The hematophagous insects are vectors of human diseases and the accumulation of knowledge, on the reproduction and development, of these vectors are essential for planning new technologies to make possible the control of their populations. In *Rhodnius prolixus*, during hatching the bug ingest the amnion and that process induce the abdominal distension and production of juvenile hormone. During the process of hatching molt also occurs. Following hatching an embryonic cuticle can be found located associated with the egg shell. In this work we describe the structure and characterize it from biochemical point of view. The morphological structures, characteristic of the cuticle, are the plates or sclerites. Under the electron microscopy the cuticle reveals a variety of morphological structures such as corrugated or smooth plates or sclerites and absence of cells. Infrared analysis, revealed the presence of N-acetil-D-glucosamine residues, characteristic of chitin. Besides that, HPLC analysis, following acid hydrolysis, also revealed the presence of N-acetil-D-glucosamine residues. Lipid analysis of the embryonic cuticle showed the presence of the neutral lipids (fatty acids, mono and triacylglycerol, cholesterol and cholesterol esters) and also phospholipids (lysophosphatidylcholine, phosphatidylcholine, phosphatidylinositol, phosphatidyl ethanolamine and phosphatidic acid). The lipids were extracted in solution containing methanol: chloroform: water (2:1:1). The protein profile of the embryonic cuticle is still being investigated. The proteins were extracted in a buffer containing 8M urea, 0,03M DTT and 0,36M Tris-HCL pH=8,4 and analysed by SDS-PAGE. It shows proteins, ranging from 20 kDa to 210 kDa. Due to the fact that the embryonic cuticle is secreted during the last stage of embryonic development, the presence of molecules with antimicrobial activity was expected to occur. The presence of antifungal activity associated with the cuticle was detected, against the fungi *Aspergillus niger*. The components responsibles for this activity is still unknown and it is now under investigation.

## BM046 - Characterization of mitochondrial oxygen consumption in the mosquito *Aedes aegypti*

Sales-Gonçalves, R.L. *(UFRJ)*; Oliveira, MF *(UFRJ)*; Galina, A *(UFRJ)*; Oliveira, PL *(UFRJ)*

The mosquito *Aedes aegypti* is a dipteran and is the vector of dengue fever virus (DV). This virus is responsible for an endemic disease that affects many people in Brazil. Understanding the mosquito metabolism may shed a light not only on its physiology but also to the interaction between DV and the mosquito. During flight, *A. aegypti* requires energy that is obtained by the ATP synthesized by oxidative phosphorylation in mitochondria. Little is known about mitochondrial physiology in insects, especially in *A. aegypti*. Therefore, in this work we investigated the respiratory functions of mitochondria isolated from sugar-fed adult *A. aegypti*. Using complex I substrates (pyruvate-proline) the state 3 respiration was 62,72 nmoles $O^2$/mg ptn/min, whereas the state 4 respiration, induced by oligomycin, was 11,23 nmoles $O^2$/mg ptn/min. Respiratory rates using complex II substrates was 37,05 nmoles $O^2$/mg ptn/min in state 3 and 36,89 nmoles $O^2$/mg ptn/min in state 4, for glycerol 3- phosphate (G3P), and 51,68 nmoles $O^2$/mg ptn/min and 33,14 nmoles $O^2$/mg ptn/min, respectively for succinate. Classical electron transport chain inhibtors, such as rotenone, antimicyn A, azide and KCN, blocked the oxygen consumption induced by both complex I and II substrates, whereas the uncoupler FCCP, promoted oxygen consumption. Additionally, western blot analysis of the *A. aegypti* isolated mitochondria, using a monoclonal antibody against mammalian UCP-1 revealed the resence of a putative UCP in this insect. A preliminary search in TIGR genome database of *A. aegypti* showed the presence of two genes with a high homology to mammalian UCPs. Taken together, these results indicate not only that mitochodria in *A. aegypti* is functional and is capable to utlize oxygen as a final acceptor in the electron transport chain, but also that G3P oxidation is not linked to mitochondrial ATP synthesis. Suported by CNPq, TWAS, HHMI,FUJB, FAPERJ.

### BM047 - Expression of the *Trypanosoma cruzi* GTPase TcRho1 in fibroblast NIH-3T3 induces phenotypic alterations.

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Rho proteins are members of the Ras superfamily of small GTPases. In higher eukaryotes these proteins play a critical role in cell movement, phagocytosis, intracellular transport, cell adhesion and maintenance of cell morphology. Other cellular processes under Rho family control are the regulation of activity of the NADPH oxidase complex, progression of the G1 phase of cell cycle, transcription regulation and metastasis induced by different oncogenes. The GTPase TcRho1 is the only member of the Rho family already described in *Trypanosoma cruzi*. The present work intends to investigate the cellular functions of TcRho1. Dominant positive mutants TcRho1-G15V and TcRho1-Q76L, obtained in a previous work by site-directed mutagenesis, were subcloned in pcDNA3.1 vector, transfected in fibroblasts NIH-3T3, selected and assayed for phenotypic modifications. The NIH-3T3 lineages expressing the dominant positive forms of TcRho1-G15V and Q76L displayed decreased levels of migration compared to the control lineage NIH-3T3 pcDNA3.1. This phenotype may reflect distinct cell-substrate adhesion properties expressed by the mutant cell lines. Cell-substrate adhesion assays confirmed this hypothesis since the mutant cell lines presented enhanced substrate-adhesion properties. Moreover, fluorescence microscopy of actin filaments stained with phalloidin revealed that fibroblasts expressing TcRho1-G15V and TcRho1-Q76L mutants exhibited numerous and thick filopods, which corroborates with the cell-substrate adhesion data. Our results suggest that TcRho1 expression in NIH-3T3 cells interferes with the cell-adhesion signaling. Furthermore, previous results revealed that a lineage of *T. cruzi* overexpressing a mutant TcRho1-T20N (negative dominant) exhibited reduced adhesion rate during metacyclogenesis process. In summary, our data suggest that TcRho1 regulates cell-substrate adhesion in NIH-3T3 cells and that this GTPase may regulate this process in *T. cruzi* during metacyclogenesis.

### BM048 - The pattern of major polymorphisms in the Duffy binding protein ligand domain among *Plasmodium vivax* isolates from the Brazilian Amazon area

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In Brazil, *Plasmodium vivax* was responsible for approximately 80% of the 400,000 malaria cases reported in 2003. The Duffy binding protein (DBP) of *P. vivax* is an important antimalaria vaccine candidate because the interaction with its receptor on host erythrocytes is absolutely required for merozoite invasion. The region II hypervariable (DBPII) contains the binding motifs necessary for DBP adherence to the erythrocyte surface receptor. Previous study using Papua New Guinea (PNG) samples has identified the major polymorphic residues which altered immune recognition of DBP and also demonstrated that some of those polymorphic residues occur frequently in association, particularly residues 417, 437 and 503, suggesting that they may compose an important discontinuous epitope in DBP which might be the main target for inhibitory antibodies. Aiming to contribute for current efforts on vaccine development, we analyze here the DBP variability in Brazilian Amazon isolates, identifying polymorphisms in those major residues of DBPII ligand domain which were previously demonstrated to being involved in the immune recognition of the protein. The gene encoding for DBPII was amplified by PCR from whole blood of *P. vivax* acute patients and the fragments were automatically sequenced. To compare the variability of DBPII from distinct geographic areas, we also compiled sequences available from PNG, Colombia and South Korea. It is demonstrated the strong association of both N417/W437 and K417/R437, but none of them could be associated with any amino acid in codon 503. By grouping the residues analyzed we constructed eight partial variant families representing the haplotypes present in Brazilian Amazon isolates. Most of our isolates (72.5%) are grouped in three families, which are unique for South American isolates. The pattern of excessive polymorphisms and the high rate of nonsynonymous polymorphisms in the ligand domain suggest that this allelic variation functions as a mechanism of immune evasion.

### BM049 - Mutations in the Human Mannan Binding Lectin gene and *Leishmania chagasi* infection

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In Brazil visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania chagasi* protozoan. Epidemiological studies in endemic regions show that only a small proportion of infected individuals develop clinic symptoms. Most infections will remain cryptic unless immunological suppression occurs. However, other host susceptibility factors remain unknown. In humans, mannan binding lectin (MBL) is a protein that might play a major role in disease progression. As a serum lectin and a component of innate immunity, it binds to carbohydrates present in cellular membrane outer surface of many pathogens, acting as an opsonin in phagocytosis process and so, enhancing infections caused by intracellular parasites, including *Leishmania sp.*. Low levels of serum MBL are strongly associated with the presence of three independent mutations at exon 1 in human MBL gene. These mutations result in disruption of a collagenous region

of the polypeptide, which hinder assembly of subunits into the functional trimeric structure, rendering them vulnerable to degradation. Furthermore, two additional mutations in MBL gene promoter seem to down regulate transcription rates of MBL gene. Gene sequencing and SNPs analysis were undertaken in a case-control study of a population from Teresina, an endemic VL area in Piauí State, Brazil, in order to correlate the presence of specific mutations at exon 1 and promoter of MBL gene to disease resistance. The genotypes found for the structural mutants only, could not explain resistance to *L. chagasi* infection, however, when associated to promoter genotypes they point to a clear correlation between MBL levels and VL outcome.

## BM050 - Non-coding RNAs from *Leishmania major.*

Nogueira, K. C. *(FMRP-USP)*; Graminha, M. A. S. *(FCF-UNESP)*; Mangini, R. S. *(FMRP-USP)*; Cruz, A. K. *(FMRP-USP)*

Sequencing of the *Leishmania* genome has led to the identification of genes based on the predicted proteins they encode. However, sequences that lack open reading frames and encode RNAs as their final product might be overlooked during the annotation process. These noncoding RNAs (ncRNA), described in many organisms from worm to man, affect a large variety of processes including transcriptional regulation, chromosome replication, RNA processing/modification, mRNA stability and translation. We are currently investigating three putative *Leishmania* ncRNAs genes: *ODD1*, *ODD2* and *ODD3*, whose secondary structure presents hairpin-like motif resembling those found in microRNAs. We generated constructs of each of the *ODD* genes and they were transfected into *Leishmania* to obtain parasites overexpressing each *ODD* gene. Also, to identify putative mRNA targets, sense oligonucleotides corresponding to the hairpin region were used as probes in Northern blot and primers in reverse transcription-polymerase chain reaction (RT-PCR) experiments. Transfectants were recovered, analyzed for phenotypic changes and potential changes of the target RNA levels. Overexpression of *ODD3* had a cytostatic effect on *Leishmania* transfectants and the morphology of promastigotes was altered. Phenotypic changes were not observed for *ODD1* and *ODD2* overexpressors. We are performing further experiments to understand *ODD* genes possible role and their correlation with the expression of their potential mRNA targets. Financial support from: CNPq and FAPESP

## BM051 - IDENTIFICATION OF THE HEAT SHOCK ELEMENT(S) IN THE POST-TRANSCRIPTIONAL REGULATION OF HSP70 GENES OF *Trypanosoma cruzi.*

de Carvalho Rodrigues *(UFRJ)*; Silva R *(UFRJ)*; Rondinelli E *(UFRJ)*; Ürmenyi TP *(UFRJ)*

Kinetoplastid protozoa show unusual mechanisms of RNA processing, such as trans-splicing, polycistronic transcription and RNA editing, and a predominance of regulation of gene expression at the post-transcriptional level. Post-transcriptional control of gene expression have been shown in kinetoplastids, as in other eukaryotes, to be mediated by sequence elements present in untranslated regions (UTRs) of mRNAs and/or intergenic regions. The HSP70 genes of *Trypanosoma cruzi* are organized as 7-10 copies arranged in tandem, and the protein is synthesized at normal temperatures. Upon heat shock, both HSP70 synthesis and mRNA levels are increased in a transcription-independent manner. As a first step to identify the heat shock responsive elements, the HSP70 trans-splicing acceptor and polyadenylation sites were identified. We found two alternatives trans-splicing acceptor sites, and three distinct cleavage/polyadenylation sites. In addition, analysis of several 3'UTR sequences cloned by RT-PCR shows polymorphism of the length of a central TTA repeat region. To understand the role of HSP70 UTRs in its gene expression control, plasmids containing the chloranfenicol acetyltransferase (CAT) reporter gene were constructed. In these constructs the CAT gene is flanked by segments of the HSP70 intergenic region containing either the 5' UTR or 3' UTR and their respective regulatory sequences. Rab7 UTRs containing sequences were used as control plasmids. The reporter genes will be under the control of the 18S ribosomal RNA promoter. Preliminary results suggest that heat-shock responsive elements are present in the 5' and 3' UTRs of the HSP70 mRNA. The CAT mRNA levels resulting from transfection of the plasmid constructs will be determined to assess the contribution of mRNA stability and translation to the CAT enzyme induction. We are also determining the half-life of the endogenous *T. cruzi* HSP70 mRNA under stress and non-stress conditions. Supported by CNPq, FAPERJ and FUJB.

## BM052 - GENE REGULATION OF HSP10 AND HSP60 OF Trypanosoma cruzi

Fernandes, M. *(UFRJ)*; Silva, R. *(UFRJ)*; Urmenyi, T, P. *(UFRJ)*; Rondinelli, E. *(UFRJ)*

HSP60 and HSP10 proteins comprise a multisubunit chaperone machine responsible for helping cellular proteins to reach their stable three-dimensional conformation. Both HSPs are inducible proteins and must be coordinately regulated, and are, therefore, good models for studying kinetoplastid gene regulation. The organization of the *Trypanosoma cruzi* HSP60 and HSP10 genes have been previously characterized in our laboratory, and shown to be present in tandemly repeated copies in two separate *loci*. The gene expression pattern of the HSP60 has been previously characterized. In order to draw a full picture of gene expression of chaperone machine and its possible coordinated regulation, we are further characterizing the gene expression pattern of HSP10. We have shown that the level of HSP10 mRNA, of about 0.5 Kb, does not increase upon heat shock at $37^o$ C and $40^o$ C. However, a smaller mRNA is induced at higher tempera-

tures due to alternative polyadenilation. The elucidation of the pattern of gene expression of the members of this chaperone machine is necessary as a first step in our efforts to investigate the mechanisms of post-transcriptional regulation, whereby trypanosomatids govern the fate of most of their genes. Therefore, to identify the heat shock-responsive elements in the HSP10 and HSP60 mRNAs, we are using CAT reporter gene assays in transiently transfected epimastigotes. Reporter plasmids containing the 5' and 3' UTRs of HSP10 and HSP60 genes are being constructed, since these regions usually contain the regulatory elements involved in control of gene expression. Transient transfections and CAT enzyme assays are currently being performed. Supported by CNPq, FAPERJ, FUJB.

## BM053 - Cell division in the endosymbiotic bacterium of the trypanosomatid Crithidia deanei

Lucía Yim *(IBMP)*; Rosana Gonçalves *(IBMP)*; Cristina Motta *(UFRJ)*; Leonardo Foti *(IBMP)*; Paulo Arauco *(IBMP)*; Christian M. Probst *(IBMP)*; Daniele Y. Sunaga *(IBMP)*; Samuel Goldenberg *(IBMP)*; Marco A. Krieger *(IBMP)*; Stenio P. Fragoso *(IBMP)*

The trypanosomatid *Crithidia deanei* harbors an endosymbiotic bacterium in its cytoplasm, which divides synchronously with the host cell and supplies it with important nutrients. Cell division in prokaryotes has been widely studied in free living organisms, but little is known about the molecular mechanisms of cell division in obligatory intracellular bacteria. Taking into account the prokaryotic origin of eukaryotic organelles such as mitochondria and chloroplasts, the study of endosymbiotic bacteria of relatively recent incorporation by their hosts can be of great help for the study of the evolution of organelles. In bacteria, a multiprotein complex called divisome assembles in the cell center before division and is responsible for the completion of the process. Many proteins have been identified as components of the divisome in *E. coli*, all of them being essential for cell viability. FtsZ, the structural homolog of eukaryotic tubulin is the earliest acting component of the division machinery, forming the so called Z ring in the cell center and is found in bacteria as well as in Archea, chloroplasts and mitochondria of primitive eukaryotes. By using a genomic library of *C. deanei* endosymbiont, we have identified the *ftsZ* gene and determined its chromosomal location into a highly conserved cluster in bacteria, the *dcw* cluster. The *ftsZ* gene was cloned and expressed in *E. coli* and functional and biochemical studies were performed. In addition, the mechanism of regulation of the position of the Z ring was studied in this bacterium by identifying, cloning and expressing the *minCDE* genes, components of the *min* system, which acts avoiding inappropriate division at cell poles. Our results indicate that the trypanosomatid endosymbiont is more closely related to bacteria from the genus *Bordetella*. Financial support from CNPq, Fiocruz.

## BM054 - The conserved gp85/*trans*-sialidase FLY domain binds to the CK18 amino-terminus.

M. R. Fessel *(IQ/USP)*; M. H. Magdesian *(UERFJ)*; R. I. Schumacher *(IQ/USP)*; W. Colli *(IQ/USP)*; M. J. M. Alves *(IQ/USP)*

All members of the *T. cruzi* gp85/*trans*-sialidase glycoproteins contain the FLY domain, a conserved motif with unknown function localized at the carboxyl-terminus. Peptide J, a FLY domain-containing peptide, was shown to be involved in adhesion of trypomastigotes to host cells and, interestingly, enhanced *T. cruzi* infection by 40%. Cytokeratin 18 (CK18) was characterized as its major receptor in epithelial cells (Magdesian *et al.*, JBC 276:19382, 2001). A monoclonal antibody (A5-5) was developed against a peptide previously selected from a phage display library by its strong interaction with peptide J. After sequencing, it was shown that it shares some identity with CK18. A5-5 recognized CK18 by immunoprecipitation and Western blotting. To characterize further the binding of peptide J to CK18, two HisTag-constructions spanning the amino- and carboxyl-terminus of CK18 were prepared. A5-5 recognized the fusion protein corresponding to the amino- but not the carboxyl-portion of CK18. Similarly, binding tests of these proteins to membrane-immobilized peptide J showed similar results, indicating that the amino-terminus of CK18 is the receptor for the FLY-domain containing peptide J. A5-5 recognized in extracts of epithelial cells only one polypeptide with the same molecular mass of CK18, showing the specificity of the antibody. Furthermore, A5-5 inhibited both the trypomastigote invasion of LLC-MK2 cells by 20% and the enhancement of *T. cruzi* infection stimulated by peptide J. The data suggest that the interaction of the FLY domain with the amino portion of CK18 may play a role in the process of trypomastigote invasion. Financial support: FAPESP and CNPq

## BM055 - Population structure of Trypanosoma cruzi defined by two hybridization events

Scott J. Westenberger *(UCLA)*; Carmen Zelaya *(UCLA)*; Helena K. Toma *(UCLA)*; Christian Barnabé *(CEPM)*; David A. Campbell *(UCLA)*; Nancy R. Sturm *(UCLA)*

Trypanosoma cruzi was divided previously into two major lineages, or three zymodemes, and further subdivided into six groups (DTUs) by Brisse, et al. (2000): DTUs I, IIa, IIb, IIc, IId, and IIe. We sought to investigate the evolutionary relationships among these six DTU groups using molecular markers. Here we provide evidence of DTU-specific SNPs in protein coding genes and intergenic regions between single-copy and tandemly-arrayed multicopy genes. In comparative analyses of 20 genes, the hybrid DTUs IId and IIe display homozygosity at four loci and heterozygosity at 16 loci on

at least six different chromosomes. Markers analyzed include the intergenic regions of the tandemly-arrayed, multicopy genes 5S rRNA, Histone 1, Histone 3, Histone 2B, Histone 2A, and Heat Shock Protein 60. SNPs in protein-coding genes for cytoplasmic and mitochondrial Isocitrate Dehydrogenase, TcMSH2 and Glucose Phosphate Isomerase and the intergenic region between the genes tcp17 and tcpgp2 were identified. We find that DTUs IIa and IIc are homozygous hybrids of DTUs I and IIb, while DTUs IId and IIe are heterozygous hybrids of DTUs IIb and IIc. Our conclusion is consistent with the extensive heterozygosity observed in the genome project strain, CL Brener, from DTU IIe. Recombination between parental sequences in hybrid strains has produced new & ≠8220;mosaic & ≠ 8221; genotypes at some loci. Mosaic Histone 2A sequences from DTU IIe strain TU18 were also observed in CL Brener genome project sequences. Bioinformatic analyses of the CL Brener and Esmeraldo (DTU IIb) genome sequence reads from TIGR revealed that two ancestral alleles of the 5S rRNA gene are present in separate arrays in CL Brener; CL Brener chromosome analysis shows that these arrays are on chromosomes of distinct sizes. Overall, four classes of sequence variation were observed for all loci, supporting the distinction of five clades or DTUs of T. cruzi.

## BM056 - *In silico* identification of putative chromosomal rearrangements in *Leishmania* species

Patrícia de Cássia Ruy *(FMRP)*; Romulo Vitor *(FMRP)*; Loislene Brito *(FMRP)*; Ângela Kaysel Cruz *(FMRP)*; Jeronimo Conceição Ruiz *(FMRP)*

Protozoan parasites of the genus *Leishmania* are the causative agents of leishmaniasis, a spectrum of diseases with different clinical manifestations ranging from mild cutaneous lesions to severe fatal visceral form. Leishmaniasis is widely distributed in Tropical and Sub-Tropical areas of the world and one of the major parasitic diseases targeted by the World Health Organization (WHO). The complete genome sequence of *Leishmania* reference strain published in July/2005 is a landmark in our knowledge of the parasite biology and the development of functional genomics will be required to fully explore this new source of information. A potential approach would be the comparative analysis of genome sequence information from other *Leishmania* species. In this study, using information from *Leishmania major*, *Leishmania infantum* and *Leishmania braziliensis* genome sequencing projects, currently underway, we are characterizing sequences involved in chromosomal rearrangements and the level of synteny (conservation of gene order) among these three organisms. We are currently in the process of mapping genome rearrangement events in the three *Leishmania* species and searching for retrotransposon-like elements associated with genomic rearrangement events. Specific programs had been written in PERL to parse, analyze and format the results from similarities searches reports and for the production of graphical representation of putative recombination events. Preliminary results of mapped rearrangements and the genes families involved will be presented.

## BM057 - Localization and expression of housekeeping genes in human non infective Leishmania

Aquino, JG *(UERJ)*; Rocha, RP *(UERJ)*; Tavares, CC *(UERJ)*; Sampaio, MCR *(UERJ)*

*Leishmania* species not involved in human infection were isolated from pets, marsupials, rodents and sloths (Lainson, R. Mem. Inst. Oswaldo Cruz.1997.92(3)377-387). An intriguing fact is that some species infect sylvatic mammals seen as important hosts of pathogenic *Leishmania*. Among these species of Leishmania, described as "enigmatic", the majority belongs to the sub-genus *Leishmania* and only one is placed in *Viannia* sub-genus. *L. enriettii* was the first described (Muniz& Medina, 1948), followed by *L. hertigi* (Herrer, 1971), *L. deanei* (Lainson& Shaw, 1977), *L. aristidesi* (Lainson& Shaw, 1979), *L. equatorensis* (Grimaldi et al, 1992) and *L. forattinii* (Yoshida et al., 1993). The undetected infectivity in man is probably associated to an inadaptability to anthropophilic vectors.Another possibility is that organisms inherent characteristics could hinder the invertebrate host-parasite interaction. The knowledge of basic molecular aspects of these "enigmatic leishmanias"are important to the understanding of their metabolism, stablishing a relationship with the known aspects of the well-studied pathogenic species of the same genus. In this study we have analyzed the poorly-studied *Leishmania* species by analysis of their molecular karyotype and the genomic localization of housekeeping genes and others ones related to the infective capacity. A comparative analysis was performed between the RNAs expressed from the pathogenic species and non-pathogenic ones. Leishmaniasis is considered by the World Health Organization and important disease due to its large incidence in Brazil and other developing countries. Therefore, it is important to study those species that are not infective to man because they were isolated from animals that live close to man and may potentially undergo adaptation to an anthropophilic invertebrate host.

## BM058 - HETEROLOGOUS EXPRESSION AND PURIFICATION OF ACTIVE RECOMBINANT APYRASE (NTPDASE I) FROM *Trypanosoma cruzi*

Bastos MS *(UFOP)*; Santos RF *(UFOP)*; DeMarco R *(USP)*; Bahia MT *(UFOP)*; Verjovski-Almeida S *(USP)* Fietto JLR *(UFOP)*

An ecto-NTP diphosphohydrolase (NTPDase I) activity, insensitive to inhibitors of ATPases and phosphatases, 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* NTPDase I we performed the 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, thus suggesting that NTPDase I could be produced as a soluble exported protein. Using this information we design a strategy to express the soluble NTPDase I. Full-lenght NTPDase I cloned in pGEM vector was used as template to amplify a 1700 bp DNA fragment that was transferred to pET21b vector (that codes for Hexa-HIS at the carboxy terminal of the recombinant fusion protein). 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 using Ni-NTA-agarose and showed specific activity for ATP hydrolysis between 2-17 nmols/mg protein.h. We concluded that the rNTPDase I was produced in an active form. We are now evaluating rNTPDase I additional biochemical parameters (substrate specificity, cation dependence, activators and inhibitors). Supported by: UFOP, FAPEMIG, CNPq.

### BM059 - Analysis of protein expression of *Trypanosoma cruzi* Zimodeme III strains

Kikuchi S.A. *(Fiocruz)*; Sodré C.L. *(Fiocruz)*; Lima L.M. *(Fiocruz)*; Fernandes O. *(Fiocruz)*

The protozoan *Trypanosoma cruzi* is the agent of Chagas disease, one of the most important public health problems throughout Latin America. Studies based on enzyme electrophoresis profile clustered *T.cruzi* isolates into 3 zymodemes (Z1, Z2 and Z3). Z1 and Z3 are related to the sylvatic cycle and Z2 to the domestic cycle of the parasite. More recently, diverse molecular techniques showed a dimorphism among the isolates, grouping them into *T.cruzi I* (related to Z1) and *T.cruzi II* (related to Z2). Strains belonging to zymodeme III (Z3) couldnbe unambiguously classified as either *T. cruzi I* or*II*. Ecological and molecular data suggest that Z3 strains are more related to *T. cruzi I* than *T. cruzi II*, but their exact phylogeny is an unresolved issue. An analysis of the protein profile of Z3 strains compared to *T. cruzi I* and *II* (data from our laboratory) could contribute to elucidate this question. This work describes the optimization of protocols used to obtain reproducible extraction and separation of proteins by two dimensional gel electrophoresis. These optimized protocols will be used to analyze the expression of proteins in two different Z3 isolates presenting different genomic segments of the ribosomal 24S$\alpha$ locus. The preliminary results show that the best condition to protein extraction was 3X108 parasites cultured in LIT medium. The cells were lysed by four cycles of alternate freezing-thawing in presence of PBS and protease inhibitor cocktail. The protein extract was precipitated with TCA and then washed with cold acetone. Finally, the pellet was resuspended in buffer containing Urea, CHAPS and DTT. These conditions yield gels suitable for image analysis and futher identification of the proteins by mass spectrometry. We hope these optimized protocols can be routinely used to investigate, at the protein expression level, the interaction of *T.cruzi* isolates and their distinct hosts

### BM060 - Molecular Characterization of *Trypanosoma cruzi* U2, U4, U5 and U6 snRNAs

Ambrósio, D. L. *(UNESP)*; Silva, M. T. A. *(UNESP)*; Cicarelli, R. M. B. *(UNESP)*

Some important factors in functioning of the eucariotic cells are the small complexes of RNA and proteins; these particles of ribonucleoproteins (UsnRNPs) have an essential role in the pre-mRNA processing, mainly during splicing. UsnRNP presents a common protein core associates between itself and with the snRNA, called Sm proteins, and specific proteins of each snRNP. Even though they are well defined in mammals, snRNPs are still not characterized in certain Trypanosomatids, as well, *Trypanosoma cruzi*, the causative agent of Chagas' disease. So, this work proposed the molecular characterization of the snRNAs (U2, U4, U5 and U6), by PCR and RT-PCR with *T. cruzi* epimastigote forms (Y strain). These amplified sequences were cloned, sequenced and compared among the Trypanosomatids and the multiple alignment (GeneDoc program) presented more than 70% of identity, except for U5 snRNA, which was less conserved. Phylogenetic trees (MEGA program) showed the evolutionary proximity between the *Trypanosoma brucei* and *Trypanosoma cruzi* snRNAs analyzed. The respective secondary structures were predicted (RNA mfold) and also confirmed similarity with *T. brucei* structures. The alignment of *T. cruzi* snRNAs with *Homo sapiens* sequences showed unique regions in U2, U4 and U5 snRNAs in this species, while U6 was strongly conserved. Experiments of Southern blot showed that these snRNAs have only one copy in *T. cruzi* genome. Studies are in progress in our lab to characterize the involvement of these structures in *T. cruzi* trans-splicing reaction and to search any therapeutic and/or autoimmunity response targets. Supported by: CAPES*, FAPESP (04/01630-9) and FUNDUNESP (850/03).

### BM061 - Comparative analysis of the surface glycoprotein *GP82* transcripts of *Trypanosoma cruzi*

Genti, L.G. *(Unifesp)*; Moraes-Barros, R.R *(Unifesp)*; Santos, M.R.M. *(Unifesp)*; Cordero, E.M. *(Unifesp)*; Franco da Silveira, J. *(Unifesp)*

Gene expression in trypanosomatids is essentially regulated at the post-transcriptional level. In *Trypanosoma cruzi*, it has been suggested that mRNA mobilization to polysomes is an alternative mechanism of post-transcriptional gene expression regulation. Therefore, we are investigating the

mechanisms involved in *GP82* gene expression, a developmentally regulated 82-kDa surface glycoprotein expressed by metacyclic trypomastigotes implicated in host cell invasion. We previously determined the levels of *GP82* transcripts in polysomal mRNA fraction of metacyclic trypomastigotes and epimastigotes by real-time PCR. We found that *GP82* gene is 128-fold more transcribed in metacyclic trypomastigotes than in epimastigotes. Here we present further characterization of *GP82* transcripts isolated from the polysomal fraction of metacyclic trypomastigotes and epimastigotes. We sequenced RT-PCR products (500 bp long) amplified with specific primers derived from the region encoding the carboxy-terminal domain of GP82. Analysis of 72 clones (37 from metacyclic trypomastigotes and 35 from epimastigotes) showed that this region is well conserved. We have also compared these sequences with those from 13 clones selected from a *T. cruzi* metacyclic trypomastigotes cDNA library (G strain) by screening either with *GP82* gene probe or with polysomal $^{3}$2P-cDNA from metacyclic trypomastigotes and epimastigotes. There are no significant differences at amino acid level between total and polysomal transcripts. Taken together, our data suggest that the carboxy-terminal domain of GP82 is conserved among the members of the family expressed in the different developmental stages. Besides, there is no apparent difference between total and polysomal transcripts, suggesting that other post-transcriptional mechanisms should be involved in *GP82* gene expression. As it has been proposed for several genes in *T. cruzi*, regulatory elements present in the $3^{'}$ UTR (untranslated region) could be responsible for differential gene expression. Thus, our next step is to investigate differences on this region. Supported by: FAPESP, CNPq, CAPES.

## BM062 - Rational design of selective inhibitors of the *trans*-sialidase from *Trypanosoma cruzi* with potential therapeutic applications in Chagas' disease.

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Studies on the biology of *Trypanosoma cruzi* have allowed the identification of a large family of *trans*-sialidase (TS) proteins. The parasite TS uses host sialic acid to sialylated its surface glycoproteins and in the absence of a suitable acceptor substrate, the TS functions as a sialidase. TS plays a central role in parasite-host cell interaction and modulation of host immunoresponse. In addition, TS is not expressed in mammal cells representing a good target for rational design of new drugs against Chagas' disease. Our goal is to associate computational modelling and nuclear magnetic resonance (NMR) spectroscopy for the rational design and synthesis of compounds able to disrupt TS/host interaction. In this work we identified by computational screening potential inhibitors of TS and assayed the inhibition of sialidase activity. Furthermore, we used NMR spectroscopy to verified inhibitor/TS interactions. The program FlexX docking was used to screen a database of chemical structures available in the LASSBio for steric fit and to predict binding with the active site of the X-ray crystal of the TS from *T. cruzi* (PDB ID: IMS1A). Our results pointed for a family of aryl-sulfonamide derivatives as potential inhibitors. Analysis of the predicted binding models allowed identifying hydrogen bonds between the Arg35, Arg245, Arg314 (NH) and the oxygen atoms of the sulfonamide group. The composts LASSBio 332, 331, 694, 752 presented high percentage of inhibition during the hydrolyze of the 4-methyl-umbelliferyl-N-acetylneuraminic acid (0.2 mM) by the TS (1 mU) with IC_50 de 4.0, 4.0, 4.0 and 5.0 $\mu$M, respectively. The binding of active compounds was further studied by STD-NMR spectroscopy. Information obtained in this work will be used for rational design and synthesis of highly affinity selective TS inhibitors with potential therapeutic applications. Supported: CNPq, TWAS, FAPERJ.

## BM063 - Characterization of *T. brucei* eIF2$\alpha$ kinases

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Little is known regarding translational control in trypanosomatids despite its relevance in regulating gene expression. The phosphorylation of translation initiation factor eIF2$\alpha$ signals for programs of cell recovery from a variety of stresses in eukaryotes. In yeast eIF2$\alpha$ is phosphorylated by protein kinase GCN2, which is activated by amino acid starvation. Mammals have 4 kinases: GCN2, PERK, HRI and PKR. Tb-eIF2$\alpha$ is larger than its known orthologues due to an extension of 117aa at the N-terminus. It contains a threonine in place of the Ser51 that is phosphorylated in all other eukaryotes. We have already shown that this sequence is expressed in *T. brucei* by cDNA sequencing and immunoblot using antibodies raised against recombinant Tb-eIF2$\alpha$. Here, we identified three putative kinases in *T. brucei*, which we named TbeIF2K1, TbeIF2K2 and TbeIF2K3, with regulatory domains that are not similar to any of the other known eIF2$\alpha$ kinases. We show that K2 but not K1 is capable of phosphorylating yeast eIF2$\alpha$. RNA interference (RNAi) assays indicate that the silencing of TbeIF2K 1 mRNA results in parasites with a slow growth phenotype. RNAi assays with the other two kinases are underway. Since the known eIF2$\alpha$ kinases are activated in stress conditions, we are testing several potential stresses in *T. brucei*, both in procyclic and bloodstream forms, for the activation of these kinases. Supported by FAPESP.

### BM064 - Analysis of detergent-resistant membrane (DRM) from *Leishmania (Viannia) braziliensis* promastigotes

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In the membrane bilayer different lipid species are asymmetrically distributed on the leaflets constituting together with specific protein microdomains. Different microdomain functions may be related with their diversity in lipid and protein composition. These microdomains are termed detergent-resistant membranes (DRMs) and are usually enriched in cholesterol and sphingolipids. Recent works have shown that DRMs are present in *Leishmania*. In our lab DRMs in *Leishmania (Viannia) braziliensis* promastigotes were isolated as low density membranes by their non-solubility in Triton X-100 at 4°C and by sucrose density gradient centrifugation. The lipid composition of *L. (V.) braziliensis* promastigote DRMs was analyzed. Lysated parasites were centrifuged at 800 x *g* for 10 min, the supernatant incubated for 30 min with 1% Triton X-100 and fractionated by sucrose density gradient ultracentrifugation (5 to 45% sucrose). Six fractions were collected and numbered from the top to bottom. Phospholipids, cholesterol/ergosterol and glycolipids were analyzed by HPTLC and the different components were quantified by densitometry. About 67% of glycoinositolphospholipids (GIPLs), 70% of inositol phosphorylceramide (IPC) and 67% of cholesterol/ergosterol were found in fractions 3 and 4, corresponding to low density membranes. On the other hand, lipids such as *lyso* phosphatidylinositol, phsophatidylserine and phosphatidylcholine were found exclusively in fractions 5 and 6 (corresponding to soluble components). Lipid molecules of *L. (V.) braziliensis* were analyzed by GC/MS. Fatty acid methyl esters analysis showed that GIPLs and IPC are mainly constitute by saturated fatty acids. These findings suggest that saturated fatty acids are essential to optimize packing of GIPLs and IPC in DRMs. Although the role of DRMs in *Leishmania* pathogenicity is unclear, it is suggestive that these microdomains could be involved in infectivity processes. *Supported by Capes, FAPESP and CNPq*

### BM065 - Saliva-induced changes on Trypanosoma rangeli protein phosphorylation profile.

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Parasite transmission by blood-sucking arthropods relies on their ability to feed on blood. Transmission is then resumed by direct injection of saliva containing parasites or through deposition of contamined feces close to the wound. Salivary gland invasion by protozoan parasites is then a required step for their transmission during feeding. Furthermore saliva imposes several molecular changes on parasite. These changes are induced by salivary gland components on the molecular plasticity of the parasite itself. The ultimate effect of such changes is the acquisition and or improvement of the parasite ability to invade and colonize host cells. Thus saliva-exposed parasites are several times more infective than non-exposed ones. Therefore these unknown mechanisms are central in order to one understand and block parasite transmission upon blood-feeding. In our lab we are interested in mapping changes imposed by saliva on intracellular signaling pathways. We are currently using *Rhodnius prolixus/Trypanosoma rangeli* model. Incubation of parasite with extracts of *R. prolixus* salivary glands induced a decrease on total protein kinase activity. *In vitro* phosphorylation assays showed a major doublet of 55 kDa phosphoproteins. Phosphorylation of these proteins is altered by exposition to saliva. In addition 12 different phosphoproteins were detected by western blotting with anti-phosphoserine antibodies. A discrete suppression of phosphorylation was observed for most of them. *In vitro* phosphorylation coupled to pharmacological screening with protein kinase inhibitors showed an increase on the phosphorylation of the 55 kDa doublet. This effect was remarkable when the following protein kinases were blocked: casein kinase II, $Ca^{2+}$ -calmodulin kinase and MEK/ERK pathway. Altogether these results show for the first time that vector saliva is able to modify parasite's intracellular signaling pathways. The identity of phosphoproteins and the role of their modifications on invasion of host cells we be next investigated. Supported by CNPq, FAPERJ, PADCT.

### BM066 - Comparative study of peptidases between *Leishmania braziliensis* infective and non-infective strains

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Parasites of genus *Leishmania* are transmitted by the bite of sand flies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts. These parasites can promote a spectrum of diseases known as leishmaniasis. The peptidases are enzymes founded in several organisms, since virus until human. They are involved in a large scale of biological reactions, such as protein metabolism, immune response and blood coagulation. These enzymes seem to be involved with a range of vital activities of parasites, as virulence, nutrition and escape mechanisms. In the present work, we have compared the pattern of peptidases in two strains of *Leishmania braziliensis*: an infective and non-infective one. The *L. braziliensis* infective strain (MHOM/BR/2002/EMM-IOC-L2535) was recently isolated from a patient. The *L. braziliensis* non-infective strain (CT-IOC-238-L566) has been kept axenically in culture for several years. The presence of E-64 (cysteine peptidase inhibitor), EGTA and phenanthroline (metallopeptidase inhibitors) drastically inhibited the cell growth of infective strain, while in the non-infective strain these inhibitors had no effect. These inhibitors also pro-

moted a decrease on the association index between *Leishmania* infective strain and macrophage. EGTA, phenantroline and E-64 inhibited 31.05%, 35.40% and 42.57% this process, respectively. After extraction, the peptidases founded in cytoplasmic content were separated in gelatin-SDS-PAGE. The profile presented by infective and non-infective promastigote forms were distinct. The infective parasites presented a pronounced peptidase activity while in non-infective strain this activity was lower. The peptidases ranged from 30 to 60 kDa. These enzymes seem to be related with important process such as cell growth and infectivity. Supported by: CNPq, TWAS (RGA No. 01-110 RG/BIO/LA) and FAPERJ.

## BM067 - Is Na, K -ATPase involved in the invasion of cardiomyocytes by *Trypanosoma cruzi*? Characterization of $\beta$ 3 subunit as "FLY domain"ligand.

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Almost all nucleated cells can be invaded by *T. cruzi*. Our laboratory has showed that peptide J, containing a conserved motif of the gp85/trans-sialidase superfamily (FLY domain), binds to the host epithelial cell and enhances T. cruzi invasion (Magdesian et al., 2001). In order to identify putative molecules from heart myocytes that interact with FLY domain, membrane extracts of rat cardiomyocytes were submitted to affinity chromatography on J-matrix. More than one polypeptide was eluted with 1 M NaCl or 4-8 M urea, what agrees with the hypothesis that peptide J contains an extremely adhesive motif. After SDS-PAGE, the major bands were removed from the gel, digested, and the fragments sequenced after HPLC purification. With special interest to our receptor-ligand recognition study, the $\beta$3 subunit of the $Na^+$,$K^+$-ATPase complex, a 35 kDa surface protein was identified. To further characterize the $\beta$3 subunit as possible ligand of the FLY domain, we amplified the portion of the protein corresponding to the extracellular segment by using rat heart mRNA and RT-PCR and cloning into pRSET vector. After expression and purification of a 30 kDa molecule, the binding of the protein was tested by overlay assay against dots containing peptide J or J-Ala (negative control). A strong response was obtained only with peptide J. Working is in progress to establish the importance of the FLY domain-$\beta$3 interaction in the invasion of cardiomyocyte by *T. cruzi*. Financial support: CNPq, FAPESP

## BM068 - Karyotype analysis of *Trypanosoma rangeli* strains isolated in Brazil and Colombia

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*Trypanosoma rangeli* is a non-pathogenic parasitic protozoa which overlaps its geographical distribution with that of *T. cruzi*. Single or mixed infections in both vertebrate and invertebrate hosts by these two trypanosome species might be expected and reinforces the needs of a correct specific identification. Karyotypic analyses were useful in the genomic characterization and in the isolation of specific sequences for a given organism. The aim of this work is to characterize the molecular karyotype of *T. rangeli* strains isolated in a formerly endemic area for Chagas disease in Minas Gerais State, Brazil (P02, P07, P18, P19, P21) and in several areas from Colombia (S018, S028, S029, S048, Cas 4). Chromosomes *T. rangeli* strains were separated in 1.0% agarose gels by pulsed-field gel electrophoresis under different running conditions. Karyotypic analysis of *T. rangeli* strains isolated demonstrated that chromosomes are size polymorphic, varying from 350kb to 1.9Mb. We have observed approximately 11 chromosomal bands in Colombian strains and 15 chromosomal bands in Brazilian strains. The intensity of ethidium bromide staining varied within each isolate indicating that each chromosomal band can present more than one chromosome. Numerical analysis (DNAPOP) based on the molecular karyotype data revealed that the Colombian strains were more polymorphic than the Brazilian strains, presenting 73% and 90% of shared bands, respectively. Interestingly, one Colombian strain, Cas-4, was grouped with the Brazilian strains. Parasite genomic DNA was digested with EcoRI and BglII and probed with an amplicon representing the LSU-rDNA from *T. rangeli*. Restriction fragments from Colombian strains presented a more intense signal, suggesting a low degree of sequence conservation of locus studied. Taken together, these results suggest differences between the strains isolated in Brazil and Colombia. These differences will be further investigated by the association of new markers to *T. rangeli* genome. Supported by CNPq.

## BM069 - *In vitro* stimulation of *Trypanosoma cruzi*, *T. cruzi*-like and *T. rangeli* growth by human urine

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*In vitro* cultivation of *Trypanosoma spp.* is important for isolation, maintenance and production of parasite forms, which can be used for biological and molecular studies. Human urine is known to stimulate growth of *Leishmania*. The aim of this work is to investigate the effect of human urine on the growth of different *Trypanosoma* species. We used strains of *T. cruzi* I (AQ, Alv, PV), *T. cruzi* II (L1G, L2G) and *T. cruzi* I/II (Mutum), five strains of *T. cruzi*-like parasites isolated from bats (EM001, EM082, EM085, EM120, RM1) and five strains of *T. rangeli* isolated from opossums (P02, P07, P18, P19 and P21). Strains PV of *T. cruzi* and P07 of *T. rangeli* were inoculated in LIT (Liver Infusion Tryptose) medium and LIT supplemented with 1%, 2%, 3% and 5% of sterile human urine (LIT-U). They were counted daily for at least 20 days to determine the optimal concentration

for growth parasites. The maximum density detected for PV strain was 1.8x$10^7$ epimastigotes/mL in LIT and 5.0x10 epimastigotes/mL in LIT-U ($p < 0,05$). The P07 strain reached 2.4x$10^6$ epimastigotes/mL in LIT and of 2.6x$10^7$ epimastigotes/mL in LIT-U ($p < 0,05$). No significant differences in parasite growth were observed when cultured under different concentrations of urine, so we further investigated the effect of 3% human urine on the growth of other parasite strains. Growth of all strains was stimulated, and it was greater ($p < 0,05$) in AQ, Alv, L1G and L2G of *T. cruzi* strains and in P18 and P21 of *T. rangeli*. All *T. cruzi*-like strains presented significantly greater ($p < 0,05$) growth in LIT-U, except RM1, which grew well in LIT. These results show that human urine can be used as a nutritional supplement in the LIT medium to stimulate *in vitro* growth of trypanosomes.

## BM070 - Seeking for a role for casein kinase 2 (CK2) in *Trypanosoma cruzi*

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Chagas disease is the major endemic disease in Latin America. Its etiological agent is *Trypanosoma cruzi* that undergoes complex morphological changes throughout its life cycle in both the insect vector and the vertebrate host. This cell differentiation is highly regulated and includes significant changes in biochemical pathways. Therefore, studies related to enzymes responsible for the phosphorylation and dephosphorylation of proteins present on the external surface of these parasites are extremely important. Casein kinase 2 (CK2) activities have been described on the cell surface and as secreted enzymes of *Leishmania major*, *L. braziliensis*, *L. tropica* and *L. amazonensis*. These enzyme activities seem to be involved with cell growth, morphology and infectivity of the parasites. Here we show CK2 activities in *T. cruzi* (Colombiana strain CTC-IOC 004) present on the surface of this parasite, in the cytoplasmic content and as a secreted form. The addition of dephosphorylated casein promoted an increase of 53% in the secreted CK2 activity of *T. cruzi*, but it had no effeect on the other activities. The addition of CK2 inhibitors, heparin and TBB, completely inhibited the growth of the parasites, while the addition of CK2 activators, spermine, spermidine and putrescine, stimulated their growth, showing the importance of this enzyme for their life cycle. The secreted CK2 showed a specific activity of 1.37 nmoles Pi per mg x min after purification by HPLC. This activity was abrogated by heparin. We have tested the modulation of CK2 secretion by the addition of protein extract from BALB c peritoneal macrophages, BSA, FCS and inactivated human serum. Only protein extracts from macrophages and from human serum were able to promote an enhancement (by 67% and 36%, respectively) on the secreted CK2 activity. Supported by: TWAS (RGA No. 01-110 RG/BIO/LA), CNPq, FAPERJ, CNPq/PIBIC-UERJ and PRONEX (0885).

## BM071 - Members of the *Trypanosoma cruzi* Tc85 family bind to extracellular matrix and cell surface

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It has been previously shown by our laboratory that one of the members of the Tc85 family/gp85/trans-sialidase superfamily - Tc85-11 - binds to laminin and cytokeratin-18 on the host cell. Cloning and sequencing of 30 new members (30-90% identity) confirmed the intense polymorphism among the Tc85 family genes and raised the hypothesis that the family encodes glycoproteins with multi-adhesion properties that help the parasite to overcome the different barriers imposed by the host. To check whether different members of the family show distinct interaction patterns, adhesion of two new recombinant proteins of the Tc85 family, Tc85-12p and Tc85-45p, to both extra cellular matrix (ECM) and LLC-MK2 cells, was studied. Both proteins bind to ECM-immobilized surfaces in a dose-dependent manner with binding, measured at the saturation point of the curve, of $89.51 \pm 5.39\%$ and $54.84 \pm 5.11\%$ to Tc85-12p and Tc85-45p, respectively. No differences in the binding of these proteins to laminin and fibronectin, two well characterized ligands of *T. cruzi*, were observed. Since Tc85-45p, but not Tc85-12p, contains the RGD sequence, frequently involved in the interaction of extra cellular elements with integrins, binding of the these proteins to the surface of LLC-MK2 cells in the presence or absence of synthetic RGD was measured. The assay showed that RGD inhibits 98% the adhesion of Tc85-45p to the cell surface in contrast to 60% inhibition observed with Tc85-12p. The results highlight the differences in putative adhesion motifs and binding capacity of the recombinant proteins to both the host cell and the extra cellular matrix that may be important to the parasite during vertebrate host colonization. Supported by: FAPESP and CNPq

## BM072 - Identification and characterization of P-bodies genes in *Trypanosoma cruzi*.

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In trypanosomatids regulation of gene expression occurs mainly at the post-transcriptional level by mechanisms involving either changes in stability, localization or ribosome occupancy of mRNAs. Whereas a lot of information is available about eukaryotic gene expression at the level of transcription, the knowledge about the coordination of what happens after transcription is poorly understood. The regulation of mRNA turnover plays a significant role in controlling gene expression and recent experiments in yeast and mammalian have defined cytoplasmic foci, referred as processing bodies

(P-bodies), wherein mRNA decay factors are concentrated and where mRNA decay occurs. P-bodies are dependent on mRNA for their formation and include the decapping enzyme (Dcp1/Dcp2), activators of decapping, Dhh1p, Pat1p, Lsm1-7p, Edc3p, 3‘ deadenylase Ccr4, 5‘ - 3‘ exonuclease Xrn1p, and the cap-binding protein eIF4E. In this study, we identified the putative *Trypanosoma cruzi* genes that codify for the proteins probably involved in mRNA decay. The putative *T. cruzi* Lsm4, Lsm7, Lsm8, Dhh1, Xrn1, eIF4E and Ccr4 proteins share over 40% similarities with the yeast and/or human related proteins. The gene encoding Lsm1 has not been found in *T. brucei* and it is believed that Lsm8 fulfills its function in the P-bodies. Lsm 2-8 putative proteins from *T. cruzi* share more than 61% similarities with the *T. brucei* ones. Interestingly, the decapping enzyme Dcp1/Dcp2 was not identified in the genome database of kinetoplastidae probably due to the fact that in trypanosomatids the mRNA cap is structurally differrent. To characterize P-bodies in *T. cruzi* we are currently cloning the identified genes in expression vectors and analyzing their mRNA levels in epimastigotes and stressed epimastigotes. Although some P-bodies proteins seems to be absent in *T. cruzi*, most of the different functions inherent to this structures are covered with the identified genes. Financial support CNPq, Fundação Araucária (Pronex), Fiocruz.

## BM073 - The trypanosomatid *Crithidia deanei* is unable to uptake D-glucose but it actively takes up L-proline.

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*Crithidia deanei* is a monoxenic protozoan which parasites invertebrates during their life cycle, usually an insect host. In these organisms, an endosymbiotic bacterium has maintained a mutualist relantionship with the host protozoan, making this a valuable model to understand the co-evolution of metabolisms. In the present work we studied the transport of glucose and proline in *Crithidia deanei*. No glucose transport was detected when [$^{14}$C-U]-D-glucose or its non metabolizable analogue D-[2-$^{3}$H]-2-deoxy-glucose were used as tracers. Due to the fact that proline is the main energy source in insect vector stages of most trypanosomatids we measured and further characterized the activity of a high affinity L-proline transporter. Results showed a $K_m$ of 0.13 mM and a $V_{max}$ of 2.32 pmoles x min $^{-1}$ per 40 x $10^6$ cells and also revealed a second low affinity system activity. The high affinity proline transporter showed no sensitive to $Na^+$ and $K^+$ but is sensitive to $H^+$, with an optimum pH of 5. Finally, when the proline uptake was measured in the normal and aposymbiotic strains of *C. deanei*, it was shown that this transport was not dependent on the presence of the endosymbiont. Supported by: CNPq, FAPERJ, CAPES, FAPESP Mail: Ariel M. Silber: asilber@iq.usp.br

## BM074 - Characterization of a *Trypanosoma cruzi* gene encoding an antigen involved with cellular trafficking

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In this study we characterize two distinct cDNAs, isolated from a *Trypanosoma cruzi* library screened with sera from chagasic patients that present homology with mammalian mannose lectins. Full length sequences of the cDNAs, denominated TcAGL1 and TcAGL2 (for *Trypanosoma cruzi* Antigen Lectin) were determined and shown to present homology to VIP36 and ERGIC-53, which are mammalian proteins involved with cellular sorting or recycling. Both cDNAs contain a microssatelite element that encodes a poli-Glu region located at the C-end of the protein. PCR amplifications with DNA from different strains of parasite revealed a polymorphism encompassing the microssatelite region of the gene. According to southern blot and sequence analyses using the *T. cruzi* genome database (www.geneDB.org), these cDNAs represent two polymorphic alleles of a single copy gene. Northern blot analysis revealed that TcAGL is constitutively transcribed into a 2.5 kb mRNA, which is slightly more abundant in amastigote than epimastigote and trypomastigote forms. Polyclonal antibodies, obtained from mice immunize with a recombinant GST::pTcAGL2 fusion protein, recognize two isoforms in protein extracts from several strains of the parasite. The antigenicity of pTcAGL was confirmed by ELISA and immunoprecipitation assays using the recombinant protein and sera from chagasic patients. The localization of this antigen in the parasite was also investigated using anti-pTcAGL2 antibodies in immunofluorescence assays as well as by transfecting epimastigote with a vector encoding the protein tagged with GFP $pTcAGL1 :: GFP$. In both experiments, a distribution near the flagellar pocket, co-localized with the kinetoplast and Golgi region, was determined. Presently, we are investigating the involvement of pTcAGL with intracellular trafficking in the parasite, by testing its capacity to interact with mannose residues, and characterizing possible components of early secretory pathway machinery that might be associated with this protein. Support FAPEMIG

## BM075 - CaM Kinase in Cell Signalling mediated by Heme (Fe-protoporphyrin IX) in *Trypanosoma cruzi*

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*Trypanosoma cruzi*, the ethiologic agent of Chagas disease, is transmitted through triatomine vectors during blood-meal on vertebrate host. These hematophagous insects usually ingest

in a single meal about 10 mM heme bound to hemoglobin. We have investigated the role of heme at cell proliferation. *T. cruzi* epimastigotes Dm28c strain were maintained in BHI supplemented with 10% FCS at 28°C without addition of heme for 14 days. Afterwards cells were incubated in the absence or in the presence of differents concentrations of heme. The addition of heme increased significatively the parasite proliferation in a dose-dependent manner. To investigate whether the proliferative effect of heme was determinated by a protein kinase (PK) mediated signaling pathway, we performed western blotting analysis with polyclonal phosphoserine antibody. The results showed a increased phosphorylation on serine residues of a 40 kDa band. To determine which enzymes were envolved in this process, we evaluated the effect of several PK inhibitors. The parasites were incubated in the absence or in presence of 30$\mu$M heme and in the absence or presence of PK inhibitors. Among all inhibitors tested, only KN93 had a signifficative effect at cell proliferation mediated by heme. *T. cruzi* epimastigotes showed a phosphatase activity able to hydrolyze pNPP at pH 7.0, 37°C. Okadaic acid had no effect on this activity. These results are complementary to those of western blotting in which the sample incubated with heme 30 $\mu$M presented a lower intensity of phosphorylation and a higher phosphatase activity. On the other hand, the sample incubated with 1 mM heme showed the opposite effect. In conclusion, heme increases epimastigotes proliferation, and our results suggest that CaM kinase pathway is important for the signaling mediated by heme. We demonstrated that heme-induced parasite proliferation occurs through the modulation protein phosphorylation cascades. Supported by PIBIC/UERJ, FAPERJ, CNPq.

## BM076 - Expression of tubulin gene in Trypanosoma cruzi: a model of autoregulation

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*Trypanosoma cruzi* alpha- and beta-tubulin mRNAs are 3- to 6-fold more abundant in epimastigotes than in trypomastigotes and amastigote forms (Bartholomeu *et al.*, 2002). It has also been shown that the increased abundance of alpha- and beta-tubulin mRNAs found in epimastigotes is due to an increase in their half-lives. By analyzing soluble and cytoskeletal protein fractions of the parasite, we found an inverse correlation between tubulin mRNA and the levels of free alpha- and beta-tubulin subunits which are more abundant in trypomastigotes and amastigotes than in epimastigotes. We investigate a possible autoregulatory mechanism responsible for the differential accumulation of tubulin mRNAs in *T. cruzi* by treating epimastigotes with vinblastine and taxol, drugs that disrupt microtubule dynamics by opposite mechanisms. Vinblastine treatment causes significant morphological alterations in epimastigotes whereas taxol does not alter the parasite morphology. Accordingly, vinblastine causes significant depolymerization of tubulin whereas taxol stabilizes microtubules. Vinblastine, but not taxol, has a specific effect on the levels of alpha- and beta-tubulin mRNAs, causing a 5- to 9-fold reduction in the steady-state levels of both mRNA populations, whereas the levels of other mRNAs, such as *gapdh* and *TCR27*, remain unchanged. The RNA reduction caused by vinblastine treatment is mediated by changes in tubulin mRNA half-lives. In an attempt to identify regulatory elements within tubulin mRNAs, plasmids containing luciferase reporter gene associated with 5'-UTR, 3'-UTR and part of coding sequence of the tubulin genes were constructed and used for transient DNA transfections of epimastigotes. Determination of luciferase activity in transfected parasites cultured in the presence and absence of vinblastine indicates that sequences located within the alpha-tubulin 3'-UTR and coding region may be involved in modulating the stability of these transcripts in response to changes in the dynamics of *T. cruzi* microtubules.

## BM077 - Clathrin in *Trypanosoma cruzi*: *in silico* and experimental identification

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During endocytosis, several receptors [e.g. the low density lipoprotein (LDL) receptor, the transferrin (TR) receptor, and the mannose-6-phosphate receptor] are found located in clathrin coated pits, which exclude other membrane proteins. These structures bud into the cytoplasm as coated vesicles, the coats disassemble, and the vesicles, packed with specific receptors, fuse with other compartments delivering their cargo. Ultra-structural analysis and the identification of protein factors associated with transport vesicles suggest that a similar machinery may operate in trypanosomatid protozoa such as *Leishmania* and *Trypanosoma*. Trypanosomatids speciation occurred three billion years ago, making them a divergent ancestor within the Eukaryota superkingdom with unusual features in gene expression and cell biology mechanisms. These parasites are able to ingest macromolecules through receptor-mediated-endocytosis, but the molecules involved in this process are still poorly known. Bioinformatic techniques were here used for proteins identification in *T. cruzi* genomic data base and allowed us to discriminate a set of genes involved in clathrin coat assembly. Different experimental approaches were employed to study the clathrin protein expression in *T. cruzi* epimastigote forms. Cells were maintained at 28°C in axenic culture medium (LIT with 10% inactivated fetal calf serum). Five-day-old culture parasites were submitted to DNA and protein extraction. The DNA was amplified by PCR (Polymerase chain reaction) with *T. brucei* clathrin primers, resulting in a 350 base pairs amplicon. The protein extract was used in western blot with a monoclonal antibody against *Bos bovis* clathrin, which was also used to immunollabel the cells by flow cytometry analysis. Both analyses evidenced the clathrin expression in epimastigote forms. This is the first time that clathrin expression is demonstrated in *T. cruzi*. Further studies are necessary to understand if coated vesicles occur in endocytic, exocytic or both pathways.

## BM078 - Analysis of the molecular karyotype of clone D11, a single cell-derived clone of the *Trypanosoma cruzi* G strain

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Chromosomal rearrangements are very frequent in protozoan parasites, including *Trypanosoma cruzi*. These events can contribute to the karyotype variability found in these parasites and provide evidence of their genome plasticity. Clone D11 is a single cell-derived clone of the *T. cruzi* G strain obtained by the minimal dilution method. Vero cells were infected with metacyclic trypomastigotes (G strain), and the selected clones were expanded by infecting naive Vero cells. The molecular karyotype of clone D11 differs from that of the parental strain in both number and size of chromosomes. Here we present further characterization of the molecular karyotype of clone D11. Analysis of the distribution of microsatellites (Macedo et al. 2001, Mem Inst Oswaldo Cruz 96, 407) indicated that the G strain is a monoclonal population, and suggested that the clone D11 appeared during the cloning process. PCR amplification of D7 rRNA 24S region showed that both parental strain and clone D11 belong to the *T. cruzi* I. We identified 19 and 21 chromosomal bands in the G strain and clone D11, respectively, by scanning of pulsed-field gels stained with SYBR-Green. We have determined the chromosomal band location of 12 *T. cruzi* genetic markers. We found that the tubulin gene is located in the chromosomal bands of 1.08 and 1.90 Mb of G strain, whereas it was mapped in chromosomal bands of 2.21 and 2.42 Mb in clone D11, suggesting the rearrangement of large chromosomal fragments. Markers TEUF 0002 and TEUF 0080 showed an interesting hybridization profile. In the G strain, they were located in a chromosomal band, while in the clone D11 they were mapped in two chromosomal bands. We are trying to elaborate a mathematical model to estimate the number of chromosomes per band and consequently the total nuclear genome sizes of the parental strain and clone D11.

## BM079 - Intergenic region of Tcp28, the intercalated gene of TcRRMs of Trypanosoma cruzi

Beatriz Brum *(UFRJ)*; Giselle Guimarães Gomes *(UFRJ)*; Turan Peter Urmenyi *(UFRJ)*; Edson Rondinelli *(UFRJ)*; Rosane Silva *(UFRJ)*

The function of Tcp28 is unknown, nevertheless the Tcp28 transcripts accumulate in trypomastigotes cells when compared to epimastigotes and spheromastigotes forms analyzed in northern blot assays. These results suggest that Tcp28 may play a specific function in these different cell stages. Control of gene expression in trypanosomatids usually is exerted posttranscriptionally. We are analyzing the intergenic region of these genes looking for sequences that regulates expression in these stages. Initially we are mapping trans splicing and polyadenylation sites in vivo. We have cloned and sequenced more than twenty 3' UTR of the Tcp28. Apparently there is only one kind of 3'UTR analyzed so far, and a major polyadenylation site. Tcp28 was identified and is located at the TcRRM locus. It is present as a multicopy gene, in at least 8 copies. Tcp28 is intercalated between copies of TcRRM 1 and TcRRM2 (RNA Recognition Motif) genes which are also regulated during stage differentiation. Supported by FAPERJ, CNPQ, CAPES

## BM080 - TcRRM (RNA Recognition Motif) is differentially expressed in Trypanosoma cruzi life cycle

Giselle Guimarães Gomes *(UFRJ)*; Turan Peter Urmenyi *(UFRJ)*; Edson Rondinelli *(UFRJ)*; Rosane Silva *(UFRJ)*

Trypanosomes are a group of eukaryotic organisms with many unusual characteristics in their molecular biology; so the identification and characterization of RNA binding proteins in T. cruzi is particularly relevant as they play key roles in the regulatory mechanisms of gene expression. In this work, we have identified coding sequences for the proteins, named TcRRM1 and TcRRM2, in the EST database generated by the T. cruzi Genomic Initiative. TcRRM1 and TcRRM2 contain two RNA binding domains (RRM) and are very similar to two T. brucei RNA binding proteins previously reported, Tbp34 and Tbp37 and to a not yet annotated ORF in Leishmania major genome project. The T. cruzi RRM genes are organized in a tandem of at least 8 copies, alternating with copies of Tcp28, a gene of unknown function. However TcRRM transcripts accumulation is higher in the spheromastigote stage, while Tcp28 transcripts accumulate more in the trypomastigote stage suggesting developmental regulation. Functional binding assays are being performed in order to verify the specificity of the ligation to ribonucleotides. Supported by FAPERJ, CNPQ, CAPES

## BM081 - Investigating the role of untranslated regions of phosphoglycerate kinase genes in the control of their expression in *Leishmania major*

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The protozoan parasite *Leishmania major* contain genes corresponding to two isoforms of phosphoglycerate kinase (PGK): the PGKB is found in the cytosol and the PGKC in the glycosome. These isoenzymes are simultaneously present in both promastigotes and amastigotes in a ratio of approximately 4/1. Preliminary results suggested a deleterious effect of PGK overexpression in *Leishmania*. In order to address

this question, genomic fragments bearing either *PGKB* or *PGKC* genes were subcloned into pX63Neo and transfected in *Leishmania major*. RNA and protein levels of PGK isoforms analyses indicated that an element present either in the 3 or 5 UTR of *PGKC* might be implicated in regulation of gene expression. To test this hypothesis we constructed four PGK recombinant chimeras: the 5 UTR of one of the PGKs was ligated to the 3 UTR of the other gene and vice versa. They were transfected into *L. major* promastigotes and results obtained suggest that the 3 UTR *PGKC* is involved in the control of transcript levels. Additionally, we introduced the hygromycin phosphotransferase (HPT) gene within *PGKB* and *PGKC* recombinants keeping their flanking regions intact. We confirmed gene insertion in the correct direction and transfected the circular molecules into *L. major*. Transfectants were recovered from plates and are currently under analysis. Furthermore, we are investigating the role of *PGKC* 3 UTR in the genomic context. Linearized versions of the recombinants bearing HPT were transfected in *L. major* to replace the corresponding resident *PGK*. The transfectants HPT RNA and protein levels will be evaluated under no drug pressure. These transfectants will be an important tool to understand the mode of regulation of the investigated locus. Supported by FAPESP and CNPq.

## BM082 - *Leishmania (L.) amazonensis* AP-Endonuclease gene (*lamap*) restores the survival of *E. coli* mutant strains challenged with menadione

Oliveira, M.Y.K. *(UERJ)*; Oliveira , C.A. *(UERJ)*; Bello, A.R. *(UERJ)*

Apurinic or apyrimidinic abasic sites may be generated by oxygen or nitrogen reactive species as well as by alkylating agents. These sites must be repaired to assure the survival of any organism, including the parasitic trypanosomatids that multiply in an environment rich at these molecular species. AP-endonuclease family enzymes actively involved in the repair of some of these lesions have already been described in *Leishmania major* and *Trypanosoma cruzi*. Our preliminary work showed that the *lamap* gene, an AP-endonuclease homologue of *Leishmania (L.) amazonensis* is an enzyme able of complementing *E. coli* mutants deficient in the repair of oxidative (mostly Endonuclease III) and alkylating lesions. In this work, the BW535 *E. coli* mutant strain (*xth-* , *nfo-*, *nth-*) was transfected or not with the pLamap plasmid (*lamap* gene cloned into the TOPO vector) and challenged with menadione sodium bisulfite (MSB) a superoxide-generating agent. The non transfected strain presented minimal growth at 62.5 $\mu$M of MSB displaying hypersensitivity to this drug. On opposition, the transfected ones exhibited a higher survival rate, growing at 10.0 mM of MSB, a concentration 80x higher than that observed for the non transfected ones, although a decrease was observed when compared to the control ones. We have extended the scope of action of the *lamap* gene, showing that its expression increases significantly the survival of *E. coli* deficient strains challenged with MSB. Peroxidation of lipids and DNA damage are intrinsically associated with the mode of action of MSB. It is also well established that trypanothione, a glutathione analog in trypanosomatids display an important role in the antioxidant defence. In this regard the completion of this panel of functional studies carried out momentarily in *E. coli* strains, as *Leishmania* mutants are not yet readily available, might suggest novel responses of these trypanosomatids when submitted to oxidative stress.

## BM083 - Analysis of transcripts from *GP82* gene of *Trypanosoma cruzi* isolated from metacyclic trypomastigotes found in naturally infected triatomines.

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*Trypanosoma cruzi* undergoes a differentiation process prior to become infective to the mammalian hosts. The entire process of differentiation, called metacyclogenesis, occurs in the digestive tract of triatomine vector. Metacyclic trypomastigotes express stage-specific surface glycoproteins such as GP82, GP90, and mucins. In this work, nymphs of *Rhodnius prolixus* were naturally-infected with *T. cruzi* (G strain) after have been fed on infected mouse. After 40 days elapsed from the initial meal, the insects were dissected and its digestive tract isolated. The different sections (stomach, mid-gut and ampulla) were homogenized to release the parasites contained in the intestinal fluid. mRNA was extracted from each aliquot and employed in: a) RT-PCR assays, to clone members of the *GP82* repertoire; b) quantitative RT-PCR in real time-PCR reaction, to determine the *GP82* transcripts levels and compared them with those found in parasites at the early infection. We sequenced the products amplified with specific primers derived from the carboxy-terminal domain of GP82. Analysis of 60 clones (300-500 bp long) showed that this region is well conserved. They did not show any significant difference when compared with those isolated from the metacyclic trypomastigotes grown in axenic cultures. The level of transcription of *GP82* gene in metacyclic trypomastigotes, which are found in the rectal ampulla, is 10.9-fold more than those shown by the parasites collected at the early infection. This increase was coincident with the enrichment in metacyclic forms from $15^th$ to 40 days after infection as determined by Giemsa‘ stain of slides of intestinal content from infected bug. Our results suggest that one member of the *GP82* gene family is preferentially transcribed in the insect vector. But the impossibility to detect anothers variants of *GP82* may be due to the specificity of the primers employed in this analysis. Supported by FAPESP, CNPq, CAPES, BIOLAC/The United Nations University

## BM084 - The 68-amino acid repeats from the immunodominant antigen H49 of *Trypanosoma cruzi* are associated with the calpain-like cysteine peptidase sequences

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*Trypanosoma cruzi* antigen genes have been cloned by screening of expression libraries with sera from chagasic patients or *T. cruzi*-infected animals. We have isolated a DNA fragment, termed clone H49, which encodes tandemly arranged repeats of 68-amino acids. These repeats are conserved among strains and isolates of *T. cruzi*. The native H49 antigen is a large structural protein (greater than 300 kDa) involved in the attachment of the flagellum to cell body of the parasite (Cotrim *et al.* Mol Biochem Parasitol, 1995, 71, 89). We took advantage of the recently completed draft of the *T. cruzi* to identify complete copies of H49 gene. TBLAST search of *T. cruzi* dabases with the 68-amino acids repeat produced 8 alignments that could be considered significant. All of H49 repeats are associated with the calpain-like cysteine peptidase sequences. These proteins show the CysPC-calpain domains IIa and IIb, characteristics of calcium-dependent cytoplasmic cysteine proteinases, papain-like. Of the 53 *T. cruzi* calpain-like sequences deposited in GenBank, eight were found associated to H49 sequences. One of the annotated *T. cruzi* calpain has 4,571 amino acids ($\tilde{5}$20 kDa), and the 68-amino acid repeats are distributed in the central domain of the molecule. The association between H49 and calpain sequences was further confirmed by Southern blot hybridization, PCR amplification and analysis of YAC clones. The possible role of calpain/H49 in the cytoskeletal remodeling processes will be investigated in our laboratory. Supported by FAPESP, CNPq and CAPES.

## BM085 - Characterization of two novel ribonucleoproteic antigens of *Trypanosoma cruzi*

Pais, F.S. *(UFMG)*; DaRocha, W.D. *(UNI-BH)*; Gazzinelli, R.T. *(UFMG)*; Teixeira, S.M. *(UFMG)*

Previously, we described the isolation of several cDNAs by immunoscreening an amastigote cDNA library of *T.cruzi* with sera from chagasic patients. Sequence analysis revealed that ribonucleoprotein and proteins containing repetitive amino acids sequence were the most frequently selected clones. Two cDNAs encoding RNA binding proteins with repetitive amino acid sequences were chosen for further characterization. Sequence analyses of the cDNA denominated TcRBP48, show high homology with two recently characterized *T.cruzi* RNA binding proteins and two *T.brucei* proteins named p34 and p37, with the *T.cruzi* gene encoding a repetitive amino acid sequences in the N-terminal region. The cDNA named TcRpL7a presents homology to eukaryotic L7a ribosomal protein. However, sequence alignments with various L7a proteins indicate that TcRpL7 is the only one containing long extension of repetitive amino acids in its N-terminal region. The repetitive sequences of TcRpL7a and TcRBP48 are also similar. The entire TcRBP48 and TcRpL7a proteins, as well as the N-terminal and C-terminal fragments of TcRpL7a, were cloned and expressed as GST fusion proteins. Purified proteins had their reactivity evaluated in Western blot and ELISA assays with sera from chagasic patients. Results indicated that the reactivity with antibodies from infected individuals occurs exclusively against the repetitive fragment in TcRpL7a. ELISA assays indicated that 70% of a panel of twenty chagasic patients sera reacted with the repetitive region of TcRpL7a and 65% reacted with TcRBP48. Interestingly, we found similar pattern of reaction with the two antigens when each serum sample was compared, suggesting that they may share similar immuno epitopes present in their repetitive regions. In addition, synthetic peptide covering the repetitive region of TcRpL7a has been produced and is being presently used in ELISA assays. Also, mice immunized with the recombinant proteins generated antibodies that are being used to detect the antigens in parasite extracts and in immunolocalization.Support: FAPEMIG

## BM086 - The characterization of Trypanosoma cruzi classes I and II hypothetical proteins involved in the interaction with vertebrate and invertebrate hosts.

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*Trypanosoma cruzi* presents a heteroxenous life cycle including invertebrate and vertebrate hosts. The parasite surface molecules have important role in *T. cruzi* interaction with a great variety of tissues and cell types. The gp85-transsialidades and mucins glycoproteins family members are some examples of well characterized surface molecules that participate at the interaction of the parasite with the different hosts. However this process is complex for different reasons. First, *T. cruzi* strains are heterogeneous, being classified in two phylogenetic groups: class I, mainly found in marsupial mammals; and class II, in placental mammals preferentially. Second, around 50% of *T. cruzi* genes are being classified as unknown genes or hypothetical proteins. The identification and characterization of hypothetical proteins as surface molecules should be interesting to study the role of these molecules in biological process. Our goal is to identify hypothetical proteins present in *T. cruzi* surface involved in the parasite interaction with mammal and insect hosts. The first step was defining 1810 sequences as *T. cruzi* hypothetical proteins bank (http://www.genedb.org/). These were analyzed by Proto-GIM (http://www.biowebdb.org/protogim/) being found 898 sequences, but only 554 presented the transamidation site at the C terminus. TMHMM analysis (http://www.cbs.dtu.dk/services/TMHMM) excluded

protein with transmembrane domains. 402 resulting sequences were searched for signal peptide motives at SignalP (http://www.cbs.dtu.dk/services/SignalP/) and N-glycosilation motives, using Proto-Gim. 6-10 proteins were chosen with signal peptide and GPI anchor signals, 20-40 KDa mass, and localized near to highly transcribed stage-specific RNAs. Primers were designed to amplify each gene ORF by PCR from DNA. RT-PCR and northern blotting from epimastigotes and metacyclic tripomastigotes mRNA were performed to detect differentially expressed genes. These genes codifying hypothetical proteins will be cloned in pTEX and overexpressed into epimastigotes forms. Biological assays comparing transgenic and wild-type will be done to determine hypothetical proteins function. Supported by: CNPq& FAPERJ.

## BM087 - Long SAGE (Serial Analysis of Gene Expression) in *Eimeria tenella* - A preliminary study

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*Eimeria tenella* is one of the most relevant causing agents of poultry coccidiosis and is the model species for coccidiosis research. A central topic to better understand the biology of the parasite is the transcriptome expression level and, most important, the differential gene expression. In order to address this point, we decided to perform serial analysis of gene expression (SAGE) on second-generation merozoites and sporozoites, both representing invasive developmental stages of the parasite. In this work, we employed the long SAGE method, a modified protocol that generates 21-bp tags, thus allowing a better tag mapping than the conventional SAGE. We have obtained so far a total of 8,096 tags (3,457 unique tags) from a merozoite-derived library. Analysis of individual tag abundance showed that 2,464 tags (30%) occurred only once, 425 tags occurred 2 times (10%), 206 tags 3 times (8%), 322 tags repeat from 4 to 20 times (30%), 29 tags from 21 to 36 times (10%) and 11 tags from 38 to 186 times (12%). These results suggest that a very small number of genes is highly expressed. In another preliminary analysis, the 3,457 unique tags were mapped onto the *E. tenella* genome sequence. From this set, 46.6% (1,612 tags) did not present any hit on the genome. A frequency distribution of the unique tags presenting genome hits revealed that 85% (1,561 tags) mapped to a single site, 12% (218 tags) to two sites, whereas 3% (66 tags) mapped to multiple sites. We intend to map the tags on EST clusters in order to improve the corresponding gene assignment. We are now constructing other SAGE libraries from sporozoite and merozoite mRNAs, whose tags will be incorporated into the study. Statistical modeling and analysis will be used to evaluate the differential gene expression.

## BM088 - Molecular and phylogenetic features of two distinct dsRNA virus families infecting *Eimeria* spp. of domestic fowl

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Virus-like particles (VLPs) classified in the Totiviridae family have been described in distinct protozoan hosts. Here we report the complete genome sequencing of five distinct viruses of *E. brunetti*, *E. maxima*, *E. mitis* and *E. praecox*. A comparative analysis permitted to classify them into two groups: Group 1, composed by Eb-RV1, Ep-RV1 and Emt-RV1, presenting a genome size of 5.3-6.2 kb; and group 2, composed by Ep-RV2 and Em-RV1, with a genome size of 4.2-4.3 kb. Cesium chloride gradients of parasite lysates were performed and fractions containing dsRNA bands were analyzed by electron microscopy (EM). Group 1 revealed icosaedric particles similar to the morphology of Totiviridae. Despite experimental evidences that Group 2 dsRNA genomes are encapsidated, we were not able to evidentiate viral particles by EM. Finally, 0.7 kb dsRNA bands were also observed in co-infection with two distinct Group 1 *Eimeria* viruses. These small elements are encapsidated and may correspond to satellite viruses. Similarity searches of Group 1 sequences showed significant hits to Totiviridade, whereas Group 2 sequences presented only a small similarity block to an unclassified virus of *Zygosaccharomyces bailii*. No similarity was observed between viruses of groups 1 and 2. Thus, Group 2 viruses may represent a novel family of protozoan viruses. Phylogenetic inference of Group 1 viruses confirmed that these viruses constitute a monophyletic group and are more closely related to fungal viruses than to other protozoan viruses. These results may suggest that a progenitor of these viruses might have been a non-infectious virus of a cell type that predated the differentiation of protozoa and fungi. However, the finding of two distinct and non-related virus groups, infecting the same protozoan host cells, may provide evidence that more than one single progenitor may have originated the viruses currently found in *Eimeria*. **Financial support:** FAPESP and CNPq

## BM089 - CLONING AND HETEROLOGOUS EXPRESSION OF TWO PUTATIVE APYRASES FROM *Leishmania*

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*Leishmania major* has two mapped apyrase genes in its genome, named NTPDase and possible guanosine diphosphatase (accession numbers BK005083 and AL359683 respectively). Apyrase function, characterized as tri and dinucleotide hydrolysis capacity, insensitive to others ATPases

inhibitors, were previously demonstrated in intact *L. amazonensis*, *L. braziliensis* (Maioli *et al.*, 2004) and *L. major* cells (unpublished data). This ecto-nucleotidase capacity has been suggested to be involved with *Leishmania* virulence and control of host-immune responses (Maioli *et al.*, 2004). In silico molecular comparisons between *Leishmania* apyrase deduced proteins showed only one possible transmembrane region localized at amino-terminal domain of both putative proteins. Furthermore, possible GDPase has a signal peptide at aminoacid position 45 (predicted by SIGNAL P program) , suggesting a possible soluble excreted protein. On the other hand, the possible n-terminal transmembrane domain in NTPDase and the presence of ecto-apyrase activity on live cells, could be suggestive of an ecto-membrane localization. Despite both predicted proteins have ACRs (Apyrase Conserved Regions), align between these predicted proteins reveled a very low identity (19,9%). In order to study the biochemical characteristics of these proteins we designed end primers and amplified both genes in these three species of *Leishmania* and initially only L. major genes were cloned into TOPO vector. The identity of these genes were evaluated by DNA sequencing. The possible GDPase cloned in TOPO was then used as template to amplified full-length message and transferred to pET21b bacterial expression vector. The GDPase expression and purification had been performed and NTPDase gene was transferred to expression vector. As showed to recombinant apyrases from *T. cruzi* (unpublished data) and *Toxoplasma gondii* we expected to produce active *Leishmania* apyrases that will be used in biochemical characterizations.Supported by: UFOP, FAPEMIG.

## BM090 - Trypanosoma cruzi MSH2 knock-outs and the role of mismatch repair machinery in generating genomic variability in the parasite

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*T. cruzi* has an heterogeneous population, composed of strains presenting distinct morphological, genetic and biochemical characteristics and two major lineages of the parasite have been identified, with distinct ecological environments: *T.cruzi* I in the silvatic cycle and *T. cruzi II* in the domestic cycle. The *T. cruzi* II lineage presents a higher genetic variability than *T. cruzi I*. We have described a gene involved with DNA mismatch repair (MMR), named TcMSH2, which codifies three MSH2 isoforms in the *T. cruzi* population. According to SNP analyses of the TcMSH2 gene, we are able to distinguish three haplogrups in the *T. cruzi* population: haplogroup A (which corresponds to *T. cruzi* I) and haplogroups B and C (all of them having *T. cruzi* II and hybrid markers). Moreover, we found evidences indicating that parasites belonging to TcMSH2 haplogoup A present higher MMR efficiency than strains belonging to haplogroups B and C. In this work, we analyzed TcMSH2 sequences from several strains and compared the efficiency of MMR of cultures of Silvio X-10 cl1 and Col1.7G2 (TcMSH2 haplogroup A) and Esmeraldo cl3 and JG strains (haplogroup C) by treating them with different concentrations of cisplatin and H_2O_2. In order to investigate the role of MSH2 related to the genomic variability in *T. cruzi*, we generate parasite knock-outs for the MSH2 gene. Stable lineages derived from the clone CL Brener (which presents MSH2 alleles corresponding to haplogroups B and C) with one deleted alele C ($msh2^{+/-}$) were assessed for survival in response to genotoxic agents. In accordance with the role of MSH2 in MMR, CL Brener $msh2^{+/-}$ clones presented a 10% higher survival to cisplatin 40% lower survival to H_2O_2 than the CL Brener wild type culture. The are currently generating double knock-out parasites to perform complementation experiments with the three distinct MSH2 isoforms. Support-FAPEMIG

## BM091 - Characterization of the gene encoding the dihydrolipoamide dehydrogenase (TcLipDH) in *Trypanosoma cruzi* populations susceptible and resistant to benzonidazole

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Dihydrolipoamide dehydrogenase (LipDH) is a FAD disulphide oxidureductase that in *Trypanosoma cruzi* catalyses the reduction of disulphide substrate dependent of NADH. This enzyme is a promising target for the structure-based development of new antiparasitic drugs because of its reduction function and structural difference between human and *T. cruzi* LipDH. Murta *et al.* (2002), using the differential display methodology selected TcLipDH gene which was overexpressed in the *T. cruzi* population resistant to benzonidazole (BZ). In this study, TcLipDH was characterized in *T. cruzi* populations with *in vitro*-induced resistance (17LER) and *in vivo*-selected resistance (BZR) to BZ. The northern blot profile of total RNA from drug-resistant and susceptible *T. cruzi* samples, hybridized with TcLipDH radiolabelled probe revealed one transcript of 2.6Kb. Quantitative analyzes revealed that the *T. cruzi* drug-resistant population 17LER expressed 2-fold more TcLipDH mRNA than drug susceptible 17WTS. Other *T. cruzi* populations BZS and BZR expressed the same level of TcLipDH mRNA independent of their drug resistance phenotype. Southern blot of DNAs digested with three restriction enzymes were hybridized with TcLipDH gene probe to evaluate the amplification of this gene. Quantitative analyzes of the fragments showed 2-fold more copies in 17LER than 17WTS population. The TcLipDH encoding region (1.431bp) was cloned into pGEX expression vector and expressed in *E. coli* BL21 as a GST-fusion recombinant TcLipDH protein. Its relative molecular weight was 82KDa: 53KDa for the rTcLipDH and 29KDa for GST. Further studies are underway to determine the TcLipDH protein expression levels in *T. cruzi* strains susceptible and resistant to BZ. Supported by FAPEMIG, CNPq and PAPES-3/FIOCRUZ.

## BM092 - Characterization of the gene encoding the hexose transporter protein (TcrHT) in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole

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The trypanosomatids hexose transporters (HTs) have been put in evidence as a rational target for chemotherapy. Murta *et al.* (2002), using the Differential Display methodology, found that the TcrHT gene is more expressed in some *T. cruzi* benznidazole (BZ) resistant strains than in its drug-susceptible pairs. In the present study, mRNA level, number of copies, polymorphism and chromosomal location of the TcrHT were analyzed in *T. cruzi* strains susceptible (S) and resistant (R) to BZ. The TcrHT encoding region (1634 bp) was cloned and the recombinant protein was expressed. Northern blot profile showed one transcript of 2.6 Kb for a population with *in vitro*-induced resistance (17LER) and its susceptible counterpart (17WTS). An additional transcript of 1.9 Kb was detected in a population with *in vivo*-selected resistance (BZR) and its susceptible counterpart (BZS). Quantitative analyses of these transcripts indicate that the TcrHT is 2-fold more expressed in population 17LER than in 17WTS. No differences in expression were observed in BZS and BZR *T. cruzi* populations. Southern blot analyses of DNA digested with *Sal*I and *Eco*RI restriction enzymes suggested that the TcrHT gene is not amplified in the genome of R parasites. Karyotype analyses showed that the TcrHT gene is present in the 1.85 and 2.02 Mb chromosomes of *T. cruzi* Z1 and in the 1.94 and 2.21 Mb chromosomes of *T. cruzi* Z2 and ZB. Nucleotide polymorphisms and chromosomal location were associated with zymodemes and not with drug-resistance phenotype. Attempts to express the complete recombinant protein (12 hydrophobic domains) were unsuccessful. However, we were able to clone and express separately the domains 1 and 2 (453 bp) and 7 to 12 (563 bp). At present, we are investigating the level of HT protein expression in BZ resistant and susceptible *T. cruzi* strains.

## BM093 - Characterization of monoclonal antibody directed to inositol phosphorylceramide of *Leishmania (Leishmania) amazonensis* promastigotes

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*Leishmania* species multiply as intracellular amastigotes within macrophages of their vertebrate hosts and as extracellular promastigotes in the midgut of their sandfly vector. Both forms are adapted to proliferate and survive in hosts expressing specific molecules on the parasite surface. In order to better characterize the differences between these forms, it was produced a monoclonal antibody (mAb) against lipid fraction of *L. amazonensis* promastigotes. Parasite lipids were extracted with mixtures of isopropyl alcohol/hexane/water (IHW) (55:20:25) and chloroform/methanol (CM) (2:1), and chromatographed on Silica gel 60 column. Lipids were eluted with different proportions of CM (9:1; 8:2; 6:4; 1:9), and IHW. The fractions eluted with CM 1:9 and IHW were combined and used to immunize BALB/c mice. One hybridoma was cloned and termed LST-1 (IgM). By high performance thin layer chromatography (HPTLC) immunostaining it was verified that LST-1 recognizes an acidic component, which was visualized in the HPTLC by primulin and Dittmer-Lester reagent, but not by orcinol. These results indicate that LST-1 recognizes a phospholipid, which does not contain carbohydrate residues. This antigen showed to resistant to alkaline hydrolysis, and it was purified by preparative HPTLC. By GC/MS analysis it was detected peaks corresponding to sphingosine, fatty acid and inositol indicating that LST-1 recognizes an inositol phosphorylceramide (IPC). By HPTLC immunostaining of lipid extracts of different trypanosomatids it was observed that LST-1 also recognizes IPC in promastigotes of *L. major, L. chagasi*, and *L. (V.) braziliensis*, and in *T. cruzi* epimastigotes. Immunofluorescence of fixed promastigotes of *L. amazonensis* showed a strong LST-1 label in all parasite plasma membrane. No LST-1 reactivity was detected with *L. amazonensis* amastigotes. MAb LST-1 could be useful to identify and to understand the biological roles of IPC in parasites and other cells. Supported by: CAPES, FAPESP and CNPq

## BM094 - CrATP: a novel inhibitor of ecto-ATPases of trypanosomatids.

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The Trypanosomatidae family has singular importance due to its capacity to infect several organisms, including men. The ecto-enzymes of these parasites are known to be involved in many cellular processes, such as protection of parasites against host immune defense and transport of nutrients and ions. CrATP, a complex formed by the stable binding of ATP and $Cr^{3+}$ ion, has been utilized in several kinetic studies due to its inhibitory properties on ATPases. In the present study we intend to investigate the effect of CrATP in ecto-ATPase activity of *Trypanosoma cruzi*, *Trypanossoma rangeli*, *Leishmania amazonensis* and *Herpetomonas sp.* To investigate inhibition by CrATP in *Herpetomonas sp.*, intact cells ($1{,}0\times10^8$ cells/mL) were incubated with different concentrations of CrATP by 1 hour at room temperature in the presence of 0.2 mM and 2 mM of $[\gamma^{32}P]$ATP. CrATP reversibly inhibited the ecto-ATPase activity with Ki= $0.33 \pm 0.1$mM and Ki= $4,76 \pm 1.0$ mM, respectively. We also observed that maximal inhibition was 60% when 2

mM ATP were used and 90% when 0.2 mM ATP was used. Higher concentrations of ATP protected the ecto-ATPase against inhibition by CrATP. DIDS, a known inhibitor of ecto-ATPases of trypanosomatids, gave inhibitions similar to the obtained with CrATP. Furthermore, an aditive effect of DIDS and CrATP in inhibition of ecto-ATPase activity was observed. Similar experiments performed using *p*-NPP (5 mM) as a substrate, showed no inhibition by CrATP of the *p*-NPPase activity of Herpetomonas sp. In *Trypanosoma cruzi*, *Trypanossoma rangeli* and *Leishmania amazonensis* similar dose-dependent inhibitory effect of CrATP on ecto-ATPase activity were observed. Taking together, our results show that CrATP is a good tool to investigate the physiological role of ecto-ATPases in trypanosomatids.

## BM095 - Using Real-time PCR to study gene expression in *Trypanosoma cruzi*: evaluation of different control genes

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Several techniques allow quantifying mRNA, and among them real time PCR (qRT-PCR) has been shown to be very useful by virtue of its accuracy, dynamic range and sensibility. This last characteristic makes RT-PCR the only available approach to quantify RNAs in some organisms, e. g., some forms of *T. cruzi*. It is essential to choose a control gene (reference or normalizer gene) to be used to normalize quantitative and qualitative differences of RNA input among the samples. Universal housekeeping genes extensively used in several organisms (e. g., GAPDH, ribosomal proteins, actin, tubulin) have been shown to be variable in other organisms or depending on the cell physiological condition. Nowadays, the general concept is that a control gene chosen for some organisms in some experiment will not necessarily serve to other organisms or in another experiment. In order to identify control genes in *T. cruzi*, we tested 21 genes in real-time PCR, comprising 8 hypothetical proteins (selected from microarray experiments), 3 actin, 3 GAPDH, 2 HGPRT, eIF3, H2A, H2B, alfa-tubulin and ribosomal protein L9. RNA of different developmental stages of *T. cruzi* (epimastigote, epimastigote after nutritional stress, differentiating-epimastigotes, metacyclic trypomastigote and cell derived trypomastigote) was extracted and retro-transcribed. The reactions were performed in 20 $\mu$l with 8 pg of cDNA, 4 pmol of each primer and SybrGreen PCR Master Mix. The comparison of the expression of each of these genes and their use as control gene to normalize the expression of the others, revealed a rather complex scenario, reinforcing the difficulty to identify an optimal control gene in *T.cruzi*. Financial support from CNPq, Pronex (CNPq-Fundação Araucaria), Fiocruz, NIH.

## BM096 - Effects of gene expression inhibition on *Trypanosoma brucei* cells induced by RNAi of mitochondrial tRNA synthetases

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Trypanosomatids have a characteristic mitochondrial genome organization. The genetic code of the kinetoplast (mitochondria) deviates from the universal code where a UGA stop codon is used as a Trp codon. All organelle tRNAs are nuclear encoded and have to be imported into the mitochondria by an as yet not fully understood process. A single nuclear-encoded tRNA Trp (CCA), that can decode the canonical Trp codon, is used by both the nucleus and mitochondria genes. A C to U editing event at position 34 of this tRNA Trp changes the anticodon of this tRNA from CCA to UCA allowing the decoding of the UGA stop codon to Trp. This editing event is restricted to the kinetoplast. We have identified two different nuclear-encoded tryptophanyl-tRNA-synthetase (*WARSs*) genes. One is localized to the cytoplasm (*WARS1*) and the other to the kinetoplast (*WARS2*) in both *Leishmania* and *Trypanosoma* cells. With the purpose to validate the mitochondrial WARS enzymes of trypanosomatids as potential drug target and elucidate their tRNA discriminatory mechanism, we performed RNA interference (RNAi) gene silencing experiments of this enzyme from *Trypanosoma brucei* (*TbWARS2*). The full length ORF was cloned into the tetracycline-regulated RNAi vector, pZJM, and stable transfectant procyclic (PCF) *T. brucei* 2913 cells, were obtained. In the conditions tested, the expression of dsRNA from a dual promoter system generated potent RNA silencing, leading to clear phenotypes characterized by morphologic alterations, severe growth inhibition, reduction in the levels of oxygen consumed and cellular death. The efficiency and specificity of silencing were estimated by semi-quantitative RT-PCR and significant modifications of the specific amount of *TbWARS2* mRNA was evidenced. Our results show unambiguously the central role that *WARS2* play in cell viability, validating this protein as targets for inhibitor development. **Supported by: CAPES, CEPID-FAPESP**

## BM097 - Comparative analysis of the Trypanosoma cruzi maxicircle genome and RNA editing events in strains CL Brener and Esmeraldo

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The implication of kinetoplast DNA (kDNA) in pathogenesis of Chagas Disease makes an understanding of the kDNA sequence composition of critical importance. In order to determine the primary sequence of the maxicircle, the TIGR *Trypanosoma cruzi* Genome Project database was examined for sequences originating from the kinetoplast DNA component of the cell. Continuous contigs were created for the CL Brener (DTU IIe) maxicircle gene coding region and for the

Esmeraldo (DTU IIb) equivalent. The overall arrangement of the gene coding regions was conserved between the CL Brener and Esmeraldo strains, and among the related kinetoplastids *Trypanosoma brucei* and *Leishmania tarentolae*. The predicted regions of RNA editing were similar to that seen in T. brucei, however frameshifts and deletions were seen within several coding regions. The potential resolution of these differential gene sequences through RNA editing events specific to Esmeraldo will be explored. The variable portion of the *T. cruzi* maxicircles contained unique repeated sequence elements, as seen in *T. brucei*. Previously reported cDNA sequences provided starting points for the determination of many of the initial RNA editing events in CL Brener. The complete cataloging of RNA editing events in CL Brener and Esmeraldo is underway. In addition to maxicircle sequences, an abundance of minicircle sequence fragments were retrieved from the Genome Project database, despite their small size relative to the technical size selection cut-off for cloning. These sequences will be examined for the presence of guide RNAs based on the editing events described in their cognate strain. To further assess the role of minicircles in pathogenesis, the relative abundance of different minicircle classes will be determined using empirical analysis from a broad selection of strains.

## BM098 - Molecular cloning and characterization of a gene encoding the caspase like proteasome subunit from *Trypanosoma cruzi*

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Proteasome are found in the nucleus and cytoplasm of all eukaryotic cells and are responsible for degradation of ubiquitin tagged protein in a ATP dependent manner. In higher eukaryotic, the break of cell proteins are degraded by this ubiquitin-proteasome pathway, and the lysosomal pathway is involved primarily in the degradation of membrane associated proteins and extra cellular proteins taken up by endocytosis. Regulation of proteins mediated by ubiquitin-proteasome pathway is an essential cellular tool in many biological process in which the life span of specific proteins controls cell cycle progression, transcriptional activation, signal transduction, or metabolic regulation. In the present study, we report the partial characterization of the caspase like proteasome subunit from *Trypanosoma cruzi*. This cDNA contain a 855- bp ORF, coding for a 284 amino acid protein, with a predicted molecular mass of 31kDa. Pairwise comparisons of amino acid sequence within the beta proteasome gene family (20-38%; 17-20%, respectively) was lower than that between *T. cruzi* subunits and corresponding orthologs from *S. cerevisae* and human (45-68%). Next, we examined the expression of the beta-1 gene, that encoding a caspase-like subunit, in different developmental stages of *T. cruzi* by traditional semiquantitative RT-PCR. Our results demonstrate that whereas levels of beta-1 remained constant in both amastigote and trypomastigote, the levels increased approximately threefold in epimastigote. Our future research will be address to investigate the expression pattern of a collection of six others beta subunits from proteasome 20S in different developmental stages and strains of this parasite. Support FAPEMIG

## BM099 - Are hypothetical proteins good targets for diagnosis in visceral leishmaniasis?

Ingrid Evans *(FIOCRUZ)*; Nicolas Marchon *(FIOCRUZ)*; Marcel Ivan Ramirez *(FIOCRUZ)*

Protozoan parasites of *Leishmania* genus cause a wide spectrum of diseases including cutaneous, mucocutaneous and visceral leishmaniasis. The latter is essentially caused by world distributed *Leishmania infantum* (also called *L. chagasi* in Latin America). Visceral leishmaniasis diagnosis is based on *in vitro* culture, Giemsa - stained smears and serological tests, methods that present serious limitations. Serological tests are not helpful, because false negative results cannot be excluded due to the immunological status of the patient. Direct detection from bone marrow, lymph node or spleen aspirates, requires invasive procedures. Diagnostic tests based on molecular biology techniques, proved to be more sensitive and specific than classical methods. PCR is most suitable for diagnosis, as it can be applied on blood, bone marrow and skin, with increased sensitivity and specificity. We have established single and multiple PCRs using primers to amplify two conserved sequences of *L. chagasi*: Pia3 and Pia4 (260pb repetitive sequence of *L. infantum* genome) and DEB8 (809pb kDNA minicircle) determining their sensibility and specificity. The sensibility of PIA3/PIA4 was 10pg and for DEB8 was 1pg; 100pg for multiple PCR. Amplification of PIA3/PIA4 and DEB8 in single and multiple PCR was specific for *L. chagasi* when comparing Genomic DNA panel from different tripanosomatids. We are testing hypothetical proteins as targets for leishmaniasis diagnosis. On-line tools were used to obtain 4450 sequences as *L. infantum* hypothetical proteins bank (http://www.genedb.org). We selected three hypothetical proteins specific to *L. chagasi* to amplify by PCR assay. The best conditions were established, as MgCl2 concentration, melting temperature in order to determinate sensibility and specificity. PIA3-4 and DEB8 will be compared to define the best target for visceral leishmaniasis diagnosis. At the future we will test single and multiple PCR in human samples to validate the method and implement it at northeast hospitals. Supported by: CNPq& FAPERJ.

## BM100 - The expression of alpha and beta tubulin genes in *Leishmania amazonensis*

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An autoregulatory mechanism of gene expression has been observed for alpha and beta tubulin genes in mammal cells

and may also occur in *Trypanosoma cruzi*. According to this model, an increase in non-polymerized tubulin results in the reduction of the levels of alpha and beta tubulin mRNAs. In *T. cruzi*, epimastigotes present lower levels of soluble tubulin, compared to other forms of the parasite. In contrast, the levels of tubulin mRNAs are higher in epimastigotes than in amastigotes and trypomastigotes. In different species of *Leishmania*, variations on the levels of alpha and beta tubulin mRNAs have also been observed throughout the life cycle of the parasite: in some *Leishmania* species, promastigotes present higher levels of tubulin mRNAs when compared with amastigotes. Here we analyzed the expression of alpha and beta tubulin genes, as well as the levels of non-polymerized tubulins during the life cycle of *Leishmania amazonensis*, using a protocol to cultivate axenic amastigotes. With this protocol, amastigote-like parasites were obtained 3 days after transferring promastigotes to a medium with reduced pH and increased amounts of FBS and incubating the cultures at higher temperatures. The differentiation process was analyzed through Western blot assays using antibodies anti-A2, an amastigotes specific protein. Western blot assays of total parasite extracts indicated that amastigotes present higher levels of non-polymerized alpha tubulin when compared to promastigotes. Futhermore, Northern blot results indicate that amastigotes present lower levels of alpha tubulin mRNA. Experiments in progress, aimed to testing the auto-regulatory model using drugs that disrupt the tubulin polymerization dynamics, indicated that *L. amazonensis* is highly sensitive to vinblastine. Promastigotes cultured for 48 hours in the presence of 30$\mu$M vinblastine display morphological alterations compatible with the disruption of microtubules. To test our model, Northern blots analyses with RNA extracted from amastigotes and vinblastine treated promastigotes will be conducted.

## BM101 - CHARACTERIZATION OF A TGF-$\beta$ FROM LUTZOMYIA LONGIPALPIS

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*Lutzomyia longipalpis* is the main vector of visceral leishmaniasis in Brazil. The prospective to control illnesses transmitted by insects depends greatly on our capacity to control the insect vector or to intervene with the parasite-vector interactions. While this has been well studied in malaria, with the development of transgenic mosquitoes incapable of transmitting the parasite, little is known about the interaction leishmania-sand fly. We are studying molecules potentially involved in feeding and infection by leishmania in *L. longipalpis*. Through DDRT-PCR we have previously identified some genes potentially involved in insect immune response. An *L. longipalpis* TGF-$\beta$ gene was identified from RNA of sand flies fed with blood containing *Leishmania chagasi*, indicating a potential immune response role for this molecule in this vector as well. The *L. longipalpis* TGF-$\beta$ cDNA was completely sequenced. This gene contains the seven cysteines that are conserved among the members of the TGF-$\beta$ superfamily and the typical catalytic domain RXXR. A fragment of the gene was cloned into an expression vector and the recombinant protein was used for the production of a polyclonal antibody. Western blots were performed using this antibody and demonstrated the constitutive expression of the protein in *L. longipalpis* LL5 cells challenged or not with *E. coli*, *M. luteus* and *L. chagasi*. Expression is now being verified in insects. Preliminary results indicate an increase in protein expression upon blood feeding. A conserved portion of the *L. longipalpis* TGF-$\beta$ was compared to the analogous sequence of others family members. Individual members of the superfamily were clustered with the orthologous genes of insect, mammal, bird, amphibian and fish. The present sequence appears to be most closely related to the family of activins/inhibins.

## BM102 - Mass spectrometry analysis of *Trypanosoma cruzi* histone H4 modifications

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Histones of Trypanosoma cruzi show a high degree of divergence when compared with histones of higher eukaryotes, mainly at the N-terminal domains. As the N-terminus of histones are subjected to extensive post-translational modifications, implicated in gene expression control and chromatin structure, here we provide a detailed characterization of the T. cruzi histone H4 post-translational modifications by mass spectrometry. Epimastigote forms of this parasite were acid extracted, and the soluble proteins enriched in histones were fractionated by reverse phase chromatography. The fractions corresponding to histone H4 were identified and digested with endoproteases ArgC and GluC. The resulting peptides were then analyzed using MALDI-TOF and LC-MS-TOF. The presence of molecules with masses corresponding to the N-terminal portion of the histone H4 (AKGKKSGEAKGTQKRQKKILRE, 2-23) in GluC digests indicates that the first methionine is removed. More important, this peptide, as well as peptides derived of GluC digests (peptide 10-23), or ArgC digests (peptide 2-16) were found to contain 14, 42, 56, 70, 98 Da additions, while more internal peptides were much less modified, suggesting the presence of several post-translational modifications in the N-terminal portion of T. cruzi histone H4. Sequencing analysis using LC-MS/MS-TOF unequivocally detected acetylation in K2, K4, K5, K10 and K14, methylation in K4 and K5, dimethylation in R54 and K58. Taken together, these results indicate that although the N-terminus of T. cruzi histone H4 is different from the terminus of histone H4 from other eukaryotes, it contains similar methylation and acetylation modifications. Supported by FAPESP/CNPq

### BM103 - Analysis of Phosphorylation Profile During Nutritional Stress in *Trypanosoma cruzi* Using 2D Electrophoresis

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The proteome complexity derives not only from the number of individual proteins present in a cell, but also from their post-translational modifications. Additionally, splice variants may be present, adding further to the complexity of the system. One of the most common post-translational modifications of proteins is phosphorylation. In trypanosomatids the post-translational modifications analysis is particularly important because these organisms do not use transcription initiation as a regulatory step to control gene expression. The transformation of epimastigotes into metacyclic trypomastigotes is induced by factors like nutritional stress, differences in temperature and pH resulting in important morphological and functional differences between these developmental forms of *Trypanosoma cruzi*. Very little is known about the first signals at very early time points that will induce the process of metacyclogenesis. In the present work we compared the phosphorylation profile differences between epimastigotes that suffered nutritional stress (incubation at different time points in the chemically defined TAU medium) and control parasites. The differences in phosphorylated and total protein profiles were visualized by performing second dimension electrophoresis followed by specific gel stains for phosphorylated proteins and total proteins respectively (Pro-Q®Diamond phosphoprotein gel stain followed by Sypro®Ruby). We could identify proteins differentially expressed and phosphorylated by *T. cruzi* at 30 minutes and 1 hour after induction of nutritional stress. Supported by FAPESP/CNPq

### BM104 - Maintenance of inverted repeats within noncoding sequences of *Leishmania* (*Viannia*) *braziliensis* H locus.

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Gene amplification is a common phenomenon observed in *Leishmania* cell lines subjected to drug pressure. In species of the *Leishmania* subgenus, the mechanism is characterized by formation of circular extrachromosomal DNA. The H locus of *Leishmania major* is normally found amplified in cell lines selected in unrelated drugs. We investigated the level of conservation and the maintenance of gene order across the H locus between *L. (V.) braziliensis* and the reference strain of *L. (L.) major*. The assembly and annotation of *Viannia* subgenus H region also uncovered part of the intergenic sequences within this locus. As expected, conservation between the two species was poor within these noncoding sequences. However, a set of inverted repeated sequences was identified at the right end of the *L. (V.) braziliensis* H locus. The position of these inverted elements is equivalent to the location of inverted repeats annotated as RIME elements within *L. (L.) major* H region. These sequences are believed to participate in the amplification of the H region of *Leishmania* subgenus species. The *L. (V.) braziliensis* repeated element is 443 bps-long and comparison between the two copies revealed a sequence identity of 98%. However, the comparison between *L. (L.) major* and *L. (V.) braziliensis* repeats did not reveal significant sequence identity. These data indicated that these noncoding repeated elements might be under functional constraints. It is well established that noncoding regions of genomes contain a higher degree of variation. However, noncoding sequences imparted with a higher sensitivity to evolutionary drift, insertions or deletions might be involved in the maintenance of genome organization and expression. Supported by CAPES, FAPESP and CNPq.

### BM105 - Identification and characterization of the Recombination Segment (RS), ribosomal promoter region, from *Trypanosoma rangeli*

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*Trypanosoma rangeli* is a hemoflagellate parasite sharing both vertebrate hosts and triatomine vectors with *T. cruzi* in a wide geographical area in Central and South America. Transfection studies in *T. cruzi* revealed a 101bp fragment, named as Segment Recombination (SR), from the ribosomal promoter sequence, which mediates a stable and integrative transfection, even using circular plasmidial forms. The aim of this work was to verify the existence of a similar sequence in the *T. rangeli* genome. A pair of primers was designed based on the alignment of *T. cruzi* strains and the pRIBOTEX plasmid sequence and used for PCR amplification of the SR segment from genomic DNA of the *T. rangeli* SC-58 and Choachi strains. As observed for the pRIBOTEX plasmid DNA used as control, an expected band of approximately 380bp was amplified in both *T. rangeli* strains, as well as for *T. cruzi* Y and CL strains. However, a similar pattern of unspecific amplification products was observed for both *T. cruzi* and *T. rangeli* strains. After gel purification and re-amplification, the 380bp fragment from *T. rangeli* was sequenced in order to confirm the product identity. Blast analysis consistently resulted in two distinct results, one revealing a high similarity with *T. cruzi* SR segment (89% identity), and the other presenting similarity with *T. cruzi* telomeric regions. Thus, these amplification products are being cloned in order to allow the insertion of the *T. rangeli* SR in the pTEX-GFPmut plasmid. Since the episomal form this plasmid proved to be effective on driving the GFP expression by the parasite, the possibility of integration of the plasmid DNA on the parasite genome will be an important tool for study the parasite biology in its hosts and vectors.

### BM106 - Effectiveness of post-transcriptional suppression of nitrophorin 2 expressed in the salivary glands of *Rhodnius prolixus* (Reduviidae, Triatominae).

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A variety of compounds have been detected in triatomine saliva, including anticoagulants, inhibitors of platelet aggregation, vasodilators, anti-histamines, a sialidase, a protease, a pore-forming, immunosuppressant, a sodium channel blocker and a complement inhibitor. In addition to these compounds, mass sequencing of cDNA libraries from salivary glands of triatomines have been identifying several novel genes with no homology in genebank which functions remain unknown. To elucidate the role of those unknown genes in blood feeding, the RNAi technique emerges as one of the most promising approaches. The aim of the present study was the standardization of the RNAi in the triatomine bug *Rhodnius prolixus*, in order to use this technique in future experiments of functional genomics. The nitrophorin 2 (NP2), an anticoagulant lipocalin expressed at the salivary gland, was used as target gene. The gene was PCR amplified from cDNA of the glands and the products were used to produce double strand RNA (dsRNA) that was introduced in the bug by two injections separated by a 48 hours interval. The level of silencing was verified using RT-PCR and the phenotype was accessed by the recalcification time assay and by the intensity of the salivary gland color, which is characteristically red. Results showed that two injections of 15$\mu$g of NP2 dsRNA in fourth instar ninths reduced about 80% of gene expression. Phenotype analysis showed that the saliva of the normal bugs prolonged 989 seconds in recalcification time while silenced bugs saliva prolonged only 161 seconds. Photos taken from the salivary glands of silenced insects showed the loss of the reddish color of the content of the gland compared to normal controls, suggesting the reducing expression of nitrophorins. These results indicate that the RNAi can be a useful technique in studding gene function in triatomine bugs. Supported by CNPq, FAPEMIG, ECLAT, Wellcome trust.

### BM107 - The role of Pteridine Reductase 1 in *Leishmania (Viannia) braziliensis* virulence and drug resistance.

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The Pteridine reductase 1 (PTR1) of *Leishmania spp* participates in the reduction of biopterin into its active form tetrahydrobiopterin (H4B). The levels of H4B were shown to play an important role in the metacyclogenesis of *Leishmania major*. Decreased levels of H4B, observed in *L. major* mutants lacking PTR1, resulted in increased virulence. The altered virulence phenotype of these mutants was a consequence of an increase in the number of metacyclic forms. PTR1 gene of *L. major* is located in a locus that is easily amplified when the parasite is cultivated in the presence of unrelated drugs. Therefore, gene amplification of PTR1 could be used to modulate the parasite virulence by controlling the differentiation into its infective forms. In order to investigate the role of PTR1 in a Viannia subgenus parasite, the *L. (V.) braziliensis* PTR1 was isolated and cloned into a shuttle vector. The LbPTR1 locus was subject to the in vitro transposition of the transposable element ELSATKO. The resulting insertion event was used to generate a reagent for the disruption of the genomic copy of PTR1 gene by homologous recombination. In order to obtain a null mutant, the heterozygotic transfectant was used to induce a gene conversion event using high concentrations of the selective drug (400$\mu$g/ml nourseotricin). The knockout transfectants are being used to investigate the role of LbPTR1 in the metacyclogenesis and virulence. It was shown that in spite of mediating an increase in methotrexate (MTX) resistance, the overexpression of the PTR1 had no effect on virulence of *L. (L.) major*. The overexpression of the LbPTR1 and its possible participation in virulence and MTX resistance is currently being investigated. The analysis of PTR1 knockouts and overexpressors will allow the study of the role of PTR1 in *L. (V.) braziliensis*. Supported by FAPESP, CAPES and CNPq

### BM108 - Functional genomic analysis of ubiquitin-proteosome pathway during T. cruzi metacyclogenesis.

Josiane Cardoso *(IBMP)*; Domitila Bonato *(IBMP)*; Carla Lima *(IBMP)*; Thiago Stolf *(IBMP)*; Vanessa Sotomaior *(IBMP)*; Christian Probst *(IBMP)*; Celina Poersh *(IBMP)*; Rozenn Le Bloas *(IBMP)*; Samuel Goldenberg *(IBMP)*; Marco Krieger *(IBMP)*

*T. cruzi* metacyclogenesis occurs naturally in the vector hindgut very likely as a response of the parasite to environmental changes such as the decrease of available nutrients. Although this biological process must be driven mainly by post-transcriptional/translational changes in gene expression, few is known about the specific mechanisms involved. Ubiquitin-mediated proteolysis should play a role in the remarkable adaptative protein turnover which leads to new morphology, physiology and behavioral features. Competitive hybridization experiments were performed on *T. cruzi* microarrays carrying more than 6.000 amplified products which represent almost 5.500 gene ID from *T. cruzi* covering 45% for ubiquitin-proteasome genes, using cDNA synthesized from RNA extracted at different times of the metacyclogenesis process. The results indicated complex gene expression regulation for genes included in this pathway. Some of these ubiquitin-proteasome genes share the same expression pattern during metacyclogenesis, for instance one activating-ubiquitin enzyme , two conjugating-ubiquitin enzyme and a ligase enzyme . These results open new perspectives in order to investigate the putative interactions between these genes and their role during the cellular differentiation of *T. cruzi*.

### BM109 - Promoter regions of Plasmodium vivax are unable to recruit the transcriptional initiation complex of P. falciparum.

Azevedo, M.F. *(ICB-USP)*; del Portillo, H.A. *(ICB-USP)*

Transcriptional analyses of P. falciparum suggest that control of gene expression is unique among eukaryotes. Gene regulation seems largely post-transcriptional with anti-sense transcription and silencing mechanisms, which involve cooperative interactions of introns and upstream regions. Furthermore, malaria parasites seems to have significantly fewer transcription factors than other eukaryotes and enhancers and promoter elements are difficult to predict based on sequence similarity due to the high AT-richness of intergenic regions. Despite these difficulties, promoters are bipartite and contain conserved regulatory elements as promoters from P.berghei and P.falciparum can drive expression of different reporter genes in heterologous transfections systems. Unexpectedly, luciferase reporter plasmids containing promoter regions with different AT-content from the msp1, dhfr, and vir genes of P.vivax were unable to drive luc activity in heterologous transfections of P. falciparum. To exclude the possibly that some essential regulatory element was missing in these reporter plasmids, we constructed a plasmid with the entire intergenic region of the two ef1-a genes of P. vivax. Like the other promoters tested, this one was unable to drive detectable luciferase activity. In an attempt to determine which elements, essential for transcription in P.falciparum, were not present in these P.vivax promoters, a minimal promoter based on the P.berghei ef1-a intergenic region was cloned between the entire Pv ef1-a intergenic region and the luciferase gene. Of notice, this minimal promoter alone cannot drive detectable luciferase activity in transient transfections. Significantly, this construct was capable of driving luciferase activity albeit to lower levels than those reported by a plasmid containing the full length P. berghei ef1-a promoter region. Together, this data indicates that promoter regions of P. vivax are unable to recruit the transcription initiation complex of P.falciparum. Present efforts are guided to identify unique or lacking cis-acting elements in P. vivax promoters.

### BM110 - Short but not long epimastigotes of *Trypanosoma rangeli* modulate ecto-enzymes on the epithelium surface of salivary glands of *Rhodnius prolixus*.

Gomes SAO *(UFRJ)*; Kiffer TM *(UFRJ)*; Fonseca de Souza AL *(UFRJ)*; Meyer-Fernandes JR *(UFRJ)*

*Trypanosoma rangeli* has a characteristic life cycle in its vector, *Rhodnius prolixus*, invading the hemolymph and then the salivary glands, where a large number of infective metacyclic trypomastigote are generated. The finding of *T. rangeli* in the salivary glands of triatomines demonstrates the importance of these organs in the transmission of this protozoon. Invasion of salivary glands by *T. rangeli* likely to be mediated by specific receptor-ligand interactions. In the present study we investigated ecto-phosphatase and ecto-ATPase activities on the surface of *R. prolixus* salivary glands. These enzymes are able to hydrolyze phosphorilated substrates in the extracellular medium. In this context, the characterization of these ecto-enzymes activities detected on the salivary glands surface was employed to investigate *T. rangeli-R. prolixus* salivary glands interaction. Salivary glands present a lower level of hydrolytic activities, $4.3 \pm 0.35$, $3.45 \pm 0.28$ and $8.21 \pm 1.15$ nmol Pi x $h^{-1}$ x gland $pair^{-1}$ for phosphatase, $Mg^{2+}$-independent ATPase and $Mg^{2+}$-dependent ATPase, respectively. Our results demonstrated *in vitro* and *in vivo* interaction assays that short but not long induced an inhibitory effect in salivary glands ecto-phosphatase activity (short forms inhibited 75% of salivary gland ecto-phosphatase activity). On the other hand, short forms were efficient to increase to 257.3% ecto-ATPase activity while long were not. *T. rangeli* epimastigotes also present these phosphohydrolase activities. Short forms present higher ecto- phosphohydrolase activities, $25.57 \pm 2.03$, $35.03 \pm 8.86$ and $209.49 \pm 26.29$ nmol Pi x $h^{-1}$ x $10^{-7}$ cells than the long forms, $10.09 \pm 0.93$, $25.01 \pm 3.89$ and $117.79 \pm 28.3$ nmol Pi x $h^{-1}$ x $10^{-7}$ cells for phosphatase, ATPase and $Mg^{2+}$-dependent ATPase activities, respectively. If the effects on the salivary glands ecto-phosphatase and ecto-ATPase activities could be consequences of differences found in enzymatic activities of both forms of the parasites, remains to be elucidated.

### BM111 - Functional analysis of the multidrug resistance gene *mdr1* of *Plasmodium vivax* through heterologous over-expression in *P. falciparum*

Orjuela, P. *(ICB-USP)*; del Portillo, H.A. *(ICB-USP)*

Emergency of chloroquine resistance (CQR) in *Plasmodium vivax*, the most widely distributed human malaria parasite, is treating to render this cheap and widely used first-line antimalarial drug, ineffective. Moreover, resistance to primaquine has also been reported for *P.vivax* strongly indicating that this human malaria parasite is developing multidrug resistance (MDR). CQR and MDR in *P.falciparum*, the most deadly species, have been associated with the *pfmdr1* gene. This gene codes for an energy-dependent membrane transport protein and mutations as well as expression and gene amplifications have been strongly, though not completely, associated with the CQR phenotype. To initiate studies on MDR in *P.vivax*, we previously cloned and characterized the orthologous gene, *pvmdr1*, in *P.vivax*. Strikingly, there were no mutations associated with CQR suggesting that a different mechanism should be involved in this resistance in *P.vivax*. Functional studies through reverse genetics of *pvmdr1* are difficult to conduct because of the present inviability of continuously growing *P.vivax in vitro*. To overcome

this difficulty in studying the role of *pvmdr1* in MDR, we have generated a stable transgenic line of the *P.falciparum* CQ sensitive clone 3D7 over-expressing this gene. Western blot analysis and drug sensitivity assays are currently being conducted in order to determine whether the over-expression of this transgene can alter the IC50 of the recipient 3D7 to CQ and other antimalarials.

## BM112 - Different Phosphatase Activities On The Cell Surface Of Short And Long Epimastigotes Of Trypanosoma rangeli

Fonseca de Souza, A. L. *(UFRJ)*; Kiffer, T. M. *(UFRJ)*; Gomes, S. A. O. *(UFRJ)*; Meyer-Fernandes, J. R. *(UFRJ)*

*Trypanosoma rangeli* is a digenetic hemoflagelated parasite able to infect several animal groups, as well as humans. The life cycle begins with the ingestion of trypomastigote forms present in vertebrate bloodstream by the triatominae. Inside of the vector gut, parasites differentiate to epimastigotes forms and pass through the gut epithelium, achieving the hemocoel and migrate to the salivary glands where trypomastigote is formed. The detection of kinases and phosphatases activities on the parasites surface is of great relevance to the comprehension of its physiological roles, such as cell adhesion, nutrients acquisition and virulence. Protein phosphatases are classified in two major families: the phosphotyrosine phosphatases and the phosphoserine / phosphothreonine phosphatases, with different molecular structures, catalytic mechanisms and substrate specificity. This work shows the presence of ecto-phosphatase activities on the surface of *T. rangeli*. Our results demonstrate that epimastigotes short and long living cells of T. rangeli hydrolyze the artificial substrate p-nitrophenylphosphate (p-NPP), presenting a Michaelian kinetic with different kinetic parameters, apparent Km of $3.48 \pm 0.62$ mM and Vmax values and $26.27 \pm 1.10$ nmol p-NP / h x $10^7$ cells for short and apparent Km of $0.25 \pm 0.045$ mM and Vmax $17.63 \pm 0.56$ nmol p-NP / h x $10^7$ cells for long epimastigotes. The pH range more significantly affected ecto-phosphatase activity of long epimastigotes. Divalent cations, such as $Mg^{2+}$, $Cu^{2+}$ and $Ni^{2+}$, stimulated strongly the ecto-phosphatase activity of long epimastigotes while $Cu^{2+}$ and $Ni^{2+}$ inhibited phosphatase activity of the short epimastigotes. These data could be indicating the presence of two different enzymes with phosphatase activity in long epimastigotes and only one activity in short epimastigotes. Our findings support the association of surface molecules changes with the evolution of the parasite cycle, from short to long epimastigotes. This work was supported by CNPq and FAPERJ.

## BM113 - Modulation of Ecto-Phosphatase Activity of Trypanosoma rangeli by Oxidative Stress

Cosentino, D. G. *(UFRJ)*; Gomes, S. A. O. *(UFRJ)*; Fonseca de Souza, A. L. *(UFRJ)*; Meyer-Fernandes, J. R. *(UFRJ)*

*Trypanosoma rangeli* is a trypanosomatid parasite that lives in two different host cells: vertebrate and invertebrate hosts. The life cycle in triatominae vectors begins with the acquisition of metacyclic trypomastigotes during blood ingestion. In the midgut, parasites differentiate to short epimastigotes forms. These stages pass through the intestinal barrier and achieve the hemolymph, where the short epimastigotes become long forms. After this, parasites migrate to the salivary gland and perform metacyclogenesis, origining metacyclic forms, which are infective stages to the mammalian cells. Cell division, adhesion and nutrients acquisition are functions suggested for ecto-phosphatases. These enzymes present their catalytic site faced to the external medium, hydrolyzing phosphomonoesters in extracellular environment producing free inorganic phosphate, an important nutrient to cellular functions. In this study we investigated the regulation of ecto-phosphatases activities in *T. rangeli* by oxidative stress. Reducing agents are not capable to influence this activity and only oxidized glutathione was efficient to induce effects on the activity, in a dual manner. The activity was enhanced 4-fold at 5mM oxidized glutathione and decreased to basal levels ($5.98 \pm 0,48$ nmol p-NP x $h^{-1}$ x $10^{-7}$ cells) at concentrations higher than 12 mM. This activation was prevented by 1 mM DTT and 1mM $\beta$-mercaptoethanol, antioxidant agents. Moreover 1mM hydrogen peroxide and 0.5 mM $Fe^{2+}$ inhibited 38% and 49% respectively. In addition, $Fe^{2+}$ plus ascorbate (ascorbyl production) inhibited activity in 48%, while ascorbate alone was not capable to influence it. We are now evaluating effects of oxi-redox reactions on the ecto-phosphatase activities. This work was supported by CNPq and FAPERJ.

## BM114 - LIPID RAFTS FROM MOSQUITOES CELLS

Vargas, C.S. *(UFRJ)*; Nimrichter, L *(UFRJ)*; Conceição,T.M. *(UFRJ)*; Da Poian, A.T *(UFRJ)*; Atella, G.C. *(UFRJ)*

Dengue is a serious viral disease transmitted by the bite of the mosquito, *Aedes aegypti*. It is spread by the bite of an infected female which has got the dengue virus by taking a blood meal on an person who is ill with dengue. The infected mosquito transmits the disease through its saliva. Lipid rafts are cholesterol-enriched microdomains that are detergent-resistant at low temperatures and are involved in a number of cellular processes such as trafficking and cell signaling. The objective of this work is the purification and characterization of lipid rafts to define their role on dengue virus entry into the cell. Sucrose density centrifugation is the standard

method for lipid rafts isolation. The $^{35}$S-methionine labeled C6/36 cells were lysed, homogenized and incubated at 4$^o$C in Triton X-100 1%. After 30 minutes the homogenate was subjected to a sucrose gradient. Protein content was measured by scintillation counting. Cholera toxin B subunit (CTB) is a specific ligand for ganglioside GM1 and can be used for the detection of GM1 containing microdomains. In order to investigate the presence of GM1, the fractions were submitted to a dot-blot using cholera toxin. We identified a large GM1 content in the corresponding fractions 5 to 8 of the sucrose gradient. The raft containing fractions were subjected to lipid extraction and TLC.The spots were analyzed by densitometry. The results showed that mosquito lipid rafts are riched in cholesterol-ester (71,86%), cholesterol (9,25%) and phospholipids (12,90%). Supported by CNPq, FAPERJ, IFS

## BM115 - Protein Targets For Structural Studies Selected by Subcellular Proteome of *Leishmania major*

de Oliveira, A.H.C. *(FFCLRP-USP)*; Rosa, J.C. *(FMRP-USP)*; Greene, L.J. *(FMRP-USP)*; Ruiz, J.C. *(FMRP-USP)*; Cruz, A.K. *(FMRP-USP)*; Ward, R.J. *(FFCLRP-USP)*

Leishmaniasis is considered to be a problem of world-wide health and the objective of this work was to identify, clone and express vesicular proteins in the promastigote form of *Leishmania major* for investigation as potential drug targets. A vesicule-rich sub-cellular fraction was obtained by differential centrifugation of promastigote extracts, and after rupture of these vesicles the soluble protein content was separated by 2D gel electrophoresis. MALDI-TOF/MS fingerprinting identified 16 protein spots from the *Leishmania major* extract which suggests that the strategy of subcellular proteome characterization may be useful for the identification of novel protein targets for molecular biological and biochemical studies. Three targets were therefore selected for further functional studies: the Nucleoside Diphosphate Kinase b (NDKb), Calpain-like Protease (CLP) and Thermostable Carboxypeptidase (TCP). The genes encoding these proteins were identified in the *Leishmania major* genome, and were amplified from total *Leishmania major* genomic DNA by PCR using oligonucleotides to the flanking DNA sequences, cloned into the vector pET28a, and expressed in *E. coli*BL21(DE3)pLysS. All three recombinant proteins showed high levels of cytoplasmic expression as estimated by SDS-PAGE, and the NDK and the CLP were expressed in a soluble form. The recombinant NDK and CLP proteins were partially purified by affinity chromatography, and a high specific nucleoside transferase activity in the case of NDK suggests that this enzyme is produced in the native conformation. All three DNA coding sequences were cloned into the pX63NEO vector, and transfection of promastigotes with these constructs will further the understanding of processes involved in the host/parasite interaction. Support by FAPESP, CNPq, PRP-USP and PRONEX.

## BM116 - THE SUBSTRATE SPECIFICITY OF THE CYSTEINE PEPTIDASE OF *Trypanosoma b. brucei*, BRUCIPAIN AND ITS INACTIVATION BY NATURAL CYSTEINE PEPTIDASE INHIBITORS.

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The Clan CA cysteine peptidases (CPs) include the papain-related proteases of plants, mammalian cells and pathogenic protozoa. Studies using synthetic irreversible CP inhibitors in several animal models of protozoan-caused diseases have indicated that these peptidases are potential virulence factors. CP inhibitors designed based on the tri-dimensional structure of the *Trypanosoma cruzi* CP, cruzipain, drastically affect the survival of *Trypanosoma b. brucei*, the causative agent of Nagana disease in live stock in vitro. However, the primary target of these drugs, brucipain, is not characterized in detail. We have previously described the cloning and expression of active recombinant brucipain, followed by its biochemical characterization. Here, we studied the substrate specificity of brucipain using synthetic 7-mer peptidyl substrates containing systematic substitutions in the $P_3$-$P_3$' positions. We also determined the inactivation constants for the inhibition of brucipain by different protein inhibitors of Clan CA peptidases. We found that brucipain shows preference for small hydrophobic residues at the $P_2$ position and is also capable of hydrolyzing substrates presenting Pro at this position. Unexpectedly, brucipain hydrolyzes 80% less efficiently the substrate containing Phe in $P_2$, contrasting with the substrate specificity of the *T. cruzi* CP, cruzipain that prefers Phe and Arg at $P_2$. Brucipain was strongly inactivated by the propeptide of cruzipain ($K_i$ 16pM) through a slow-binding kinetic mechanism and by human cystatin C ($K_i$ 7pM). Taken together, our results indicate that brucipain and cruzipain are strikingly similar regarding their inactivation by proteinacious CP inhibitors but present remarkably different substrate specificity. The investigation of the structural basis for the substrate specificity of brucipain may aid in the design of drugs tailored for efficient inhibition of this *T.b.brucei* cysteine peptidase.

## BM117 - POTENT INHIBITION OF CATHEPSIN F AND OF CYSTEINE PROTEASES FROM TRYPANOSOMES (CRUZIPAIN AND BRUCIPAIN) BY THE PROPEPTIDE OF CRUZIPAIN

Reis, F.C.G. *(UFRJ)*; Costa, T.F.R. *(UFRJ)*; Abreu,M.F. *(UFRJ)*; Sulea, T. *(BRI)*; Mezzetti, A. *(BRI)*; Ménard, R. *(BRI)*; Brömme, D. *(UBC)*; Scharfstein, J. *(UFRJ)*; Lima, A.P.C.A. *(UFRJ)*

Papain-like cysteine proteases of pathogenic protozoa play

important roles in parasite growth, differentiation and host cell invasion. The main cysteine proteases of *Trypanosoma cruzi* (cruzipain) and of *Trypanosoma brucei* (brucipain) have been validated as targets for the development of new chemotherapies. These proteases are synthesized as precursors that require the removal of the N-terminal pro-domain to acquire enzymatic activity. Here we report the potent inhibition of cruzipain and of brucipain by the recombinant full length pro-domain (propeptide) of cruzipain. The propeptide displayed selectivity for the trypanosomal enzymes, since we did not detect inhibition of human cathepsins S, K, B or papain at the tested concentrations. However, human cathepsin F was inhibited with a $K_i$ of 32 pM, which was a surprising and interesting finding indicating that the propeptide is able to distinguish cathepsin F from other cathepsin L-like enzymes. Human cathepsins V and L were likewise inhibited, albeit about 100-fold less efficiently. The propeptide partially inhibited cysteine peptidase activity in living mammalian cells and impaired their infection by *T. cruzi*. Comparative structure modeling and analysis identified the interaction between the loop $\beta$1p-$\alpha$3p of the propeptide and the propeptide binding loop (PBL) of the mature enzyme as structural entities for the partial selectivity of the cruzipain propeptide for the inhibition of cathepsin F, cruzipain and brucipain versus cathepsins V, L, S, and K.

## BM118 - PHOSPHOLIPASE $A_2$ ACTIVITY IN SALIVARY GLANDS OF DIFFERENT HEMIPTERANS SPECIES.

Rocha,F.F *(UFRJ)*; Dias,F.A *(UFRJ)*; Pereira,m.A. *(UFMG)*; Melo ,A.L *(UFMG)*; Lopes,A.H.C.S. *(UFRJ)*; Golodne,D.M. *(UFRJ)*; Atella,G. *(UFRJ)*

Phospholipase $A_2$ ($PLA_2$, EC 3.1.1.4) catalyzes the hydrolysis of the sn-2 fatty acyl bond of phospholipids which releases free fatty acids and lysophospholipids. In this work, we verified the presence of this enzyme in the saliva of hemipterans with different feeding habits. In *Rhodnius prolixus* (a hematophagous hempiteran) salivary lysophosphatidylcholine (lysoPC) displays anti-haemostatic properties and $PLA_2$ is involved in the production of salivary lysoPC. The possibility of $PLA_2$ to display anti-hemostatic effects itself is currently under investigation. The presence of such enzyme on hemipteran saliva was studied in the following species: *Oncopeltus fasciatus* (a phytophagous hemipteran) and *Belostoma anurum* (an aquatic hemipteran with predator habits). Saliva obtained from the above sources including *Rhodnius prolixus* were analyzed as follows. Samples were incubated in 100 mM Tris, 5 mM CaCl2, 100 mM NaCl, pH 8.5 containing 500 ng PED6 (a flurogenic substrate for $PLA_2$). After incubation the media were collected and subjected to lipid extraction, thin-layer chromatography, followed by scanning on a Storm 860 laser scanner and quantification of fluorescent fatty acids. In *R. prolixus*luminal glands showed a $PLA_2$activity (128.291 arbitraries units (A.U.)) which was time- and substrate concentration-dependent but partially calcium-independent. *O. fasciatus* luminal glands showed a $PLA_2$ activity of 55.082 A.U.. *B. anurum* saliva showed a high level of $PLA_2$ activity (2.808.756 A.U.) which accounts for the highest activity detected in hemipterans studies. The characterization and developmental profile of such enzymes is under investigation in order to define their role in blood feeding. Supported by CNPq, FAPERJ and IFS.

## BM119 - Phylogenetic analysis of trypanosome alternative oxidase (TAO) compared to alternative oxidases from species of different taxa.

Guimarães A. C. R. *(FIOCRUZ)*; Degrave W. M. *(FIOCRUZ)*; Ferreira M. A. *(FIOCRUZ)*

Plants, some fungi, and protists contain a cyanide-resistant, alternative mitochondrial respiratory pathway. This pathway possesses an enzyme known as "alternative oxidase"(AOX), which is encoded by the gene nuclear *aox1*. Some trypanosomes also possess a similar enzyme which is called TAO (Trypanosome Alternative Oxidase). The TAO is part of the terminal step of the mitochondrial oxidative process involved in the energy metabolism of different life-cycle forms of these parasites. The objective of this work is to verify the similarity of this trypanosome enzyme in relation to others organisms. The phylogenetic position of the trypanosomes is of great importance, to the extent that these enzymes can represent a potential target for chemotherapy. Multiple alignments of sequences of homologous AOX proteins were used for comparison of this enzyme in vast taxa. In this evaluation, we considered the taxonomic position of the species studied, and performed a phylogenetic analysis using MEGA version 1.8 1, with Poisson model and gamma parameter. The phylogenetic tree was constructed with neighbor-joining method. These analyses showed that TAO from trypanosome species grouped more closely to plants than to fungi. Similar behavior for other proteins has already been observed in the literature. An explanation for such event would be the possibility of horizontal transfer of genes coding for this protein. Supported by: FAPERJ, CNPq, FIOCRUZ PAPES, PDTIS.

## BM120 - FATTY ACID METABOLISM IN *RHODNIUS PROLIXUS* MIDGUT INFECTED WITH *TRYPANOSOMA CRUZI*

Cunha, PRBB *(UFRJ)*; Folly, E *(CEFETEQ)*; Paiva-Silva, GO *(UFRJ)*; Atella, GC *(UFRJ)*

*Trypanosoma cruzi* is hemoflagellate that employs a wide variety of mammalian hosts and hematophogus insects in its life cycle. In humans, *T. cruzi* is found as both an intracellular form, the amastigote, and as a trypomastigote form in the blood. Under natural condition this parasite is transmitted to the vertebrate host by triatomine insects. In the vector the parasite reproduces asexually. The *T. cruzi* development is confined to the insect gut. We have previously demonstrated

that *Rhodnius* midgut is the main organ in fatty acid absorption. Now we are studying the capacity of infected midgut incorporate and synthesize lipids. Additionally, the presence of the fatty acid binding-protein (FABP) was also investigated. $^{14}$C-Oleic acid was injected in *R. prolixus* females in the first day after a blood meal. The fatty acid incorporation was linear up to 30 min. The incorporation increased (40%) when the midgut was infected with the *T. cruzi*. The $^{14}$C-oleic acid was used by the infected midguts to synthesize phospholipids and neutral lipids. In order to investigate the presence of FABP, adult females were fed with blood of rabbits containing *T. cruzi*. After one day, five midguts of adult females were dissected and RNA was extracted. RT-PCR analysis revealed that FABP is expressed in midgut. Preliminary results suggest that FABP expression is increased in the midgut by *Trypanosoma cruzi* infection. It corroborates the results of lipid incorporation. Supported by: CNPQ, FINEP, FAPERJ

## BM121 - Effect of *Bauhinia bauhinioides* Inhibitors on *Trypanosoma cruzi* Infection

Santana, L A *(UNIFESP/EPM)*; Yoshida, N *(UNIFESP/EPM)*; Ferreira, D *(UNIFESP/EPM)*; Hyane, MI *(UNIFESP/EPM)*; Hansen, D *(UNIFESP/EPM)*; Andrade, SS *(UNIFESP/EPM)*; Lopes. GS *(UNIFESP/EPM)*; Sampaio, MU *(UNIFESP/EPM)*; Oliva, MLV *(UNIFESP/EPM)*

*B.bauhinoides* Cruzipain Inhibitor (BbCI) and Kallikrein Inhibitor (BbKI) are cysteine and serine peptidase inhibitors. BbCI is effective in cruzipain/cruzain inhibition while BbKI is a kallikrein inhibitor. This work aims to analyze the efect of these inhibitors on *T. cruzi* infection and invasion. Preliminary results revealed that pre-treated trypomastigote forms of *T.cruzi*, Y stain, with BbKI (27 nM) for 30 min flowing by incubation with HeLa cell line for 20 hours, reduced significantly the infection (60%) and the invasion (50%) level comparing with PBS buffer as control. BbCI does not affect the cells invasion. Analyzing the effect on metacyclic trypomastigote forms of *T.cruzi*, CL stain, it was observed an inhibitory effect by BbCI (around 50%). The inhibitory specificity of these inhibitors should be considerated for *T. cruzi* invasion and infection investigation. Supported by FAPESP, CNPq, SPDM/FADA, Probal (CAPES/DAAD).

## BM122 - Mapping and identification of a Leishmania (L.) major gene-locus related to Tubercidin resistance

Aoki, J.I *(IMT)*; Coelho, A. C. *(IMT)*; Yamashiro-Kanashiro, E.H *(IMT)*; Cotrim, P.C. *(IMT)*

Leishmania is the causative agent of leishmaniasis, a worldwide parasitic disease with high prevalence. The design of selective anti parasitic drugs depends on the exploitation of fundamental biochemical differences between parasite and host. Perhaps one of the most remarkable metabolic discrepancies between parasite and their host is that the former are incapable of de novo synthesis of the purine ring. Based on that, we first studied the potent anti parasite action of the purine analogue Tubercidin (TUB) against Leishmania in association with the nucleoside transport inhibitor S-(4-Nitrobenzyl)-6-Thioinosine. We verified that this association does not alter the anti parasite effect of TUB, besides decrease the TUB toxicity in both promastigotes and amastigotes leishmania cultures. Starting from a L.(L.)major Friedlin A1 strain (LmFA1) genomic library constructed into the cLHYG shuttle vector, DNA was transfected on promastigotes and cells submitted to overexpression / selection experiments in the presence of increasing concentrations of TUB. One out of the two selected cosmids capable to render TUB resistance to LmFA1 was chosen for further analysis. The cTub1 (approximately 30kb) was mapped and deleted based upon a restriction patterns, aiming at localization of the gene-locus related with TUB resistance. Deleted DNAs were then transfected into LmFA1 wild type cells and submitted to functional tests in the presence of TUB, after DNA copy number amplification. Using this strategy, we expected to isolate small fragments from original insert cTub1, related with TUB resistance. To identify the gene(s) present in the resistant locus, nucleotide-sequencing reactions followed by comparative analysis with the leishmania genome project database will be held. From these data, in association with functional tests in the presence of TUB and TUB transport inhibitor, hypotheses about TUB resistance can be formulated, pointing to a better understanding of TUB action aiming at anti-leishmania chemotherapy. Supported by FAPESP, CNPq, LIM-48.

## BM123 - Identification of the gene *PRP1* related to Pentamidine resistance in three *Leishmania* species

Coelho, A. C. *(IMT-USP)*; Cotrim, P.C. *(IMT-USP)*

ABC transporters are involved in the drug resistance of various pathogenic protozoa. In *Leishmania* spp., several members of the ABC transporter superfamily are involved in drug resistance. We have previously characterized the ABC transporter gene *PRP1 (Pentamidine Resistance Protein 1)* able to mediate pentamidine and trivalent antimonials resistance in *Leishmania (L.) major* (Coelho *et al.*, 2003). We used polymerase chain reaction with degenerate oligonucleotide primers corresponding to the conserved sequence of the first nucleotide binding domain of *PRP1* gene to probe the genome of *Leishmania (L.) amazonensis, L. (L.) chagasi* and *L. (V.) braziliensis* for the presence of the ABC transporter PRP1. We identified sequence segments encoding the first ATP-binding cassettes of PRP1 of *L. (L.) amazonensis* and *L. (V.) braziliensis* but not in *L. (L.) chagasi*, although analysis of genomic DNA by Southern blot indicated the presence of related PRP1 sequences in the three *Leishmania* species. Sequence analysis of PRP1 of both *Leishma-*

*nia* species indicated that they are highly identity to *L. (L.) major* PRP1 although some differences in restriction analysis exist between the isolated segments. These degenerate primers also amplified sequence segments of three *Leishmania* spp. homologous to PGPA of *L. (L.) major*, related to pentavalent and trivalent antimonials resistance. Our data suggest that both identified genes may be involved in drug resistance in these *Leishmania* species due to their high identity to the respectively ABC transporters PRP1 and PGPA of *Leishmania*. Supported by FAPESP, CNPq and LIM-48.

## BM124 - PROTEIN DISULFIDE ISOMERASE, AN ENDOPLASMIC RETICULUM REDOX CHAPERONE, IS REQUIRED FOR EFFICIENT PARASITE PHAGOCYTOSIS

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NAD(P)H oxidase is the major source of Reactive Oxygen Species in phagocytes and its overactivity is associated with parasite infection control. Our laboratory showed that protein disulfide isomerase (PDI), an oxireductase of Endoplasmic Reticulum (ER), regulates NAD(P)H oxidase in phagocytes and vascular cells during ER stress. Moreover, PDI has been proposed to participate in ER-mediated phagocytosis (recent proteomic studies). We thus hypothesized that PDI has a role in parasite-host interaction. Therefore, we studied in vitro infection of macrophages J774 by Saccharomyces cerevisae (Sc) (multiplicity 1/5) and by amastigotes/promastigotes of Leishmania chagasi (multiplicity 1/5). Microscopy showed that macrophages preincubated with PDI inhibitors DTNB or Bacitracin, or with a monoclonal antibody against PDI (d=1/100) resulted in significant decrease (ca. 50-60%) in Infection Index (II) of Sc. Similar results were obtained using neutrophil. The II was dependent on Sc redox state, as indicated by phagocytosis assays employing fungus pre-treated with 1mM DTT or 1 mM H2O2. In these conditions, the II was higher (ca. 30% vs. control) when Sc was in the reduced state. In the case of Leismania chagasi, promastigote phagocytosis was greatly inhibited (50-60%) by PDI inhibitors (including 1 $\mu$M PAO, a dithiol-alkylating agent) while in case of amastigotes there was no effect. To gain insight into ER Stress-mediated phagocytosis, we studied NAD(P)H oxidase activation in macrophages by ER stressors (5 $\mu$M Thapsigargin, 5 $\mu$g/ml Tunicamycin or 20 $\mu$g/ml 7-keto-Cholesterol). All agents induced significant oxygen burst in a time dependent-manner at similar levels or grater than PMA stimulus, as assayed by the fluorescence of dihydroethidiun products. Oxygen burst was significantly inhibited by 5 $\mu$g/ml BrefeldinA, a Golgidisrupting agent, thus indicating a role for protein traffic in oxidase activation. Together, these data suggest that PDI may be associated not only in controlling NAD(P)H oxidase activity, but also in parasite-host interaction and infection.

## BM125 - Automated identification of analogous enzymes and in silico reconstruction of the biochemical pathways of the amino acid metabolism of *Trypanosoma cruzi*

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Analogous enzymes are interesting entities from an evolutionary point of view besides being potential drug targets, often overlooked. We have developed an automated workflow designed to i) extract information (primary sequences) for each E.C. number; ii) construct clusters for each E.C.; iii) identify putative analogous enzymes and iv) annotate proteins with cluster-related information. This approach was used to annotate and reconstruct metabolic pathways, in particular the amino acid metabolism of *Trypanosoma cruzi* as a model in the present study. First, we compiled a list of E.C.'s and the protein sequences of all enzymes involved in the amino acid metabolism from KEGG. Using a similarity-based approach, we have clustered the proteins belonging to each E.C. For each cluster we constructed statistical profiles using Hmmer. These were employed to query a dataset composed of predicted proteins of *T. cruzi*. Cut-off values were determined using an annotated dataset composed of *Plasmodium falciparum* proteins. 570 E.C.'s corresponding to 25 different pathways in the amino acid metabolism are represented in KEGG. From these, 394 have at least one sequence. For these, we have obtained 1341 clusters. 39% of the enzymes have only one cluster, the mean value being 3.4. Analogous enzymes were found in 61% of the pathways. For instance, 255 sequences are annotated with the E.C. 1.11.1.6, belonging to three different metabolic maps, and forming four separated clusters. The utilization of Hmmer models constructed for each cluster allowed us to identify 304 *T. cruzi* proteins probably involved in the metabolism of amino acids. Crossing these results with similar data obtained for human sequences will allow us to identify instances where analogous sequences not shared between human and *T. cruzi* may represent potential drug targets. A generalization of this approach may be used for other enzyme groups and pathways.

## BM126 - LmRjl a putative target for cis-splicing *Leishmania* spp.

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It was long believed that kinetoplastid genomes lacked introns in their protein coding genes and that the only splicing reaction occurring in these organisms was trans-splicing. A few years ago cis-splicing was reported in the genus *Trypanosoma*, in the poly-A polymerase gene. Here we present suggesting evidence for a cis-splicing mRNA target in *Leishmania*. We have previously reported the description of the RJL family of GTPases and the characterization of *TcRjl*,

the *T. cruzi* orthologue. When blasting *Leishmania major* genome in search for *Rjl* orthologues we could identify a putative *LmRjl* gene. Curiously its open reading frame has two insertions, of 66 bp and 120 bp. Such insertions become evident when *LmRjl* is aligned to trypanosomatid and non-trypanosomatid *Rjl* orthologues. The insertions are present in the same positions of smaller ones in *T. cruzi* and *T. brucei* genes and coincide with intronic borders of the algae *Chlamydomonas reinhardtii Rjl* orthologue. *LmRjl* insertions resemble introns by their low sequence complexity, high GC bias (70% to 86%, in contrast to 65% of the whole gene), by the presence of pyrimidine tracts and putative splicing acceptors sites. Although resembling introns in such ways, they do not interrupt the open reading frame. The same insertions are also present in *Rjl* orthologues from *L. infantum* and *L. braziliensis*, although their length diverge. We are establishing a RT-PCR approach to access if *LmRjl* gene insertions are spliced out of *LmRjl* pre-mRNA or whether they are simple gene insertions, reminiscent of an ancestral intronic segment. PCR from genomic DNA amplifies a single band of around 840 bp, corresponding to the genomic sequence found on database. RT-PCR against promastigote mRNA reveals an 840 bp band and some smaller fragments, which are being cloned and sequenced to investigate the hypothesis of a cis-splicing reaction occurring in *Leishmania Rjl* genes.

## BM127 - Characterization of *Trypanosoma cruzi* strains isolated from an acute outbreak of human Chagas disease in Santa Catarina State, Southern Brazil.

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During March 2005 twenty four cases of human acute Chagas disease were detected in Santa Catarina State, southern Brazil, all of them related to the ingestion of sugar cane juice at the Navegantes Municipality. Hemoculture in LIT medium allowed the isolation of 09 strains from humans (SC94-SC102). Furthermore, field studies in the surrounding areas of the outbreak spot allowed the capture of *Didelphis aurita* opossums and *Triatoma tibiamaculata*, from which 4 strains were isolated, two from opossums (SC90, SC91) and two from triatomines (SC92, SC93). The aim of this work was to characterize these *T. cruzi* strains. For that, all 13 strains were characterized by isoenzyme gel electrophoresis (GPI, ME, G6PD, PGM, ASAT and ALAT) and by analysis of the spliced-leader gene as described elsewhere. As controls *T. cruzi* SC28 (TcI) and Y (TcII) strains, as well as, the *T. rangeli* Choachi strain were used for spliced-leader PCR assays and standard Z1 (Barra Seca), Z2 (Y), ZB (CL), ZD (150D) and Z3 (CANIII) *T. cruzi* strains for isoenzyme assays. Both techniques revealed that all strains isolated from humans belong to the TcII group, but revealing a Z2 variant pattern for the PGM enzyme. Strains SC90 and 91 showed a Z1 profile in all analyzed enzymes, but PCR assays indicated a mixed profile (TcI/TcII). Strains SC92 and 93 revealed a mixed TcI/TcII profile in both isoenzyme and spliced-leader assays and no indication of the presence of *T. rangeli* was observed in any assay. Considering that mixed strains (TcI/TcII) were isolated from both triatomines and opossums and that all strains isolated from humans belong to TcII group, our results suggest a fast selection towards TcII group in humans. Further characterization of these strains by analysis of rDNA ITS and 24S rDNA are in progress. Supported by CNPq, CPqRR/Fiocruz, SES/SC and UFSC.

## BM128 - Classification of Transporter Families in *Trypanosoma cruzi.*

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*Trypanosoma cruzi*, the aetiological agent of Chagas disease, undergoes a series of morphological and physiological adaptations in order to survive within the insect and mammalian host. In this respect, transporters can act as sensors of nutrient availability and environmental insults; also they have an essential role in cellular homeostasis, metabolism and maintenance of membrane potential. Transporters can be classified in classes and families based on i) the mechanism of substrate translocation, ii) the mechanism of energy coupling and iii) sequence similarity. Little is known about the type and distribution in families of transporters in *T.cruzi*. In this work, we used a computational approach to identify and classify putative *T.cruzi* transporters. First, sequences of transporters from 177 organisms, previously classified in 130 families, were downloaded from TransportDB database. Sequences of each family were clustered, a HMMER model was built for each cluster and used as query against a cleaned dataset composed of potential *T.cruzi* transmembrane proteins, as predicted by TMHMM. Annotated proteins from *P.falciparum* were used to determine the cut off values of similarity. Transporters belonging to 33 families, whose putative sequences share high identity and similarity with families in TransportDB, were identified in *T.cruzi*. Some families have a higher representation, ranging from 7 to 15 % of the total putative transporters: AAAP; ABC; APC; DMT; F-ATPase; MC; MFS; P-ATPase. 97 families were not detected by our approach, suggesting that they are not present in *T.cruzi* genome. Interestingly, some families found in *T.cruzi* are not present in human genome, according to Transport DB: H+Ppase; MerTP; AEC; MIT; SSPTS. In contrast, VIC family is highly represented in Humans but not in *T.cruzi*. A detailed analysis of the information generated by this study will give new insights about the biochemistry of *T.cruzi* and relevant information for the identification of new targets for therapy.

### BM129 - Leishmanicidal activity of primary S-nitrosothiols against *L. (L.) amazonensis*

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Nitric oxide (NO) inhibits the development of intracellular (*Trypanosoma, Leishmania, Plasmodium* and *Toxoplasma*) and extracellular (*Entamoeba*) protozoa and helminths (*Schistosoma*) in mammalian hosts. NO releasing drugs as S-nitrosoglutathione (GSNO), S-nitrosoalbumin, S-nitroso-L-cysteine and S-nitrosoacetylpenicillamine (SNAP) kill *Trypanosoma cruzi* and *Plasmodium falciparum* in a dose-dependent inhibitory effect. Glyceryl trinitrate (GTN) and SNAP were successfully used in the treatment of cutaneous leishmaniasis in man. In the present study, we investigated the leishmanicidal activity of the NO donors GSNO and S-N-acetyl-cysteine (SNAC) against *Leishmania* promastigotes and the effect of the reducing agents dithiothreitol (DTT) and L-ascorbic acid as inhibitors of the NO donors activity. GSNO and SNAC were incubated with *L. (L.) amazonensis* promastigote cultures resulting in antileishmanial activity with IC50 values of $50.13 \pm 2.08$ $\mu$M and $238.56 \pm 24.52$ $\mu$M, respectively. DTT alone (50 or 100 $\mu$M) was mildly toxic to parasites while concentrations of L-ascorbic acid up to 10 mM resulted in a slight increase in promastigotes growth. Simultaneous incubation of parasites with GSNO and L-ascorbic acid was able to block completely the GSNO effect. This reversal of effect was also observed with DTT. On the other hand, incubation of *Leishmania* with SNAC plus L-ascorbic acid or DTT was completely ineffective in blocking parasite killing. These data indicate that killing of promastigotes by GSNO or SNAC proceeds through different mechanisms. GSNO is likely to act via S-transnitrosation reactions involving vital substrates for the promastigote survival. This toxic effect is probably reversed by both ascorbic acid and DTT through their ability to reduce SNO groups back to their parent active free thiols. Supported by FAPESP.

### BM130 - Characterization of mechanisms of antimonial resistance in *Leishmania (Viannia) braziliensis*

Wilton C. Z. Lopes *(USP)*; Fabrício C. Dias *(USP)*; Luiz R. O. Tosi *(USP)*

Species of the protozoan *Leishmania* are the causative agents of a wide range of pathologies called leishmaniasis. The treatment for the diseases consists of pentavalent antimonials, whose collateral effects are dose-cumulative. The study of the ability of *Leishmania* to evade chemotherapy is important to help the design of effective treatments. When subjected to high concentration of antimonials, or other unrelated drugs, *L. major* amplifies the H locus. The P-glicoprotein A (PGPA) is among the genes coded in the H region. PGPA confers antimonial resistance and is associated to vesicular elements of the parasite exocytic pathway. We have investigated the role in resistance of *Leishmania (Viannia) braziliensis PGPA*. Using a stepwise selection protocol we isolated *L. braziliensis* cell lines resistant to 10mg/ml of the pentavalent antimonial (SbV). Our results indicated that resistance to antimonial in these cell lines does not seem to be associated to an altered influx or reduction of the drug. Southern analysis revealed that *PGPA* was not amplified in resistant cell line and PFGE analysis showed that it did not carry amplified episomal molecules. Cell lines of *L. major* subjected to the same selection protocol clearly showed the formation of amplicons and a higher copy number of the *PGPA* gene. The *L. major PGPA* gene was able to confer resistance in *L. (V.) braziliensis* when transfected in this *Viannia* species, indicating that all the other components in PGPA-related antimonial resistance are present in *L. (V.) braziliensis*. Cross resistance experiments revealed that in spite of the fact that *PGPA* or *HTBF* are not amplified, antimonial resistant *L. (V.) braziliensis* cell lines are also resistant to terbinafine. Althogether our results suggest that *L. (V.) braziliensis* does not seem to favor gene amplification as a mechanism underlying drug resistance. Supported by FAPESP and CNPq.

### BM131 - Correlation between the *Trypanosoma cruzi* drug-resistance phenotype and the subversion of the INF-$\gamma$ mediated immune response in human fibroblasts.

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The problematic of Chagas chemotherapy is mainly due to it low cure rates, its high toxicity and to the existence of naturally drug-resistant parasite strains. In the present study we have investigated if the drug-resistance phenotype in *Trypanosoma cruzi* is associated with the modulation of interferon-gamma (IFN-$\gamma$) mediated response, one of the most important immune responses against parasitic infections. The major IFN-g mediated response in human fibroblasts is the induction of indoleamine-2,3-dioxygenase (IDO) expression which results in the degradation of the essential amino acid tryptophan. The IFN-$\gamma$ signaling pathway is regulated by protein tyrosine-phosphatases (PTPs) as well as by the IRF family of transcription factors. In this study we used a *T. cruzi* BZ resistant population (BZR) selected in vivo which has shown to be 2-fold more resistant than its susceptible counterpart (BZS) in our in vitro BZ susceptibility assay. To investigate the possible mechanisms accounting for the impaired responses to IFN-$\gamma$ used by these populations, we have quantified by Real Time Reverse Transcriptase PCR, the mRNA expression of IDO, IRF-1, IRF-2 genes in human fibroblasts infected with *T. cruzi* populations followed by rIFN-$\gamma$ stimulation. Our results showed that the infection of fibroblasts with the BZS population reduced the IDO mRNA

expression. Nevertheless, the infection of fibroblasts with the BZR population has not only reduced the IDO but also the IRF-1 mRNA expression. Moreover, infection of cells with the resistant population has also raised IRF-2 mRNA expression. rIFN-$\gamma$ stimulation of BZS or BZR infected fibroblasts caused no effect in the *T. cruzi* protein tyrosine phosphatase (TcPTP) mRNA expression. Taken together, our results show that drug-resistant parasites present a higher evasive potential against the IFN-$\gamma$ mediated response as compared to susceptible ones, suggesting a correlation between the *T. cruzi* drug-resistance phenotype and the subversion of IFN-$\gamma$ mediated response in human fibroblasts.

## BM132 - Anti- *T. cruzi* Activity of *Pterodon pubescens* extracts

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There is an obvious need for new medicine for the treatment of Chagas disease. The only drug available produces toxic side effects and has limited efficacy for treatment of chronic patients. Development of anti-parasite chemotherapy could emerge, among other efforts, from screening natural products, with recognized activity for other diseases, as the case for *Pterodon pubescens* (sucupira branca). Its extracts have shown immunomodulatory and anti-inflamatory properties and devoid of toxic effects, when assayed in the murine model of arthritis. Experimentally, powdered seeds were extracted with ethanol for 15 days. Solvent was removed under vacuum and the crude extract (OEP) was sequentially fractionated by liquid-liquid extraction with hexane (PF1), dichloromethane (PF2) and ethyl acetate (PF3). The hexane fraction PF1 was further fractionated by HPLC, yielding the fractions PF1.1, PF1.2 and PF1.3. The crude extract and all the fractions, albeit with different potencies, induced a dose-dependent effect on growth of the extracellular epimastigote form of *T. cruzi* .PF1.2 was ten-fold more active than PF1 and caused complete growth arrest and cell lysis at 5 $\mu$ g/ml. The same pattern of cytotoxic and cytolytic effects of the extracts was observed in the clinically relevant trypomastigotes in a 24hr assay. The fraction PF1.2 was the most active one, ensuing complete trypomastigotes lysis with the $IC_{50}$ of 20 $\mu$ g/ml . These findings are promising and merit further investigation in the pursuit of new drugs against *T. cruzi*. Supported by FAPERJ, CNPq and IOC/FIOCRUZ . J.M.L.S. e A.N.D. were fellows of the "Programa Aristides Pacheco Leão de Estímulo a Vocações Científicas" da Academia Brasileira de Ciências/CNPq.

## BM133 - Association of increased expression of iron superoxide dismutase-A (TcFeSOD-A) protein with the *in vitro*-induced resistance to benznidazole in *Trypanosoma cruzi*.

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The enzyme Superoxide Dismutase (SOD) has the function to remove excess superoxide radicals via dismutation to oxygen and hydrogen peroxide. Murta et al. (2002), using the Representation of Differential Expression methodology selected the TcFeSOD gene, which was overexpressed in the *T. cruzi* population resistant to benznidazole (BZ). In this work, TcFeSOD was characterized in *T. cruzi* populations susceptible, naturally resistant, *in vitro*-induced resistance (17LER) and *in vivo*-selected (BZR) resistance to BZ. Quantification of TcFeSOD-A mRNA by real time RT-PCR assay (qPCR) showed that the drug-resistant population 17LER expressed 3-fold more TcFeSOD-A mRNA compared to its susceptible pair 17WTS. Quantification of this gene by qPCR shows that the 17LER population has 2-fold more TcFeSOD-A gene copies than 17WTS. Western blot of two-dimensional gel of *T. cruzi* protein extracts probed with a rabbit anti-recombinant TcFeSOD-A polyclonal serum revealed that the protein is 2-fold more expressed in the 17LER than 17WTS. Mass espectrometric analysis confirmed the presence of the TcFeSOD-A protein and its overexpression in the 17LER. In addition, enzymatic activity of TcFeSOD-A in the *T. cruzi* sample was associated to the increased protein expression level, being its activity 2-fold higher in the 17LER population than the 17WTS. The multi-alignment of the TcFeSOD-A nucleotide and amino acid sequences from 9 *T. cruzi* samples showed 11 nucleotide mutations leading to two amino acids substituitions. No association of nucleotide mutations or amino acid substitutions with resistance phenotype was observed. On the other hand, all mutations were associated to zymodeme of the *T. cruzi* strains. Our results show that the TcFeSOD-A protein is overexpressed only in *T. cruzi* population with *in vitro*-induced resistance to BZ. Interestingly, the mechanism of resistance to metronidazole in *Entamoeba histolytica* is also associated with increased expression of the FeSOD protein (Wassmann et al., 1999). Support FAPEMIG

## BM134 - Evaluation of leishmanicidal effect induzed by proteins of crude venom of rattlesnake *Crotalus durissus cascavella*.

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Snake venom of the species *Crotalus durissus (C.d)* has high crotoxin content, a neurotoxin, composed by fosfolipase a2 an crotapotin. In previous studies, we showed that the crude

venom of *C.d.cascavella, C.d.collilineatus* and *C.d.terrificus* present different profiles eletrophoretics, profiles cromatographics and different effects as leishmanicidal. The objective of this work was to isolate the different fractions that compose the crude venom of *C.d.cascavella* and to evaluate his biological activity in promastigotas of *L.(L.)amazonensis*. The crude venom of *C.d.cascavella* was breaking in fractions using a combination of two methods cromatographic in HPLC (molecular exclusion and reverse phase), and the degree of molecular homogeneity was evaluated by eletrophoresis in SDS-PAGE. The identification of the fractions was made using standardized experimental protocols. Promastigotes of *L.(L.)amazonensis* in stationary phase were washed and adjusted to concentration of $10^6$ parasites/ml. Concentrations of the protein fractions varying from 3,12 to 100,00 $\mu$g/ml were added to the culture. The activity of the fractions was evaluated by counting in Neubauer chain in 24, 48 and 72 hours after the plating and dear IC50 through the statistical software Origin 5.0. Four protein fractions were isolated and purified through HPLC (molecular exclusion and reverse phase), being characterized as: crotamin, giroxin (L-amino acid oxidase) crotoxin and convulxin. Through electrophoreses in SDS-PAGE verified that the fractions were isolated with high degree of purity. IC50 was of 20,00 $\mu$g/ml for to crotamin and 2,39$\mu$g/ml for to L-amino acid oxidase. The convulxin presented, when counting in Neubauer chamber, the same leishmanicidal activities for all used concentrations, not being possible to calculate his IC50, the crotoxin didn't show any activity in the promastigotas. Therefore, we found that the crude venom when purifyed, showed three protein fractions with leishmanicidal activities and one fraction that didn't show any activity in the promastigotas. Supported: FAPESP and LIM-50 HCFMUSP

## BM135 - Luminescence-based assay for Antimalarial drug Screening

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The rapid spread of multidrug resistance in the most deadly human malaria parasite, Plasmodium falciparum, has motivated worldwide efforts to look for new antimalarial drugs and to develop high-throughput screenings for these new compounds. Indeed, the microscopic test based on morphological estimation of parasite growth cannot be use for massive screening; yet, it remains a "gold-standard"to validate new tests. The first highthroughput test to evaluate parasite sensitivity was developed based on the measurement of the metabolic activity by radioactive marker, [3H]-labelled hypoxanthine. Although this method is highly accurate and precise, it poses the problems of handling radioisotopes. To overcome this limitation, two other non-isotopic tests that measure parasite antigens by immune detection, namely lactate dehydrogenase, pLDH and Histidine-Rich Protein II, HRP2, were developed. Although these methods have been validated through comparisons with the microscopic and isotope methods, the present cost-per-reaction limits their value in large-scale studies. More recently, a new test based on SYBER Green I-based fluorescence has been developed. Although it offers several advantages as low cost and easily handling, SYBER green is an intercalating agent that will detect indiscriminately any contaminant DNA. We report here the development of an alternative, luminescence-based in vitro assay to screen new drugs against P. falciparum. The assay is based on a transgenic P. falciparum parasite clone stably and highly expressing luciferase throughout the asexual blood stages. Thus, after drug exposure the parasite survival is evaluated by remaining luciferase activity. Preliminary results demonstrated high sensitivity and reproducibility of the test when compared to the isotopic method suggesting that this assay could be convenient for high-throughput screening of antimalarial drugs against P. falciparum. In addition it is simple, fast and inexpensive. Further validations of this assay will be presented.

# Epidemiologia - Epidemiology

## EP01 - Molecular typing of *Trypanosoma cruzi* isolates from *Panstrongylus geniculatus* captured inside houses in rural area at the northeastern of Venezuela

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Chagas disease is a serious public health problem in Latin America (WHO Report, 1998). At northeastern of Venezuela in previous seroepidemiological study carried out in 2002 by active search in San Pedro, rural village, we found a high seroprevalence (26%) of Chagas disease associated to all age groups of people living in this area, predominantly in individuals younger than 20 years old (24%), suggesting an active transmission of the disease. In this sense in order to establish the presence and molecular typing of *Trypanosoma cruzi* parasites circulating in the region we carried out a capture of Triatomine bugs at houses where seropositives cases were detected between march-june of the present year. After taxonomic identification of Triatomine bugs, the insects were examined for the presence and isolation of parasites. RAPD and miniexon analysis were applied for species identification and main group typing. 22 *Panstrongylus geniculatus* attracted by ligh were collected at San Pedro and 15 (68%) of them were found infected with *T. cruzi*. All the isolates were identified as corresponding to the Tc I or zimodeme 1 main group. These finding reveal a high infection index of *P. geniculatus* with *T. cruzi* and suggest changes in the pattern of behavior of this sylvatic specie of Triatomine bug, possibly associated with high seroprevalence of Chagas disease at San Pedro village.

## EP02 - Phylogeny of Apicomplexa parasites and oomycetes, as detected by small ribosomal RNA sequences.

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Apicomplexa parasites, as Plasmodium, Toxoplasma and Cryptosporidium, are highly prevalent intracellular pathogens presenting a characteristic apical complex, machinery for host cell invasion. Their cycle are complex, involving two parts, one asexual, with sporogonic division, including endodiogeny, and another sexual, involving gamete formation with meiosis and zygotes, usually in different host species. Recently, a non-photosynthetic chloroplast like organel, the apicoplast, was also described associated to specific amylopectin granules, chemically and structurally similar to those produced by red algae. These complex characteristics distinguish them from other parasitic protozoa, without sexual division, as Kynetoplastidae, resulting in diverse origin and phylogeny. One interesting group of disseminated plant pathogens, the Oomycota, presented some similar intriguing features, as biflagellate gametes similar as apicomplexan microgametes, similar storage sugars, and also active plant cell invasion. Here, we compare the sequences of rRNAs of several organisms including Phytophthora, other oomycetes and several apicomplexa, looking for their phylogenetic distance. We recovered the published sequences in the GeneBank, using the software MegAlign 4.00 expert sequence analysis software (DNAstar Inc.) with the ClustalV algorithm for alignment and dendrogram construction. The dendrogram of the 5S rRNA showed some clustering between oomycetes and Apicomplexan sequences, without including other tested Kynetoplastidae parasitic protozoa, similar to published reports analyzing tubulin genes. We also tested other sequences of complete large and small rRNAs with not so clear results. Our preliminary data argue for a common ancestor between Apicomplexa and Oomycota parasites, probably Algae, as the red algae elsewhere described. This evolution as parasitic agents was successful for the Apicomplexa, specially with the surge of complex metazoans, with intestinal tubes and large bodies. This diverse origin of parasitic protozoa could explain different susceptibility to therapeutic drugs and offers alternatives for prevention and control of those important parasitic diseases. Supported by LIMHC/FMUSP.

## EP03 - TOXOPLASMOSIS SEROPREVALENCE IN SMALL AND LARGE CITIES IS NOT RELATED TO RURAL AND URBAN AREAS IN SÃO PAULO, BRAZIL

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Toxoplasmosis is an widely distributed zoonoses in humans and animals, caused by an obligate intracellular parasite, Toxoplasma gondii. The disease is transmitted by ingestion of oocysts shed in cat stools or by cysts in undercooked meat. Usually, the transmission is also attributed to low sanitation, suggesting that people who live in rural areas, without sanitation, must have higher incidence of this disease. In the present study, we analyzed 133 samples from Pinhalzinho, São Paulo, a typical 15.000 hab small city in South-East Brazil, with a large rural population, comparing to the seroprevalence in 873 samples from a large urban ten million city, nearly without rural areas and adequate sanitation. The urban area of the small city presented good sanitation, with sewage system and treated water but people who live in rural areas presented only self promoted sanitation. We evaluated the seropositivity for toxoplasmosis in samples by IFI or using an in house IgG ELISA, with IFI checked standardization. Samples were divided by sex, or in ten years age groups, starting after adolescence. Results: The age-corrected prevalence was no gender difference in both cities, but was higher in the small city, over 75% after age 40, as

related to the stable 60% found in the same age group in the large city population. There are no differences between rural or urban people in the small city, who presented similar distribution. This data suggests that the transmission of toxoplasmosis is more efficient in a small city with large rural areas, probably due to local production and distribution of most meat or food products, resulting in higher transmission to the whole small city, which is prevented in large cities. This work was supported by LIMHCFMUSP and fellowships (HFAJr.-CNPq, MSM CAPES, MAMM& TACS-FUNDAP)

## EP04 - Determining the repertoire of the *var*, *rifin* and *stevor* gene families of Amazonian *Plasmodium falciparum* isolates

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The *Plasmodium falciparum* genome contains the multigene families *var*, *stevor* and *rif*, which code for antigens responsible for the pathology of this organism. The *var* gene family encodes highly polymorphic *P. falciparum* erythrocyte membrane proteins 1 (PfEMP1). PfEMP1s have an important role in cytoadherence and immune evasion of infected erythrocytes. While plenty information regarding the diversity and diversity-creating mechanisms of the *var* genes was accumulated, almost no data are available for the *stevor* (subtelomeric variant open reading frame) and *rif* (repetitive interspersed family) genes. In this study, we simultaneously analyze the repertoire of *var*, *stevor* and *rif* genes in cloned isolates from the Western Brazilian Amazon (Porto Velho, RO). We amplified and sequenced variable regions from each of the three multigene families (*var* DBLalpha region, exon 2 of *stevor* and *rif* genes) and analyzed 50 clones of *var* and *rif*, and 40 clones of *stevor* genes. Sequence analysis of nine isolates of the *var* gene family showed 16-28 unique DBLalpha sequences per isolate. The number of shared sequences between each of the clones was 4,7/- 2,42, ranging from 3 to 10 shared sequences. When comparing each sequence identified in one parasite clone, the average diversity was 36,6%. From 450 sequences, 103 were different. For *stevor*, we found 11-21 unique sequences for each parasite clone, and 57 unique sequences from nine clones (360 sequences). The average diversity of *stevor* genes of each clone was 23,4%, indicating a lower diversity than found for *var* genes. One *stevor* sequence was present in all isolates and 29 sequences were present in more that one isolate. This indicates that despite of comparable genomic localization, the variation of *stevor* genes is significantly lower than that of *var* genes. The *rif* analysis is currently being concluded and results will be shown. Supported by FAPESP

## EP05 - A cheaper test for *Leishmania* identification adapted from SSU-based methodology.

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PCR assays have been widely used to identify pathogenic organisms such as virus, bacteria, fungi and protozoa. For the genus *Leishmania* these assays have employed primers based on the sequences of kDNA, SSU, mini-exons, sub-telomeric sequences, alfa-tubulin, G6PD and RAPD generated fragments. However, their specificity depends on the target and they may identify the parasite to the generic, subgeneric [*L. (Viannia)* and *L. (Leishmania)*] or species level.

The SSU-based test initially described by Uliana *et al*, 1994 and subsequently improved by Uliana *et al*, 2000 relies on a two step procedure: an initial PCR assay is followed by a hybridization step with four oligonucleotide probes. The second hybridization is essential since the primary PCR is positive with the DNA of other trypanosomatids, some of which are sympatric with *Leishmania* species.

Exploring these same SSU nucleotide differences, we designed new primers to perform a nested PCR assay followed by the sequence determination of the amplicon. This PCR is positive with the DNA of *Leishmania* species of both subgenera and negative with the DNA of *Crithidia fasciculata*, *Trypanosoma cruzi* (Y strain), *Phytomonas macgheei*, *Endotrypanum schaudinni* and human. DNA sequencing of the PCR products distinguished *L. (L.) amazonensis*, *L. (L.) chagasi*, *L. (L.) mexicana* and *L. (Viannia)* sub-genus species. In recent years DNA sequencing has become a routine technique in many research centers which has greatly decreased the cost. Nowadays, it is cheaper to perform a single sequencing reaction than several radioactive hybridizations. Besides, this DNA sequencing can be extremely fast and is safer than hybridization with radioactive probes. We have successfully used the nested SSU PCR with DNA extracted from naturally infected sand flies, human paraffin-embedded biopsies and biopsies from dogs, including a dog that was also infected with *Trypanosoma evansi*.

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