Biologia Molecular - Molecular Biology

BM001 - THE eIF4F COMPLEX IN TRYPANOSOMATIDS: MAPPING INTERACTIONS THROUGH PULL-DOWN AND IMMUNOPRECIPITATION ASSAYS

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A LIKELY TARGET FOR MECHANISMS REGULATING GENE EXPRESSION IN TRYPANOSOMATIDS IS THE INITIATION STAGE OF PROTEIN SYNTHESIS, OR TRANSLATION, WHEREAS THE COMPLEX EIF4F (FORMED BY THE SUBUNITS EIF4A, EIF4E AND EIF4G) PERFORMS A CRUCIAL ROLE BY FACILITATING THE RECOGNITION OF THE MRNA BY THE RIBOSOMES. SURPRISINGLY, SEVERAL HOMOLOGUES OF EIF4E (EIF4E1-4) AND EIF4G (EIF4G1-5) HAVE BEEN IDENTIFIED IN THE TRYPANOSOMATIDS AND WE HAVE PREVIOUSLY STARTED TO INVESTIGATE THEIR FUNCTION IN *L. MAJOR* AND *T. BRUCEI*. AS PART OF THIS STUDY, WE HAVE HERE INVESTIGATED POSSIBLE INTERACTIONS BETWEEN SEVERAL OF THESE HOMOLOGUES. FIRST, THROUGH PULL-DOWN ASSAYS, WE HAVE CONFIRMED THAT AT LEAST TWO OF THE *L. MAJOR* EIF4GS (EIF4G3-4) BIND SIGNIFICANTLY TO THE EIF4A HOMOLOGUE (EIF4AI). THESE SAME TWO PROTEINS HAVE STRIKINGLY DIFFERENT AFFINITIES FOR SOME OF THE EIF4E HOMOLOGUES. THUS, EIF4G3 BINDS SPECIFICALLY TO EIF4E4 WHILST EIF4G4 BINDS TO EIF4E3. USING SITE-DIRECTED MUTAGENESIS OF EIF4G3-4 WE WENT ON TO MAP THE BINDING SITES FOR THE EIF4A AND EIF4E HOMOLOGUES. MUTAGENESIS OF THE CONSERVED RESIDUES LNK TO AAA IN BOTH PROTEINS DRASTICALLY REDUCED THEIR INTERACTION WITH EIF4AI. IN CONTRAST, DIFFERENT RESIDUES WERE IMPLICATED IN THE BINDING TO EIF4E3-4. WHILST THE SUBSTITUTION OF THE RESIDUES FSL (ALSO CONSERVED IN BOTH PROTEINS) TO AAA IN EIF4G3 PREVENTED ITS BINDING TO EIF4E4, MUTAGENESIS OF A SECOND SET OF RESIDUES, I25L26, WAS REQUIRED ON ITS OWN TO ABOLISH THE BINDING OF EIF4G4 TO EIF4E3, IMPLYING DIFFERENT MODES OF EIF4E RECOGNITION BY THE TWO PROTEINS. THE INTERACTIONS OBSERVED WITH THE *L. MAJOR* PROTEINS WERE ALSO CONFIRMED FOR THEIR *T. BRUCEI* ORTHOLOGUES *IN VITRO*, THROUGH A NEW SET OF PULL-DOWN ASSAYS, AND *IN VIVO*, THROUGH IMMUNOPRECIPITATION USING POLYCLONAL SERA DIRECTED AGAINST THE VARIOUS PROTEINS. THESE RESULTS REINFORCE THE EXISTENCE OF AT LEAST TWO EIF4F COMPLEXES IN TRYPANOSOMATIDS, WITH FUNCTIONAL ROLES IN TRANSLATION YET TO BE DEFINED.

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BM002 - TRANSCRIPTION AND EXPRESSION PROFILE OF A TGF-BETA FROM *LUTZOMYIA LONGIPALPIS*, IN IMMATURE DEVELOPMENTAL STAGES, THROUGH ADULT BLOOD DIGESTION, AND *LEISHMANIA* INFECTION

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Lutzomyia longipalpis is the main vector of visceral leishmaniasis in Brazil. We are studying sand fly molecules potentially involved in insect-parasite interaction to help to understand the mechanisms under which *Leishmania* must endure in order to establish infection in the insect midgut. Through DDRT-PCR we have previously identified a *L. longipalpis* TGF-beta gene from
RNA of sand flies fed with blood containing *Leishmania infantum chagasi*, and its cDNA was sequenced. Its identity was assumed by similarity with other TGF-beta sequences, and was closely related to the family of activins/inhibins. The transcriptional profile of this gene was studied by RT-PCR with specific TGF-beta primers. We amplified cDNA samples obtained from immature developmental stages (egg, L1, L2, L3, L4 and pupae), non fed adult insects (male and female) and blood fed females (2h to 72h post blood meal - PBM). Samples obtained from females artificially infected with *L. i. chagasi* were also studied. Dissected midguts and carcasses were analyzed separately whenever was possible. Midgut samples showed an increase of gene transcription in 2h and 48h PBM, while carcass samples showed a peak at 72h PBM. Comparing samples obtained from whole insects at 72h PBM and post artificial infection, we found that TGF-beta transcription is increased six times on infected insects. A fragment of the protein was previously expressed and used for the production of a polyclonal antibody. Western-blot experiments using this antibody detected two bands, one of approximately 66 kDa and another of 30 kDa. Curiously the band with higher molecular weight was seen only between 2h and 24h PBM and the other is present in every sample. Experiments with midgut are still being performed. This same antibody will be used in immunolocalization experiments, with confocal microscopy. We believe that TGF-beta production is modulated upon blood digestion process and increased as an insect response to *Leishmania* infection.
Supported by CNPq, CAPES, FAPERJ.

BM003 - THE *LEISHMANIA MAJOR* Sc-Sir2 HOMOLOGUES AND THEIR POSSIBLE ROLE IN GENE SILENCING

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An important aspect of the organization and expression of eukaryotic genomes is the demarcation of transcriptionally active and inert regions. Therefore, posttranslational histone modifications, such as acetylation, methylation or phosporylation play an important role in transcriptional regulation. Sir-2 belongs to the NAD^+-dependent histone deacetylase (HDAC) family and its implication in histone modification has been widely documented. In silico analysis revealed that Sir-2 has three homologues in *Leishmania major* genome. From these, LmSIR-2 RP3 is the best candidate to be involved in genomic silencing mechanisms. This is based on our observations that: (i) the product of this gene is localized to the nucleus; (ii) its overexpression confers resistance to DNA-targeting drugs suggesting its involvement in DNA repair, as described in *Saccharomyces cereviasae* and *Trypanosoma brucei*; and (iii) its expression can modulate the expression of a telomere-located gene. This last observation came from experiments in which LmSIR-2 was expressed into a parasite cell line bearing a selectable marker integrated at the end of chromosome 20. In this lineage, high levels of SAT (streptotrycin acetyl transferase) marker expression is observed after previous cultivation in the presence of the selectable drug (nourseothricin). SAT expression is notably reduced after cultivation in the absence of the drug. Following transfection and cloning, the expression of LmSIR-2 led to a reduced expression of SAT, even after cultivation in the presence of nourseotrhicin. Our results are indicative that LmSIR-2 RP3 may play a role in epigenetic mechanisms of gene expression regulation in the protozoan *Leishmania*.
Supported by FAPESP and CNPq.

BM004 – RIBOSOMAL PROTEIN L19 OF *LEISHMANIA SPP*: CHARACTERIZATION OF ITS PATTERN OF EXPRESSION DURING THE LIFE CYCLE AND INVESTIGATION OF AN EXTRA-RIBOSOMAL FUNCTION

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There are different reports showing that ribosomal proteins may play extra-ribosomal functions. Little is known about the ribosomal proteins in the Trypanosomatidae family and in our laboratory we have observed that the transcript for the ribosomal protein L19 (RPL19) is amastigotes present at different levels during the parasite life cycle. Therefore, we decided to investigate a possible extra-ribosomal role for RPL19. We generated transfectant parasites of *L. major* (LV39) and *L. braziliensis* (Lb2904) that overexpresses the transcript and L19 protein. Promastigotes of independent clones present an impaired growth and reach the stationary phase one day after the parental line. We evaluated levels of transcript and protein L19 throughout the parasite life cycle of *L. major*, *L. braziliensis, L. donovani* (HU3), *L. amazonensis* (M2269) and transfectants. Northern blots probed with the L19 gene and Western blots probed with polyclonal antibodies raised against *L. major* RPL19 revealed that the transcript and protein levels are higher in the early phases of the culture and suffer a marked decay in stationary phase promastigotes. The comparison of RPL19 levels between *L. major* and *L. braziliensis* reveals that *L. braziliensis* presents about 3.5 times less protein than *L. major.* In addition, considering that an extra-ribosomal role of L19 could be to control levels of expression of a gene or a group of genes, *L. major* transfectants and the parental line were submitted to comparative proteomic analysis in order to identify possible targets of control by the ribosomal protein. The differentially expressed proteins detected in 2D gels were identified by mass spectrometry. The identified differentially expressed genes will be tested regarding a possible interaction of their transcripts with RPL19
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BM0005 - Pre-rRNA *trans*-splicing: new targets for old mechanisms

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Trypanosomatids regulate gene expression mainly at the post-transcriptional level. Large pre-mRNA polycistronic units are converted to monocistronic mature messages by accomplished *trans*-splicing and polyadenylation. Preliminary results suggested that *trans*-splicing is not an exclusive pre-mRNA processing mechanism, but it can also use 5'ETS pre-rRNA sequences as substrates. In fact, the SL addition *trans*-splicing could be detected at the 5' ETS region of pre-rRNAs from four trypanosomatid species (*T. brucei, T. cruzi, L. (L.)* amazonensis and *C. fasciculata*) that obeyed the same basic rules described for pre-mRNA *trans*-splicing. RNase protection and primer extension experiments strengthened that SL-5'ETS molecules indeed correspond to native products in *L.(L.)amazonensis.* Moreover, real-time RT-PCR experiments (qRTPCR) using *L.(L.)amazonensis* total RNA extracted from cells treated with *trans*-splicing inhibitor sinefungin showed that SL-5'ETS products diminish in concentration while non-spliced 5'ETS molecules remained constant. qRTPCR also revealed that SL-5'ETS hybrid molecules are present in a low copy number. Treatment of the cells with exosome inhibitor 5-fluoruracyl resulted in accumulation of the SL-5'ETS molecules in a dose dependent manner, suggesting they can be discarded, at least in part, by exosome degradation route. If *trans*-splicing is used to address 5'ETS processing products to a degradation route, we could expect to find the same process in other pre-rRNA spacers. In fact, RT-PCR experiments addressing acceptor splice sites in *L.(L.) amazonensis* internal transcribed spacer I (ITSI) showed other three different pre-rRNA *trans*-splice sites. Finally, we detected polyadenylation products upstream from the 5'ETS SL addition sites. This polyadenylation event was expected since *trans*-splicing and polyadenylation are accomplished mechanisms in trypanosomatids. We are currently investigating if these polyadenylation could function as flags for 5'ETS degradation, as proposed for SL sequences. Here, for the first time we show that trypanosomatids' pre-rRNA molecules can also be processed by SL-addition *trans*-splicing as well as by polyadenylation. Supported by:FAPESP, CNPq.

BM006 - AN ATYPICAL PEPTIDIL-PROLYL *CIS/TRANS* ISOMERASE IS REQUIRED FOR *Trypanosoma brucei* CELL PROLIFERATION

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The parvulin family of peptidyl-prolyl *cis/trans* isomerases (PPIases) catalyzes the *cis/trans* isomerization of the peptide bonds preceding Pro residues and it has been suggested they play a critical role controlling cell-cycle progression. A novel member of the parvulin family of PPIases named Par45 was identified in the TriTryp genome. Analysis of the sequence of Par45 showed the existence of the sequence motifs typical of the parvulin catalytic core. Like most other parvulins, Par45 has an N-terminal extension but in contrast to hPin1, Par45 contains a FHA instead a WW domain at N-terminal end. A comparison of the relative values of the specificity constants for various substrates shows a general pattern as it was found for Par14 with a strong preference for a substrate with the basic Arg residue preceding Pro (Suc-Ala-Arg-Pro-Phe-NH-Np: k_{cat}/K_M = 97.1 /M/s). In contrast to Pin1 but like Par14, Par45 does not accelerate the cis/trans interconversion of acidic substrates containing Glu-Pro bonds. By using a proteomic approach and YTH analysis we found that Par45 associate with proteins involved in transcription and/or RNA processing. Immunolocalization of Par45 corroborates the presence of the parvulin within the nuclei of parasites. Single RNA interference (RNAi)-mediated knock-down of Par45 suggest lethality and thus a required function shared by others Pin1-type PPIases. These findings coupled with the other results suggest that Par45 is linked to the RNA processing machinery and is necessary for normal cell proliferation in *T. brucei*.
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BM007 - CHARACTERIZATION AND UTILIZATION FOR PHYLOGEOGRAPHICAL ANALYSIS OF SPLICED LEADER GENES FROM *TRYPANOSOMA* (*MEGATRYPANUM*) *THEILERI*

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Trypanosoma (*Megatrypanum*) *theileri* and related species are non-pathogenic and widespread parasite of artiodactyls, with high incidence in cattle, sheep, buffalo, antelopes and cervids. Despite alleged host-specificity, species from distinct hosts clustered together in phylogenetic analyses. Moreover, phylogenies based on SSU and ITSrDNA revealed a complex branching pattern, with lineages of cattle isolates apparently related to geographic origin. Analysis of more variable sequences of a larger number of trypanosomes from broader geographic origins can help to understand segregation of *T. theileri* lineages. In this study, we determined sequences of spliced leader (SL) gene of cattle and water buffalo isolates from distant Brazilian regions. Sequences of whole SL repeats of *T. theileri* showed the 5SrRNA gene inserted within the intergenic region. These sequences were employed to assess phylogeographical patterns of 22 Brazilian *T. theileri* isolates based on polymorphisms of intron and intergenic sequences. Results showed small length variability but relevant sequence divergence among isolates of *T. theileri* established in culture, revealing at least 5 genotypes distributed in the major lineages I and II. Lineage I comprise three genotypes, IA and IB respectively from buffalo and cattle from Southeast and Central regions, while genotype IC is restricted to cattle from Southern region. Lineage II comprises 2 major genotypes: IIA, found in cattle from Central, Western, Northern and Northeast regions; IIB restricted to isolates from Northern and Northeast regions. In addition, mixed genotypes of lineage II could be found from primary cultures, evidencing selection of genotypes by successive passages in culture. PCR-RFLP of SL gene revealed to be valuable tool for genotyping *T. theileri* isolates. Results from this study based on SL polymorphic sequences corroborate the complexity and geographical population structure of *T. theileri* lineages.
Supported by CNPq.

BM008 - HETEROLOGOUS EXPRESSION OF *Trypanosoma cruzi* GP82 IN THE LIFE-CYCLE OF *Trypanosoma rangel*
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The mystery of *T. rangeli* propagation in the mammalian host is long-standing. Two possibilities exist, either *T. rangeli* trypomastigotes are able to propagate unobserved in an extracellular niche – or a replicative amastigotes form can replicate intracellularly in cell types so far unobserved. *T. cruzi* is a sibling species of *T. rangeli* in which the latter mechanism is well established. The gp82 protein is paradigmatically involved in cell recognition/invasion for *T. cruzi*. We reasoned that trans-expression of this molecule in *T. rangeli* could facilitate invasion and test the hypothesis that propagation can occur by amastigogenesis in cultured cell lines readily tractable to investigation. *T. cruzi* gp82 gene was included in a pTEX modified construct that also contains the GFP gene. After transformation, the stable expression was followed by observation of GFP fluorescence and western blot using *T. cruzi* gp82 3F6 monoclonal antibody. The gp82-expressing parasites were able to complete the metacyclogenesis and remained infective to mice and triatomine bugs (cyclic passages), showed no difference on parasitemia levels when compared to wild type cells. The levels of protein expression were maintained during the infection time course in absence of selective drug (G-418), being observed green fluorescent parasites in digestive tracts of infected bugs and hemocultures three months after mice infection. Further molecular and microscopy studies are in progress to assess the outcomes of the *T. cruzi* gp82 expression by *T. rangeli*, including interaction assays between transfected trypomastigotes and primary cell lineages. Supported by CNPq, CAPES, BMRC/UEA and UFSC.

BM009 - PARTIAL GENOMIC ANALYSES OF *TRYPANOSOMA RANGELI* BY GSS (GENOMIC SEQUENCE SURVEY)

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The hemoflagellate protozoan parasite *Trypanosoma rangeli* shares several invertebrate and vertebrate hosts as well as antigens with *T. cruzi*. Despite extensive studies on the genomes of the trypanosomatids with importance in human health (*T. cruzi*, *T. brucei*, *Leishmania major*, *L. braziliensis*), non-pathogenic species such as *T. rangeli* have not been systematically studied. Distinct approaches have been used on genomics of these species, specially GSS (*Genome Sequence Survey*), EST (*Expressed Sequence Tags*) or ORESTES (*Open Reading Frame EST*). In this study, GSS libraries from *T. rangeli* SC-58 and Choachí strains were prepared and sequenced. A total of 3,284 genomic sequences generated and analyzed using the STINGRAY system. After quality analysis by Phred/Phrap software (phred ≥20), a total of 1,342 sequences were grouped by CAP3 in 719 non-redundant sequences (GSS-nr), consisting in 544 singlets and 175 clusters. The G+C content of the GSS-nr and the coding regions were 50% and 56% respectively, being similar to *T. cruzi* (53,4%) than to *T. brucei* (50,9%). Similarity searches based on BLAST and Interpro were positive to 635 of the sequences, 47% of which with trypanosomatids, mainly *T. cruzi* (333). Among these sequences, 42% were annotated as coding sequences and around 70% of these were annotated as hypothetical proteins. For 84 sequences it was not possible to infer functions based on similarity searches and these may represent unknown sequences, *T. rangeli* specific sequences or even intergenic regions. Comparison of the GSS-nr sequences with the *T. rangeli* EST database from our group, which contains around 12,000 EST sequences, only 27% revealed similarity. These results have quantitatively and qualitatively increased the *T. rangeli* genomic database, resolving sequencing quality issues on common genes such as sialidase, surface proteases or glycoproteins, as well as pointing out new genes for this taxon through the combined use of GSS and EST.
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BM010 - Molecular markers for phylogenetic analysis, diagnosis and genotyping of bat trypanosomes based on Cathepsin L-like and ribosomal genes, and comparison with morphological and biological parameters

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Chiropterans are commonly infected by trypanosomes, showing high prevalence of species of the subgenus *Schizotrypanum* including *T. cruzi* and species exclusive of bats denominated *T. cruzi*-like (*T. cruzi marinkellei*, *T. dionisii* and *T. vespertilionis*). *T. cruzi* and *T. c. marinkellei* occur in Central and South Americas where they share hosts and overlapping regions with *T. dionisii* and *T. vespertilionis* found in the New and Old World. Reliable taxonomical parameters and methods for identification are required for studies of prevalence, distribution, diversity and evolutionary history of bat trypanosomes. In this study, we determined Cathepsin L-like sequences homologous to cruzipain from isolates of Brazilian *Schizotrypanum,* and inferred phylogenies with sequences from other bat trypanosomes of this subgenus from Europe, *Megatrypanum* species from Brazil and Africa, and *T. rangeli* from Brazilian bats. Trees were congruent with those of SSUrRNA. All *Schizotrypanum* trypanosomes nested within the clade *T. cruzi,* which was more closely related to *T. rangeli* than to other bat trypanosomes. Polymorphisms of all genes allowed the definition of markers useful for identification and genotyping of bat trypanosomes. CatL-like and SSUrRNA sequences proved to be good targets for PCR-RFLP assays for easy and species identification. Comparison of molecular and morphological data demonstrated that morphology of blood and culture forms and intracellular behavior could help to classify bat trypanosomes in subgenera, but do not allow a definitive classification nor differentiate between *T. cruzi* and *T. cruzi*-like species, which could be achieved by behavior in mice and triatomine bugs. Morphological and biological characterization, besides laborious and time-consuming, was not enough to discriminate *T. cruzi*-like trypanosomes. Therefore, molecular methods developed in this study are valuable tools for analysis of genetic diversity and specific and easy identification of bat trypanosomes. Supported by CNPq and Fapesp (Pronex)

BM011 - REGULATORY ELEMENTS IN HSP70 mRNA MODULATE STABILITY AND TRANSLATION DURING HEAT SHOCK IN *Trypanosoma cruzi.*

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The understanding of control of gene expression of members of the chaperone family will shed light on the mechanisms of post-transcriptional regulation through which trypanosomatids govern the fate of most of their mRNAs. We have determined that at 29ºC the HSP70 mRNA shows a half-life of 60 minutes in an exponential kinetics, while at 37ºC the half-life is increased to 120 minutes. In addition, the stabilization of HSP70 mRNA at 37ºC is lost when protein synthesis is inhibited. We have also determined the contribution of untranslated regions (UTRs) of the mRNA to HSP70 gene regulation during heat shock. Plasmids containing the CAT reporter gene, flanked by intergenic regions containing either the 5' or 3' UTR and their respective regulatory sequences of the HSP70 mRNA were constructed. Our results show that both the 5'- and 3'-UTRs confer temperature-dependent regulation to a chloramphenicol acetyltransferase (CAT) reporter gene, and that the effects of each on CAT induction appear to be additive. We also show that, while each UTR separately is unable to affect CAT mRNA stability at 37ºC, the presence of both UTRs lead to a two-fold increase in CAT mRNA levels, indicating that the 5'- and 3'-UTRs act cooperatively to stabilize HSP70 mRNA during heat shock, and both UTRs can contribute individually and in combination to increase translational efficiency at 37ºC. We have also investigated the function of an AU-rich element present in the 3`-UTR of the HSP70 mRNA, and we found that it may act as a destabilizing element of its mRNA at 29ºC.
Supported by CNPq and FAPERJ.

BM012 - INVESTIGATING THE ROLE OF *TRYPANOSOMA CRUZI* AMASTINS IN HOST-PARASITE PROTEIN INTERACTIONS WITHIN THE CYTOPLASM OF INFECTED CELLS.

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T. cruzi amastigotes express on their surface stage-specific molecules that are likely to be involved in protein interactions with host cell molecules present in the cytoplasm. Amastins are 170-200 amino acids, highly hydrophobic surface glycoproteins expressed not only in T. cruzi amastigotes but also in amastigotes from several species of *Leishmania*. The *T. cruzi* genome contains several copies of amastin genes organized in a cluster containing alternated copies of tuzin genes, which encode a G-like protein. Although they are constitutively transcribed, amastins mRNAs are 50-fold more abundant in amastigotes when compared to epimastigotes or trypomastigotes. Analyses of amastin genes present in the genome of various strains showed that they encode highly divergent proteins with increased amino acid variability in the protein domain that is likely in contact with the host cell cytoplasm. In order to gain new insights into their function, we investigated the effect of over-expressing amastins in the parasite and performed yeast two-hybrid assays to identify human proteins that are able to interact with them. In tissue culture infection assays, transfected *T. cruzi* cell lines over-expressing amastins showed lower infectivity and higher intracellular multiplication compared to the wild type. Transfected epimastigotes also showed increased cell division rates in LIT medium. Using a yeast two-hybrid screening assay we obtained evidences indicating that amastins interact with various human proteins including those involved with intracellular trafficking and cell signaling. Currently we are in the process of validating these interactions using in vitro and in vivo pull-down experiments, co-immunoprecipitation assays and cellular localization to further investigate the role of host cell proteins/amastin interactions in the course of the *T. cruzi* infection. Supported by: CNPq, FAPEMIG and HHMI.

BM013 - *Trypanosoma cruzi* produces a novel population of tRNA-derived small RNAs which are recruited in specific cytoplasmic granules.

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Until recently most of the known non-coding RNAs (ncRNAs) have relatively generic functions in cells. Over the years, an expanding family of regulators ncRNAs do not having these generic functions have been discovered. In the last decade there has been described a family of small RNAs ("sRNAs") of 20-30 nt whose biological roll is the defense of the genome and the regulation of gene expression at transcriptional and post-transcriptional level. This novel form of regulation of the gene expression has been found in almost organisms (early and higher eukaryotes). The enzymatic machinery associated with the biogenesis and effectors mechanisms of these "sRNAs" has been found in the majority of the organisms studied. The exceptions for this are some species of trypanosomatids including *Trypanosoma cruzi* (etiological agent of Chagas), and *Leishmania major* (Leishmaniasis). This accounts for the absence of interference of RNA in the above mentioned parasites. In the present work we have analyzed by cloning the "sRNA" population of total RNA of *T. cruzi*. Our results showed a highly represented population of small RNAs derived from de cleavage of mature tRNAs representing about 30% of small RNA population which we named "mini-tRNAs". Surprisingly, more than 98% of mini-tRNAs derived from the 5' half of tRNA for Asp and Glu and localize in particular granular structures in the cytoplasm of *T. cruzi* at all stages of its life cycle. Whereas the biological significance is currently unknown this "mini-tRNAs" population ~~it~~ has been described in the last year in other organism as *Giardia lamblia, Aspergillus fumigatus* and many mammalian cancer cells. This could represent a new family of small RNAs with relevance in gene expression regulation in particular for these unicellular parasites which gene regulation is principally at post-transcriptional level.

BM014 - *Trypanosoma cruzi* BDF2, a protein that senses histone acetylation and could participate in chromatin dynamics.

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Histone tail post-translational modifications regulate many cellular processes. Among these modifications, phosphorylation, methylation and acetylation have already been described in trypanosomatid histones. Bromodomains, together with chromodomains and histone-binding SANT domains, were proposed to be responsible for the ''histone code" reading. The *Trypanosoma cruzi* genome encodes four coding sequences (CDSs) that contain a bromodomain, named TcBDF1-4. We have shown that one of those, TcBDF2, is expressed in discrete regions inside the nucleus of all the parasite life cycle stages, between dense and less dense chromatin regions. TcBDF2 binds H4 (with preference for K10 and K14 acetylated residues) and H2A purified histones from *T. cruzi*. Also, this protein was accumulated after UV irradiation of epimastigotes. These results suggest that TcBDF2 could be implicated in chromatin remodelling as part of a larger protein complex as other known bromodomain proteins. This kind of complexes has a broad range of functions participating in genome expression, maintenance and duplication in other organisms, A partial TcBDF2 colocalization with RNA polymerase II was observed in immunolocalization experiments. In order to analyze TcBDF2 function we attempted expression of a dominant negative TcBDF2. TcBDF2 fused to c-myc tag and C-terminal deleted TcBDF2 (TcBDF2ΔC) transgenes were expressed from a tetracycline regulated promoter using pTcINDEX-I vector. An important reduction in growth rate was observed in parasites expressing TcBDF2ΔC as well as cells having abnormal combinations of kineto-nucleo. In addition, these parasites presented diminished resistance to UV irradiation compared to wild type. Although more results are needed to understand the role of TcBDF2 in trypanosomes biology, TcBDF2 accumulation after UV irradiation and its ability to interact with acetylated histones support the hypothesis that it could have a role in chromatin dynamics as a member of a chromatin remodelling complex. Supported by ANPCyT, CONICET, CNPq and FAPESP.

BM015 - FUNCTIONAL CHARACTERIZATION OF A NOVEL ARGININE TRANSPORTER FROM TRYPANOSOMA CRUZI

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Transporters play an important role in trypanosomatids, keeping cellular homeostasis, importing nutrients, exporting toxic substances and in drug resistance. *Trypanosome cruzi* is auxotrophic for arginine and its requirement is supplied from the host through specific transporters. Our purpose was to perform the characterization of a novel arginine transporter from *T. cruzi*, TcCAT1.1, in heterologous systems. Substrate saturation curves were made in *S.cerevisiae* and an apparent K_m of 85 □ 36,37 □M was inferred for [^{3}H]-arginine. Competition assays were performed with 100 fold competitor over [^{3}H]-arginine, from the compounds tested, uptake was significantly inhibited by canavanine (70%). Ornithine, showed low affinity for TcCAT1.1, displayed 10% inhibition of [^{3}H]-arginine uptake and Km of 1,7 □ 0,24 mM, similar to other cationic amino acids. Trans-stimulation was observed in *X.laevis* oocytes expressing TcCAT1.1 pre-loaded just with arginine, whose [^{3}H]-arginine uptake increased 7 fold. Oocytes pre-loaded with [^{3}H]- arginine displayed 16 fold higher efflux of [^{3}H]-arginine, than control. *TcCAT1.1* is a member of a family composed by at least four copies in the genome, sequencing alignment and comparison analysis between TcCAT family and human CAT demonstrated low identity, 10%, at the amino acid sequence level. To investigate cellular localization of TcCAT isoforms, TcCAT1.1 and TcCAT1.2 were fused with EGFP. EGFP-TcCAT1.1 labeling was observed by confocal microscopy, in vesicles at the anterior region of epimastigotes. EGFP-TcCAT1.2, the isoform with 76 amino acids missing at the amino terminal region of arginine transporter is under investigation and the precise localization of both isoforms will be elucidated by immunocytochemistry. Microarray analysis demonstrated that the arginine transporter family, TcCAT, is upregulated under nutritional stress and down regulated in metacyclic. These results demonstrate that TcCAT1.1 operates as an exchanger and its physiological role for *T.cruzi* homeostasis and cycle is our next goal.
Supported by University of Pittsburgh, FAPERJ and CNPq.

BM016 - Genetic variability of surface glycoprotein GP85 gene family of *Trypanosoma cruzi*: the involvement of telomeric regions and rearrangements after DNA double strand breaks

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Members of GP85 gene family of *T. cruzi* display great sequence diversity and encode many surface glycoproteins related with cell invasion and evasion from the host immune system. Subtelomeric regions of *T. cruzi* chromosomes are rich in GP85 (pseudo)genes. We have previously suggested that the preferred telomeric location of GP85 genes could be connected to the generation of new variants by recombination. Recently, Levin and co-workers (unpublished results) developed a *T. cruzi* artificial chromosome (pTAC) as a tool to explore structural and functional aspects of chromosomes in this parasite. pTAC contains the neomycin gene for selection of transfectants. Digestion with Hind *III* exposes *T. cruzi* telomeric repeats at both ends of the vector. To investigate the role of subtelomeric regions in recombination of GP85 genes, epimastigotes (CL Brener) were transfected with pTAC vector carrying a GP85 pseudogene (φGP85) isolated from the subtelomeric region. pTAC-φGP85 construct was generated using φGP85 and its 5´ flanking sequences, a truncated retrotransposon hot spot (RHS) protein and SIRE, in fusion with the Green Fluorescent Protein gene. In order to obtain single cell-derived clones, epimastigotes stably transfected were cloned by the minimal dilution method. Eleven pTAC-φGP85 clones were analyzed by hybridization and PCR. No recombination events involving displacement of pTAC-φGP85 sequences to different chromosomes of the parasite could be detected. Rearrangements of DNA in trypanosomes may occur in the repair of DNA double strand breaks (DSBs) that can be induced with ionizing radiation. To test the feasibility of generating recombination between pTAC- φGP85 sequences and chromosomes by DSBs, transfectants were submitted to radiation and cloned again by the minimal dilution method. When chromosomal DNA was examined after Southern blotting of PFGE gels, chromosome rearrangements could be detected by miniexon probes. These rearrangements will be analyzed through PCR assays using specific primers for pTAC and GP85 sequences. Supported by FAPESP, CNPq and UNU-Biolac.

BM017 - Characterization of *Crithidia deanei* endosymbiont porin: the prokariotic origin of bacterial outer membrane

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Crithidia deanei is a monoxenic protozoan of the Trypanosomatidae family that presents a symbiotic bacterium in the cytoplasm. This mutualistic association constitutes an excellent model to study the origin of organelles and cellular evolution. The symbiont is enclosed by two units of membrane like Gram-negative bacteria, however the peptidoglycan layer is reduced and the septum is absent during the cellular division. There is controversy about the origin of the outer membrane: some authors consider that it is derived from the host cell membrane, while others believe in prokaryotic nature. Molecular analysis showed that this symbiont is closely related to *Bordetella parapertussis*, which is classified in the β division of Proteobacteria. In the present study, we used bioinformatics, molecular and biochemical approaches to identify porins, which are typical outer membrane proteins, in the *C. deanei* endosymbiont envelope. A search on the endosymbiont genome annotation database identified a sequence that shares homology with porin genes of Gram-negative bacteria. The protein primary sequence presents some porins features and the secondary structure prediction indicates a β-barrel format with 18 transmembrane domains connected by hydrophilic loops. A recombinant protein of approximately 47 kDa was superexpressed, purified and used to produce a mouse antiserum. This antiserum was purified and the antibodies obtained were used in immunocytochemical approaches that revealed labeling at the endosymbiont envelope. Ultrastructural analysis by transmission electron microscopy supports the outer membrane localization for the protein. Further studies are necessary to analyse the structure and functions of the porin-like protein in the symbiont of trypanosomatids, but from now on, we can propose the prokaryotic origin of this bacterium outer membrane. Support by: CNPq, FAPERJ and CAPES.

BM018 - Gregarines (Apicomplexa) parasitic in marine invertebrates from the West Coast of British Columbia, Canada

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Gregarine apicomplexans are a diverse group of parasites that inhabit the intestines, coeloms and reproductive vesicles of marine, terrestrial and freshwater invertebrates. There are around 1400 known species and perhaps millions more have yet to be described. Comparative morphology indicates that some gregarines, the archigregarines such as *Selenidium* spp., possess several characteristics that have been retained from the most recent ancestor of all apicomplexans, such as extracellular feeding stages, a monoxenous life cycle, and a prevailing presence in marine environments. Most gregarines, however, represent highly derived parasites with novel ultrastructural and behavioural adaptations. An improved understanding of gregarine diversity will provide important insights into the molecular, cellular and life history properties of apicomplexans as a whole. Moreover, molecular phylogenetic studies on gregarine diversity are expected to help pinpoint the closest living relatives of *Cryptosporidium*. Comparative morphology and molecular phylogeny of several novel gregarine lineages isolated from different marine invertebrate host groups, such as sipunculids, tunicates, polychaetes and crustaceans collected from the West Coast of British Columbia, Canada will be addressed. These data help to understand the diversity and evolutionary history of gregarines and the novel ways in which these parasites have solved fundamental biological problems (e.g. nutrition, locomotion and reproduction).
Supported by Tula Foundation (Centre for Microbial Diversity and Evolution), NSERC 283091-04 and CIFAR (Program in Integrated Microbial Biodiversity).

BM019 - Phylogeny of the 'forgotten slime mold' *Fonticula alba,* reveals a key evolutionary position within Opisthokonta.

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Fonticula alba is a cellular slime mold that forms multicellular fruiting bodies by aggregation and limited differentiation of individual amoebae. Over the course of eukaryotic evolution multicellularity has independently arisen numerous times in nearly every eukaryotic supergroup, and is manifested in various degrees complexity and fashions of cooperation. Two of the most striking examples of multicellular lineages are found within Opisthokonta that contains the Metazoa and the Fungi, as well as several unicellular protist lineages. Amoebae with the ability to form fruiting structures are found within Amoebozoa (i.e., myxomycetes, dictyostelids, and protosteloid amoeba), Excavata (i.e., Acrasis within Heterolobosea), and possibly elsewhere in the Eukaryote Tree of Life. We set out to determine the phylogenetic affinity of *Fonticula* and to determine if it is specifically related to other fruiting amoeba. Phylogenetic analyses of 5 genes (nuclear-encoded genes; SSU rRNA, actin, beta-tubulin, elongation factor-1-alpha, and the cytosolic isoform of heat shock 70 gene) robustly place *Fonticula* within Opisthokonta as a specific relative to nucleariid amoebae. Together *Fonticula* + *Nuclearia* are sister to the Fungi. All other unicellular protists fall on the Metazoan (e.g., Holozoan) side of Opisthokonta. Single gene, SSU rRNA analysis shows that *Fonticula* does not branch with a monophyletic *Nuclearia*, consistent with the validity of its taxonomic status. *Fonticula* represents a 3rd type of multicellularity within Opisthokonta and minimally a 3rd independent derivation of multicellularity within this supergroup. *Fonticula* will likely be a key taxon to further elucidate the opisthokont genomic 'tool-kit' involved in multicellular development that has been suggested to aid in the group's success.

BM020 - Genomic aspects of initial speciation in *Paramecium* (Ciliophora)

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Chromosomal mutations are known as one of the main mechanisms leading to reproductive isolation. Though it is quite possible that divergence of syngens in ciliates (and, in particular, in *Paramecium*) is caused by chromosomal rearrangements, this issue remained completely unknown. There is no sufficient approach to study karyotype of micronucleus; as to macronuclear "karyotype", its existence was itself questionable due to very specific molecular organization of this nucleus genome. However, we performed molecular karyotyping of macronucleus by PFGE; more than 80 strains representing all 15 sibling species of *P. aurelia* complex, when possible – from geographically isolated populations, were analyzed. PFGE profile of the macronucleus reflects indirectly the frequency and distribution of fragmentation sites in the micronuclear chromosomes. Thus, any change of disposition, appearance or loss of a fragmentation site (detectable as a change in macronuclear molecular karyotype) may be not only a result of some point mutation, but also a consequence (and a marker) of a certain chromosomal aberration in micronucleus: inversion, deletion, transposition, etc. In total 12 main variants of electrophoretic macronuclear karyotypes were observed in *P. aurelia* species complex. 9 of them appeared to be species-specific (restricted to all or part of the strains of a certain species), while 3 other variants could be found in several species each. Significant intraspecific polymorphism was observed for several species (*P. biaurelia, P. septaurelia, P. novaurelia, P. decaurelia, P. dodecaurelia*); in some of these cases (*P. novaurelia, P. decaurelia, P. dodecaurelia*), a certain variant of macronuclear karyotype was specific for all strains from the same continent. Sharing of the same macronuclear karyotype variant between different species well corresponds to the data on possibility of interspecies conjugation. Distribution of the macronuclear karyotype variants along the most comprehensive molecular phylogenetic tree of *P. aurelia* complex (Hori et al., 2006) allows to conclude that isolation of each species or group of species of *P. aurelia* complex was accompanied by the appearance of differences in macronuclear genome molecular organization.

BM021 - DESCRIPTION OF A NEW SPECIES OF TRICHODINID (CILIOPHORA: PERITRICHIDA) BASED ON MORPHOLOGICAL AND MOLECULAR DATA

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Trichodina paraheterodentata sp. nov. was collected from the skin of *Siniperca chuatsi* in Chongqing. The new species, which belongs to genus, *Trichodina* Ehrenberg, 1830 (Ciliophora: Peritrichida), was identified by its entirely robust blade in adhesive disc and body. Moreover, the small subunit ribosomal DNA (SSU rDNA) of the new organism was also acquired and sequenced. Additional sequences were included based on Basic Local Alignment Search Tool (BLAST) searches. According to the phylogenetic trees using neighbor joining (NJ) conducted by Phylip software, our species is clustering with *Trichodina heterodentata* Duncan, 1977 and *Trichodina nobilis* Chen, 1963, which almost conforms to the morphological features.
Supported by the NSFCC (Project No. CSTC-2006BA1019) and founding of Chongqing Normal University (Project No. 08XLZ10).

BM22 - Characterization of mitochondrion-like organelle in *Mastigamoeba balamuthi*

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Mastigamoeba balamuthi is a free-living protist closely related to the human parasite *Entamoeba histolytica*. Both organisms are adapted for life in oxygen-poor environment. While mastigamoeba thrives in anaerobic freshwater, which is likely an ancestral trait, entamoeba underwent an adaptation to parasitic lifestyle. In the latter organisms, the adaptation resulted in the reduction of mitochondria to mitosomes, the organelles that lost most of mitochondrial functions including ATP synthesis, and function of which is still unknown in *E. histolytica*. In contrast, several components of mitochondrial pathways were recently predicted, based on EST survey, to be present in mitochondrion-like organelle (MLO) of *M. balamuthi* (Gill et al., 2007). Here we investigated the function of mastigamoeba MLO by means of cellular fractionation, Western blot analyses and enzymatic assays. In MLO-rich fraction we detected the activity of an NAD-specific malate dehydrogenase (MDH) and NADP-specific glutamate dehydrogenase. The presence of MDH in MLO-rich fraction was also confirmed by Western blot analysis. We also identified the activity of D-lactate dehydrogenase in the organelles. Importantly, the MLO-rich fraction exhibited ADP-phosphorylating activity, indicative of the presence of energy metabolism. However, we were unable to detect any activity of citric acid cycle enzymes. Enzymes known to be present in anaerobic mitochondria (hydrogenosomes) of trichomonads including pyruvate:ferredoxin oxidoreductase, hydrogenase and malic enzyme were in mastigamoeba found in the cytosolic fraction. Finally, NifS and NifU components of FeS cluster assembly machinery of e-proteobacterial origin were also detected in the cytosol and not in the organelles. These results indicate that mastigamoeba MLO are metabolically active, ATP-producing organelles. Formation of FeS clusters within the MLO remains enigmatic.

Gill et al., 2007. Mol. Microbiol. 66:1306-1320,

BM023 - GENOMICS APPROACH FOR THE ISOLATION OF SPECIES-SPECIFIC SEQUENCES IN *TRYPANOSOMA RANGELI*

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Trypanosoma cruzi and *Trypanosoma rangeli* are infectious agents which are able to infect humans. *T. cruzi* is the etiological agent of Chagas disease, whereas *T. rangeli* is non-pathogenic to humans. However, serological diagnosis of Chagas disease might be confused with infection by *T. rangeli*, generating a marked socioeconomic impact in countries where the disease is endemic. Accordingly, methods based on parasite DNA detection can be useful to determine the intraspecific polymorphism in these parasites and would constitute in an alternative for the differential diagnosis of infections caused by different trypanosomatids species. The aim of this work was to isolate *T. rangeli*-specific sequences and evaluate their use in the development of PCR reactions able to discriminate the infections caused by *T. cruzi* and *T. rangeli*. The analysis of 222 genomic sequences the P07 strain of *T. rangeli* allowed the identification of 84.2% sequences conserved between *T. rangeli* and other trypanosomatids and 15.8% of non-conserved sequences, potentially *T. rangeli*-specific. The primers ALP-Tr05/ALP-Tr06 and ALP-Tr07/ALP-Tr08 were designed from sequences with no sequence identity predicted in sequence databanks. Amplicons were observed in all *T. rangeli* strains and the amplification pattern allowed the discrimination between the KP1(+) and KP1(-) genotypes of the parasite, as defined by kDNA analysis. None of the *T. cruzi* strains used, with representatives of genotypes I and II and hybrids strains, showed amplification products with these primers. As control of DNA integrity, the primers ALP-Tr03/ALP-Tr04 were designed from a predicted Chaperone protein, highly conserved in *T. rangeli* and *T. cruzi*, and provided a 240bp amplicon in both species. The analysis allowed the specific amplification of *T. rangeli* sequences and, also, the discrimination between the different genotypes of the parasite, generating new perspectives for the molecular diagnosis of the infection caused by *T. rangeli*.
Supported by FAPEMIG and CNPq.

BM024 - Analysis of Genome Size, Repetitive DNA Sequences Content and Molecular Karyotype from *Trypanosoma cruzi* stocks – Conserved Features and Differences

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Genome size of stocks from Tc group I (G and Trycc:1161 strains; clone DM28c), Tc group II (Esmeraldo-cl3) and hybrid clones (CL Brener and SO3-cl5) were determined by different approaches. Total DNA content was estimated in hydroxyurea-arrested epimastigotes using fluorescent nucleic acid stain for dsDNA. The reliability of the assay was demonstrated using unbroken genomic DNA molecules and short DNA fragments obtained by sonication. The total DNA content and genome size, including the kDNA, were calculated for each *T. cruzi* isolate. Our data is agreement with CL Brener genome data considering 20-25% of total DNA is kDNA (~30-38 Mb).This study confirms *Tc*-I isolates genome size is smaller than *Tc*-II (Pedroso et al. 2003) except for Trycc:1161. We determined the relative amount of minisatellite sequences were two- to three -fold more abundant in *Tc*-II isolates than *Tc*-I. However, L1Tc is more abundant in hybrid clones, clone DM28c and Trycc:1161, a different pattern to literature data that shown repetitive sequences as less abundant in *Tc*-I isolates. Molecular karyotype and chromosome size-variation was analyzed. Clone DM28c and G strain showed 17 and 19 chromosomal-bands comprising between 0.60-2.69 Mb. Trycc:1161 displayed 20 chromosomal-bands between 0.55-3.03 Mb. This pattern is similar to others *T. cruzi* II genomes (19 to 20 bands ranging from 0.50-3.27 Mb). Despite the karyotype polymorphism, we have found several syntenic and linkage groups conserved among all isolates analyzed in this study. Probably, there is a selective pressure to keep gene order.
Supported by FAPESP and CNPq.

BM025 - INVOLVEMENT OF SERINE PROTEASES IN THE EXCYSTATION AND METACYSTIC DEVELOPMENT OF *ENTAMOEBA INVADENS*

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Although the functions of cysteine proteases involved in the pathogenicity and differentiation of *Entamoeba histolytica* have been demonstrated, little is known about the functions of serine proteases. We examined the involvement of serine proteases in amoebic excystation and metacystic development using inhibitors specific for serine proteases. *E. invadens* IP-1 strain was used as the model of excystation and metacystic development of *E. histolytica*. Four serine protease inhibitors, PMSF, AEBSF, DCI, and TPCK, decreased the number of metacystic amoebae in a dose-dependent manner, without showing cytotoxicity to cysts. PMSF inhibited not only the increase, but also the development of metacystic amoebae as determined by the change of nucleus number from 4-nucleate to 1-nucleate amoebae. The protease activity in cyst lysates was also inhibited by PMSF and the band of protease on gelatin SDS-PAGE was weaker than controls when treated with PMSF. Three serine protease families, S28 (three types), S9 (two), and S26 (one) were retrieved from the database of *E. invadens*. The expression levels of these serine proteases in cysts 5 hours after the induction of excystation as assessed by real-time RT-PCR were higher than those observed prior to induction assayed by real-time RT-PCR; the increase in one type of S9 (named S9-3) expression was highest. The expression of S9 enzymes also increased from cysts to trophozoites higher than the other family serine proteases. Phylogenetic analysis revealed that amebic enzymes from the serine protease families formed different clades from those from other organisms. Thus the results show that *Entamoeba* uses their serine proteases in the excystation and metacystic development, which leads to successful infection.

BM026 - Molecular modelling and function prediction of 14 hypothetical proteins of *Leishmania Major*

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Leishmania sp is a protozoary that belongs to Trypanosomatidae family and presents two main forms: a flagellate or promastigote, found in the gut of insect vector and in some artificial culture media, and other aflagellate or amastigote, seen in the tissues of vertebrate hosts (human and other higher animals)(Manual de enzymatic, 2000). Leishmania´s amastigotes are found parasitizing cells of the phagocytic ***mononuclear system*** (SMF) of vertebrate host, mainly macrophages resident in the skin (Neves, 2005). To simplify, the disease can be classified as Cutaneous Leishmaniasis (CL) or visceral leishmaniasis (VL). The disease is endemic in 88 countries from four different continents, and is, according to assessments of TDR / WHO, one of the six endemic diseases worldwide priority. These data reinforce the need of strategies to eradicate the disease. These include the development of more efficient and safer chemotherapics, and the development of new diagnostic tools and vaccines. With this objective, fourteen hypothetical proteins of *Leishmania Major* were selected to through molecular modeling and structural alignment have their functions predicted. ***Primary amino acid sequence contains all the information*** to form the secondary and ***tertiary*** structure. Amino acid sequences of hypothetical proteins selected for this study were used to predict their structure. Structural modeling was carried out using the MODELLER program (copyright 1989 – 2009, Andrej Sali), this program is used for homology or comparative modeling of protein three-dimensional structures. HHPRED server was used for the sequences alignment and an alignment of our template and a protein with known three dimensional structure with more than 30% of identity was used as an input to MODELLER that automatically calculates a model containing all non-hydrogen atoms. The accuracy of the built models was evaluated with Ramachandran plot and Verify 3D (Luethy et al, 1992), the best model was used as a probe to structural alignment and protein function prediction through DALI (Holm et al, 2008), SSM-EBI (Krissinel & Henrick, 2004) and ProFunc (Laskowski et al, 2005). Our results using the bioinfomartics tools described above can suggest that some proteins ***annotated*** as hypothetical proteins in databanks have enzymatic functions or known folds. The knowlogde of protein function is important to understand parasite biology and is useful for identification and selection of new targets for the development of ***Chemotherapics***, vaccines and techniques for rapid diagnosis.

BM027 - TANDEM REPEAT PROTEINS PROTOZOAN PARASITES

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Several studies have shown that repeat-containing proteins in protozoan parasites are highly immunogenic, involved in immune evasion, cytoadherence and/or protein-protein interactions. Here we have performed an *in silico* analysis searching for perfect and degenerated repeats in the predicted proteome of nine protozoan parasites. Five of these parasites have intracellular stages and four are exclusively extracellular. The repetitive content of B cell linear epitopes of *Trypanosoma cruzi* was also analyzed. To identify aminoacid repeats, we have developed the program AA-RepeatFinder written in Pascal Language and the linear epitopes of B cell were searched using the Bepipred program. In our analysis, parasites with intracellular stage have higher repetitive content than those exclusively extracellular. Four out of five intracellular parasites have at least one amino acid enriched in the repeats which are those known to be involved in O- and N-glycosylation. We observed a positive correlation between the number of codons and the frequency of amino acid enriched in degenerated repetitive sequences. Aliphatic amino acids are more enriched in degenerated sequences, especially leucine and alanine. In *T. cruzi*, the large multigene families of surface proteins have high repetitive content. Analysis of each family showed that the largest content of perfect repetitive segments is derived from TcMUC mucin followed by MASP, but these profile reverses when degenerate sequences are compared. The MASP family showed higher variability of its repetitive sequences. Although there is no significant difference in the repetitive content of both CL Brener haplotypes, the predicted Esmeraldo-like proteome is less variable than the Non-Esmeraldo one: 53,4% and 62,5% respectively. Also, around 25% of the *T. cruzi* proteins with predicted linear B cell epitopes have repeats.
Supported by FAPEMIG, CNPq and WHO.

BM028 - A DNA BASED APPROACH FOR LEISHMANIA TYPIFICATION IMPROVEMENT AND FOR A DEEPER INVESTIGATION OVER LEISHMANIA POPULATION GENETICS

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Multi-locus sequence typing (MLST) was proposed in 1998 as an approach to provide accurate and portable data for epidemiological investigation of bacterial pathogens reflecting evolutionary routes and population biology. MLST was recently introduced to the study of *Leishmania* and may become a molecular tool useful to help overcome MLEE drawbacks (such as distinct allozymes having similar mobility, few characters available and the fact that parasites must be cultured in bulk). MLST is based on the same principle of MLEE but it takes advantage of a DNA based approach, analyzing DNA sequences and not enzyme mobility. Therefore MLST moves towards an improvement of *Leishmania* typing processes, contributing to a deeper investigation of *Leishmania* population genetics. For this purpose we have so far sequenced the enzyme-coding genes MPI (589 bp), ICD (914 bp) and 6PGDH (716 bp) directly from PCR purified products of *L. braziliensis* (n=43), *L. lainsoni* (n=3), *L. naiffi* (n=7), *L. shawi* (n=3) and *L. guyanensis* (n=2) isolates. Editing and consensus sequences were performed in ***Phred*****,** Phrap**,** ***Consed*** software packages ***using*** two forward and two reverse strands for each of the 58 samples. The alignment in MEGA and analysis of data in DNAsp allowed the detection of polymorphisms which generated species-specific and non-specific alleles for the three target locus. Heterozygous samples were identified through chromatogram observation in the *L. braziliensis* group in MPI, ICD e 6PGDH genes, and in *L. lainsoni* and *L. shawi* in ICD only. The finding of specific alleles encourages the construction of a database containing the allelic profile for the different species, giving rise to a cheaper, faster, more accurate and standardized way for *Leishmania* typing. Further work is needed to increase the number of enzyme-coding genes and isolates to be sequenced.
Suported by Leishepinet-SA project (EU-FP6 : INCO-CT2005-015407), CNPq and FAPERJ

BM029 - EXPRESSION OF GP82 SURFACE GLYCOPROTEIN OF *TRYPANOSOMA CRUZI* DURING *IN VITRO* METACYCLOGENESIS

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The surface glycoprotein GP82 is a developmentally regulated adhesin expressed in *T. cruzi* metacyclic trypomastigotes, which has been implicated in mammalian cell invasion. Here we investigate the GP82 expression pattern during metacyclogenesis. Epimastigotes of G strain and CL Brener clone were submitted to *in vitro* differentiation using TAU3AAG medium. Parasites adhered to culture flask, epimastigotes and intermediate forms undergoing differentiation, were analyzed at 24 h, 48 h and 72 h after the inoculum in TAU3AAG. As a control, we used a parasite sample collected before the inoculum and metacyclic trypomastigotes purified by DEAE-cellulose chromatography. Western blot analysis using a specific monoclonal antibody against GP82 (MAb3F6) showed that GP82 translation begins at 48 h in both G and CL Brener. Immunofluorescence assays with MAb3F6 showed that GP82 is localized in vesicles at the posterior region of epimastigotes and intermediate forms. These vesicles co-localize with the cysteine proteinase cruzipain, which is known to accumulate in reservosomes. Adhered parasites collected at 24 h and 48 h were also analyzed by FACS using MAb3F6, and an increase in the fluorescence signal was detected in parasites permeabilized when compared to non-permeabilized. Increased levels of GP82 transcripts were accompanied by morphological differentiation from epimastigotes into metacyclic forms. These results indicate that GP82 transcripts are translated before the morphological differentiation from epimastigotes into metacyclic forms under the conditions described. Moreover the localization of GP82 in reservosomes suggests that vesicles from the secretory and endocytic pathways fuse at the posterior region of the parasite and specific sorting segregate molecules, like GP82 with GPI anchor, to their destiny.
Supported by FAPESP and CNPq.

BM030 - *Trypanosoma cruzi* interaction with host cells: identification and purification of cellular adhesion ligands using DNA aptamers

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The interaction between *T. cruzi* surface proteins and host cell proteins - including extracellular matrix proteins - is important for the progression of the infection. Our group was the first to discover in the infective trypomastigote forms, 85 kDa parasite specific surface membrane glycoproteins, collectively called Tc85, and implicate their role on the parasite invasion process in tissue cultured cells (Katzin & Colli, 1983; Andrews et al., 1984; Alves et al., 1986; Abuin et al., 1989). Later these glycoproteins have been included in a large protein superfamily called gp/85-trans-sialidase. In this work the SELEX procedure (Systematic Evolution of Ligands by EXponential enrichment) has been used in an attempt to develop DNA aptamers capable of being displaced by a peptide common to the majority of the superfamily members and containing the FLY domain which was demonstrated by our group to be a domain of cellular adhesion (Magdesian et al., 2001). Based on nucleotide sequencing, the obtained DNA aptamers were distributed in five different families and have no homology with DNA aptamers belonging to an initial unselected round. They also had no structural relationship with previously developed aptamers with affinity for extracellular matrix components (Ulrich et al., 2002). The secondary structure of each family as well as their consensus sequences was determined. These selected aptamers show an increased affinity for monkey kidney epithelial cells employed both in binding and adhesion inhibition assays. These aptamers will be used to isolate putative receptors at the host-cell surface. Also they could be an important tool for *in vivo* analysis of the parasite-host cell interaction using animal models.
Supported by FAPESP and CNPq.

BM031 - MOLECULAR CHARACTERIZATION OF SAP (SERINE-, ALANINE-, AND PROLINE-RICH PROTEIN) OF *TRYPANOSOMA CRUZI* BY MURINE MONOCLONAL ANTIBODIES

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SAP peptides display several serine-, alanine-, and proline-containing repeats. SAP is involved in the invasion of mammalian cells by *T. cruzi* metacyclic forms. The recombinant SAP exhibited an adhesive capacity toward mammalian cells, where binding was dose-dependent and saturable, indicating a possible ligand-receptor interaction. SAP triggered the host cell Ca^{2+} response required for parasite internalization. Our aim is generation and characterization of murine monoclonal antibodies (MAbs) against recombinant SAP and their use for epitope mapping of the antigen. The central domain of SAP (155 amino acids) was cloned into the pGEX vector and expressed as GST fusion protein. Hybridoma cells were established from Balb/c mice immunized with recombinant SAP. Specificity of MAbs was determined by ELISA and immunoblotting. Three hybridoma clones producing MAbs specific for the immunogen were established. The Mabs could bind with recombinant antigen, as well as native antigens in ELISA. The antibodies were then used to localize the endogenous SAP proteins and determine the expression pattern in epimastigotes and metacyclic trypomastigotes by immunoblotting and indirect immunofluorescence assay. Two of the MAbs (SAP2 and SAP4) showed stronger reactivity with a 55-kDa protein in epimastigotes and metacyclic forms in Western blot analysis, whereas MAbSAP5 showed lower affinity with the native antigen. The 55-kDa protein corresponds to a SAP molecule shed into the extra-cellular medium by metacyclic forms. To determine the cellular localization of SAP, epimastigotes and metacyclic trypomastigotes were analyzed by immunofluorescence. MAbs reacted with cytoplasmic components of parasites permeabilized with saponin and fixed with formaldehyde, and also reacted at low intensity with intact live parasites. To map continuous epitopes of the SAP, overlapping sequences from the central domain were subcloned into pGEX vector. The reactivity of these regions with MAb and polyclonal antibodies has been analyzed. First two authors contributed equally to the work.
Supported by CNPq and FAPESP.

BM032 - First identification, isolation in culture and molecular characterization of *Trypanosoma* (*Megatrypanum*) *theileri* in Croatia

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Typanosoma theileri is described as ubiquitous non-pathogenic trypanosomatid parasite of bovines, despite some reports about its potential pathogenicity. This is the only bovine trypanosome known to exist in Europe. This species is transmitted by tabanid flies and, maybe, also by ticks. Bovine blood samples have been used to supplement culture media employed for routine microbiological diagnoses. Sterility of bovine blood needs to be previously tested and for this purpose in our laboratory we employed blood cultures. This procedure revealed a blood samples that was positive for *T. theileri,* which has been reported as contaminant in routine bovine blood sterility testing. The culture of trypanosome obtained has been successfully maintained for more than 6 years. Culture smears stained by Giemsa revealed epimastigote forms characteristic of *T. theileri*. Cultured trypanosomes were used to prepare DNA for molecular diagnosis using a PCR assay based on ITSrDNA, and results confirmed the identification of this trypanosome as *T. theileri.* In addition, we determined the SSUrDNA sequence of this isolate and aligned its sequence with sequences from *T. theileri* isolates of bovines from Germany, Brazil, USA and Japan, *T. theileri*-trypanosomes from water-buffalo, antelopes and cervids, besides a sequence from *T. melophagium* of sheep keds from Croatia. The isolate of *T. theileri* from Croatia showed to be highly similar to all bovid isolates, whereas significantly differed from *T. melophagium* also from Croatia. This result corroborated that although *T. melophagium* is very closely related to *T. theileri*, these two trypanosome species circulate in different host species and are transmitted by different vectors, sheep keds and tabanids, respectively. This is the first report of *T. theileri* in bovine from Croatia.
Supported by CNPq

BM033 - Phylogenetic position and description of *Rhytidocystis cyamus* sp. n. (Apicomplexa, Rhytidocystidae): A novel intestinal parasite of the North-eastern Pacific 'stink worm'

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A new *Rhytidocystis* species is described from the North-eastern Pacific Coast. Trophozoites of *R. cyamus* sp. n. were isolated from the intestines of the opheliid polychaete *Travisia pupa*, collected from mud dredged at a depth of 80 m. The trophozoites of *R. cyamus* sp. n. were relatively small (40-64 µm long, 27-30 µm wide) and bean shaped with a centrally located nucleus. The trophozoite surface was inscribed by 10-12 longitudinal rows of short transverse folds and less conspicuous grooves with an irregular pattern. Micropores were observed in association with the transverse folds. A mucron or apical complex was not observed with either light or scanning electron microscopy. The trophozoites did not show any degree of motility. The SSU rDNA sequence obtained from *R. cyamus* sp. n. clustered strongly with *R. polygordiae* within the rhytidocystid clade. Although the precise phylogenetic position of the rhytidocystid clade within the Apicomplexa remains uncertain, the rhytidocystid sequences diverged with a weak affinity to a terrestrial clade containing cryptosporidians, neogregarines and monocystids.
Supported by Tula Foundation (Centre for Microbial Diversity and Evolution), NSERC 283091-04 and CIFAR (Program in Integrated Microbial Biodiversity).

BM034 - Analysis of molecular karyotype and genome size reveals significant differences among clones CL Brener and CL14 derived from the CL strain of *Trypanosoma cruzi*

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Protozoan undergoes chromosomal rearrangements, which are the main cause of karyotype variability among the populations. Chromosomal rearrangements have been detected in different isolates of *T. cruzi* as well as among clones originating from the same isolate. In this study, we aimed to (i) estimate the genome size and analyze the chromosome size-variation among two cloned stocks, CL Brener and CL14, and CL strain; (ii) identify chromosomal rearrangements and study the process of chromosome formation. Total DNA content was estimated in hydroxyurea-arrested epimastigotes using a fluorescent nucleic acid stain for dsDNA. We found significant difference in the DNA content between CL14 and the parental strain (140 to 175 Mb). Analysis of chromosomes by PFGE showed the existence of both size and number polymorphisms that was confirmed by hybridization with 30 genetic markers, including 10 chromosome-specific probes. Rearrangements in the location of genes for tubulin, SL sequence, TS proteins, retrotranposons and repetitive DNA were observed. We show that CL Brener and the parental strain present a 2.3 Mb long form of chromosome XVI, whereas a smaller form of this chromosome about 23% shorter (2.0 Mb) was found in CL14. This finding suggests that the 2.3 Mb-band underwent a large deletion giving a band of 2.0 Mb. No difference was found in the length of telomeric regions of 2.0 and 2.3 Mb bands, suggesting that the deletion occurred within the chromosome XVI. This was confirmed by analysis of an integrated partial physical map of chromosome XVI using genome sequencing data, YAC contigs and hybridization with chromosome-specific markers. We suggest that a DNA fragment of 300 kb, produced by breakages within the chromosome XVI, was eliminated and the remaining chromosomal arms underwent fusion resulting in a 2.0 Mb-long chromosome.

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BM035 - A HYDROGENOSOME IN THE FREE-LIVING EXCAVATE *ANDALUCIA INCARCERATA*

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A wide range of unrelated anaerobic protists that lack conventional mitochondria have been shown to contain independently-derived organelles of mitochondrial origin. The mechanisms of mitochondrial reduction and the acquisition of novel functions permitting survival under anaerobic conditions are therefore of great interest. We describe the mitochondrion-derived organelle of *Andalucia incarcerata*, a free-living anaerobic excavate. EST data from *A. incarcerata* has revealed the presence of putative mitochondrial proteins, including components of energy generation, iron-sulfur cluster biosynthesis and amino acid metabolism pathways, as well as the citric acid cycle. Putative mitochondrial targeting sequences have been identified for a number of these proteins. The localization of an iron hydrogenase to the organelle confirms the organelle's role in anaerobic energy generation, and permits its classification as a hydrogenosome.
This work is supported by the Fonds National de la Recherche du Luxembourg, the Canadian Institutes of Health Research, the Canadian Institute for Advanced Research, the Natural Sciences and Engineering Research Council of Canada, and the Tula Foundation

BM036 - First data on molecular phylogeny of microsporidian family Metchnikovellidae

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Five microsporidian nucleotide sequences of the SSU rRNA gene were PCR amplified from DNA samples of gregarines parasitic in White Sea polychaetes. Three sequences were obtained from gregarine *Ancora sagittata* parasitic in the gut of *Capitella capitata* (two of them were almost identical and differed by only a few substitutions); and two more sequences were obtained from gregarines parasitic in *Lumbrineris fragilis*: one from *Lecudina* cf. *elongata* and another from *L.* cf. *longissima*. All the sequences formed a stable monophyletic clade (with a posterior probability of 0.99) at the base of a Bayesian tree of 70 microsporidian species, which represent all branches commonly resolved in the SSU rDNA phylogenetic analysis. Some representatives of the hyperparasitic microsporidian family Metchnikovellidae are morphologically described from gregarine hosts mentioned above or from their close relatives: two *Amphiamblys* species from *Ancora sagittata* and two *Amphiacantha* species from *Lecudina* spp. parasitic in *Lumbrineris* spp. (including *L. elongata*). Our previous light and electron microscopic studies on the gregarines, from which the microsporidian sequences were obtained, also often demonstrated vegetative stages of microsporidians and characteristic spore-containing cysts similar to those of *Amphiamblys* and *Amphiacantha*. This suggests that the obtained sequences belong to these microsporidians too. Thus, molecular phylogenetic analysis confirms the affiliation of Metchnikovellidae to microsporidians and demonstrates that they are the most plesiomorphic group of modern Microsporidia.

BM037 - Expression and sub-cellular localization of a *Trypanosoma cruzi* UDP-GlcNAc:Polypeptide O-α-*N*-acetyl-D-glucosaminyltransferase (*Tc-OGNT2*) in *Leishmania tarentolae* and *T. cruzi*

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Biosynthesis of *O*-glycans present in mucin-like glycoproteins that cover all stages of *T. cruzi* begins through addition of α-GlcNAc to threonine, reaction catalysed by a Golgi UDP-GlcNAc:polypeptide O-α-*N*-acetyl-D-glucosaminyltransferase (pp-αGlcNAcT). Previously, we identified three genes in the parasite genome (*Tc-OGNT1*, *2* and *L*) that might encode pp-αGlcNAcTs. When full-length versions of the former two were independently expressed in *L. tarentolae*, extracts exhibited increased ppαGlcNAcT and UDP-GlcNAc hydrolysis activities. Here, we used a highly purified mono-specific polyclonal antibody raised against Tc-OGNT2 (anti-TcOGNT2) to study its overexpression and sub-cellular localization in *L. tarentolae* and *T. cruzi*. A band at ~55 kDa, the expected size of the full-length Tc-OGNT2, was recognized by anti-TcOGNT2 by Western blot only in total extracts of *L. tarentolae* transfected with pF4SPX1.4-TcOGNT2, but not the empty vector. A similar construct, with a c-myc tag fused to the C-terminus of the recombinant protein, gave rise to a band at ~63 kDa that was recognized by both anti-TcOGNT2 and MAb-9E10 in Western blots of total *T. cruzi* epimastigote extracts transfected with pTEX-TcOGNT2-c-myc, but not with pTEX. Sub-cellular fractionation by differential centrifugation, followed by Western blotting with anti-TcOGNT2 and MAb-9E10, identified two bands (~57 and ~63 kDa) associated only with the microsomal fraction of pTEX-TcOGNT2-c-myc transfected parasites. No signal was observed in mitochondrial, glycosomal or cytosolic fractions, supporting previous immunofluorescence observations that Tc-OGNT2 was located in the Golgi. Despite the strong signal detected by Western blotting, microsomes from pTEX-TcOGNT2-c-myc transfected cells showed only 1.5x higher ppGlcNAcT activity than equivalent fractions from pTEX- or non-transfected parasites. However, the UDP-[^{3}H]GlcNAc hydrolysis increased 5x relative to controls. The 9:1 ratio of hydrolysis:transferase activity of the expressed relative to endogenous activity suggests that the tag, or deficiency of a cofactor, impairs the proper assembly and activity of the overexpressed Tc-OGNT2 in its own host.
Support: CNPq, FAPERJ, OCAST.

BM038 - CLONING AND EXPRESSION OF MEMBERS OF THE *Trypanosoma cruzi* MUCIN-ASSOCIATED SURFACE PROTEIN (MASP) FAMILY FOR STRUCTURAL BIOLOGY STUDIES

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The *Trypanosoma cruzi masp* gene family was identified during the annotation of the genome as the second largest gene family in this human pathogen, consisting of approximately 1400 members. MASP members contain N- and C- terminal conserved domains that encode a putative signal peptide and a GPI-anchor addition site, respectively. The central region is variable both in length and in amino acid sequence and contains a large repertoire of repetitive motifs, which suggests MASP may be involved in parasite-host cell interaction. Since no member of the MASP family has been characterized to date, we have decided to study the secondary and tertiary structures of distinct MASPs. For crystallization purposes, we have decided to clone and express in *Escherichia coli* only the central region of four MASP genes. For this, some features of the target proteins were considered such as short amino acid sequence length, around 170 residues, low content of potential glycosylation sites and low intrinsic disorder degree. Knowing that sequence regions with low complexity nearly always correspond to nonfolding segments or extended structures, we have used a web server to predict disordered regions in MASPs. The sequences were PCR amplified and cloned in two expression vectors derived from pET-28a vector (Novagen), which were modified in the Center of Molecular and Structural Biology (CeBiME, Campinas/SP). In both vectors, the thrombin ***recognition site*** was replaced by a TEV (*tobacco etch virus*) protease recognition site. *E. coli* BL-21 Star strain transformed with the constructs were cultured for 3 hours and overnight, in 37ºC and 30ºC, respectively, in the presence of 1mM IPTG. The N-terminal fusion His-tag MASP was expressed in the soluble form in both temperatures, whereas the GST fusion MASP was expressed as insoluble inclusion bodies.
Supported by FAPEMIG, CNPq and WHO.

BM039 - Characterization of putative Hemolysins type III genes in *Leishmania major*

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Previous works from our group had shown that *Leishmania amazonensis* promastigote has a pore forming protein (PFP) that lyses erythrocytes and damages macrophages, which we named leishporin. This cytolysin has already been characterized in a number of biochemical aspects. However, its molecular identity is still unknown. Since the genome of the *Leishmania major* is now completely sequenced, we have performed a data mining effort to identify putative hemolysin genes in this species, in attempt to identify leishporin gene. We found four sequences containing the Pfam domain PF03006, named hemolysins type III-related. It is also worth noting that these sequences showed a significant homology to other characterized PFPs. To verify the expression of these genes in the parasite, total RNA was isolated from *L. major* promastigotes and used in a RT-PCR reaction using specific primers for the LmjF36.5520 gene which contains the PF03006 domain. Preliminary results show that this gene is expressed in promastigotes of *L. major*. We are currently verifying whether the other three sequences annotated as hemolysins type III are also expressed in promastigotes. In the next step, we will perform real-time RT-PCT assays to quantify the expression level of these four genes in the promastigotes and amastigotes. Stable transfected parasites, overexpressing the hemolysins type III genes will be generated in order to verify the hemolytic activity of the proteins encoded by these genes. In addition, the cellular localization of the protein will be investigated by transfection experiments in *L. major* with a vector harboring the genes in fusion with GFP.
Supported by CAPES and CNPq.

BM040 - EXPRESSION PROFILE ANALYSIS OF *TRYPANOSOMA CRUZI* MUCIN-ASSOCIATED SURFACE PROTEIN (MASP) GENE FAMILY

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A novel multigene family of the *Trypanosoma cruzi* was identified during the annotation of the genome and was named mucin-associated surface proteins (MASP). MASP is the second largest *T. cruzi* gene family, presenting approximately 1400 members, and is characterized by conserved N- and C-terminal domains that encode a signal peptide and a GPI-anchor addition site. The central region is highly variable in length and sequence and contains a large repertoire of repetitive motifs. Although its function is unknown, its high variability, massive expansion in the genome and surface location in the circulating trypomastigotes may suggest that MASP participates in immune evasion mechanisms and/or cell invasion and adhesion. The aim of this work is to analyze the MASP expression profile in blood trypomastigotes and culture trypomastigotes derived from distinct host cell lines from different passages. CL Brener strain trypomastigotes from the 0, 1^a, 4^a, 7^a and 14^a passages derived from L6 and LLCMK2 cell lines were collected and purified. Blood trypomastigotes from two passages in mice during the acute phase were also collected and purified. Part of the blood parasites were used to infect L6 and LLCMK2 cell cultures and trypomastigotes derived from the fourth passage were also collected. Total RNA from the different samples was extracted and cDNA libraries from each sample were constructed by RT-PCR using MASP specific primers. The libraries have been cloned in TOPO system and sequenced in MegaBase sequencer. The cDNA sequences have been analyzed using Phred-Phrap-Consed package and searched against a local *T. cruzi* coding sequence database using BLASTN algorithm to identify the MASP expressed members.
Financial support: FAPEMIG/WHO/CNPQ

BM041 - MOLECULAR CHARACTERIZATION OF *Trypanosoma cruzi* MEXICAN STRAINS AFTER EXPERIMENTAL INFECTION

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The Chagas' disease is widely distributed in Mexico, in which Jalisco is one of the most affected states. The prevalence of seropositivity for *Trypanosoma cruzi* in this state is approximately 17%, but few epidemiological studies, control blood donors and human cases of the disease have been performed. The objective of this study was to observe the behavior in mice infected with Mexican strains of *Trypanosoma cruzi* isolated from different hosts and genetically characterize them. These strains were obtained from peridomestic triatomine vectors (*Triatoma pallidipennis, T. longipennis, T. picturata*) and from human cases in the acute and chronic phases of the disease. The strains had been grown in LIT medium for parasite mass producing and subsequent DNA extraction. The strain classification was performed by PCR analysis of the subunit rRNA, mini-exon, D7 domain of 24Sα and 18s of genes´ region. The haplotypes of *Trypanosoma cruzi* I strains were determined by analysis the mini-exon gene intergenic region. The results of amplifications were observed on a 6% polyacrylamide gel by silver staining. The parasitamia curves profile was accompanied by the microhematocrit method when necessary or by method of Brener in non isogonic mice inoculated with $3x10^5$ trypomastigotes of each strain and the mortality was followed daily. There was low, medium or high which mortality that was not always associated with parasitemia curves ranging from a subpatente way (positive only in microhematocrit) to a patent with maximum peak between $4.6x10^6$ and $1.0x10^7$ parasites/ml of blood. The existence of *T. rangeli* was ruled out by molecular analysis and all samples were classified as *Trypanosoma cruzi I* corresponding Ia (250pb) – Id (200pb) haplotype. These results lead us to conclude that the *Trypanosoma cruzi I* Mexican strains presented different biological characteristics, but all showed the same haplotype.
Financial Support: FAPEMIG, CAPES, CNPq and FUNEPU-UFTM

BM042 - GENOMIC LOCALIZATION OF PHOSPHATIDYLINOSITOL– AND RELATED- KINASES SUBTYPES IN G STRAIN AND CL BRENER CLONE OF *TRYPANOSOMA CRUZI*

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Phosphatidylinositol-kinase (PIK) pathway is one of the most popular targets for the development of new cancer treatment and this is why several pharmaceutical companies regard its components as potential drug targets. PIK and its lipid products-phosphoinositides-have proved to play a pivotal role in a wide range of cellular processes such as cell growth, cell survival, vesicle trafficking, cytoskeletal reorganisation and chemotaxis, cell adhesion, superoxide production and glucose transport, by resorting to specific inhibitors like LY294002 and wortmannin, which are mechanistically and structurally distinct. Trypomastigote and amastigote treatments with PIK inhibitors prevent parasite cell entry, which indicates that PIK is essential for invasion. In a previous study, we presented a survey of PIK and -related kinases (PIK-related) made by similarities searches against *Trypanosoma cruzi* genome available to date. PIK and PIK-related were classified according to five models 1, 2, 3, 4 and 5. We found that *Trypanosoma cruzi* displays twelve PIK genes divided into these five PKs models. In the present study, we have mapped four different PIK members belonging to models 2, 3 and 5 on the chromosomal bands of *T. cruzi* strains in order to understand the genomic organization of the PIK family. PIK genes were cloned and used as molecular markers. Chromosomes of CL Brener clone and G strain were separated by pulsed-field gel electrophoresis (PFGE) and hybridized with PIK-radiolabeled probes. All selected PIKs hybridized with two chromosomal bands in CL Brener and with one chromosomal band in G strain. One of the PIK genes corresponding to Model 1 displayed a FYVE domain located at the N-terminus. FYVE, Mod5.120 and Mod5.230 genes hybridized with chromosomal band XIX (2.88 Mb) in CL Brener and in G strain; FYVE and Mod5.120 are located on band 11 (1.40 Mb). These results indicated that PIK members are not randomly widespread on the genome of *T. cruzi*, and thus suggesting a clusterization of PIK in some chromosomes. This chromosomal polymorphism between different parasite stocks may be due to the hybrid origin of the CL Brener genome. Antibodies to these proteins would enable us to localize PIK in cells and study their possible role in host cell invasion. Support: FAPESP, CNPq

BM043 - PROTEOMIC IDENTIFICATION OF MOLECULES INVOLVED ON ESTABLISHMENT AND CONTROL OF CUTANEOUS LEISHMANIASIS

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CBA/J mice are resistant to *Leishmania major* and susceptible to *Leishmania amazonensis*. Also, CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*, suggesting an important role for macrophages on the determination of *Leishmania* infection outcome. Using microarray approach we showed that *L. amazonensis*- and *L. major*-infected macrophages differentially express very few numbers of genes related to early cell-*Leishmania* interaction and immune-inflammatory response. Although microarray is useful to identify molecules at transcriptional level, it is necessary to associate expressed genes to theirs respective proteins. Proteomic experiments using inflammatory macrophages showed a small number of differently expressed proteins after *L. amazonensis* or *L. major* infection. In order to better evaluate the macrophage response to *Leishmania* infection, we used bone marrow derived macrophages (BMM□), since these macrophages are not pre-activated by thioglycollate and BMM□ cultures are synchronized and homogeneous. First, we performed kinetic studies and compared the capacity of *L. amazonensis* and *L. major* to infect BMM□. In this study, using proteomic approach, we evaluated the differential expressed proteins at 6 and 24h of *L. major* or *L. amazonensis* infection on CBA/J BMM□. Protein extracts were obtained to identify peptides by LC-MS/MS in a MudPIT approach. The results from 6 independent experiments were analyzed to identify the differentially expressed proteins. The results show that 234 proteins were differently expressed in *L. amazonensis*- or *L. major*-infected cells. These proteins are involved in cell death, post-translational modification, protein folding, lipid metabolism, protein synthesis, cellular assembly and organization, molecular transport and DNA replication, recombination and repair. Current western-blot analyses are been performed to corroborate the differences on expression at protein level detected by high throughput proteomic studies. Taken together, the data point out to a pivotal role for the parasite on determining the subsequent immune response and course of infection.
Supported by CNPq.

BM044 - DISRUPTION OF THE *Trypanosoma cruzi* KAP3 GENE: THE EFFECT IN THE CELL PROLIFERATION AND DIFFERENTIATION

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The kinetoplast DNA (kDNA) of trypanosomatids consists of an unusual arrangement of two types of circular molecules catenated into a single network: (1) A few maxicircles, that encode for some subunits of mitochondrial enzymes and rRNA in a cryptic pattern, and (2) thousands of minicircles that encode for the guide RNA (gRNAs). Studies in *Crithidia fasciculata* showed that the kDNA is associated with proteins, known as kinetoplast-associated proteins (KAPs), which are capable of condensing the kDNA network. However, little is known about the KAPs of *Trypanosoma cruzi*, a parasitic protozoon that shows distinct patterns of kDNA condensation during their complex morphogenetic development. In this work we have cloned a gene of *T. cruzi* which encodes the protein TcKAP3 (kinetoplast-associated protein 3). *Tckap3* is a single-copy gene that is transcribed into a 1.8 kb mRNA and expressed in all stages of the parasite. Mouse antiserum raised against the recombinant TcKPA3 recognized a 25 kDa protein which was found, by indirect immunofluorescence, to be associated with the kinetoplast of *T. cruzi*. Several features of TcKAP3 such as its small size, basic nature and similarity with CfKAP3, are consistent with a role in DNA charge neutralization and condensation, suggesting that this protein could be involved in organizing the kDNA network. Gene deletion was used to investigate the function of TcKAP3. *Tckap3* null mutants can complete the life-cycle in vitro without losing their infective capacity, with no modification in the structure and morphology of kinetoplast.
Supported by CNPq and Fundação Araucária.

BM045 - Investigating the generation of chimeric genes in *Trypanosoma cruzi*

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The largest *Trypanosoma cruzi* gene families encoding surface proteins are often clustered into large haploid and heterogeneous arrays that can be as large as 600 kb. In these clusters, members of each family are not duplicated in tandem, but instead members of different families alternate among each other in a not well defined fashion. Several lines of evidence suggest that those areas containing arrays of surface proteins are subject to intense rearrangements: (i) the average length of the directional gene clusters in these *T. cruzi* specific regions is much smaller than for syntenic regions with *T. brucei* and *L. major* genomes; (ii) the presence of large number of pseudogenes (iii) the abundance of retroelements and (iv) syntenic break between the two CL-Brener haplotypes. In fact, by analyzing the *T. cruzi* genes encoding surface proteins and cDNA sequences derived from a trypomastigote cDNA library, we have identified several chimeric genes containing segments from different surface protein-encoding genes. To investigate the molecular mechanisms involved in the generation of chimeric sequences, we are developing an integrative vector in which the GFP (green fluorescent protein) gene is flanked by conserved regions derived from a MASP-TcMUC mucin mosaic sequence identified in the *T. cruzi* genome. The neomycin resistance gene, present in the vector, will allow the selection of stable transformed parasites. The vector is designed to allow integration by homologous recombination in a MASP specific locus of the CL-Brener strain. Recombination events will be monitored by PCR using GFP and MASP and/or GFP and TcMUC mucin specific primers.
Supported by WHO, CNPq and FAPEMIG

BM046 - EMYB1, a MYB-like transcription factor, is essentially required for macronucleus development in *Euplotes aediculatus*

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Myb proto-oncogenes are characterised by their highly conserved DNA-binding domain which binds specifically to the recognition sequences of their target genes. myb genes were found in all the multi-cellular eukaryotes tested and comprise one of the largest families of transcription factor. In animals, there exist three myb members, c-myb, a-myb and b-myb, which involve in cell proliferation, differentiation, apoptosis and embryonic development. In plant, the family members of it can be up to more than 100, ex in Arabidopsis thaliana, and they function widely in regulation of cell cycling, various metabolisms and responses to environmental stresses. But the myb genes had not been reported in unicellular Ciliates before the first characterization of myb genes in Euplotes aediculatus and Sterkiella histriomuscorum by a French laboratory (Yang et al, 2004). However, it is still the only report of myb genes in ciliated protozoan up to now and their functions remain unknown..

In our present study, the DNA-binding domain of the myb gene in Euplotes aediculatus, nominated emyb1, was expressed in prokaryotic system and antibody against it was generated. Its Western blot demonstrated that EMYB1 expressed at a lower level in mature vegetative cells, while elevated obviously after starvation when conjugation took place. Immunofluorescent labelling by the antibody showed that EMYB1 was localized exclusively in the developing macronucleus (anlagen). This suggests that EMYB1 takes part in the regulation of macronucleus development in Euplotes. Further study was carried out by microinjection of the antibody into conjugants of Euplotes and the results showed that the macronucleus development was interrupted or blocked in the injected cells ($>$60%). These data suggests that EMYB1 plays an essential role in the progression of macronucleus development. This is the first transcription factor identified that is directly implicated for macronucleus development in *Ciliates*.
Note: This study is granted by the NSFC 30370213 and 30771081

BM047 - MORPHOLOGICAL AND MOLECULAR DESCRIPTION OF *BLASTOCRITHIDIA CYRTOMENI* N. SP. (KINETOPLASTEA: TRYPANOSOMATIDAE) ASSOCIATED WITH *CYRTOMENUS BERGI* FROESCHNER (HEMIPTERA: CYDNIDAE) FROM COLOMBIA

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*Cyrtomenus bergi***,** a polyphagous pest, affects a variety of tropical crops causing economical losses in Colombia as well as in other neotropical countries. In onion fields in Pereira-Risaralda (Colombia), the insects are infected with trypanosomatid flagellates found in salivary glands, intestinal tract, Malphigian tubes and haemolymph. The prevalence of infection was 100%. In order to identify the parasites, their morphology and molecular phylogeny were investigated. Predominantly epimastigotes of variable size with straphangers cysts adhered at the middle of the flagellum were observed. Kinetoplast was always observed in the anterior position with respect to the nucleus. Ultrastructure of longitudinal sections of epimastigotes showed that a flagellum arising laterally from a relatively shallow flagellar pocket near to kinetoplast. DNA was extracted from eggs and midgut of *C. bergi*. The amplification of SL RNA and 5S rRNA gene repeats were positive in all cases producing a 0.8 kb band representing a monomeric repeat unit. The amplicons sequenced were 797-803 bp long and >98.5% identical with each other, indicating that they all represent the same organism. SL repeats units were compared with the entire database of the available repeats from other Trypanosomatidae using neighbor-joining cluster analysis. Repeats from the *C. bergi* parasite were unique and clearly distinct from *Blastochritidia triatomae* (59.2% identity level) and *B. leptocorodis* (55.8% identity). SSU rRNA gene sequence from the *C. bergi* parasites was compared along with selected members of the major known phylogenetic clades of the Trypanosomatidae. By all analyses used, the *C. bergi* parasites formed a close association with *B. triatomae*. Based on the presence of epimastigotes and flagellar cysts and on the molecular phylogenetic analyses, we concluded that the parasite from *C. bergi* represents a new trypanosomatid species that is assigned to the genus *Blastocrithidia*. Work supported by COLCIENCIAS, Colombia (grant No.021-2005).

BM048 - INTERGENIC POLYMORPHIC REGION OF THE TCUMSBP LOCUS OF TRYPANOSOMA CRUZI CLBRENER: STUDIES ON THE RNA PROCESSING AND STABILIZATION

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The intergenic polymorphic region of the *TcUMSBP* locus, characterized in previous work, corresponds to two alleles that differ in 62 bp. This indel (insertion/deletion) affects the mRNA processing generating differential RNA accumulation and two sites of polyadenylation. The polycistronic RNAs derived from these alleles generate mature RNAs carrying two types of 3'UTR, which also differ in 62 bases. Our goal is to investigate if these two kinds of transcripts have different properties regarding the RNA stabilization. We cloned the two polymorphic intergenic regions downstream to the CAT (Chloramphenicol acetyl transferase) gene in plasmids having the *Hsp70* intergenic region as the upstream region. Epimastigotes were transfected, in duplicate, with 100μg of each plasmid construct, and after 48 hrs, CAT assays were performed in triplicate. CAT activity was higher (2x) in cells transfected with the 62bp insertion intergenic region. This shows that the mRNA containing the 62 indel stabilizes the RNA or turns it more efficient for translation. To answer this question we are quantifying the CAT mRNA using qRT-PCR in these experiments and determining the site of polyadenylation by cloning and sequencing the 3´UTR of the CAT mRNA. We are also preparing plasmids for permanent transfections in order to confirm these results.
Supported by: FAPERJ and CNPq

BM049 - GENOTYPING OF *GIARDIA* IN BRAZIL: A PHYLOGENETIC ANALYSIS OF HUMAN AND ANIMAL ISOLATES

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*Giardia duodena*lis (syn. *Giardia lamblia*, *Giardia intestinalis*) is a protozoan organism that can infect the intestinal tract of many animal species, including mammals. This parasite is still considered as the most common water-borne diarrhea-causing disease. *G. duodenalis* infection remains among child care workers, children attending day care centers, school aged children and international travellers. This disease usually afflicts men who have sex with men and immunocompromised patients such as HIV-positive and HIV-negative individuals accomitted to any other kind of immunocompromising situation. Genetic heterogeneity of G. *duodenalis* is well described but the zoonotic potential and the correlation between the presence of symptoms and also the *G. duodenalis* genotypes is still not clear. In order to get more insights about the different *G. duodenalis* genotypes found within Brazilian population we analyzed 100 *Giardia* DNA samples directly isolated from human (HIV positive and negative patients) and from canine stool specimens, by DNA sequencing analysis of PCR products of the beta-giardin gene. The cysts were collected in the city of São José do Rio Preto, in Brazil, from a population composed by humans (*n* = 83 , 5 HIV positive and 78 negatives) and these domestic animals, dogs (*n* = 17). All 100 samples were *G. duodenalis* positive by parasitological exams and coproantigen detection and were also subjected to molecular analysis using a Nested-PCR that amplified a fragment of the beta-giardin gene. Results showed that all human amplified isolates (62.7%, 52/83) were genotype A and could be divided into two main subtypes A1 (*n* = 33) and A2 (*n* = 44) on the basis of PCR assays specific for the beta-giardin gene. Five *Giardia* isolates from human samples were characterized only as genotype A, without subtype identification. Among canine samples amplified (*n* = 7), three isolates were identified as genotype A1, two as A2 and two undefined. The *G. duodenalis* genotype B and the dog-specific genotypes C and D were not found. The identification of genotype A in both humans and his dogs, suggests that zoonotic transmission should represent a real result.
Supported by CNPq.

BM050 - GENE REPERTOIRE AND SEQUENCE DIVERSITY OF GP82 SURFACE GLYCOPROTEIN FAMILY, A MAJOR ADHESION MOLECULE OF *TRYPANOSOMA CRUZI*

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Metacyclic trypomastigotes, the insect-derived *Trypanosoma cruzi* developmental forms, express a stage-specific surface glycoprotein (GP82) that is involved in the penetration of the parasite into mammalian cell. GP82 is a cell adhesion molecule that leads to intracellular calcium mobilization in the target cell and parasite. GP82 family belongs to the trans-sialidase (TS) like-proteins family. Here, we investigate the divergence and polymorphism in the repertoire of GP82 genes from the G strain and clone CL Brener. The protein diversity between these isolates was analyzed through the sequencing of genomic and cDNA clones and functional analysis of cDNAs with a monoclonal antibody specific for GP82. We have performed phylogenetic analyses based on the multiple alignment of GP82 proteins with top scoring sequences identified in the GenBank database by BlastP and BlastN searches. We identified 15 full-length paralogs of GP82 genes in CL Brener, a low number of genes when compared to those of other TS-like families (GP90, Tc85/GP85, ASP). There are intra-strain variations in GP82 sequences indicating the existence of several copies per strain. The inter-strain variability of GP82 agrees with the genetic subdivisions of *T. cruzi* into lineages. GP82 proteins share significant homology at their carboxy and central domains and a strong spatial conservation of cysteine residues. The phylogenetic analysis based on the full-length sequences and the carboxy and central domains alignment of GP82 revealed that the sequences from each isolate clustered together, suggesting that GP82 genes may be structured in a strain-specific manner. Our data support the view that metacyclic trypomastigotes developed a system that efficiently generates a high degree of polymorphism based on a relatively low number of GP82 genes. This contrasts with other TS –like protein families which are composed by large sets of genes such as GP90, Tc85/GP85 and ASP. Supported by FAPESP, CNPq and CAPES.

BM051 - *Entamoeba* surveys from invertebrate-animal guts reveal novel diversity: Implications for species concept(s) and *Entamoeba* phylogeny

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Entamoebae are anaerobic/microaerophilic Amoebozoans that primarily parasitize the guts of animals, with one or two species reported as free-living. Broad-scale phylogenies have mainly relied on single-gene analyses of small subunit ribosomal RNA genes (ssu) from a limited subset of described species - those that infect vertebrate animals and the free-living/human infecting (e.g., amphizoic) *Entamoeba moshkovskii*. In general molecular phylogenies support the grouping of amoebae that possess a like number of nuclei/mature cyst (i.e., monophyletic lineage of 4 nuclei/cyst species, monophyletic lineage of 8 nuclei/cyst species, etc.). However, molecular studies are needed to determine how invertebrate infecting *Entamoeba* fit into this paradigm. Thus, we have analyzed *Entamoeba* ssu rRNA genes from amoebae cultured from insect hosts as well as sequences obtained from environmental surveys of insect gut content DNA using *Entamoeba* specific ssu primers. Culturing efforts from a variety of insects have expanded the known host range of *Entamoeba moshkovskii* to at least 3-4 insect species and revealed a novel phenotype for these isolates. An ongoing environmental ssu survey from a variety of insect guts has uncovered a novel clade that shows a weak affinity to an *Entamoeba* lineage possessing 1 nucleus/cyst. These represent the first molecular data from any Entamoebae infecting invertebrates. Detailed morphologic and molecular characterization of *Entamoeba* that encompass the known and suspected biodiversity of the genus will help elucidate the evolutionary history of this lineage, assess previously unrecognized natural reservoirs of human parasites, and help establish reliable criteria for taxonomy and species delineation.
Partially supported by the University of Arkansas Honors College.

BM052 - COMPLETE GENOME OF THE ENDOSYMBIONT FROM *Crithidia deanei*

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DNA-sequencing projects have illustrated the mechanisms and consequences of severe genome size reduction among obligate bacterial associates compared with their free-living relatives. Here we investigated the genome of the endosymbiotic bacterium of the trypanosomatid *Crithidia deanei*. Like other endosymbionts, the genome of the endosymbiont of C. deanei is A+T rich (~68%) and has a reduced size (~800kb). Earlier analysis of rRNA sequences showed that the endosymbiont belongs to β-proteobacteria group. According with our dates, symbiont genome share identity with *Bordetella parapertussis*. The genome of this bacterium is composed by 721 protein-encoding genes with a 1kb medium size, which are being catalogued using Genbank (BLAST), PFAM (HMMer), KEGG and COG, among other tools/databases, to identify essential biochemical pathways. At proteomic approach we identified 204 proteins (28.7% of total amount of endosymbiont predicted protein) in at least one experiment, with a p-value of 1e10-5. Using no p-value threshold, 353 proteins (49.6%) were identified. The KEGG functional annotation of these 204 proteins is related mainly to aminoacid metabolism (n=~30), purine metabolism (n=15), aminoacyl-tRNA biosynthesis (n=12), glycolysis/gluconeogenesis (n=9), ribosome (n=8), oxidative phosphorilation (n=7), pyrimidine metabolism (n=7) and TCA cycle (n=6). We are currently constructing the endosymbiont ORFeome and microarray platform to study the differential expression of endosymbiont genes, which will be further enhanced by a LQT-orbitrap proteomic approach.
Supported by CNPq and Fundação Araucária.

BM053 - Molecular characterization and cellular localization of Isopentenyl-Diphosphate Delta-Isomerase from *Trypanosoma cruzi*

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Trypanosoma cruzi is the etiological agent of the Chagas disease. There are some drugs that are being used to treat this disease, like nitrofurans and nitroimidazols, but they are active only against the acute phase. We used AnEnπ, a computational tool that possibilities the detection of analogous enzymes, for the identification of new possible drug targets, based on the structural differences of enzymatic activities shared by humans and *T. cruzi*. In other words, they have similar functions, but with differences on the 3D structure. One of these enzymes, isopentenyl diphosphate delta-isomerase (IPPI), catalyzes the interconversion of isopentenyl diphosphate and dimethylallyl diphosphate. This is an essential step in the mevalonate entry into the isoprenoid biosynthetic pathway. The objective of this study is the molecular characterization and the cellular localization of the IPPI, a putative analogue enzyme that can lead us to develop new kinds of drug that can inhibit or decrease the catalytic activity of this enzyme of the protozoan. In this study we have cloned and sequenced the gene of IPPI of *T. cruzi*. The amplicon, was inserted in pBadThio-TOPO vector and cloned in *Escherichia coli* TOP10 strain. After induction with arabinose 0.02% (w/v), the expression of a protein with approximately 36 kDa was obtained in insoluble form. The protein was purified by Ni^{2+}-HisTrap HP column on HPLC and after that utilized for susceptible BALB/c mice immunization to obtain anti-IPPI polyclonal antibodies. This distinct antisera reacted strongly with the respective recombinant proteins in Western blot and are currently being used to localize IPPI in *T. cruzi* using immunofluorescence and immunoelectronmicroscopy. This work is of considerable relevance for the study of the parasite metabolism and for the development of new strategies for drug design against this pathogen.
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BM54 - New isolates of *Carpediemonas*-like organisms and phylogeny of Excavata

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Recently all eukaryotic diversity was classified into several “supergroups”, of these, ‘Excavata’ is the most controversial with its monophyly/non-monophyly and internal phylogeny still debated. Diplomonads (e.g. *Giardia*) are the most problematic group of excavates as they tend to constitute extremely long branches in molecular phylogenetic or phylogenomic trees, which makes resolving their precise affinities very difficult. Diplomonads also posses extremely reduced mitochondria (mitosomes), whose only known function is iron-sulfur cluster assembly. Three poorly known anaerobes/microaerophiles, *Carpediemonas membranifera*, *Dysnectes brevis* and *Hicanonectes teleskopos,* branch at the base of the diplomonad subtree and, on the basis of the few sequenced genes, may constitute ‘shorter branches’. Seventeen new strains of *Carpediemonas*-like organisms were isolated from anaerobic marine/saline habitats and sequences for SSU rRNA genes were obtained. Phylogenetic analyses suggest that all new *Carpediemonas*-like isolates, together with *C. membranifera*, *D. brevis* and *H. teleskopos*, form a series of 6 distinct groups at the base of diplomonads plus retortamonads, although the branching pattern amongst these groups is mostly unclear. Most of these groups have not been detected in prior environmental PCR studies of oxygen-poor marine habitats. Preliminary analyses of ~100 protein coding genes confirmed that *Carpediemonas*-like organisms really tend to constitute short branches in comparison to diplomonads. This makes them promising candidates for resolving the position of the diplomonad lineage relative to other excavates and eukaryotes. More detailed knowledge of putative mitochondrion-related organelles of *Carpediemonas*-like organisms may help to reconstruct the evolution of diplomonad mitosomes. Supported by NSHRF and NSERC

BM055 - GENETIC DIVERSITY AND PHYLOGENETIC POSITION OF THE SUBCLASS ASTOMATIA (CILIOPHORA) BASED ON A SAMPLING OF SEVEN GENERA FROM WEST AFRICAN OLIGOCHAETES

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The astome ciliates have long been considered a monophyletic group, derived from hymenostome- or scuticociliate-like ancestors. The small subunit (SSU) rRNA sequence of *Anoplophrya marylandensis* supported their assignment to the Class OLIGOHYMENOPHOREA. However, a single sequence is insufficient to confidently assess relationships of the larger group. Therefore, we obtained SSU rRNA sequences from seven additional species distributed in six genera and three families: *Almophrya bivacuolata*, *Eudrilophrya complanata*, *Metaracoelophrya* sp1, *Metaracoelophrya intermedia*, *Metaradiophrya simplex*, *Njinella prolifera*, and a proposed new clausilocolid genus. The intestinal tracts of oligochaetes (Families Megascolecidae and Glossoscolecidae), collected in the vicinity of Yaoundé, Cameroun, were removed from the worms and their contents transferred to Ringer's solution. The ciliates were picked by micropipette, rinsed thoroughly in sterile Ringer's solution, and fixed in ethanol. The DNA of fixed ciliates was extracted with a Chelex procedure and the SSU rRNA genes were PCR-amplified using standard protocols and primers. Sequences were aligned in our DCSE alignment, which was refined in relation to secondary structure. Phylogenetic analyses were undertaken using neighbor-joining, Bayesian, maximum likelihood, and maximum parsimony. The eight species of astomes formed a strongly supported clade as a weakly supported sister clade to the scuticociliates. There were two strongly supported clades within the astomes. However, genera assigned to the same family were found in different clades, and genera assigned to the same order, as proposed by de Puytorac (1994), were found in both clades. While species assigned to the same genus (e.g., *Metaracoelophrya*) were found in the same larger clade, they did not form a monophyletic group. Thus, if our identifications are correct, astome taxa appear to be paraphyletic when morphology is used to assign species to genera. However, the Subclass Astomatia remains monophyletic.
Supported by a Natural and Engineering Research Council of Canada Discovery Grant awarded to DHL.

BM56 - Functional complementation of the acyl-CoA dependent Ceramide Synthase (CerS) in yeast using an ortholog gene from *Trypanosoma cruzi* (*TcCerS*).

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Ceramide is both an intermediate in the biosynthetic pathway of sphingolipids and an important bioactive lipid involved in a number of molecular signaling pathways. Ceramide is synthesized mainly from the reaction of a fatty acyl-CoA with a sphingoid long-chain base by an acyl-CoA-dependent and Fumonisin B_1-sensitive ceramide synthase (CerS). The first CerS to be characterized was the yeast enzyme, Lag1/Lac1, which lead to the subsequent discovery of six mammalian genes. In yeast, the single deletion of either *LAG1* or *LAC1* does not seem to cause growth defects. However, the concomitant deletion of *LAG1* and *LAC1* causes a significant growth defect in the genetic background of W303 cells and the same double deletion does not yield viable cells in the YPK9 background, which was used for the present study. Lethality of YPK9.*lag1*□*lac1*□ can be overcome by expression of a CerS ortholog gene from *T. cruzi* (*TcCerS*), a gene that shows 22% identity to yeast *LAG1*. Southern blot analysis suggests that *T. cruzi* has more than one copy of *TcCerS* in the genome, and northern blot analysis shows two mRNA species of 1.8 and 3.0 Kb in epimastigotes. In addition, *TcCerS* 5'-*trans*-splicing and 3'-polyadenylation sites were mapped by RT-PCR. Similar to the yeast CerS, the parasite enzymatic activity detected in epimastigotes' crude microsomal membranes, exhibited a preference for fatty acyl-CoA rather than free fatty acid as donor substrate, was blocked by Fumonisin B_1 and was inhibited at higher protein concentrations. Also, unlike the predilection of the yeast CerS for long-chain length (>C20:0) fatty acyl-CoA's, TcCerS favored almost exclusively the use of C16:0-CoA. Our results suggest that TcCerS is an authentic acyl-CoA dependent ceramide synthase from *T. cruzi*. Support: CAPES, CNPq, FAPERJ.

BM057 - TRANSCRIPTOME-WIDE ANALYSIS OF MICROSATELLITE REPEATS IN *Trypanosoma rangeli*

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Microsatellite (MS) repeats are valuable DNA markers for a wide range of applications due their polymorphism despite being based on time-consuming, hard-working and expensive methods for isolating repeats and flanking sequences. The availability of sequences from genomic/transcriptomic libraries have exponentially increasing, providing an easy and efficient data source for MS identification. Despite the importance of MS on genomic organization and the fact that these repeats may be associated with virulence factors in a range of organisms, no systematic analysis have been reported in trypanosomatids so far. In this work we have examined the distribution and density of microsatellites (1-6bp) in *T. rangeli* transcriptomic sequences. Using the TRF and TRAP software, around 2.45Mb of EST/ORESTES sequences from epimastigote and trypomastigote forms of *T. rangeli* SC-58 and Choachí strains were examined. A total of 2,132 MS repeats were found distributed in 418 distinct classes. The average of frequency was 1/1.24Kb; and the average of density was 9,600pb/Mb, presenting an uneven distribution among distinct parasite forms and strains. Hexanucleotide repeats were the most abundant class of MS, following trimeric (20.8%) monomeric (18.5%), pentameric (10.9%), dimeric (9.9%) and tetrameric (7.3%) repeats, with significant variation between strains in some classes. The prevalence of hexameric and trimeric repeats could be explained based on the suppression of non-trimeric repeats in coding regions due to the risk of occurrence of reading frame shifts. Additionally, A-rich repeats are predominant in each MS class, whereas G-rich repeats are rare in coding regions (except for trimeric repeats). Based on the MS identified among the *T. rangeli* sequences, nine loci were selected for genotyping assays using distinct parasite strains in order to assess the parasite populational structure. Supported by FINEP, CNPq, CAPES and UFSC.

BM058 - *Trypanosoma* (*Herpetosoma*) *lewisi* and allied species: Analyses of multiple gene sequences support close phylogenetic relationships and host-switching of *T. lewisi* from rats to captive monkeys

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We characterized four trypanosomes from domestic rats and three from captive non-human primates, isolated by hemoculture and showing epi- and trypomastigotes similar to *T. lewisi,* a non-pathogenic rodent species transmitted by fleas and non-infective to humans. Isolates were identified as *T. lewisi*-trypanosomes by barcoding using V7-V8 SSUrDNA. Phylogeny using SSUrDNA sequences tightly clustered all new isolates with *T. lewisi* and allied species from other hosts into the homogeneous clade *T. lewisi.* Identical SSUrDNA sequences were shared by all new isolates from rats and monkeys, which were highly similar to European *T. lewisi* whereas significant divergences separate other trypanosomes from diverse hosts and geographic origins. Aiming to resolve relationships within clade *T. lewisi,* polymorphic spliced leader and ITS2rDNA sequences from new isolates were analyzed. Results corroborated that isolates from rats and monkeys were indeed quite close. Behavior of monkey isolates in culture and experimentally infected *Rattus norvergicus* was also compatible with *T. lewisi*. Therefore, all results supported that new isolates from monkeys are indeed *T. lewisi*. *T. lewisi*-trypanosomes are considered highly host-restricted despite host-switching mediated by fleas reported among genera/subfamilies of rodents living in sympatry. Only three of more than 150 captive monkeys examined were found infected with *T. lewisi* whereas free-ranging monkeys were never infected with this species although showing high prevalence of *T. rangeli* and *T. cruzi* infections. The existence of infected rats and exposition of captive monkeys to infective fleas should be responsible for switching of *T. lewisi* from rats to monkeys. Cases of sick infants infected by *T. lewisi*-like in Thailand and Malaysia were also attributed to rat reservoirs and flea-vectors, suggesting that *T. lewisi* trypanosomes can be opportunistic parasites of primates, what could explain infection of phylogenetically distant host species and our results from captive-monkeys.
Supported by CNPq and Capes (PNPD)

BM059 - Phylogeography and new hosts of *Trypanosoma cruzi* lineage TCIIc

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The evolutionary history of *T. cruzi* lineages is far from being clearly understood. The phylogenetic placement of lineage TCIIc in relation to other lineages is weakly supported in most studies, which are based on a few isolates from humans and triatomines. TCIIc has been sporadically reported in humans and is relatively poorly understood despite a widespread distribution in sylvatic cycles, occurring from Amazonia to southern Brazil, and in Colombia, Argentina, and Paraguay. We characterized 28 new isolates of TCIIc of mammals and triatomines from Northern to Southern Brazil, confirming the widespread distribution of this lineage. Phylogenetic analyses based on SSU rDNA and cytochrome b sequences separated TCIIc from TCIIa according to terrestrial and arboreal ecotopes of their preferential mammalian hosts and vectors. TCIIc was more closely related to TCIId/e and separated by large distances from TCIIb and TCI. We provide evidence that TCIIa from South America (arboreal ecotopes of Amazonia), TCIIa from North America (raccoon and dogs) and TCIIc correspond to three independent lineages that circulate in distinct hosts and ecological niches. In addition, armadillos, terrestrial didelphids and rodents, and domestic dogs were found infected by TCIIc in Brazil. Together, habitat, mammalian host and vector association corroborated the link between TCIIc and terrestrial transmission cycles/ecological niches. We believe that, in Brazil, this is the first description of TCIIc from rodents and domestic dogs. Terrestrial triatomines of genera *Panstrongylus* and *Triatoma* were confirmed as vectors of TCIIc in Brazil. In addition, phylogeographical analysis using ITS1 rDNA sequences disclosed clusters of TCIIc isolates in accordance with their geographic origin, independent of their host species. Results from this study confirm the need for phylogeographical studies, in addition to traditional genotyping, to understand the association of *T. cruzi* lineages with mammals, vectors and ecotopes.
Supported by CNPq and FAPESP (PRONEX)

BM060 - Phylogeographycal analysis based on nuclear and mitochondrial genes of *Schizotrypanum* trypanosomes isolated from Brazilian bats

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Several species of trypanosomes, most of subgenera *Schizotrypanum* and *Megatrypanum,* have been recorded in chiropterans throughout the world. The subgenus *Schizotrypanum* comprises highly prevalent species restricted to bats, excepting *T. cruzi* that is a generalist parasite in mammals of the American continent. The evolutionary processes that have led to the current phylogenetic structure of *Schizotrypanum* trypanosomes are poorly understood. In this study, we evaluated the genetic diversity and phylo- and biogeographical patterns of trypanosomes from Brazilian bats by examining 1043 bats (63 species of 7 families) from Amazonia, the Pantanal, Cerrado and the Atlantic Forest biomes. The prevalence of trypanosome-infected bats, as estimated by haemoculture, was 12.9%, resulting in 77 cultures. However, the real prevalence is much higher as indicate by analysis of bat blood samples by microhaematocrit. Most of isolates were identified as *Schizotrypanum*, and classified as *T. cruzi* (14)*, T. c. marinkellei* (38) or *T. dionisii*-like (25). Phylogenies using nuclear SSUrDNA and mitochondrial cytb sequences generated 3 clades corresponding to these species, which formed the clade *Schizotrypanum. T. dionisii*-like from Brazil clustered with *T. dionisii* from Europe despite relevant genetic distance. Bat trypanosomes were associated with phylogeographical and ecobiological patterns. *T. dionisii*-like (32.4%) infected 12 species from 4 bat families captured in all biomes, from North to South Brazil. *T. c. marinkellei* (49.3%) was restricted to Phyllostomidae bats from Amazonia to the Pantanal. *T. cruzi* (18.2%) was mainly from Vespertilionidae and Phyllostomidae from the Pantanal/Cerrado and the Atlantic Forest, besides some isolates from Amazonia. Knowledge of *Schizotrypanum* trypanosomes are important to improve our understanding of host-parasite relationships and the evolution of these closely related trypanosomes, which range from generalist to highly bat-restricted species, are transmitted by different vectors, and vary from non-pathogenic species to the pathogenic *T. cruzi*. Supported by FAPESP (PRONEX) and CNPq

BM061 - A potentially pathogenic *Acanthamoeba sp.* isolated from tap water in Brazil: genotyping, biological features and proteolytic activities

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Free-living amoebae of the genus *Acanthamoeba* are distributed worldwide, and frequently isolated from the environment. The organisms may cause human keratitis, and more severe, often lethal infections (granulomatous encephalitis, disseminated cutaneous diseases) in immunosupressed hosts. Infections by *Acanthamoeba* remain poorly investigated in Brazil as well as the genetic diversity, and the pathogenic potential of species prevalent in our country. We report here for the first time isolation of an *Acanthamoeba sp.* (ACC01) from tap water in São Paulo City (São Paulo State, Brazil). Following axenic culture establishment, the organism could be serially maintained without antibiotics in ATCC 712 (PYG) growth medium. *Acanthamoeba* ACC01 was molecularly identified by analysis of a ~900 bp SSU rRNA gene sequence aligned with 25 sequences retrieved from GenBank: 21 were from *Acanthamoeba* reference species, and four (U/E5, U/E6, U/E8R U/E10) from brazilian human cases of keratitis. Phylogenetic analysis (using MP, ML, and NJ) based on partial SSU rRNA gene sequencing assigned the tap water isolate in genotype T4, and revealed high similarity (~ 99%) with *A. polyphaga* ATCC 30461 that was originally isolated from a patient suffering from chronic keratitis. The Brazilian and the keratitis isolate (ATCC 30461) exhibited morphology typical of group II *Acanthamoeba* species, shared the ability to grow at 37 ^{o}C (temperature tolerance), and to multiply and/or resist in hiperosmolar media (osmotolerance). Both isolates were additionally shown to secrete comparable amounts of proteolytic activities, particularly serine peptidases optimally active at a near neutral/alkaline pH, and resolving identically in gelatin gels. Incubation of substrate gels at pH 4.0 with 2 mM DTT also indicated secretion of similar, E-64 susceptible cysteine peptidases. Altogether, the results indicate the pathogenic potential of isolate ACC01.
FAPESP and CNPq supported this work.

BM062 - Phylogenetic analysis using *SSU*rRNA and *gGAPDH* genes and ultrastructural characterization of snake trypanosomes

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The description of species of snake trypanosomes has relied on host origin and morphology of blood forms. Consequently, the validity of these species requires confirmation. The two snake trypanosomes characterized in this study were isolated by hemoculture from Brazilian *Crotalus durissus* and *Pseudoboa nigra,* cultured over layered insect cells and their morphometrical and ultrastructural features were analyzed by light, and scanning and transmission electron microscopies. Both trypanosomes displayed very large and wide trypomastigotes with a highly curved shape and developed undulant membrane. Culture forms showed a remarkable ramified mitochondrion, a large Golgi complex with many cisternae and a nucleus with an unusual number of pores. We sequenced the *SSU*rDNA and *gGAPDH* genes of these trypanosomes and inferred phylogenetic trees using independent and combined data sets. This is the first time that snake trypanosomes were included in the phylogenetic trees of Trypanosomatidae, which always clustered tightly together the snake trypanosomes in a clade closest to terrestrial lizard trypanosomes. The fact that snake and lizard trypanosomes could be closely related is not unexpected since they share squamate hosts and both could be transmitted by phlebotomines. However, *C. durissus* is from the Cerrado biome and *P. nigra* from wetlands of the Pantanal biome, suggesting that trypanosomes from these snakes should have distinct vector species. The positioning in the phylogenetic trees and the barcoding using *SSU*rDNA, which showed high sequence divergence, allowed the classification of the isolates from the distinct snake species as separate species: The isolate from *P. nigra* is described as a new species whereas the isolate from *C. durissus* is redescribed as *T. cascavelli*.
Supported by CAPES and CNPq

BM063 - ESTABLISHMENT OF *UNICAPSULOCAUDATA MUGILIS* GEN. NOV. SP. NOV. (MYXOZOA: CERATOMYXIDAE) BASED ON MORPHOLOGICAL AND MOLECULAR DATA

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Unicapsulocaudata mugilis gen. nov. sp. nov., which parasitizes in the gall bladder of *Mugil cephalus* Linnaeus, 1758, was isolated from the East China Sea. Spores with bluntly pointed apex and tapered posterior are slim and long, measuring (n=15) 25.4 ± 3.8 (18 ~ 29) μm long × 2.9 ± 0.3 (2 ~ 3) μm wide. Single polar capsule is pyriform, measuring 5.2 ± 1.2 (3 ~ 7) μm long × 2.6 ± 0.3 (2 ~ 3) μm wide, containing polar filaments coiled 3 – 4 turns. Spore shape of this species differs from that of any other myxozoan species. Furthermore, the small subunit ribosomal DNA (SSU rDNA) of *Unicapsulocaudata mugilis* sp. n. is unique among myxozoans sequenced to date. Phylogenetically, the new species fell within a well supported clade of *Ceratomyxa* species that infect the gall bladders. The results of our study has supported that gene sequencing and identification of morphological characters will enhance the taxonomy and systematics of Myxosporea.
Supported by the NSFC (Project No. 30570221)

BM064 - PARTICIPATION OF THE THROMBOSPONDIN RELATED ANONYMOUS PROTEIN 2 (TRAP 2) OF THE APICOMPLEXAN *NEOSPORA CANINUM* IN THE CELL INVASION PROCESS

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Neospora caninum is an Apicomplexan protozoan, obligatory intracellular parasite that has the dogs as definitive hosts and especially cattle as intermediate hosts, causing in the first ones encephalopathy and the last ones abortion with fertility impairment. Such facts lead to significant losses to livestock worldwide. The parasite must invade the cells for development, using the discharge of proteins contained in the phylum-specific organelles, like the micronemes, rhoptries and dense granules. This work was performed on a micronemal protein named thrombospondin-related anonymous protein (TRAP), related with adhesion and connection with the intracellular motor responsible for the active process of invasion. Based on the homologue previously described in the literature, TRAP1, the aim of this study was the characterization of the unknown TRAP2 homologue, including expression of its recombinant forms, use of its anti-sera for functional assays and detection of its native form. Based on ESTs and genomic data from *N. caninum*, and using the RLM-RACE (5´ RACE System for Rapid Amplification of cDNA Ends) the complete sequence of NcTRAP2 was obtained. As NcTRAP1, the protein sequence is formed by a signal peptide, an integrin, five thrombospondin, a transmembrane region and a cytoplasmatic tail. The integrin and thrombospondin motifs are related to adhesion for initiation of the invasive process and were the targets of this work. The recombinant fragment from the functional core (thrombospondin and integrin), with 52 kDa was utilized for antiserum production. The antiserum localized the native form of NcTRAP2 with 80 kDa and its processed forms in ESA (Excreted/Secreted Antigen) with 70 kDa, both in western blot 1D and 2D. The purified antibody had the ability to inhibit the invasive process up to 61%. NcTRAP2 was localized at the apical complex of the parasite of the tachyzoites by confocal immunofluorescence, confirming its putative micronemal localization.
Keywords: *Neospora caninum*, Apicomplexa, Thrombospondin-Related Anonymous Protein
Supported by FAPESP (project and fellowship) and CNPq (project).

BM065 - Characterization of a protein with apple domains from the Apicomplexan *Neospora caninum*

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Neospora caninum (Apicomplexa: Eimeriina: Sarcocystidae) is a protozoan parasite which can cause the disease neosporosis, a major cause of bovine fetal loss worldwide. Due to its association to abortion, neosporosis has a huge economic impact. An essential process of *N. caninum* cycle is the host cell invasion, i.e., the interaction parasite/host cell which is conserved to Apicomplexan members. Surface receptors associated to a series of proteins released by the secretory organelles micronemes, rhoptries and dense granules participate of the invasion process. Some microneme proteins found in other Apicomplexan parasites, such as MIC4 from *Toxoplasma gondii* and EtMIC5 from *Eimeria tenella*, contain apple domains, which are composed of six conserved cysteine residues. These proteins are likely to have an important conserved function, possibly in binding interactions between the parasite and host cell. The aim of the present work was the investigation of genes with Apple domains from *N. caninum* followed by their cloning, expression and purification of the recombinant forms. Based on ESTs it was possible to screen the non-annotated genomic database of *N. caninum* and find three flanking fragments localized in a single contig. Each of these fragments contained three apple domains and the sequencing data revealed a high level of similarity among the three fragments. Two of these fragments were cloned and expressed in pET28 with molecular weight of 35 kDa and 40 KDa, both consistent with the theoretical molecular weights. We are currently immunizing animals for raising antisera and we are also investigating the gene organization of this (these) protein (s), which indicates to be very different from TgMIC4 and EtMIC5.
Supported by FAPESP (project) and CAPES (fellowship).

BM066 - SELENOCYSTEINE PATHWAY AND ITS PRODUCTS: RELEVANCE IN THE OXIDATIVE PROTECTION OF *TRYPANOSOMA BRUCEI*.

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Selenoproteins result from the incorporation of selenocysteine (Sec-U) at an in-frame UGA stop codons. These proteins, as well as the Sec synthesis pathway are present in many representatives from Bacteria, Archaobacteria and Eukaryotes, and are mostly involved in oxidative pathways. In Kinetoplastida the presence of the Sec pathway was identified and is been characterized. The inhibition of cell growth by the antirheumatic agent Auranofin, a known inhibitor of selenoproteins, opens the possibility for the use of the Sec pathway as a target for Kinetoplastid drug development. RNA interference experiments of *SPS2* in procyclic *T. brucei* form causes significant growth inhibition and an apoptotic-like phenotype, contrasting with similar experiments recently published (Aeby, E. *et al*, 2009). We are investigating the reason for such discrepancy and our results indicate that the pathway is essential during oxidative stress. We are now investigating the effect of *SPS2* knock-down on a non-oxidative stress state and in the bloodstream form of *T. brucei*, to clarify the importance of selenoproteins in oxidative pathways.
Supported by FAPESP.

BM067 - Molecular Characterization of two SECp43 mutants and RNAi studies in *Trypanosoma brucei*

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The focus of our work is the selenocysteine synthesis pathway in the parasite *Trypanosoma brucei*. This pathway is responsible for decoding in-frame UGA-stop codons into the 21th amino acid selenocisteína in a set of proteins known as selenoproteins. These amino acid represent the major biological form of selenium and its synthesis and incorporation into selenoproteins requires complex molecular machinery.
Trypanosomatids are a major cause of mortality in tropical and sub-tropical regions of the world and are affected by the lack of effective treatments. Several intriguing molecular pathways are found in these parasites, rendering them particularly attractive for biochemical investigation. In kinetoplastida, Secp43 appears to be a key enzyme in the $tRNA^{Sec}$ modification and its presentation to the translation machinery. In eukaryotes the full repertoire of genes involved in this route of synthesis has recently been uncovered, but still several answers remain unchecked.

This gene is intriguing due to its architecture, resulting from a gene duplication and divergent evolution event. The counterparts of SECp43 of various organisms are conserved with each other but posses a single domain. We have cloned and characterized two *T. brucei* SECp43 constructs. To provide new insights on the relationship between the selenoprotein synthesis pathway and their biological role in *T. brucei*, we used the RNA interference technique to knock down the gene expression in both procyclic and bloodstream forms. The mutant was also cloned and an expression and purification protocol established. Mouse polyclonal antibodies were obtained and revealed the presence of the translated Secp43 protein in *T. brucei* extracts. A detailed biochemical characterization of this protein and its interaction with $tRNA^{Sec}$ is under way.

Supported by FAPESP, CNPq

BM068 - DNA and histone amounts during transformation of tissue culture derived trypomastigotes to epimastigotes from *Trypanosoma cruzi*

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Trypanosoma cruzi, the agent of Chagas disease presents a complex life cycle involving two hosts, the insect Reduviidae and the mammalian. The infective forms are the trypomastigotes (metacyclic and bloodstream), while epimastigotes and amastigotes proliferate by binary fission. We found a two to four increase in the amount of histones in epimastigotes compared to tissue culture derived trypomastigotes, the form that corresponds to the bloodstream stage. Consequently, we asked if this was due to a different chromatin organization or to variations in the total DNA of in these forms. By quantifying satellite DNA extracted from these forms, which corresponds to 10% of the total DNA, we found evidences that epimastigotes has more than twice satellite DNA than the trypomastigotes, suggesting that DNA levels varies in different *T.* cruzi stages. Flow cytometry analyses of cells stained by propidium iodide also indicate a different DNA staining. When these trypomastigotes were incubated in liver infusion tryptose they transformed in round forms and later on epimastigotes, a process that normally occurs in the insect vector. After 36 hours in liver infusion medium, we noticed that round forms aggregate and their flagellum started to growth. After 72 hours, some parasites were much larger presenting several nuclei and kinetoplasts. After 132 hours most of population transforms in typical epimastigotes that starts to proliferate. The amount of DNA extracted from the originating trypomastigotes was also smaller when compared to the obtained epimastigotes, considering the same amount of cells. These results suggest that during the transformation of trypomastigotes to epimastigotes DNA levels can change in the parasite. We are further investigating the mechanism that governs these changes.
Supported by FAPESP and CNPq.

BM069 - *TRYPANOSOMA CRUZI* MSH2: A DUAL ROLE PROTEIN INVOLVED IN NUCLEAR DNA MISMATCH REPAIR AND MITOCHONDRIAL OXIDATIVE STRESS RESPONSE?

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The taxon *T. cruzi* presents large intra-specific genetic variation, with strains showing distinct characteristics including virulence, sensitivity to drugs, antigenic profile and histotropism. In spite of this genetic diversity, three major lineages of the parasite, named *T. cruzi* I, II and III have been identified, with *T. cruzi* II strains being preferentially associated with the human infection. Several studies have also indicated that *T. cruzi* II strains present higher levels of genomic sequence variability compared to *T. cruzi* I strains. Early studies from our group suggested that TcMSH2, a protein that plays a major role in DNA mismatch repair (MMR) could be part of the molecular mechanisms underlying the differences in *T. cruzi* genetic diversity. Here we present evidences suggesting that, in *T. cruzi* MSH2 may be also involved in the response to oxidative stress. *Tcmsh2* gene polymorphisms generate A,B and C isoforms of the protein, which are specific for *T. cruzi* I, III and II strains, respectively. We postulated that differences in TcMSH2 activity may have a role in generating differences in genetic variability amongst *T. cruzi* strains. Levels of oxidative stress-induced DNA damage, oxidative metabolism, as well as the response of cell cultures to different genotoxic agents were determined in epimastigote cultures of *T. cruzi* I and II strains. Our analyses support the hypothesis that strains belonging to *T. cruzi* I lineage (which express the TcMSH2a isoform) present higher DNA repair capacity. Similar analyses performed in *Tcmsh2* single knockouts as well as in *T. brucei* null mutants transfected with *Tcmsh2* also indicate that, in addition to its role in MMR, TcMSH2 may be also directly involved with the response to oxidative stress. Several attempts to knockout the *Tcmsh2* gene indicated that this gene may be multifunctional and, consequently, essential.
Support: CNPq, FAPEMIG and HHMI.

BM070 – *Trypanosoma rangeli*: Phylogenetic analysis and markers for diagnosis and genotyping based on cathepsin L-like genes

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Trypanosoma rangeli is a non-pathogenic parasite of man and domestic and wild animals that shares with *T. cruzi* mammalian hosts, triatomine vectors and an overlapping geographic distribution in Central and South America. To understand the evolutionary relationship among cathepsin L-like (CatL-like) genes of *T. rangeli,* other trypanosomes, *Leishmania* spp. and bodonids, all available sequences encoding the mature CatL-like proteases were used for phylogenetic analysis. In all inferred phylogenetic trees, sequences of *T. rangeli* positioned closest to homologous sequences from *T. cruzi* (cruzipain)*,* and distant from CatL-like from *T. brucei*, thus corroborating the phylogeny of kinetoplastids based on SSUrDNA. Moreover, sequences corresponding to the catalytic domain of CatL-like (cdCatL-like) genes were determined for 17 isolates of *T. rangeli* from humans, wild mammals and triatomines of the genus *Rhodnius* from Central and South America. Analyses of all sequences from these isolates of *T. rangeli* suggest the existence of one major CatL-like enzyme homologous to cruzipain. However, polymorphism analysis of cdCatL-like sequences revealed a relevant heterogeneity within *T. rangeli* contrasting with the homogeneity of cruzipain sequences from *T. cruzi* isolates of distinct lineages. Phylogenetic analysis of sequences from isolates representative of the overall phylogenetic diversity and geographical range of *T. rangeli* supported all lineages previously established using ribosomal and spliced leader genes. Congruence of lineage divergence using three independent molecular markers corroborate clonal evolution and independent transmission cycles of *T. rangeli* lineages associated to sympatric species of *Rhodnius*. This is the first comprehensive study using protein-encoding genes to infer phylogenetic relationships among lineages within *T. rangeli*. Sequences of CatL-like genes were used as targets to species-specific PCR assays for *T. rangeli* and *T. cruzi*, and for a multiplex-PCR for *T. rangeli* genotyping.
Supported by FAPESP (PRONEX) and CNPq.

BM071 - PHYLOGENETIC RELATIONSHIPS AND MORPHOLOGICAL FEATURES OF BRAZILIAN ISOLATES REVEALED NEW CLADES AND SPECIES OF *EUGLENA*

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Flagellates of the genus *Euglena* are abundant in soil, freshwater and marine environments. The phylogenetic relationships within the genus are far from understood and vary according to taxa, genes and methodologies used. For a better appraisal of the diversity and resolution of relationships within *Euglena* it would be necessary to analyze a large number of isolates from different habitats and geographical origins. Until now, species of *Euglena* from South America had not been included in any phylogenetic study. In this study, we characterized isolates of this genus from the Brazilian biomes of the Atlantic Forest, the Pantanal, Amazonia and Cerrado. Flagellates from 20 samples of soil and water were identified as *Euglena* by traditional taxonomic parameters, and distributed in 5 main morphological groups. Analyses of SSU rDNA sequences of the new flagellates disclosed 25 distinct sequences, indicating that 5 were mixed cultures containing two isolates. These sequences were used to infer phylogenetic relationships among Brazilian and foreign isolates. Phylogenetic trees supported the classification of all Brazilian isolates as *Euglena* and distributed them in seven clades, most nested within the major "Group 3" of *Euglena,* thus disclosing cryptic genetic diversity between morphologically indistinguishable isolates. One isolate representative of each phylogenetically defined clade was selected for ultrastructural characterization through scanning electron microscopy. Differences among isolates from distinct clades included: shape of posterior and anterior regions, arrangement of pellicle strips varying from four to eight strips between pores, and two patterns of strip reduction. Together, molecular and morphological features revealed a high diversity among *Euglena* isolates from Brazil and disclosed new species. Results greatly enhanced the knowledge about the genetic diversity of *Euglena* and improved the understanding of the phylogenetic relationships among clades within this genus.
Supported by CNPq (CT-Hidro).

BM072 - RIBOSE 5-PHOSPHATE ISOMERASE FROM *LEISHMANIA MAJOR*: MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF NATIVE ENZYME

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Leishmaniasis is a widespread disease that is endemic in several parts of the world and is caused by some species of *Leishmania* genera. There are drugs that are being used to treat this disease, but the parasite is becoming resistant to many of them. Then, it is essential importance the development of new effective drugs against this parasite. We used the AnEnπ tool for the identification of possible drug targets, based on the structural differences of enzymatic activities shared by humans and *Leishmania major.* One of these enzymes is ribose-5-phosphate isomerase (R5PI) that catalyzes an important step of pentose phosphate pathway. Our group has already cloned the R5PI gene from *L. major* in pBadThio/TOPO® vector, expressed the protein in insoluble form, purified it and produced polyclonal antibodies against the protein. For identification of native enzyme we performed a 2D-PAGE with soluble proteins from *L. major* promastigotes, followed by western-blot using our polyclonal antibodies. We identificated a spot with MW = 18.27 and PI = 6.52, that are very close to expected values (18.61 and 6.40 respectively). We have also done a RT-PCR followed by sequencing of the PCR product, and our data shows that R5PI's mRNA is present in promastigotes cells. Future works will be done to make immunocitochemistry assays. Our data corroborates with genomics annotation, showing that this enzyme is expressed at least in promastigotes of *L. major*.
Supported by: PDTIS – FIOCRUZ; CNPq, FAPERJ.
Keywords: *Leishmania major*, ribose 5-phosphate isomerase, trypanosomatids

BM073 - *Mantamonas* gen. n., a major new deep-branching eukaryotic lineage of marine gliding zooflagellates

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Mantamonas is a new genus of deep-branching marine gliding zooflagellates, exhibiting highly distinctive morphological traits. These include a strongly flattened, asymmetric, plastic cell shape, characterized by a conspicuous extensible pseudopodium on the left-hand side of the cell (viewed dorsally); a short and nearly invisible anterior cilium; and a longer, more obvious, acronematic posterior cilium that is used for smooth gliding along the substratum. We cultivated the type species *Mantamonas plastica* from marine sediment in Walney Island, England and studied it by differential interference contrast and phase contrast microscopy. *Mantamonas* plastica is bacterivorous and ~4 µm long and ~4 µm wide. We later isolated a second morphologically similar strain from Dar-Es Salam Beach, Tanzania, and extracted its DNA, but the culture died before we could take pictures. We sequenced 18S, ITS and 28S rDNA from both isolates. Phylogenetic analysis shows that they represent two genetically radically different but robustly related species. This *Mantamonas* clade is one the deepest branching lineages in the eukaryote tree. As it is a fairly short branch on the tree we do not suspect serious long-branch artifacts. However, its closest relatives are unclear based on 18S and 28S rDNA alone; *Mantamonas* could be the sister lineage to planomonads or apusomonads, which are also gliding flagellates. It may be of special significance for reconstructing the morphology of the earliest eukaryotic cells.

BM074 - COMPARISON OF *Leishmania chagasi* GENOTYPES ISOLATED FROM DOGS OF TWO REGIONS OF BRAZIL

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The study of polymorphism using tools based on the sequence of DNA shows that the presence of genetic differences between the parasites of *Leishmania infantum / chagasi* species of Old World is not associated to preferences for a particular host or to the disease outcome. On the other hand, differences on parasite genotype can be associated to geographical origin. The aim of our study was to compare the genotypic profiles of *L. infantum / chagasi* isolated from dogs of Jequié-BA and Campo Grande-MS. *Leishmania* isolates were obtained from 48 dogs with visceral leishmaniasis presenting different degrees of severity, between the years of 2006 and 2008. Genomic DNA was randomly amplified by RAPD. Polymorphism was observed in the profiles generated by 4 of the 7 primers employed in the method. A similarity matrix was generated containing approximately 2,352 characters that ranged from ~550 bp to ~2100 bp. All isolates showed profiles identical to the reference strain of *L. chagasi* (MHOM/BR00/MER02). The little polymorphism observed in the isolates was not associated with geographical origin, clinical presentation of the disease, or the year in which isolates have been obtained. These data suggest that similarities among strains of *L. chagasi* circulating among dogs could be correlated with the absence of clinical polymorphism observed in canine visceral leishmaniasis in Brazil, and can explain the closed adaptation of the parasite with a single specie of vector.

Supported by FAPESB and CNPq.

BM075 - MOLECULAR CHARACTERIZATION OF TELOMERIC AND SUBTELOMERIC REGIONS OF KP1(+) AND KP1(-) STRAINS OF *TRYPANOSOMA RANGELI*

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The analysis of telomeric and subtelomeric regions has contributed to the knowledge of the biology of different trypanossomatids and the characterization of these regions in *Trypanosoma rangeli* can be useful in the elucidation of the genetic and biological diversity presented by this parasite. In this work, we aimed to investigate the organization of sequences present in the chromosomal extremities of KP1(+) and KP1(-) strains of *T. rangeli*. Southern blots containing samples of *Rsa*I-digested genomic DNA of four KP1(+) strains and six KP1(-) strains of the parasite were probed with the previously described subtelomeric sequences of *T. rangeli* and with the hexameric-telomere repetition of *Trypanosoma brucei*. Southern hybridization experiments revealed two distinct hybridization patterns entirely coincident with the *T. rangeli* genotypes defined by kDNA analysis. The range of Terminal Restriction Fragments was 1.3kb-4.0kb for KP1(+) strains and 0.3kb-3.0kb for KP1(-) strains of *T. rangeli*. The 170bp subtelomeric amplicons of *T. rangeli* were cloned in pCR4-TOPO and sequenced. Sequence analysis was performed by BLASTN and ClustalW and revealed the occurrence of sequence polymorphisms in the subtelomeric region of *T. rangeli* that were, also, coincident with the parasite KP1(+) and KP1(-) genotypes. Furthermore, we observed the presence of sequence polymorphisms within groups, as demonstrated by the analysis of one sequence obtained from a KP1(-) strain, which appeared isolated in one branch of the phenogram. This result is noteworthy, since some authors have suggested that *T. rangeli* strains can be divided in more than two subpopulations. Concluding, our results extend the genotypic differences observed by both kDNA and karyotypic analysis in *T. rangeli* to the chromosomal extremities. Further studies on these regions may reveal novel features of *T. rangeli* genetics and biology.
Supported by CNPq, FUNEPU and FAPEMIG.

BM076 - GENE CHARACTERIZATION AND MOLECULAR MODELLING OF THE CHAPERONE ClpB/HSP104 OF *TRYPANOSOMA CRUZI*

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The Kinetoplastid *Trypanosoma cruzi* is the causative agent of Chagas' disease, infecting mammalian and insect hosts. Temperature is considered to be a triggering factor of the developmental program allowing the adaptation of the parasite to the mammalian host conditions. The heat shock response protects cells from the deleterious effects of environmental stresses such as heat. The heat shock protein (HSP) 100 family is a group of chaperones crucial to maintaining the native protein conformation and preventing nonspecific protein aggregation. Our aim is to study ClpB/HSP104 gene structure, expression in *T. cruzi*. The amino acid sequence of ClpB/HSP104 of *T. brucei* was used to search for orthologous sequences in TcruziDB. An assembly error was detected and an internal 10 amino acid gap in the draft genome was filled through PCR amplification and sequencing to obtain the complete coding region of 2577 bp and 859 amino acids. Genomic southern blot analysis suggests that the ClpB/HSP104 gene is present in just one copy in the genome. A recombinant ClpB/HSP104 will be used to generate specific polyclonal antibodies to determine the subcellular localization of the protein. Northern blot analysis using poly(A)+ RNA to identify the ClpB/HSP104 mRNA(s) are currently under way, and preliminary qRT-PCR analysis showed a 3-fold increase in mRNA levels in epimastigote after incubation at 37□C for 3h. In addition, a 3D structure of *T. cruzi* ClpB/HSP104 is being generated through molecular modelling. The 3D model is being constructed with the Modeller 9v3 software using ClpB of *Thermus thermophilus* as a template (PDB code 1QVR). The first results showed e-value = 0.0, identity = 51%, positives = 70% and more than 99% of sequence coverage, indicating a consistent putative structure. Supported by CNPq and FAPERJ.

BM077 - MODULATION OF GENE EXPRESSION IN *LEISHMANIA BRAZILIENSIS* AND *LEISHMANIA GUYANENSIS* CLINICAL ISOLATES WITH DIFFERENT TREATMENT OUTCOME FROM BRAZIL

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In Brazil, the cutaneous leishmaniasis represents an important problem for the public health and chemotherapy is an important element of the control arsenal. However, treatment efficacy is variable, a phenomenon that might be due to host factors as well as parasite drug resistance. We previously observed that Brazilian patients infected with *Leishmania guyanensis* (LG) showed a higher risk of antimony treatment failure than patients infected with *L. braziliensis* (LB). In order to better understand the possible contribution of parasite factors to this phenomenon, we characterized 12 LB and 25 LG isolates collected from patients presenting different treatment outcome. For each isolate, promastigotes culture duplicates were grown and harvested in two points of the growth curve related to the late log and stationary phase. Four genes encoding proteins possibly involved in antimony-resistance, including transport (AQP1 and MRPA) and redox metabolism (γ-GCS and TR), were analyzed through gene expression profiling. We found out that in general, MRPA and γ-GCS were more expressed in LG than in LB. Within LB, we did not find statistically significant differences between isolates from different treatment outcome. However, in LG, the gene γ-GCS was overexpressed in therapeutic failure isolates regardless of the growth curve phase. To the best of our knowledge, this is the first report in which someone approached gene expression profiling of two *Leishmania* species at the same analysis providing further insights. The results indicate that treatment failure might be related to a change in thiol metabolism and increase of the efflux of the drug by the parasite. Therefore, the present work enlightens the potential of expression profiling of the genes MRPA and γ-GCS to be used in the prognosis of cutaneous leishmaniasis caused by LB and LG in Brazil.
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BM078 - STUDY ON DIVERSITY OF STOP CODON IN CILIATES AND STOP-CODON RECOGNITION BY CILIATE ERF1S

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The genetic code for nuclear genes in certain ciliates are known to be reassigned in UGA, UAG, and UAA codons, which are assigned as stop codons in the standard genetic code. In some ciliate species, such as *Euplotes* , UAA and UAG encode glutamine. In other ciliates, such as *Blepharisma*, UGA appears to be translated as tryptophan. Molecular mechanisms for the variant genetic codes for the universal stop codons remain unsolved. Since eukaryotic release factor 1 (eRF1) is known to recognize all stop codon recognition, it is assumed that molecular evolution of specific tRNAs for canonical stop codons and codon recognition of eRF1 are tightly coupled in ciliates with variant stop codons. The crystal structure of human eRF1 has been determined, and it is composed of three structurally separated domains. Thus, convenient assay systems for codon specificity are established using chimeric proteins in domain 1 of eRF1.
In *Blepharisma*, the universal stop codon UGA is reassigned to tryptophan. The reassignment of the codon UGA implies that eRF1 may lose specificity for UGA. We examined the stop codon recognizing capacity of eRF1 domain 1 from *Blepharisma musculus* by an *in vivo* yeast complementation assay system. Unexpectedly, the chimeric eRF1, which contained *Blepharisma* domain 1, was able to replace the endogenous yeast eRF1, i.e. *Blepharisma* eRF1 and recognized all three stop codons (UAA, UAG and UGA). Our result leads to a speculation that the stop codon reassignment in *Blepharisma* has occurred by a mechanism that might require the appearance of a novel $tRNA^{Trp}$ molecule that is exclusively specific to UGA. We also constructed the chimeric eRF1s, which contained *Loxodes striatus* domain1 and other heterotrich ciliate domain 1, and examined the stop codon recognizing capacity.

BM079 - ORAL TRANSMISSION OF CHAGAS DISEASE IN SANTA CATARINA: MOLECULAR CHARACTERIZATION OF *Trypanosoma cruzi* ISOLATED FROM HUMANS BEFORE AND AFTER TREATMENT FAILURE.

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An outbreak of acute human Chagas disease due to ingestion of *Trypanosoma cruzi*-contaminated sugar cane juice occurred in 2005 in Santa Catarina State, southern Brazil, when 24 people were infected. After diagnosis, patients received treatment with Rochagan® (5mg/kg/60 to 90 days) and were included in a five-year follow-up protocol using clinical, serological and parasitological methods. The present study aims to characterize 13 *T. cruzi* isolates from humans before (n= 9) and after treatment failure (n= 4) using distinct molecular markers. Single-gene analysis of the mini-exon, 24Sα rRNA and 18S rRNA genes were performed using TC/TC1/TCII, D71/D72 and V1/V2 primers, respectively. We have found that all studied patients' isolates before or after treatment failure belong to the TcIIb subgroup. In addition, genomic variability assessed by means of random amplification of DNA (RAPD) using primers 3303/3304/3305/3306/AB1/AB5 as well as UPGMA analysis revealed a high genetic homogeneity among all isolates. This study demonstrates that all patients were infected with the same *T. cruzi* lineage and it suggests that TcIIb parasites are maintained during human infection.
Supported by CNPq, FAPESC, CAPES and UFSC.

BM080 - CORRELATION OF THE *Trypanosoma cruzi* RFLP's PROFILES (SCHIZODEMES) AND THE CLINICAL FORMS OF CHAGAS´ DISEASE: PROBING THE PARASITE KDNA SEQUENCES VARIABILITY DIRECTLY FROM TISSUES FROM CHRONIC CHAGASIC PATIENTS

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Geographical variations in the pathogenesis and distribution of distinct CFCD (clinical forms of Chagas' disease) have been widely noticed, and it is believed that this pleomorphism is due to the relationship existent between host and parasite factors. However, many molecular methods have failed to demonstrate a correlation between *Trypanosoma cruzi* genetic variability and the CFCD. This unsuccessfulness is possibly related to the fact that these methodologies were based on previously isolated parasites, which do not exactly represent the subpopulations found in the affected tissues and probably more related with the CFCD pathogenesis. In the present work, by using four restriction enzymes we performed *T. cruzi* RFLP's analysis (schizodeme) from 330bp kDNA fragments amplified from ten heart and ten colon samples respectively obtained from chronic chagasic patients with cardiopathy (Uberaba-MG) and megacolon (five from Uberaba-MG and five from Goiânia-GO). In order to estimate the similarity existent among parasite subpopulations, the RFLP's profiles were analyzed using Jaccard's coefficient and UPGMA method. By using two of the four enzymes no significant difference among the subpopulations similarities was demonstrated. However, by using the others two enzymes a remarkable similarity among subpopulations from a same CFCD was observed, which was higher than that observed among subpopulations obtained from distinct CFCD. Based on the last results, a very homogeneous UPGMA dendrogram exhibiting two main groups was generated: one group was associated with the chagasic cardiopathy and composed of all subpopulations of hearts samples and one of a colon sample (from Uberaba-MG), while the second one was related to the chagasic megacolon and constituted by remained nine colon samples subpopulations. The clear association observed between *Trypanosoma cruzi* RFLP's and the CFCD (which wasn´t related to geographical source of the samples) suggests that genetically related subpopulations might be associated with the pathogenesis of Chagas' disease.
Supported by CNPq.

BM081 - A NEW HEMI-NESTED ASSAY AND PCR PROTOCOL OPTIMIZATION FOR *Trypanosoma cruzi* 330bp KDNA FRAGMENT DETECTION DIRECTLY FROM TISSUE SAMPLES FROM PATIENTS WITH CHRONIC CHAGAS' DISEASE: THE BENEFITS OF THE LOW-DENATURING TEMPERATURES

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PCR detection of *Trypanosoma cruzi* in tissues from chronic chagasic patients is essential to evaluate therapies' efficacy as well as to investigate the pathogenesis and the molecular epidemiology of Chagas' disease. However, this aim usually constitutes a difficult task due to the parasite scarcity observed in this phase of the disease. This work was focused on: (1) improve the PCR protocol used to amplify the *T. cruzi* 330bp kDNA fragment of DNA extracted from tissue samples (heart and colon) from chronic chagasic patients with cardiopathy and megacolon; (2) develop a new hemi-nested-PCR assay based on the 121, 122 and S67 kDNA primers. The PCR optimization was carried out by testing different reaction component concentrations, and distinct thermal cycling program temperatures. After seting out the best PCR component concentrations and using a thermal cycling program with remarkable low-denaturing temperature (86 ºC), we observed a significant increase on the intensity and yield of PCR products, as well as the decrease of unspecific fragments. The hemi-nested assay was composed of a first reaction using the primers 121/S67 followed by a second step using the 121/122 primers. The results obtained by hemi-nested were compared with those obtained by direct PCR. By using the hemi-nested assay, we were able to satisfactorily amplify the 330bp fragment using up to the 10^5 fold dilution of DNA template, while using the direct PCR a comparable result was only obtained when employing the same DNA sample thousands times concentrated. The results obtained by presented improved and inexpensive protocol were similar to those obtained by Hot-start assays. In addition, the hemi-nested here proposed significantly increased the sensitivity of parasite detection, besides allowing to amplify the whole 330bp kDNA fragment, which could be useful as template for typing techniques as the LSSP-PCR, schizodemes and hybridization approaches.
Supported by CNPq.

BM082 - STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF PUTATIVE RIBOSOMAL PROTEINS L7A AND L19 FROM *T.cruzi*.

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We have previously described the isolation and characterization of two new *T.cruzi* cDNAs encoding unusual L7a and L19 ribosomal proteins that are expressed during the *T.cruzi* entire life cycle. These proteins carry a repetitive domain at N-terminus (TcRpL7a) or at C-terminus (TcRpL19), which are absent on their respective orthologous, including *T.brucei* and Leishmania. Both proteins are highly immunogenic and its antigenicity can be attributed to the repetitive amino acid sequence, as shown by western blot using truncated versions of TcRpL7a in fusion with GST or 6xHis-Tag. Additional experiments using specific antibodies against recombinant TcRpL7a and cellular fractioning has shown the enrichment of TcRpL7a on polysomal fraction. Since the repetitive regions are present only in the potential L7a and L19 ribosomal proteins of *T.cruzi*, we decided to analyze the secondary structure of these proteins and their structures/location in the ribosome with resolved structure. Furthermore, we also decided to perform experiments for gene complementation using gene *T.brucei* as a model. The secondary structure prediction using the improved self-optimized prediction method (SOPMA) of the repetitive extensions showed that these regions are mainly forming a random coil structure. The structural analysis of the ribosome from *Canis familiaris* showed that the ribosomal proteins L19 and L7a are superficially located at the ribosome. And as expected, the extremities (N- or C-terminus), where the repeats are found in the *T.cruzi* ribosomal proteins, are facing the outside of the ribosome, probably not interfering with its probable function. To test whether these proteins are essential for the functioning of the ribosome, we have generated constructs to silence the *T.cruzi* orthologous in *T.brucei* by RNAi using the p2T7 177 vector. Currently, the parasites are being selected in culture medium for further analysis. Supported by: FAPEMIG

BM083 - Identification of mRNAs associated with TcRBP19, A new Trypanosoma cruzi RNA BINDING PROTEIN

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Trypanosoma cruzi is the causative agent of Chagas disease. Its life cycle involves at least four stages. Consequently, several proteins have to be tightly regulated to allow the rapid adaptation for the parasite survival. Gene expression regulation in trypanosomatids presents deviations from standard eukaryotic paradigms. Genes are organized in polycistronic units separated by intergenic regions containing information for mRNA processing. There are no canonical promoters identified yet and there is no evidence for regulated transcription initiation of protein coding genes.. The regulation of gene expression occurs predominantly at the post-transcriptional level. Undoubtedly, RNA binding proteins (RBP) play a major role in many aspects of RNA processing, function, and degradation of mRNA. We have described the characterization of TcRBP19, an RNA-binding protein containing an RRM in *T. cruzi*. Orthologous genes are present only in the TriTryp genomes, suggesting unique function in those parasites. TcRBP19 is a low expression protein barely detected at the amastigote stage localizing in a diffuse pattern in the cytoplasm. Epimastigotes overexpressing TcRBP19 showed no phenotypic alterations but, interestingly, those parasites have diminished their capacity of develop metacyclogenesis. Moreover, lower infection rates were observed in VERO cells with those transfectant parasites. In this work, we focused in the identification of mRNAs associated with TcRBP19, we performed RNA GST-pull down assays and we found that TcRBP19, among other interesting target RNAs, TcRBP19 also binds its own mRNA. We also investigated the effect of the TcRBP19 overexpression in the global amastigote gene expression using DNA microarray hybridization to evaluate the mRNA levels of parasites transfected

This work was supported by CSIC, PEDECIBA, CNPq

BM084 - TWO *Trypanosoma cruzi* SUBGROUPS INFECT CHRONIC CHAGASIC PATIENTS FROM THE STATE OF RIO GRANDE DO SUL, BRAZIL

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The taxon *Trypanosoma cruzi* are currently divided in two major phylogenetic lineages named as *T. cruzi* I (TcI) and *T. cruzi* II (TcII), which are found infecting chagasic patients in Brazil. Recent studies using multilocus enzyme electrophoresis (MLEE), random amplification polymorphic DNA (RAPD), mini-exon, 24Sα rRNA and 18S rRNA markers sub-divided lineage TcII in five distinct subgroups named as TcIIa–IIe. A total of eighty *T. cruzi* strains isolated from chronic chagasic patients from Rio Grande do Sul State were characterized in the present study using molecular markers based on the large and small subunit rRNA and mini-exon. Total DNA was obtained by standard phenol–chloroform method and PCR assays were performed using TC/TCI/TCII, D71/D72 and V1/V2 primers directed to the mini-exon, 24Sα rRNA and 18S rRNA genes, respectively. Amplified products were observed in silver stained 10% polyacrylamide gels. Forty-one strains (51.2%) presented a TcII profile according to the mini-exon and 24Sα rRNA genes and, according to the 18S rRNA profile, were grouped into the subgroup TcIId. The remaining 39 strains showed a hybrid profile (mini-exon TcII and 24Sα rRNA TcI), but all showing a profile compatible with the subgroup TcIIb according to the 18S rRNA assay. Our results shows, for the first time, the occurrence of 39 hybrid *T. cruzi* populations TcI / TcII infecting humans in the Rio Grande do Sul State, pointing out that the typing assays based on the mini-exon and DNAr24Sα genes may reveal contradictory results.

Supported CNPq, FEPPS, FIOCRUZ and CAPES.

BM085 - Selenocysteine incorporation in Kinetoplastid: Selenophosphate synthetase from *Leishmania major* and *Trypanosoma brucei.*

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Selenophosphate synthetase (EC 2.7.9.3) the product of the *sps2* gene, produces the biologically active selenium donor compound, monoselenophophate, from ATP and selenide, for the synthesis of selenocysteine. The Kinetoplastid *Leishmania major* and *Trypanosoma brucei sps2* genes were cloned and the SPS2 protein overexpressed and purified. The *sps2* genes in *L. major* and *T. brucei* are respectively 1197 and 1179 base pairs long encoding proteins of 399 and 393 amino acids with molecular masses of 42.7 and 43 kDa. The molecular mass of 100 kDa for both enzymes (*L. major* and *T. brucei*) is consistent with the size expected for dimeric proteins. The Kinetoplastid *sps2* complement *Escherichia coli* (WL400) *selD* deletion confirming a functional enzyme and the specific activity of these enzymes was determined. A conserved Cys residue was identified by both multiple sequence alignment as well as by functional complementation and activity assay of the mutant (Cys to Ala) forms of the SPS2 identifying this residue as essential for the catalytic function.

BM086 - PCR DETECTION OF *Trypanosoma cruzi* KDNA CONSERVED SEQUENCES DIRECTLY FROM CHRONIC CHAGASIC HUMAN TISSUES.

Silveira, L. G. F., Aguiar, A. F.[1]*, Adad, S. J.[2], Oliveira, E. C.[3], Luquetti A. O.[3], Neto S. G.[3], Reis, D. D.[1] and Vago, A. R[1].
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Trypanosoma cruzi detection in tissues from chronic chagasic patients is difficult due to the parasite scarcity in this phase of the disease. The present work reports an improved PCR protocol for amplification of the 120 bp kDNA fragment in human tissues, from which DNA was extracted by an inexpensive, simple and single-step methodology (alkaline lysis). For the protocol standardization the best amplification conditions were chosen based on the intensity and yield of PCR products, as well as on the decreasing of unspecific products. In this work we analyzed ten heart and ten colon samples obtained from patients with chagasic cardiopathy and megacolon, respectively. All the used samples were positive in a previous test using the *T. cruzi* 330bp kDNA fragment PCR amplification. By using our improved protocol we were able to demonstrate kDNA persistence in all the analyzed tissues. The 120 bp kDNA fragment was previously used to detect *T. cruzi* DNA from paraffin embedded heart sections from chronic chagasic patients (Olivares-Villagomez *et al.*, 1998), and in comparison with two others nuclear sequences tested, this kDNA fragment was considered the most sensitive target for detecting tissue parasitism. Another interesting work employed the *T. cruzi* 120 bp kDNA to detect parasite in mummified tissues originated from the northern Chile, which were dated from 2000 years BP-1400 AD (Ferreira *et al.*, 2000). However, multi-step DNA extraction methods and hybridization with radioactively labeled probes were employed in these works in order to improve the sensitivity detection of PCR products. Thus, the methodology reported in the present work, composed of a single-step DNA extraction method, followed by a conventional PCR amplification to amplify the *T. cruzi* 120 bp kDNA fragment, consists in a very simple and inexpensive alternative for detecting parasite kDNA in fresh tissues samples obtained from chronic chagasic patients.
Supported by CNPq.

BM087 - INTRASPECIFIC VARIABILITY OF *Trypanosoma cruzi I* IN BRAZILIAN ENDEMIC REGIONS OF CHAGAS DISEASE

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The intraspecific variability and the behavior of *T. cruzi* I (TcI) in the sylvatic and peridomestic cycles and its relation to the epidemiology of Chagas disease is poorly studied. Twenty nine parasite isolates, previously characterized as TcI, were analysed to establish the haplotypes from mini-exon gene intergenic region and variability of kDNA by LSSP –PCR. These populations were obtained from triatomines (*Panstrongylus megistus,Triatoma infestans*, *Triatoma sordida*), reservoir (*D. albiventris*) and humans in two Brazilian endemic areas of Chagas disease, corresponding to Minas Gerais -MG and Bahia –BA. The kDNA of TcI populations circulating in sylvatic and peridomestic cycles in MG and BA showed high genetic variability distributed in seven polymorphic groups with different populations circulating between vectors, *D. albiventris* and humans. Most of them were grouped according geographical and hosts/vectors distribution related to the presence of clones in each region. *P .megistus, D .albiventris and T. infestans* were associated with MG and *T. sordida* with Bahia State. Human isolates were more polymorphic group. For the first time in Brazil, haplotype D of TcI (amplified product of 200pb) was detected in three human isolates (BA and MG) and two sylvatic isolates: T2 (*T. infestans*) and Alvani (*P. megistus*) both from MG. In Colombia, this haplotype was associated with *T. cruzi* of sylvatic cycle and *Rhodnius prolixus* however, in this study, was also detected in association with human domestic cycle. The others TcI isolates did not show amplification probably associated with different haplotypes sequences not detected in Brazilian samples. The haplotypes A, B were not detected and C was not done .The data show high genetic kDNA variability and different epidemiological patterns of Brazilian TcI haplotypes. New haplotypes sequences, clinical impact and distribution geographic must be evaluated.
Financial Support: FAPEMIG, FUNEPU, Ministério da Saúde, FNS and REUNI

BM088 - Study of an Argonaute protein (TcPWI1) in the protozoan parasite *Trypanosoma cruzi.*

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Argonautes are a unique class of proteins required for small non-coding RNAs (emerged as important contributors to gene regulation during the past decade) to carry out their biological functions. The Argonaute proteins are characterized by having a piwi-argonaute-zwille (PAZ) and a PIWI domain. It has been shown that in appearance, in some organisms, such as the parasites *Trypanosoma cruzi* (*T. cruzi*) and *Leishmania major (L. major)* Argonaute proteins may have lost the PAZ domain and retained only a PIWI domain. In the genome of *T. cruzi* exists one candidate gene potentially involved with the Argonaute family, named TcPWI1. The function and expression of this protein is unknown, its homologue en *T. brucei* (TbPWI1) has been studied and has been showed that this protein is not involved in RNAi related phenomena. Despite the emerging view that members of the Ago family play an essential role in a variety of silencing phenomena, at this moment there is no evidence that in early divergent eukaryotes such as *T. cruzi*, this kind of proteins could carry out RNAi related functions. This work was aimed to demonstrate the expression of this putative gene TcPWI1 in *T. cruzi* strain Dm28c and to relate this candidate protein with the recently cloned small non-coding RNAs named mini-tRNAs in *T. cruzi*. Our data showed that TcPWI1 is expressed in T. cruzi in all stages of the live cycle at transcript and protein level. Bioinformatic studies of the sequence obtained by the strain Dm28c of *T. cruzi* TcPWI1 showed that this protein could present a divergent PAZ domain not reported yet. Colocalization assays of TcPWI1 protein with probes of mini-tRNAs cloned previously in *T. cruzi* showed that both elements colocalized in amastigotes stages and in nutritional stress condition of epimastigotes in cytoplasmatic regions. We hypothesized that TcPWI1 proteins could be associated either to the biogenesis or to an eventual effector function of mini-tRNA which remain to be elucidated.

BM089 - IDENTIFICATION OF PROTIST MICRORNA TARGETS BY COMPARATIVE PROTEOMICS AND TRANSCRIPTOMICS

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microRNAs are a class of highly conserved small non-coding RNAs that can either suppress protein translation or facilitate cleavage of the mRNA through complementary binding to the 3'-untranslated region (UTR) of the target mRNAs. Relatively little is known about the miRNA machinery in protist. Our previous study identified 9 mature miRNA in the parasitic protist *Trichomonas vaginalis*. In the present study, we attempted to use comparative proteomics and transcriptomics to identify protist miRNA targets. A total of 49 differentially expressed proteins in the trophozoite and amoeboid stage of *T. vaginalis* were identified by 2D gel electrophoresis. The gene expression levels of these proteins which contain putative miRNA target sites predicted by of miRanda were determined by using RT-PCR. We showed that the gene expression level of *T. vaginalis* malate dehydrogenase (Tv_MDH) was up-regulated in the amoeboid form, but the expression level of protein is down-regulated. Bioinformatics analysis revealed that the 3'UTR of Tv_MDH mRNA contain a putative target site of the *miR-1* family. The expression level of the mature *tva-miR-1* in amoeboid form was significantly higher than trophozoite form. The protein expression level of Tv_MDH was significantly downregulated in *T. vaginalis* transfected with *tva-miR-1* mimics. Based on these experimental data, we concluded that Tv_MDH is negatively regulated by *tva-miR-1*. The result of the present study demonstrated that a combination of proteomics of transcriptomics is a powerful tool to identify miRNA targets.

BM090 - PECULIAR ABUNDANCE OF POLY-DINUCLEOTIDES IN TRITRYP

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The protozoans *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*, the so called Tritryps, are important worldwide human pathogens and constitute evolutionarily ancient eukaryotes. Probably due to the early branching in eukaryotic evolution, they present unique features. Indeed, signals for the different molecular processes remain mostly elusive. Repetitive sequences though initially considered as selfish DNA, have been lately recognized as important functional elements in cell biology. In particular, the pattern of dinucleotides occurrence has been related to genome compartmentalization, gene evolution and level of gene expression. Here we present the analysis of the occurrence, length and distribution of dinucleotide repeats in the whole genomes of the Tritryps. We observed that most types of poly-dinucleotides are significantly more abundant than what is expected by chance and that complementary dinucleotide repeats usually displayed asymmetrical strand distribution, privileging TT and GT repeats in the coding strands. In addition, we found that GT poly-dinucleotides are among the longest repeats in the three parasite genomes. Finally, we showed that particular poly-dinucleotides are preferred at the vicinity of the open reading frames. The striking bias in content, strand distribution, length and location of poly-dinucleotides suggest that these sequences have an active role in the kinetoplastid genomic dynamics and might represent signals for molecular processes.
Supported by PEDECIBA and CSIC.

BM091 - Role of Natural Inhibitor of Cysteine Peptidase in *T. brucei*

Inhibitor of cysteine peptidase (ICP) is a chagasin-family from clan IX, natural tight binding inhibitor of Clan CA, family C1 cysteine peptidases (CP), such as brucipain and cathepsin B. Endogenous mammalian and plant CP inhibitors from the cystatin class share no homology with ICP, which has only been identified in protozooa and bacteria. Analysis of phenotype of ICP null mutant in monomorphic bloodstream form *T. brucei* 427 suggests that it regulates endogenous CP activity, and consequently plays a part in modulation of surface coat exchange during differentiation, intracellular proteolysis and parasite infectivity in mice. Previous studies using an in vitro model of the blood brain barrier (BBB) composed of the brain microvascular endothelial cells (BMECs) suggest that brucipain is important to the transendothelial migration of *T. b. rhodesiense* IL1852. To study the role of ICP in this process, we have generated two different pleomorphic strains lacking ICP, *T. b. rhodesiense* IL1852 and *T. b. brucei* 247. In vitro, IL1852 ICP null mutants show increased BBB transmigration and human neutrophil adhesion in BMECs compared to WT, which correlates with increased CP activity in these mutants.

BM092 - PROBING THE POLYCLONAL NATURE OF *TRYPANOSOMA CRUZI* STRAIN BERENICE-78 AFTER LONG-TERM INFECTION IN VERTEBRATE HOST

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The first human case of Chagas disease was described by Carlos Chagas (1909) in a two-year old girl, called Berenice. Two isolates of *T. cruzi* were obtained from this patient at different times: 1962 and 1978, respectively called Berenice-62 (Be-62) and Berenice-78 (Be-78). Although both strains were obtained from the same patient, they showed significant differences when they were compared using biological, biochemical and molecular criteria. The major goal of the present work was to clarify the structure population of Be-78 strain using 24Sα rDNA, the intergenic region of spliced leader genes (SL-IR), COII (cytochrome oxidase subunit 2) and ND7 (NADH dehydrogenase subunit 7) mitochondrial genes and microsatellite loci. For that purpose, Be-78 was inoculated in dogs, reisolated and then reinoculated for 25 successive passages in mice. The DNA obtained from the parental strain Be-78 and mice passages Be-78 1A, 1B, 1C, 1D, 25A, 25B, 25C and 25D were submitted to PCR analysis employing all molecular markers. All Be-78 isolates demonstrated rDNA type 1 and fragments of ~150-157bp for SL-IR. The COII-RFLP, ND7 and microsatellite profiles showed that some isolates were constituted by a populational mixture. To individually study the subpopulations, single cells of Be-78 25B isolate were sorted by FACS Cell Sorter. Microsatellite PCR on these isolate´s single cells was able to detect the presence of two distinct subpopulations: one corresponding to Be-62 parental and another subpopulation genetically distinct from Be-62 and Be-78 parental strains. These findings confirm that Be-78 strain presents a polyclonal nature constituted by three different subpopulations and that the successive passages in laboratory animals had propitiated the preferential growth of subclones with different characteristics. Furthermore, our data confirmed that Be-62 and Be-78 were indeed originated from the same infected person.

Financial support: FAPEMIG, CNPq, CAPES.

BM093 - GENETIC VARIABILITY OF *LEISHMANIA (LEISHMANIA) CHAGASI* FIELD POPULATIONS FROM BRAZIL

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Considering the clinical importance of visceral leishmanisis (VL) in Brazil and the lack of information about the genetic characteristics of its etiological agent, the aim of this work was to evaluate the genetic variability of *L. (L.) chagasi* isolates from five different areas in Brazil. Initially, all isolates were identified by PCR-RFLP as *L. (L.) chagasi*. In order to study genetic diversity within these parasites 3 molecular methods were employed, RAPD, SSR-PCR and MLMT. RAPD and SSR-PCR results showed complex profiles, allowing analyses of 181 bands. Even with minor differences, the general topology of the trees obtained for both techniques was quite similar, revealing the existence of greater genetic similarity within isolates from closely situated geographic areas. Although we observed distribution of isolates according to its geographical origin, genetic distance between them shows low levels of intra-specific variation. In our MLMT results, 16 of 54 *L. (L.) chagasi* isolates presented allelic variants, whose combination results in 8 different genotypes. We did not detect correlation between the microsatellite patterns and geographic origin, except for Espírito Santo isolates. In summary, our results showed to be sufficiently promising. However, further work should be done, including other geographic areas and a higher number of isolates, in order to better understand the hosts, reservoirs and vectors roles in the generation and maintenance of genetic diversity. Moreover, it would be possible to evaluate the existence of a correlation between intra-specific variability and drug resistance or different clinical manifestations of VL.
Supported by CAPES and UFES.

BM094 - MOLECULAR DIVERSITY OF HISTONE H1 FROM DIFFERENT *LEISHMANIA* AND *TRYPANOSOMA* SPECIES

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Histone proteins play a key role in DNA binding, chromatin condensation, and gene regulation. The linker histone H1, involved in the formation of higher-order chromatin structure, is considered the most divergent class of histones. In the Trypanosomatidae, histone H1 lacks the central hydrophobic globular domain, an essential evolutionary change with respect to its homologs in the metazoan lineage. The role of histone H1 in the Trypanosomatidae is not yet fully understood; however, it has been implicated in parasite infectivity and virulence. Furthermore, the antigenicity and immunogenicity of this protein has been demonstrated experimentally. Our main goal is to investigate the molecular diversity of histone H1 from protozoan parasites that cause disease in human and other mammals to gain insights into pathogen biology and vaccine development. Molecular sequences from different species of *Leishmania* and *Trypanosoma* were retrieved from public databases based on sequence-similarity searches. Multiple sequence alignments and evolutionary trees inferred by Bayesian methods were analyzed in detail. Our results have shown that sequences are highly conserved both at the gene and protein levels and that they form three distinct clusters (*Leishmania*, *T. cruzi*, and *T. brucei*) as inferred by sequence analysis and evolutionary tree reconstruction. The clustering pattern does not always follow the taxonomic classification or the geographic distribution of the selected species, suggesting that some relationships might reflect gene/protein evolution rather and speciation and ecology. Together, the conservation of histone H1 proteins among some Trypanosomatidae and their divergence from the other metazoan homologs (due the lack of the globular domain) support the choice of histone H1 as a target for vaccine development against diseases caused by these parasites.
Supported by CNPq and NIH.

BM095 - THE SERINE PEPTIDASE INHIBITOR (ISP2) PRODUCED BY *Trypanosoma cruzi*: INFLUENCE OF HOST CELL INVASION.

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Bacterial ecotins are high affinity inhibitors of family S1 serine peptidases with a trypsin-fold. Genes similar to ecotins were identified in the genomes of *T. cruzi, T. brucei* and *Leishmania*, and were designated Inhibitors of Serine Peptidases (ISPs). Since there is no gene encoding S1 peptidases in the *T. cruzi* genome, we raised the possibility that ISPs could modulate host enzymes. We produced *T. cruzi* recombinant ISP2 (ISP2r) and determined the inhibition constants (Ki) for trypsin, chymotrypsin, cathepsin G and human neutrophil elastase (HNE), which range from 8 to 20 nM. We produced anti-ISP2 antisera and detected the expression of ISP2 in all three life cycle stages of *T. cruzi* Dm28c, being slightly higher in epimastigotes. Immunofluorescence assays using anti-ISP2 antisera showed a punctual distribution pattern. In order to confirm ISP2 localization in *T. cruzi*, we generated constructs containing the green fluorescent protein (GFP) fused at the 3' end of *ISP*2 in pTEX and transfected epimastigotes. A possible role of ISP2 in the host-parasite interaction was analyzed by performing invasion assays of human smooth muscle cell lines with tissue culture trypomastigotes (TCT). The addition of ISP2r during the TCT-host cell interaction or the pretreatment of host cells significantly reduced parasite entry, suggesting that host serine peptidases might play a role in parasite invasion. In order to further evaluate the role of ISP2, we produced a *T. cruzi* cell line overexpressing ISP2 by transfection with a pTEX-ISP2 construct. After drug selection and plate cloning, we successfully obtained *T. cruzi* clones overexpressing functional ISP2. ISP2 overexpressing TCT were less infective than parasites containing empty vector, implicating ISP2 in the molecular pathways involved in host cell invasion by *T. cruzi*.

BM096 - Investigation of a possible angiostatic activity in *Leishmania major*'s Tryptophanyl tRNA Synthetase

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Aminoacyl tRNA Synthetases (aaTRS) catalyzes the acylation of aminoacids and subsequent ligation of this aminoacyl acid to its respective tRNA. In humans, besides its role in aminoacid activation, Tryptophanyl tRNA Synthetase (TrpTRS) has been characterized as an inhibitor of angiogenesis after proteolysis by leukocyte elastase. An antagonic function was observed for Tyrosyl tRNA Synthetase, an IL-8 like protein that mediates inflammatory response and possesses angiogenic activity.

The *Leishmania major* genome encodes two different TrpTRSs, a mitochondrial (TrpTRS1) and a citosolic (TrpTRS2) form. We set out to investigate if *L. major* heterologous TTRSs present the same angiostatic behavior observed in the processed human protein. TrpTRS1 and TrpTRS2 genes were amplified from LV39 genomic DNA and cloned into pET28a vector. The recombinant proteins were expressed in Rosetta (DE3) strains as inclusion bodies, and solubilized in 20mM Tris-Cl pH8.0, 2% sarkosyl, 1mM EDTA and 10mM β-mercaptethanol. Sarkosyl removal was accomplished by ammonium sulphate precipitation for TrpTRS1 and gel filtration for TrpTRS2. Proteins were then purified by ion exchange chromatography in DEAE-Sepharose using Tris-Cl pH 7.0 buffer with a step gradient of NaCl ranging from 300-600mM NaCl. Aminoacylation activity of both heterologous proteins was confirmed by [H3] L-Trp assay. We also produced a polyclonal antibody capable of recognizing both proteins in western blots and imunofloresce protocols. This allowed us to confirm mitochondrial and citosolic localization for TrpTRS1 and TrpTRS2, respectively, by Confocal microscopy.

Spectroscopic characterization of recombinant protein will be assayed by circular dichroism and angiostatic potential will be analyzed using chicken chorioallantoic membrane assay.
Supported by: FAPESP, CAPES and CNPq

BM097 - Proteomic analysis of *L. braziliensis* and *L. major* selected cell lines resistant to antimonials

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Clinical resistance to pentavalent antimonial has been recognized for a long time as a major problem in the treatment of leishmaniasis. However, mechanisms of natural resistance are unclear. Understanding these mechanisms is fundamental to the development of efficient strategies to monitor the emergence and spreading of (SbV) resistance. We have previously selected lineages of *L. major* and *L. braziliensis* resistant to SbIII and SbV and characterized their gene expression profile for six genes possibly involved in drug resistance: (*ACR2*) antimoniate reductase, (*TDR1*) thiol-dependent reductase 1, (*GCS*) γ-glutamylcysteine synthetase, (*ODC*) ornithine decarboxylase, (*MRPA*) multidrug resistance protein A and (*AQP1*) aquaglycerporin 1.
Based on that we set out to investigate differences in protein levels in our lineages using a proteomic approach. Promastigote protein extract was separated over non-linear pH gradients 3-10 in 12% polyacrylamide gels and visualized by Coomassie Blue G-250 staining. The images of biological triplicates were analyzed considering at least twice as significant protein expression difference compared with wild-type parasite. The 2D gels of *L. (L.) major* and *L. (V.) braziliensis* extracts defined approximately 240 well-resolved spots suitable for MALDI-Tof protein identification of which 60% presented overlapping gel coordinates between wild-type and resistant parasites. In comparison with wild-type, *L. major* resistant parasites presented 7 and 5 differentially expressed spots in the presence of SbIII and GNT compounds respectively. In *L braziliensis* resistant parasites, 5 and 3 spots were considered differentially expressed in the presence of SbIII and GNT compounds. The identification of these proteins is currently in progress.

Supported by FAPESP, CNPq and CAPES

BM098 - BIDIRECTIONAL PCR AMPLIFICATION OF A SPECIFIC ALLELE (BI-PASA) TECHNIQUE APPLIED FOR NADH DEHYDROGENASE SUBUNIT 1 (ND1) FROM *TRYPANOSOMA CRUZI*

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Chagas Disease is a tropical disease caused by the protozoan *Trypanosoma cruzi*, which still is a serious public health problem, affecting approximately 10 million individuals, causing about 14.000 deaths per year in Latin America. The molecular characterization of the major lineages in *T. cruzi* employing mitochondrial genes contained in the maxicircle of the parasite's KDNA has been largely used in the past years. Techniques such as PCR-RFLP are frequently used for this purpose, more specifically the COII (cytochrome oxidase subunit 2) gene. However, this characterization process requires the use of restriction enzymes that makes it expensive and high time consuming. Based on these difficulties, our main goal in this work is to develop a faster and cheaper method based only on PCR for *T. cruzi* strains molecular characterization. For this purpose we use an specific allele PCR technique called Bi-PASA. Initially, DNA sequences of ND1 gene were retrieved from GeneBank and submitted to multiple alignment program to verify the polymorphism presence in the sequences that allows *T. cruzi* strains characterization within major phylogenetic lineages. We found a region within the ND1 gene that presented single nucleotide polymorphisms (SNPs) that allowed the classification of *T. cruzi* strains in three different groups: *T. cruzi* I, *T. cruzi* II and *T. cruzi* III/hybrids. Subsequently, we designed three forward primers with differences on the 3'-end according to SNP's variation and a tail presenting different sizes were attached to each primer on the 5'-end and the same reverse primer was used in all situations. To validate this technique we are using a multiplex PCR system and DNA obtained from *T. cruzi* strains belonging to different phylogenetic lineages. The success of this technique will contribute to future molecular characterization studies involving *T. cruzi*.
Financial support: FAPEMIG, CNPq, CAPES.

BM099 - Intragenic recombination in *Trypanosoma cruzi* and its implications for the phylogeny of hybrid strains

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Hybrid strains of *Trypanosoma cruzi* are of great medical and epidemiological relevance. However, the phylogenetic position of these strains relative to *T. cruzi* I and *T. cruzi* II is controversial. These hybrids, such as 1/2, Zymodeme 3 (Z3) and CL Brener, seem sometimes closer to *T. cruzi* I with a set of markers whereas according to other set of markers they seem closer to *T. cruzi* II. Here we tested if intragenic recombination could explain this behavior. We used a bootscanning neighbor-joining algorithm comparing the haplotypes of hybrids CL Brener (esmo-like and non esmo-like haplotypes), NRcl3 (1/2) and MT4167 (Z3) with *T. cruzi* I and *T. cruzi* II. The haplotypes analyzed were from genes encoding Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS), Actin, Trypanothione reductase (TR) and Translational Elongation Factor 1 (EF-1). Our results show that the CL Brener DHFR-TS non esmo-like haplotype is approximately 80% *T. cruzi* I and 20% *T. cruzi* II, which explains its position closer to *T. cruzi* I in phylogenies whereas the esmo-like haplotype is 100% *T.cruzi* II. The CL Brener TR has the opposite pattern. NRcl3 the intraspecific pattern is even more pronounced and the Z3 haplotypes have more intragenic recombination in the Actin gene and *T. cruzi* II pattern in both haplotypes of EF-1. We conclude that intragenic recombination pattern in the hybrid haplotypes is relatively frequent and is a plausible cause for the ambiguous position of hybrid strains relative to *T. cruzi* I and *T. cruzi* II. Expectedly, the amount of the "parental" type in the hybrid haplotype determines its distance from *T. cruzi* I and *T.cruzi* II in phylogenies, which supports the idea that hybrid strains are polyphyletic. This is fundamental to correlate the phylogenetic pattern of *T. cruzi* with its epidemiological distribution.
Support from WHO, FAPESP and CNPq.

BM100 - GENOTIPIC DISTRIBUTION OF T. CRUZI LINEAGE I IN VECTORS, RESERVOIRS AND HUMANS FROM ENDEMIC COUNTRIES BASED ON THE SEQUENCE OF THE SPLICED LEADER INTERGENIC SPACER

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Natural populations of *T.cruzi* are classified into two major groups *T. cruzi* I and II predominating in the northern and southern areas of Latin America, respectively. *T. cruzi* II has been divided into 5 discrete typing units (a-e) but the high heterogeneity of *T.cruzi* I hampered DTUs assessment. In 2007, Herrera et al. proposed 4 T.cruzi I haplotypes in Colombia, identified by SNPs and insertions/deletions within a motif at the intergenic spacer of spliced-leader genes (SL-IR), recently named as TcI a–d by Falla et al, 2009. We aimed to characterize this polymorphism in *T.cruzi* I samples infecting faeces of Rhodnius, Pastrongylus and Triatoma bugs, wild reservoirs and blood and tissues from patients of different endemic areas.
The SL-IR was amplified by PCR from 73 samples. PCR with primers UTCC/Tc2 (475 bp) was applied in culture stocks and heminested-PCR with primers TCC/Tc2 (350 bp) in clinical samples. Purified amplicons were sequenced for alignment using MEGA 4.0. Five Argentinean stocks were Tc Ia and 11 Tc Id. It is worthnoting that Tc Ia or Id was detected in blood from Chagas Heart disease patients but only Tc Id in cardiac tissues from explants (4/4), even in a case with mixed Tc Ia/d infection. Three Brazilian stocks, 3 from French Guiana and 5 from Paraguay were Tc Id. Three from Mexico and one from Chile were Tc Ia. Among 37 Colombian strains, 6 were Tc Ia, 17 Tc Id and 14 Tc Ib. Interestingly, 4 samples from Argentina and 1 from Bolivia had a novel motif, named Tc Ie. In conclusion, we found association of Tc Ia with domestic cycles, Tc Ib mainly with sylvatic and peridomestic habitats of Colombia, 61% of Tc Id with sylvatic cycles but cardiac histotropism in Chagas heart patients, and Tc Ie with domestic cycle in Argentina and Bolivia. Tc Ic was not found in our population.
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BM101 - Identification of Conserved Intercoding Elements in *Leishmania* Genomes and Investigation of Their Role in Gene Expression Regulation

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The completed genomes of *L. braziliensis*, *L. infantum* and *L. major* are important tools to improve the understanding of these pathogens' biology. In order to identify and to map conserved intercoding elements (CICEs) in the genomes of these *Leishmania* species and to group them according to the function of their neighboring annotated genes we developed a computational pipeline. Using *L. major* Gene Ontology Family categories we grouped 680 CICE-GO: 238 in biological process term, 368 in molecular function and 74 in cellular component. To validate the results and verify the robustness of this pipeline we submitted five different CICE groups to another algorithm, MEME, obtaining similar results. Functional evaluation of the identified CICEs was conducted by Electrophoretic Mobility Shift Assays (EMSA). This approach revealed three elements which are potential targets of DNA or RNA binding proteins. They are named TXN45bp (a motif between LmjF29.1150 and LmjF29.1160), HDA63nt (a motif downstream of LmjF24.1370), and TXN50nt (a motif downstream of LmjF29.1160). Other five CICEs are currently being analyzed with EMSA. To identify the interacting protein(s) the RNA-protein bands were recovered from the gels and submitted to mass spectrometry (MS) analysis. MS peptide profiles (with a Mascot ions score threshold of 39) indicate that tryparedoxin peroxidase (TXNPx, LmjF15.1040) is the protein that binds to HDA63nt and TXN50nt. To test the identified CICEs as putative regulatory elements we are currently generating transfectants to evaluate the expression profiles of a reporter gene accompanied or not by a CICE placed at its 3'-UTRs.
Supported by FAPESP and CNPq.

BM102 - ALPHA-TUBULIN PHYLOGENY OF PERITRICHS DOES NOT SUPPORT THE CLUSTERING OF MOBILIDS AND SESSILIDS

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Systematic studies on peritrich ciliates have been traditionally based on morphological characters. Peritrichs are mainly characterized by two prominent bands of cilia that run in counter clockwise fashion and an expanded oral area, called peristome. Peritrichs are traditionally subdivided into two Orders: representatives of the Order Sessilida possess a stalk, a scopula or a lorica to attach to the substrate; representatives of the Order Mobilida are free-swimming, although parasitic (or commensal) forms possess an aboral adhesive disc to attach to their hosts. Molecular data have been used to explore phylogenetic relationships of peritrichs. Seemingly, the placement of the peritrichs within the Class Oligohymenophorea is the only assumption widely accepted and supported by both morphological characters and phylogenetics. However, the debate is currently centred on the relationships between sessilids and mobilids, since phylogenetic analyses based on small subunit ribosomic RNA (SSU rRNA) sequences suggest that peritrichs do not constitute a monophyletic group, though the hypothesis of peritrich monophyly has some statistical support in those trees. To gain more insight into the phylogeny of peritrichs, we have sequenced □-tubulin genes of four mobilids and four sessilids, and built a phylogenetic tree based on □-tubulin sequences. In ciliates, tubulins have strong structural constraints to evolve, reason by which sequences show a similar length and highly degree of conservation, making alignments highly reliable. We have then compared □-tubulin and SSU rRNA trees. Interestingly, both trees are overall congruent. The main difference between trees is seen at the Class level, since the □-tubulin tree fails to separate Nassophorea from Spirotrichea, but at the Order level, they show that mobilids and sessilids constitute two separated clusters. These data reinforce the idea that peritrich is not a monophyletic group.
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BM103 - INVOLVEMENT OF LMHUS1 IN THE RESPONSE TO DNA-TARGETING DRUGS IN LEISHMANIA MAJOR.

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In yeast and mammals, the trimeric complex 911, composed by proteins Rad9, Hus1 and Rad1, has been shown to participate in DNA damage responses triggering DNA repair and checkpoint activities. Under DNA damage, the 911 sensor complex acts in the transduction of signal to a variety of effector molecules including Chk1 and Chk2 kinases. These kinases regulate other proteins that ultimately inactivate cyclin-dependent kinases (Cdks) which inhibit cell-cycle progression. We are currently characterizing the *LmHUS1* gene of *Leishmania major* and investigating its participation in the delay or arrest of cell cycle progression under DNA damage. The gene is (i) conserved among *Leishmania* species; (ii) its product localizes to the nucleus; and when expressed in *L. major*, (iii) it confers resistance to DNA-targeting drugs such as hydroxyurea and methyl methanesulfonate. Protection against DNA damage was confirmed in TUNEL assays, in which, following DNA stress, *LmHUS1* transfectant presented DNA damage in 48% of cells against 84% of wild type cells. We are also characterizing the possible Chk1 kinase of *L. major*. The parasite genome encodes 2 candidate genes; one of them was cloned into expression vector and will be used to produce antibody. Current work is focused not only in the generation of a *LmHUS1* knockout cell line, but also in the investigation of the role of *LmCHK1* in phenotypes determined by LmHUS1 expression. These studies will provide a better understanding of the DNA repair pathways and mechanisms of genome maintenance in the protozoan parasite *Leishmania*.
Financial support: FAPESP and CNPq

BM104 - Intrinsically Unstructured Proteins: Computational identification and characterization in Schistosoma mansoni genome

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Intrinsically Unstructured Proteins (IUP) represents a special class of proteins that defies the dogma that each protein has one structure and one function. The identification of these protein disorder regions is important for protein production, protein structure prediction and determination, protein function annotation and host-parasite interactions.Despite of the existence of several different disorder prediction softwares and since there is no parameter definition for protein disorder, a wide range of methodologies exists. Our main goal is to develop a computational pipeline approach that combines key features from different methodologies together with a user pre-defined level of sensitivity and specificity for high throughput disorder prediction.As part of the developed pipeline a relational schema has been created integrating data from several disorder predictors (IUPred, GlobPipe and DisEMBL), protein physico-chemical properties, subcellular localization, functional/structural annotation and signal cleavage. The developed pipeline represents a powerful tool to establish the biological correlations between structural disorder and function and represents an original integrative study approach.The pipeline was systematically evaluated on a protein disorder dataset created from Protein Data Bank in terms of the relationship between sensitivity and specificity and applied on S.mansoni database. In total 368 IUPs were predicted for S.mansoni proteome. The integrative approach predicted that (19/368) ~5% could be classified as secreted proteins or as cell adhesion molecules and so correlated with the host/parasite interactions.
Supported by CNPq.

BM105 - The role of protein disorder in *Leishmania ssp.* genome: An overview from a database perspective.

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Proteins are composed of one or more chains of amino acids, and exhibit several levels of structure. Recently, a class of proteins called IUPs (Intrinsically Unstructured Proteins) has been discovered that do not fold into any particular configuration existing as dynamic ensembles in their native state. Due to their intrinsic adaptability, they participate in many regulatory biological processes including parasite immune escape.Using the information from *Leishmania spp.* proteomes we are developing a database aiming to establish the biological correlations between protein structural disorder and host-parasite interactions.The MySQL database was built by open source visual database designer for MySQL in Linux/OS. The main relational database tables created were: IUP table (containing structural/functional annotation and subcellular locations), DISORDER table (containing the predictions of 3 disorder predictors), TRANSMEMBRANE table (containing a combined transmembrane topology and signal peptide prediction), STATISTICS table (containing statistical protein attributes) and PATHWAY table (containing predicted metabolic pathways). All data are automatically loaded on the relational database by Perl scripts.Effective keyword searches of biological information over the relational database aggregating information from functional annotation (PFam, PRINTS, ProDom, ProSite, InterPro and Gene Ontology terms), disorder prediction, metabolic pathways and subcellular location allowed the establishment of biological implications associated with the role of protein structural disorder in parasite genomes.Since there is no pipeline or databases addressing this issue this database represents the first attempt to establish the correlations between protein function and structural disorder. The database will be freely available to academic institutions.
Supported by CNPq.

BM106 - PLACING APUSOZOA AND *MANTAMONAS* gen. n. IN THE EUKARYOTIC TREE USING MULTIGENE PHYLOGENOMIC ANALYSES.

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The phylum Apusozoa comprises an under-studied collection of gliding heterotrophic flagellates found in marine, freshwater and soil environments. The phylum has two main groups – Apusomonadida (*Apusomonas* and *Amastigomonas*) and the Planomonadida. Ultrastructural analyses and a characteristic gene fusion (in apusomonads) suggest that apusozoans are bikonts, but gene sequence trees have been poorly resolving and contradictory, so the unity and phylogenetic position(s) of Apusozoa remain unclear. Some single-gene studies place both apusozoan orders within the bikonts, while others suggest that one or both is within the unikonts. Although unresolved, their position relative to either side of the tree suggests that they occupy a deep branch near the base of all eukaryotes. Additionally, another group that is possibly related to apusozoans is the newly described genus, *Mantamonas*; a gliding, marine zooflagellate that is also deep-branching within the eukaryotic tree. Phylogenetic analyses using one or two genes weakly place *Mantamonas* as sister to either of the two main apusozoan groups, therefore its position within the tree is uncertain. To resolve the placement of the Apusozoa and *Mantamonas* among the basal eukaryotes, we have conducted large-scale, random EST sequencing on five apusozoan species (two apusomonads and three planomonads) and one member of the new genus *Mantamonas* to be compared to other eukaryote EST and genomic datasets. Multigene analyses will give a robust placement of the Apusozoa within the eukaryotic tree and establish (or disprove) its monophyly, and also define the position of *Mantamonas* with respect to the Apusozoa near the base of the eukaryotic tree. Supported by NERC, UK.

BM107 - DEFINING BIOLOGICAL SPECIES USING ITS2 (rRNA) SEQUENCES: A PROTOZOAN PERSPECTIVE

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In many unicellular eukaryotes sexual reproduction has never been observed, so that species boundaries rely solely on morphological distinctions. In many protozoans however there are so few constant morphological characters that finding a reliable molecular marker for discriminating species would be very valuable. In animals, fungi, plants, and ciliate protozoans, the internal transcribed spacer 2 (ITS2) region of ribosomal RNA (rRNA) genes seems almost ideal for this purpose. It has been shown that substitutions in key parts of the ITS2 sequences correlate extremely well with sexual incompatibility between two organisms. Because this correlation occurs independently of reproduction and mating affinities, it could be applied to protozoans whether or not they are known to be sexual. All eukaryotic groups in which such a correlation has been observed up to now possess typical ribosomal gene clusters wherein all copies in the genome are virtually identical even in their most variable regions, due to a mechanism of homogenization known as “concerted evolution”. However, we recently discovered based on clonal cultures that at least some lineages of protozoans possess a surprisingly high level of sequence variation between copies of the rRNA genes in the genome of a single cell. In this study we assess the utility of ITS2 as a species marker in some key protozoan taxa where sexual reproduction is mostly unknown (Amoebozoa, Apusozoa, Choanozoa, and Cercozoa) by comparing the levels of intraspecific vs. interspecific ITS2 sequence variability. Supported by NERC, UK.

BM108 - Molecular and Biological Aspects of *Trypanosoma cruzi* Isolates from Congenital Chagas Disease Transmitting and Non-transmitting Mothers in Minas Gerais (MG), Brazil

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After the control of vector-borne Chagas disease transmission, congenital transmission has had its epidemiologic relevance increased. A preliminary study evaluated congenital transmission rates in MG. In this study mothers who transmitted and mothers who didn’t transmit the infection to their fetuses were analyzed, indicating a 1% risk of Chagas disease congenital transmission. The factors that determine the transmission of *T. cruzi* infection to fetuses in some chagasic pregnant patients are unclear and both host and parasite characteristics may be involved. To evaluate probable factors related to the parasite we carried out molecular and biologic studies with *T. cruzi* isolates from mothers who transmitted and who didn’t transmit the infection congenitally. Molecular markers such as rDNA 24Sα, Cytochrome Oxidase II and mini-exon pointed that all the analyzed isolates belong to *T. cruzi* II (DTU IIb) lineage, which is the prevailing lineage in human infections in MG. The isolates from transmitting and non-transmitting mothers didn’t show any difference on the NADH dehydrogenase 7 mitochondrial gene. Microsatellite profiles show perfect genotypic identity between the mother-offspring pairs analyzed confirming the congenital infection diagnostic. We also evaluated the behavior of epimastigote forms through growth curves in culture medium and of trypomastigote forms in LCC-MK2 cells. All the isolates analyzed showed great similarity on growth curves although some differences were seen on LCC-MK2 infection assays, where one of the transmitting-mother isolates showed smaller trypomastigote forms with a faster differentiation to the amastigote form when compared to an isolate from a non-transmitting mother. These results suggest that parasite characteristics may be involved in congenital transmission of *T. cruzi* infection. Our next steps are analyzing more isolates form transmitting and non-transmitting mothers and also evaluate the infectivity of isolates in placenta cells.
Supportted by FAPEMIG, CAPES, CNPq.

BM109 - DNA Polymerase Rev1 from *Trypanosoma cruzi* presents catalytic properties not encountered in its described orthologs

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Key-words: Rev1, translesion synthesis, *Trypanosoma cruzi*.

Trypanosoma cruzi, the causative agent of Chagas disease, possesses a complex population structure. This parasite presents a high genetic diversity, which was reported by several studies employing distinct molecular markers. The generation of this genetic variability could be linked to replication processes carried out by low-fidelity DNA polymerases. DNA polymerase Rev1 belongs to this class of enzymes and was grouped within the Y family of DNA polymerases. It is highly specialized in incorporation of C opposite G or abasic sites, in a mechanism of DNA synthesis that employs a proteic template instead of a DNA template. Rev1 is also related with a causative fashion to mutagenic events that are induced by different types of DNA lesions. The complete sequencing of *T. cruzi* genome revealed that this organism presents a protein that is similar to Rev1, herein denominated TcRev1-like. In this report, we demonstrate that TcRev1-like is capable to partially complement a Rev1-deficient yeast strain, as well as it presented DNA polymerase activity *in vitro*. Surprisingly, our results suggest that TcRev1-like is not a deoxycytidiltransferase, since it has the ability to incorporate other nucleotides besides dCMP. The overexpression of TcRev1-like in parasite cells does not result in any phenotype regarding the resistance or sensibility to genotoxic agents. The confocal microscopy examination demonstrates that TcRev1-like fused to GFP displays nuclear localization. Either the overexpression or the residual expression of TcRev1-like within the *Escherichia coli* strain AB1157 result in inhibition of bacterial growth. On the other hand, the low-level expression of TcRev1-like containing the site-directed mutations Y120W and R394M leads to a shortening of the lag phase of this bacteria's growth curve. Nevertheless, the overexpression of this mutant DNA polymerase within Escherichia coli AB1157 resulted in the same behaviour that was observed for the overexpression of wild type TcRev1-like.

Financial support: CNPq, FAPEMIG, Howard Huges.

BM110 - DOES LEISHMANIA CALL 911 FOR DNA DAMAGE REPAIR?

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The association between Rad9, Rad1 e Hus1 forming a trimeric ring-like complex (911 complex) has been well documented in other organisms. In mammals, 911 associates with telomeres playing a relevant role in chromosome stability. It has also been shown to interact with RPA, a DNA damage repair protein that, in *Leishmania amazonensis*, associates to chromosomal ends. The overexpression of *LmHUS1* in *L. major* confers resistance to genotoxic drugs such as hydroxyurea and methyl methanesulfonate suggesting that LmHus1 is also involved in DNA repair mechanisms. We set out to investigate the existence of 911 complex in Leishmania. Recombinant GST-LmHus1 was expressed in *E. coli*> and the purified protein will be used in pull down assays in order to identify and characterize the LmHus1 interacting proteins, such as RPA1 and telomerase. A heterologous His-tagged LmHus1 version was also generated and is being used to raise anti bodies against LmHus1. We have also identified and cloned the LmRad9 gene. Current work is focused on the expression of a His-tagged version of LmRad9 that will be used to generate anti-LmRad9 antibodies. The participation of LmRad9 in DNA damage repair, its subcelular localization and its possible interaction with LmHus1 is currently being investigated in *L. major*.
Supported by: FAPESP and CNPq.

BM111 - GENERATION OF *LEISHMANIA* MUTANTS TO BE USED IN LARGE-SCALE DRUG SCREENING

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Leishmaniasis is a spectral disease that afflicts thousands of people annually around the world. However, available drugs for the treatment of leishmaniasis are far from ideal and novel leishmanicidal compounds are needed. To facilitate the use of high-throughput screening methods we have generated *Leishmania* mutants to constitutively express a reporter gene. These parasites should ideally lack a drug resistance marker to avoid potential interference with tested compounds. Therefore, we have engineered vectors bearing a positive and a negative selectable marker to be able to rescue transfectant parasites carrying the reporter gene integrated into the ribosomal locus in the absence of a drug resistance marker, using the hit and run strategy (H. Denise et al, FEMS, 2004). Mutants of *L. major, L. braziliensis* and *L. donovani* that contain either GFP (green fluorescent protein), SEAP (secreted placental alkaline phosphatase) or Luciferase have been generated. Confirmation of the genomic integration has been conducted by Southern blotting and PCR analysis. We are currently evaluating the dynamics of loss of the heterologous DNA in the available *Leishmania* mutants, because a potential problem with these transfectants is the spontaneous loss of the integrated DNA.
Supported by FAPESP and UNICEF-UNDP/World Bank/Special Programme for Research and Training in Tropical Disease.

BM112 - COMPARATIVE ANALYSIS OF THE EXPRESSED GENOME OF *Leishmania braziliensis* ISOLATED FROM TWO DISTINCT CLINICAL MANIFESTATIONS

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Leishmaniasis is a spectral disease and its clinical manifestations depend on both parasite and host factors. Understanding parasite factors that contribute to the diversity of tropism and virulence is relevant. *Leishmania braziliensis* is a major agent of human leishmaniasis in Brazil, being implicated in both Cutaneous (CL) and mucosal leishmaniasis (ML). Mucosal lesions are frequently diagnosed months or years after primary cutaneous lesions. In the present report we take advantage of a cohort study of 220 CL patients from the endemic area of Jequié (Bahia/Brazil) followed forseveral months aimed at identifying cases of CL who develop ML. In six cases we recovered parasites from both the early cutaneous lesions and from the mucosa of 6 patients. We report on the compratison of proteomes from these paired parasites obtained from two sites from the same patient. Promastigotes from these paired isolates obtained from two patients were cultured for protein extraction. Proteins were fractionated using immobilized pH linear gradient strips from 4.0 to 7.0 followed by a second dimension performed in 12%-SDS-PAGE. Two-dimensional fractionation of proteins of biological triplicates were analyzed using Image Master Platinum and the 2D gels showed approximately 400 spots, suitable for MALDI-Tof analysis. The analysis of one of the pairs revealed that thirty proteins presented significant volume differences and were sent to be identified by mass spectrometry. Comparative analysis is in course to define the molecular karyotype of different pairs. Differences in the expressed genome of these paired isolates of *L. braziliensis* may contribute to understanding tropism pattern and to identify proteins involved in this process. Supported by: CNPq, FAPESB and FAPESP.

BM113 - The role of protein disorder in *Leishmania ssp.* genome: An overview from a database perspective.

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Proteins are composed of one or more chains of amino acids, and exhibit several levels of structure. Recently, a class of proteins called IUPs (Intrinsically Unstructured Proteins) has been discovered that do not fold into any particular configuration existing as dynamic ensembles in their native state. Due to their intrinsic adaptability, they participate in many regulatory biological processes including parasite immune escape.Using the information from *Leishmania spp.* proteomes we are developing a database aiming to establish the biological correlations between protein structural disorder and host-parasite interactions.The MySQL database was built by open source visual database designer for MySQL in Linux/OS. The main relational database tables created were: IUP table (containing structural/functional annotation and subcellular locations), DISORDER table (containing the predictions of 3 disorder predictors), TRANSMEMBRANE table (containing a combined transmembrane topology and signal peptide prediction), STATISTICS table (containing statistical protein attributes) and PATHWAY table (containing predicted metabolic pathways). All data are automatically loaded on the relational database by Perl scripts.Effective keyword searches of biological information over the relational database aggregating information from functional annotation (PFam, PRINTS, ProDom, ProSite, InterPro and Gene Ontology terms), disorder prediction, metabolic pathways and subcellular location allowed the establishment of biological implications associated with the role of protein structural disorder in parasite genomes.Since there is no pipeline or databases addressing this issue this database represents the first attempt to establish the correlations between protein function and structural disorder. The database will be freely available to academic institutions.
Supported by CNPq.

BM114 - Intrinsically Unstructured Proteins: Computational identification and characterization in Schistosoma mansoni genome

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Intrinsically Unstructured Proteins (IUP) represents a special class of proteins that defies the dogma that each protein has one structure and one function. The identification of these protein disorder regions is important for protein production, protein structure prediction and determination, protein function annotation and host-parasite interactions.Despite of the existence of several different disorder prediction softwares and since there is no parameter definition for protein disorder, a wide range of methodologies exists. Our main goal is to develop a computational pipeline approach that combines key features from different methodologies together with a user pre-defined level of sensitivity and specificity for high throughput disorder prediction.As part of the developed pipeline a relational schema has been created integrating data from several disorder predictors (IUPred, GlobPipe and DisEMBL), protein physico-chemical properties, subcellular localization, functional/structural annotation and signal cleavage. The developed pipeline represents a powerful tool to establish the biological correlations between structural disorder and function and represents an original integrative study approach.The pipeline was systematically evaluated on a protein disorder dataset created from Protein Data Bank in terms of the relationship between sensitivity and specificity and applied on S.mansoni database. In total 368 IUPs were predicted for S.mansoni proteome. The integrative approach predicted that (19/368) ~5% could be classified as secreted proteins or as cell adhesion molecules and so correlated with the host/parasite interactions.
Supported by CNPq.

BM115 - Amoebozoa revisited: a phylogenomics point of view

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Amoebozoa is one of several eukaryote supergroups recently defined by molecular and ultrastructural data. Despite their ecological and medical importance, amoebae have long been largely ignored by evolutionary biologists. Available data suggest that Amoebozoa belongs to a major eukaryote clade, the unikonts, together with opisthokonts. The phylum thus occupies a key position in the eukaryote tree, as it probably diverged very early, yet would be specifically related to the common ancestor of animals and fungi. However, sequence data for Amoebozoa are limited, both for genes and taxa, so that the monophyly of the group remains questionable and the internal relationships among its members are still largely unresolved, leaving many outstanding evolutionary questions open. In particular, the phenotype of the ancestral lobose amoeba cannot yet be conclusively reconstructed, impeding that of the first eukaryote cell. In this project, we widely expand the available sampling in Amoebozoa for both genes and taxa. We sequenced cDNA libraries from 8 lobose amoebae representing all higher-level taxa within the phylum and use these data for constructing phylogenetic trees based on over 100 genes to (i) confirm the monophyly of Amoebozoa, (ii) better test the position of the phylum with respect to other eukaryotes (in particular the enigmatic amoeboflagellate *Breviata*), and (iii) assess the internal relationships within Amoebozoa and pinpoint the position of the root in the amoebozoan phylogenetic tree. In parallel, we PCR-targeted 4 genes (SSU and LSU rDNA, elongation factor 1 alpha, and myosin II) in a much larger number of amoebozoan taxa to refine relationships among some taxa previously considered *incertae sedis*. We shall compare the results of the gene-rich and taxon-rich trees and discuss the evolutionary and taxonomic significance of our findings.

BM116 - eIF3 IN TRYPANOSOMATIDS: A BIOINFORMATICAL ANALYSIS

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Regulation of gene expression in trypanosomatids is known to occur mainly at the post-transcriptional level. A key target for this regulation is the initiation stage of translation, which includes all the events leading to the assembly of the ribosome along the mRNAs. These in eukaryotes are mediated by the action of at least eleven distinct translation initiation factors (eIFs). The largest and least known of the eIFs, eIF3, participates in several essential steps in translation initiation such as AUG recognition and ribosome binding. In mammals it consists of 13 different subunits (named eIF3a-eIF3m) however in the yeast *Saccharomyces cerevisiae* a minimum core eIF3 was defined formed by 5 conserved essential subunits (eIF3a, eIF3b, eIF3c, eIF3g and eIF3i). Little is known regarding the overall translation process or eIF3 function in trypanosomatids. Here various bioinformatic tools were used in order to identify and characterize homologues of eIF3 subunits in *Leishmania major* and *Trypanosoma brucei*. These were first identified through BLAST searches using as queries sequences from human, plant and yeast subunits. The core subunit eIF3g, together with non-core subunits eIF3j, eIF3l and eIF3m were absent in trypanosomatids and a putative homologue of eIF3a (the largest of the subunits) was just identified in both species, but with very low homology. Homologues to eIF3b, eIF3c and eIF3e displayed slightly higher conservation but conserved binding domains were identified. Putative eIF3f and eIF3h homologues were found, but each was more closely related to proteasome subunits than to proper eIF3 constituents, and deserve to be better studied. Unexpectedly, the highest degree of similarity was verified for the non-core subunits eIF3d, eIF3i and eIF3k. Our data indicate that a true eIF3 complex, similar in structure to the one found in higher eukaryotes, is present in trypanosomatids but contain singular differences which may indicate new roles in translation.
Supported by FACEPE, CNPq and FIOCRUZ.

BM117 - BAT TRYPANOSOMATIDS: TISSUE TROPISM DETECTED BY PCR AND HISTOPATOLOGY IN NATURAL INFECTIONS

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Recent data has associated bats trypanosomatids with *Trypanosoma cruzi*, however, the behavior of this natural infection is unknown. This study was conducted in an endemic area for Chagas disease in Minas Gerais - Brazil where 238 bats were captured. Histopathological analysis was performed in 187 bats regardless of blood trypanosomes positivity. Using microhematocrit and blood culture in LIT medium, trypanosomatids were detected in 8% (19/238), from these, in 42.1% (8/19) tissue tropism were determined by specific *T. cruzi* PCR with 121/122 kDNA primers. Fourteen different tissues from three species of bats, *Phyllostomus hastatus* (four), *Carollia perspicillata* (three) and *Glossophaga soricina* (one), were analyzed. Histopathological analysis performed in 2618 samples showed no tissue parasitism nor inflammatory processes. In 87.5% (7/8) of animals tissue parasitism was detected by PCR. Positive results were observed in kidney and muscle chest in 50% of animals; heart, diaphragm, intercostal muscle and adrenal gland in 37.5%; smooth muscle and peritoneum in 25.0%; liver and cecum in 12.5%. *P. hastatus* presented higher frequency of tissue infected, 100% of positive animals in at least 21.4% (3/14) of tissues and 78.6% (11/14) and marked tropism for skeletal muscles and moderate for the smooth muscle and adrenal gland. From *C. perspicillata,* 66.7% (2/3) animals were PCR positive, one in the kidney and heart and the other in smooth muscle. Only one *G. soricina* bat specimen was analyzed with parasite DNA in adrenal and peritoneal muscle. The PCR demonstrated high sensibility for the detection of tissue parasites in bats. High frequency of PCR positive tissues was observed in the *P. hastatus* species, followed by *C. perspicillata* and *G. soricina*. Bat trypanosomatids showed tropism for skeletal and smooth muscles with differentiated pattern according to species examined without correlation with parasitemia or the inflammatory process.
Supported by CAPES, CNPq (301512/2008-6), FAPEMIG (CBB-APQ950/08) and FUNEPU.

BM118 - BAT TRYPANOSOMATIDS GENETIC CHARACTERIZATION USING *Trypanossoma cruzi* SPECIFIC MOLECULAR MARKERS

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Identification of wild-type trypanosomatids by biological patterns is scarce, however, the genetic characterization could provide a correct species determination. Previous reports suggest that bat trypanosomatids may belong to different species including *Trypanosoma cruzi*. In this study nine populations isolated from three species of bats, *Carollia perspicillata* (3), *Glossophaga soricina* (1) and *Phyllostomus hastatus* (5), captured at the Triangulo Mineiro and Alto Paranaíba - MG, were characterized by seven nuclear DNA and kDNA molecular markers directed to *T. cruzi* and *T. rangeli*. Were used: the D71/D72 and D72/D75 primers that amplify sequences of rRNA gene 24Sα; V1/V2 of the 18S rRNA; the intergenic region of miniexon TC/TC1/TC2; 121/122 and S35/S36 to kDNA; D75/RG3 that identifies *T. rangeli*. The *P. hastatus* and *G. soricina* isolates amplified *T. cruzi* products using both kDNA primers, and *T. cruzi* I major lineage by the markers directed to 24Sα ribosomal genes (D72/D75 and D71/72) and 18S rDNA, these populations did not amplify *T. cruzi* miniexon gene and *T. rangeli* sequences. None amplification was observed with tripanosomatid isolates from *C. perspicillata*. This data suggests that bat trypanosomatids populations isolated from these regions, may correspond to at least two different species, one of them genetically correlated to *T. cruzi* I. Althought, *T. cruzi* similarity, *P. hastatus* and *G. soricina* tripanosomatids did not amplify the intergenic gene region of the miniexon, possibly, being a different species from *T.cruzi*
Supported by CAPES, CNPq (301512/2008-6), FAPEMIG (CBB-APQ950/08) and FUNEPU.

BM119 - Proteomic analysis of benznidazole susceptible and resistant *Trypanosoma cruzi* amastigote

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After the last 30 years the treatment of Chagas' disease has been done either by 2-nitroimidazole (benznidazole) or 5-nitrofuran (nifurtimox). Both drugs have frequent side-effects, very low anti-parasitic activity in long-term chronic forms of the disease and variable efficacy according to the geographical area. Furthermore, the resistance to both drugs, already described for some populations of the *Trypanosoma cruzi*, has a considerable impact in the disease chemotherapy. Proteomic analysis has been reported as an ideal approach to study the global changes in the expression of genes in tripanosomatids. It can be useful in the knowledge of the parasite's resistance mechanism as well as the searching for biochemical targets to new drugs. Our goal is to identify proteins differentially expressed in *T. cruzi* benznidazole resistant and susceptible amastigote forms that can be involved in the benznidazole resistance mechanism. Trypomastigote forms from two *T. cruzi* populations selected *in vivo*, BZ resistant (BZR) and BZ susceptible (BZS) were maintained in Vero cell monolayers and induced to transform in amastigotes after 18h exposure at pH 5.0. The total protein extraction and dosage show that about 10^8 parasites are needed to obtain 250 μg of protein. The total protein extracts were analyzed, initially, by the one-dimensional electrophoresis (MW) followed by a bi-dimensional electrophoresis (pI and MW). The gels were Coomassie blue stained and the spots differentially expressed were selected using the software PDQuest7.3.0 (Bio-Rad). Those spots will be taken from the gels, digested with trypsine and identified by mass spectrometry.
Supported by CNPq; PDTIS-FIOCRUZ.

BM120 - IDENTIFICATION OF PROTEIN COMPLEX ASSOCIATED TO FUNCTIONAL RECOMBINANT SCAVANGER RECEPTOR MARCO USING TANDEM AFFINITY PURIFICATION METHOD

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CBA/J mice are resistant to *Leishmania major* infection but are permissive to *L. amazonensis* infection. In addition, CBA/J macrophages control *L. major* but not *L. amazonensis* infection in vitro. We have demonstrated that MARCO scavenger receptor gene is upregulated in CBA/J macrophages infected with *L. major* but not *L. amazonensis*. Mab ED31 blockage reduces in 30% macrophage infection. These data indicate that MARCO plays a role in macrophages infection by *L. major*. The goal of the present work is to identify proteins associated to MARCO during macrophage-*L. major* interaction. MARCO receptor gene was cloned into pTAPC2 vector containing the TAPTAG sequence, a dual tagging purification method that consists of a sequence containing two protein A domains, intercalated by a TEV protease site followed by a calmodulin binding domain. After cloning, MARCO sequence was confirmed by comparison in GenBank database. In order to evaluate recombinant MARCO-TAPTAG gene expression, we first transfected CHO cells with pTAPC2-MARCO. Cells were then lysed and MARCO expression analyzed by western blot. As described in previous studies, two bands were identified. In addition, to demonstrate the functionality of recombinant MARCO-TAPTAG on membrane surface we performed a binding assay with a pathogen known to interact with MARCO. Transfected CHO cells were exposed to *Escherichia coli* for 30 minutes at 4°C and submitted to immunocytochemistry. The result shows that *E. coli* colocalize with MARCO-TAPTAG-expressing CHO cells. In addition, similarly to previously described MARCO-TAPTAG-expressing CHO cell presented lamellipodia-like protrusions. This data indicate that MARCO-TAPTAG is functional. In the next step CHO cell stable transfected with pTAPC2-MARCO will be used to purify protein complex by tandem affinity purification method followed by mass spectrometry identification of the complex. The identification of proteins involved on *L. major* biding and phagocytosis will contribute to the understanding of mechanisms involved on leishmaniasis infection.

Supported by FIOCRUZ.

BM121 - Preliminary structural analysis of an essential immunophylin FKBP12 in *Trypanosoma brucei*.

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FKBPs (FK506 Binding Proteins) belong to the super family of peptidyl prolyl cis-trans isomerases (PPIase) (e.g. immunophilin). They are conserved from bacteria to mammals and function as chaperones. As the name suggests, they are able to bind immunosuppressive drugs as the two macrolactones, FK506 and rapamycin, which inhibits their PPIase activity. In mammals, this immunosupressive activity is unrelated to their PPIase activity but is due to the formation of ligand-receptor complexes that inhibit the proteine kinase TOR (rapamycin-FKBP) or the proteine phosphatase calcineurin (FK506-FKBP12). The information obtained over the last 15 years reveals that FKBP are involved in diverse biological processes affecting the function and structure of target proteins, the organism development and several signal transduction pathways. Some of them might play a role in parasite virulence (as TcMIP in *Trypanosoma cruzi*) and could therefore be the target of anti-parasitic drugs. By using a systematic knock-down by RNA interference of several FKBP genes from *T. brucei*, it was recently shown that one of them, FKBP12, is an essential protein for survival in both parasite stages (bloodstream and procyclic forms). In addition, it was reported that this lethal growth phenotype observed in bloodstream forms could be due to a strong cytokinesis deficiency. These data suggest for the first time that a FKBP might be essential for an organism. Therefore, we decided to study the 3-D structure of this protein that possesses a highly conserved catalytic domain. The ^{1}H NMR spectrum of the recombinant FKBP12 shows a good quality with sharp lines and good dispersion of the signals indicating that the protein is folded in solution. 2D spectra TOCSY and NOESY peaks indicate a conserved pattern of secondary structure characteristic of the bona fide FKBP12, composed mainly of β-sheets. Structure calculation and binding experiments with FK506 and rapamycin are currently investigated, as well as the determination of its binding site.
Supported by CAPES, CNPq and FAPERJ

BM122 - (TG/CA)n Role on Gene Expression in *Trypanosoma cruzi*

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

BM123 - Multilocus Sequence Typing: a useful typing method for differentiation of *Trypanosoma cruzi* isolates.

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The currently characterization of different isolates of *Trypanosoma cruzi* has problems due difficulties of data comparison among laboratories. Besides standard characterization techniques have a small resolution power so far. The Multilocus Sequence Typing (MLST) technique allows us the comparison of different isolates through the analysis of 400-500 bp form sequences of housekeeping genes. In this work, we proposed an MLST algorithm which allows the discrimination of the circulating genotypes of our study area (Chaco Province, Argentina) with only two sequence steps. This scheme is based on the analysis of Rho-Like GTP binding protein (RL-Gtp), Small GTP-binding protein Rab7 (Gtp) and Glucose-6-phosphate isomerase (Gpi), which together allow the identification of the 4 lineages described in our study area (*T. cruzi* I, IIc, IId and IIe). Moreover, seven genotypes were identify for *T. cruzi* I with this scheme. We work with different typing techniques (MLEE, RAPD, HVR probes, microsatellites) to identify the current variability of *T. cruzi* in our study area. So far MLEE was used as a gold standard typing method. We proppouse the used of the present scheme of MLST as suitable method of *T. cruzi* typing based on the accurate and confident features of this technique. Finally, MLST has the advantage of detect a small quantity of DNA (100 fg/ul), which is relevant when this is applied to biological samples without previous isolation as showed our results.

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