Bioquímica e Biologia Molecular-Biochem

BM001 - Contribution of proteasome for phenotype resistance to benznidazole in *Trypanosoma cruzi*

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Proteasome is the main component of ubiquitin-dependent protein degradation pathway, where substrates are marked for degradation by covalent linkage to multiple ubiquitin molecules. Specifically ubiquitin-conjugated proteins are degraded by the 26S proteasome, that consists of a 20S core particle and one or two 19S regulatory particles that modulate the peptidase activities caspase, trypsin and chymotrypsin-like. Recently, the involvement of the proteasome in the stage-specific transformation (trypomastigote into amastigote) in *Trypanosoma cruzi* was described. It was also observed that in this transition, occurs increased rates of intracellular proteasome-ubiquitin dependent proteolysis, suggesting that this pathway is essential for the changes associated with morphologic remodeling. The objective of this work was to understand the contribution of the proteasome for phenotype of resistance and sensibility to benznidazole. For this, we used sensitive and *in vitro* resistant (17WTS and 17LER) and *in vivo* (BZS and BZR) *T. cruzi* strains. The proteasome activities were analyzed using fluorogenic substrates and a proteasome enriched fraction obtained from epimastigotes by ultracentrifugation. The levels of 20S proteasome were detected by Western blotting, using antibody reactivity for α subunits. Our results showed that the levels of 20S proteasome are more abundant in 17WTS than in 17LER. Changes were also observed in both BZR and BZS strains. The results provided evidence that 20S proteasome is induced in 17WTS/17LER strains. They also showed that there is a inverse relationship between levels of proteasome subunit and activity associated with *T. cruzi* strain *in vivo*- and *in vitro*-drug resistance, suggesting that post-translation modification could be modulating the proteolytic activity of the 20S proteasome more efficiently than the transcriptional regulation. These hypotheses are now being investigated.
Financial support:FAPEMIG / CNPq

BM002 - Molecular studies on *Trypanosoma rangeli* Protein Tyrosine Phosphatase (PTP)

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Protein tyrosine phosphatases constitute a phosphatase group able to dephosphorilate phosphotyrosine residues. It is thought that these enzymes play key roles on complex events of cell cycle and differentiation of parasites, as well as interaction with host cells. Previous biochemical studies of ectoproteins on *Trypanosoma rangeli* H14 strain demonstrated a predominant PTP activity on the parasite surface. Recently, our group also verified a different PTP activity on the surface of *T. rangeli* Choachi and SC58 strains. Furthermore, the *T. rangeli* Transcriptome Project carried out by our group detected a cDNA fragment with high similarity with the *T. cruzi* homologous PTP gene. Thus, the aim of this study was to characterize the *T. rangeli* PTP gene. For that, PCR amplification was carried out from mRNA using primers directed to the conserved intergenic regions of the gene and to the exon. Amplified fragment of 941bp corresponding to the $5'$ end of the gene was obtained, cloned in pGEMT vectors and sequenced. After quality analysis, all sequences with Phred$\geq$20 were clusterized and analyzed by Blastx to confirm their identity. Based on the *T. cruzi* genome, the resultant ORF is up to now of 846bp, representing 86.5% of the predicted gene. Intraespecific analyzes carried out by ClustalW revealed an aminoacid difference at position 62 (hystidine for SC58 and tyrosine for H14 and Choachi) and also inside the catalytic domain (196-287) at position 228, where SC58 and Choachi had a proline and H14 a hystidine. These alterations may be due PCR errors or could be reflecting the previously observed biochemical differences. Interespecific evaluation with similar sequences of the TriTryps demonstrated 72% of identity with *T. cruzi*, 59% with *T. brucei* and 31% with *Leishmania major*. Additional studies are in progress to obtain the complete PTP gene sequence for these strains. Supported by:CNPq and UFSC.

BM003 - Generation of antisera against the mucin-associated surface protein (MASP) family of Trypanosoma cruzi and expression studies

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MASP, a new multigene family of surface proteins, was identified by the T. cruzi sequencing consortium (El-Sayed et al., 2005). The MASP family corresponds to approximately 6% of the T.cruzi diploid genome and is characterized by conserved N- and C-terminal domains and a central highly variable and repetitive region. In order to initiate the functional characterization of the family MASP, peptides derived

from the family were selected for synthesis and generation of the corresponding antibodies. The following selection criteria was used: 1) the most conserved motifs found in the mature protein; 2) the peptides should be immunogenic; 3) peptides should be MASP-specific and 4) peptides should represent different MASP sub-groups to allow co-expression studies. Based in these criteria three peptides were selected. Cysteine residues were added to the N- or C-terminal of each peptide to allow conjugation to the keyhole limpet haemocyanin (KLH) carrier protein, which increases the size of the antigen as well as its immunogenicity. Antibody titer was monitored by Enzyme-Linked Immunosorbent Assay. The anti-MASP affinity-purified antibodies have been used in immunofluoresence assays and in experiments of Western blot using extracts from epimastigote, tripomastigote and amastigote forms. Our results indicated that MASP is expressed on the surface of trypomastigote forms. MASP peptides and the corresponding antibodies will be used on immunogenicity and infectivity assays.

BM004 - Cloning and characterization of gene coding the proline oxidase of T. cruzi

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The flagellated parasite *Trypanosoma cruzi*, the etiological agent for Chagas` disease, can use carbohydrates or amino acids as main energy sources. The role of amino acids in trypanosomatids goes beyond protein synthesis and energy metabolism, involving several processes such as differentiation, osmoregulation, growth and stress resistance (for a review, see Silber *et al.* 2005). Biochemical evidences support the hypothesis that *T.cruzi* is able to obtain energy from L-proline by oxidizing it through the enzyme proline oxidase (PO) (EC 1.5.1.2) or the enzyme proline dehydrogenase (PD) (EC 1.5.99.8) (Sylvester and Krassner, 1976). In the present work, we searched the *T.cruzi* genome database for genes coding both enzymes. It was found a single gene coding for the *T.cruzi* PO (Systematic name Tc00.1047053510943.50 [www.genedb.org]). On the basis of the DNA sequence, specific primers were designed and, after PCR amplification, a band of 1.7 Kb was obtained. This PCR product was cloned in pGEM-T Easy ® and its identity was confirmed by sequencing. The PO activity of the enzyme coded by this gene was demonstrated by complementation a null mutant of *Saccharomyces cerevisiae*, deficient for the functional expression of the endogenous PO (strain YLR42w from EUROSCARF collection). The complemented mutant was able to grow in the presence of proline as the only nitrogen source, and in the presence of a mixture of amino acids without proline, showing that the reaction catalyzed by the cloned enzyme is reversible. These results show that the putative gene for *T. cruzi* PO really codes for this enzyme in spite of its low identity with other PO orthologues (12% identity with yeast and 23% identity with human protein). The low identity with the human enzyme leads us to propose the *T. cruzi* PO as a possible therapeutic drug target. Support: USP - FAPESP - CAPES

BM005 - Comparative genomics of metabolic genes in trypanosomatids

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Comparative genomics helps to understand organisms' evolution by comparison of similarities and differences of their genomes, improving annotation transference. The goals of the present study are detection of distant homologs in trypanosomatid metabolic pathways and analysis of genes involved in the following trypanosomatid metabolic pathways: (i) energy production and conversion; (ii) nucleotide transport and metabolism. COGs (Clusters of Orthologous Groups) belonging to the mentioned metabolic pathways were used for all analysis. For each COG, a multiple alignment (using MAFFT) then a HMM model were created using HMMER. A total of 329 COG-HMMs were used to search for distant homologs in *Trypanosoma cruzi, T. brucei* and *Leishmania major* genomes downloaded from GenBank, using a cutoff E-value of 0.1. Our preliminary results allowed us to partially re-annotate trypanosomatid genomes (when COG-HMMs had at least 1 significant hit). In T.cruzi , 0.60% (115/18939) entries were re-annotated: 78.3% (90/115) were in agreement to previous annotation; 8.7% were annotated differently, and 13.0% were annotated as hypothetical or unknown. In *L. major*, 1.46% (121/8265) entries were re-annotated: 85.2% (103/121) were in agreement to previous annotation; 7.4% were annotated differently; and 7.4% were annotated as hypothetical. In *T.brucei*, 1.32% (106/7795) entries were re-annotated: 82.1% (87/106) were in agreement to previous annotation; 7.5% were annotated with differently; and 10.4% were annotated as hypothetical. An example of annotation agreement is COG0074 (Succinyl-CoA synthetase alpha subunit) with entry 70886195 from T. cruzi. COG0127 (Xanthosine triphosphate pyrophosphatase) matched with entry 70832889 from *T. brucei*, that was previously annotated as hypothetical. Our preliminary results show that this methodology is useful for the identification of distant homologies. However, additional analyses are being done to corroborate our findings. Supported by CNPq, Fiocruz, PAPES IV

BM006 - Large chromosomal translocation in a single cell-derived clone of the *Trypanosoma cruzi* G strain

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Chromosomal rearrangements occur in different strains of *Trypanosoma cruzi* as well as among clones originating from the same strain. Large chromosomal events such as translocations enable rapid adaptation to new environments. Clone D11 is a single cell-derived clone of the G strain. Its molecular karyotype differs from that of the parental strain in both number and size of chromosomes. We have been shown previously that several chromosomes have undergone rearrangements during the isolation of clone D11. Here we present some evidence suggesting that these rearrangements may be due to chromosomal translocations. Hybridization with genetic markers showed that several chromosomes are duplicated in clone D11. Some markers hybridized with a single chromosomal band of G strain, whereas in clone D11 they hybridized with two chromosomal bands of approximately the same size (e.g., TEUF0002 hybridized with a band of 1.08 Mb in the G strain and bands of 1.18 and 1.53 Mb of clone D11). We could assume that the parental homologous chromosomes (1.08 Mb) carrying the TEUF0002 underwent telomere-telomere fusion, generating an unstable dicentric chromosome that broke to produce a chromosomal translocation, forming two novel chromosomes (1.18 and 1.53 Mb). An alternative hypothesis could be a chromosome breakage in the parental chromosome between two copies of TEUF0002 generating two fragments that underwent telomere fusion with heterologous chromosomes. Rearrangements in tubulin encoding chromosomes involve translocation of large fragments. Tubulin was mapped in bands of 1.11 and 1.88 Mb of G strain and in two bands of 2.33 and 2.47 Mb in clone D11. We suggest that a chromosome breakage occurred at a fragile site in the parental chromosome producing a large fragment carrying the tubulin loci that underwent telomere fusion with a heterologous chromosome. This could explain the presence of larger tubulin encoding chromosomes in the clone D11. Support: FAPESP, CNPq, CAPES

BM007 – Structural and transcriptional properties of TcTREZ0, a *Trypanosoma cruzi* site-specific repeated element

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We present here further characterization of TcTREZ0 (*T. cruzi* Tandem Repetitive Element Z0). It is a composite repeated element ($\sim$ 1.6 kb) since three subregions may be defined within it on the basis of sequence similarities with other *T. cruzi* sequences. We identified two subregions of the element with sequence homology to previously reported *T. cruzi* sequences. Subregion 1 ($\sim$570 nt) has 90% of identity with sequences previously described in the intergenic spacer of the *T. cruzi* genes for pyrimidine biosynthesis. Subregion 3 ($\sim$289 nt) presents a high sequence identity (87%) with *T. cruzi* U2II small nucleolar RNA gene and the untranslated region of mucin-like pseudogene. The central core of the element (subregion 2, $\sim$ 714 nt) is composed by a sequence of 446 bp followed by repeats units of 54- and 55-bp arranged in head to tail tandem arrays. Northern blots carrying total and poly-A+ RNAs of epimastigotes were hybridized with TcTREZ0. The complete element hybridized with 1.70- and 0.2 kb-transcripts in both RNA fractions. The 54- and 55-bp repeats strongly hybridized with the 0.2 kb-transcript and to a lesser degree with the 1.70-kb transcript, and the subregion 1 only hybridized with 1.70-kb transcript. Interestingly, TcTREZ0 also hybridized with a 0.2 kb-transcript in poly-A- RNA suggesting that this transcript is related to the U2II RNA. To isolate transcripts containing TcTREZ0, cDNAs were amplified by RT-PCR using a primer from the subregion 1 and an antisense primer from an internal sequence only found in TcTREZ0, and cloned into TA-vectors. Twenty recombinant sequences were found to be homologous to the TcTREZ0. Furthermore, we identified 15 ESTs, confirming that this repeated element is transcribed. Taken together, these results indicate that TcTREZ0 is expressed as oligo(A)-terminated transcripts. Isolation of full-length cDNAs carrying the complete TcTREZ0 element is underway in our laboratory. Support: FAPESP, CNPq.

BM008 - Differentially expressed genes in the metacyclic trypomastigotes of *Trypanosoma cruzi* identified by hybridization with polysomal mRNA

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The differentiation of epimastigotes into metacyclic trypomastigotes involves changes in the pattern of expressed genes. It has been demonstrated that mRNA mobilization to polysomes is an important mechanism of post-transcriptional gene expression regulation in *T. cruzi* (Ávila et al., Gen Mol Res 2003, 2:159). We constructed a cDNA library using total RNA extracted from metacyclic trypomastigotes (G strain). To identify genes differentially expressed in metacyclic forms, the library was screened with 32P-cDNAs synthesized from polysomal RNAs of epimastigotes and metacyclic trypomastigotes. We identified clones that only hybridized with either epimastigote (n=426) or metacyclic

probes (n=96). Clones with predicted or known functions were classified into putative cellular roles. Of the metacyclic-specific clones analyzed, the largest number (27%) encodes hypothetical proteins, other categories include sequences related to metabolism (25%), ribosomal proteins (13%), sequences without homology to genes available in databases (12%), cell surface proteins (8%), nucleic acids binding proteins (5%), structural proteins (3%) and heat shock proteins (3%). Among the epimastigote-specific clones, the largest number (36%) encodes hypothetical proteins followed by clones without any homology to sequences available in databases (21%), sequences related to metabolism (10%), cell surface proteins (8.9%), structural proteins (4%), ribosomal proteins (4%), heat shock proteins (2%), RHS and retrotransposon sequences (2%). Sequences related to cell surface proteins constitute the second-largest category of known functions. Some transcripts found in epimastigote polysomes are also present in the metacyclic trypomastigotes but not in the polysomal compartment, and vice-versa, RNAs found in metacyclic polysomes are also detected in the epimastigotes but not associated to the polysomal fraction. This result was confirmed by northen blotting hybridization using total and polysomal RNAS. Analysis of *T. cruzi* proteome databases showed that several stage-specific genes defined by this approach were only translated in a given parasite developmental stage. Support: FAPESP, CNPq, CAPES

BM009 - FUNCTIONAL GENOMIC ANALYSIS OF *Trypanosoma cruzi*: SUBMITTED TO DIFFERENT STRESS TYPES

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The differentiation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) involves the transformation of a replicative, non-infectious form of *T. cruzi* into a non-replicative, infectious stage. An essential event in metacyclogenesis is the nutritional stress mimicking the conditions faced by the parasites at the mid-gut of the insect host. Important changes in the gene expression program occur during this process and it is likely that they might play an important role in the physiological and morphological changes observed during the metacyclogenesis. However, little information is available concerning the ability of different stress conditions in triggering metacyclogenesis and also, about the genes involved in the regulation of these responses. We performed a comparative analysis of different stress conditions (temperature increase, pH decrease and nutritional stress) in triggering metacyclogenesis in vitro and investigated the expressed genes using microarray technology. Concomitantly, shotgun proteome LC-MS/MS analysis have been carried out in order to gain further insight into the relative abundance of *T. cruzi* proteins when different stress conditions were established. We are presently selecting sets of genes specifically expressed following each kind of stress and genes common to all stress conditions and comparing the biological properties of metacyclic trypomastigotes originated from each stress condition. Financial support from PRONEX, NIH CNPq.

BM010 - Post-transcriptional mechanisms involved in the control of expression of *GP82* gene of *Trypanosoma cruzi*

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The aim of this work is to investigate post-transcriptional regulatory mechanisms involved in the control of expression of *GP82* gene that encodes a metacyclic stage-specific surface glycoprotein of *T. cruzi*. We had previously shown that *GP82* transcripts can be found in the polysomal mRNA of metacyclic trypomastigotes (MT) but not in epimastigotes. To investigate whether mRNA stability may be responsible for the differences in the steady-state levels of *GP82* mRNA, parasites were treated with drugs that inhibit transcription (actinomycin D) or protein synthesis (cycloheximide). We found a clear difference in the levels of *GP82* mRNA between two forms after incubation with these drugs. When treated with actinomycin, the half-lives estimated for *GP82* transcripts in MT were higher than 8 h and less than 0.5 h in epimastigotes. These results suggest that, at least in part, the differences in the steady state mRNA levels are due to differential stability of *GP82* mRNA. Another explanation for differences in *GP82* mRNA stability between epimastigotes and MT could be the presence of protein factors affecting its turnover. To answer this question, half-life determinations were carried out in the presence of cicloheximide. Incubation with cicloheximide did not alter the *GP82* mRNA level in MT. However, it induced the accumulation of *GP82* transcript in epimastigotes. This suggests the existence of stabilizing and destabilizing mechanisms acting in MT and epimastigotes, respectively. As it has been proposed for several genes in *T. cruzi* (DiNoia *et al.* 2000, JBC 275:10218), regulatory elements present in the 3úntranslated region could be responsible for differential gene expression. Comparison of this region from twelve *GP82* clones revealed the presence of AU-rich elements (AREs). The presence of AREs in the *GP82* transcripts as regulatory element would direct our efforts to find out proteins involved in the stabilization of *GP82* transcripts. Support: CNPq, CAPES, FAPESP.

BM011 - Cloning of two *Trypanosoma rangeli* genes encoding type II DNA topoisomerases

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Type II DNA topoisomerases (topoII) are enzymes that catalyze structural changes of the DNA molecule by promoting transient double-strand cuts, being involved in many cellular processes, including DNA replication, transcription, recombination and chromosome segregation. Along with their role in nuclear DNA metabolism, these enzymes are also involved in replication and organization of the kinetoplast DNA in trypanosomatids, being potential targets for chemotherapy. The genome sequence of TriTryps showed the presence of two genes encoding type II DNA topoisomerases, in which a mitochondrial topoisomerase II is well described but little is known concerning its nuclear activity. In order to clone the TOP2 genes from *Trypanosoma rangeli*, a PCR approach was used with primers designed based on conserved amino acid regions deduced from available trypanosomatid TOP2 genes (TOP2mt and TOP2 α). The results revealed a 2.3 kb and 2.0 kb DNA fragments, respectively named as TOP2mt714 and TOP2α14 which were cloned, partially sequenced and revealed high similarity with topoII of other trypanosomatid species. These fragments were used to screen $2x10^4$ clones of a *T. rangeli* genomic library. Three clones of the TrTOP2mt gene and two clones of the TrTOP2α gene were selected from the EMBL3 genomic library. After CsCl gradient purification and restriction enzymes digestion, fragments were cloned into pBluescript (SK+) for automated sequencing. Further studies based on polyclonal antiserum against recombinant TrTOP2mt and TrTOP2α proteins in order to analyze the expression pattern and sub-cellular localization are in progress. Supported by CNPq, CAPES, Fiocruz and UFSC.

BM012 - Comparative analisys of *Trypanosoma rangeli* genomic sequences survey (GSS)

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The hemoflagellate protozoan parasite *Trypanosoma rangeli* Tejera, 1920 *Kinetoplastida* : *Trypanosomatidae* shares several invertebrate and vertebrate host species with *T. cruzi*, the etiological agent of Chagas disease. Despite the recent publication of the genome sequence of the TriTryps *T. cruzi*, *T. bruceiandLeishmania major*, non-pathogenic trypanosomatid species such as *T. rangeli* has not been well studied from the molecular point of view. In the present study, 1,720 genomic sequences from *T. rangeli* SC58 strain were generated by using the Genome Sequence Survey (GSS) approach. After analysis of the sequences using the GARSA *GenomicAnalysisResourcesforSequenceAnnotation* system, a total of 915 were grouped in 375 non-redundant sequences $GSS - nr$. The G+C content of the coding regions was 55%. Similarity searches based on BLAST and Interpro showed hits for 68% of the sequences, being 53% hypothetical proteins of organisms of the same family, especially *T. cruzi*. Also, sequences related to the mRNA editing process such as DEAD box helicase, as well as from the parasite coat as transsialidase, metaloproteases and mucins were found. Functional annotation based on the Gene Ontology consortia vocabulary showed sequences mostly related to molecular function and related to RNA helicase, serinopeptidases and ligands. Based on similarity searches, no possible function was attributed to 31% of the sequences, representing unknown sequences, *T. rangeli* specific sequences or even intergenic regions. Up to now there is a single report concerning the analysis of the *T. rangeli* transcriptome, indicating that the present work is originally addressing a large scale exploration of the parasite genome. The herein presented GSS data are now being analyzed along with the transcriptomic data. Supported by CNPq, IOCFiocruz and UFSC.

BM013 - BIOCHEMICAL AND BIOLOGICAL STUDIES WITH RECOMBINANT *Trypanosoma cruzi* APYRASE (NTPDASE I)

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An ecto-NTP diphosphohydrolase activity, insensitive to inhibitors of ATPases and phosphatases, was characterized on the surface of *T. cruzi* and a 2282 bp cDNA encoding a full-length NTPDase was cloned (Fietto et al., 2004). Trypomastigotes were shown to have a 2:1 ATP/ADP hydrolysis ratio, while epimastigotes presented a 1:1 ratio, suggesting a possible role for the NTPDase in the parasite/s virulence mechanism. To further characterize *T. cruzi* NTPDase I we performed the heterologous expression of active recombinant enzyme. In silico analyses of the sequence predicts a possible cleavage signal peptide at amino acid position 36, immediately following an amino-terminal predicted transmembrane segment, thus suggesting that NTPDase I could be produced

as a soluble exported protein. Using this information we design a strategy to express the soluble NTPDase I. Full-lenght NTPDase I cloned in pGEM vector was used as template to amplify a 1700 bp DNA fragment that was transferred to pET21b vector (that codes for Hexa-HIS at the carboxy terminal of the recombinant fusion protein). This construction was used to transform *E. coli* BL21 cells. Recombinant protein was expressed after 1 hour of induction. Soluble and insoluble recombinant apyrases were purified using Ni-NTA-agarose and showed specific activity for ATP hydrolysis between 2-17 nmolsmg protein^{-1} h. The substrate specificity showed preference for tri-phosphate nucleotides (ATP>GTP>UTP). The activity was higher in presence of Mg than Ca and the best renaturation pH was 7,5. We concluded that the rNTPDase I was produced in an active form. Now we are starting the production of full-rNTPDase I expressed in bacteria to compare biochemical parameters with rNTPDase (without putative signal peptide). Supported by: UFOP, FAPEMIG, CNPq.

BM014 - SSU rRNA processome proteins are translationally regulated during differentiation of *Trypanosoma cruzi*

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During the life cycle of *Trypanosoma cruzi* at least four distinct developmental stages are observed: epimastigotes and amastigotes are the replicative forms and metacyclic trypomastigotes and bloodstream trypomastigotes are the infective and non replicative forms. The process by which epimastigotes differentiate into metacyclic trypomastigotes is called metacyclogenesis and takes place in the insect vector. This event can be mimicked in vitro under chemically defined conditions. We used differential display to select genes differentially expressed during differentiation of epimastigotes into metacyclic trypomastigotes in the protozoan parasite *T. cruzi*. One of the selected clones had a sequence similar to that of the SSU processome protein Sof1p involved in rRNA processing. The corresponding *T. cruzi* protein, TcSof1, displayed a nuclear localization and is downregulated during metacyclogenesis. Heterologous RNA interference assays showed that depletion of this protein impaired growth but did not affect progression through the cell cycle, suggesting that ribosome synthesis regulation and the cell cycle are uncoupled in this parasite. Quantitative PCR assays of several identified SSU processome-specific genes in *T. cruzi* also showed that most were regulated posttranscriptionally, in a process involving the accumulation of mRNA in the polysome-associated fraction. Northern blot analyses of TcSof1 mRNA associated with polysomes confirmed the qPCR data, suggesting that the mechanism of regulation involves the blocking of translational elongation. Financial support: FIOCRUZ; PRONEX/Fundação Araucária; CNPq and FAPESP.

BM015 - Ecto-Phosphatase Activities in *Trypanosoma cruzi* sylvatic isolates from the State of Rio de Janeiro

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Trypanosoma cruzi Chagas 1909, ethiologic agent of Chagas disease, is represented by parasites that circulate between men, vectors, reservoirs and domestic servants. The phenotypic and genotypic variability presented by *T. cruzi* strains, may justify different forms of Chagas disease manifestations observed in different geographic regions. In this study, we characterized the profile of ecto-phosphatase activities of wild samples of *T. cruzi* isolated from the locality of Triunfo, Santa Maria Madalena (RJ) and its possible role in cell proliferation and differentiation. Previous reports of our group demonstrated some differences on the expression of cell-surface enzymes such as ecto-phosphatase activities in trypanosomatids. In general, parasite surface proteins are involved in host cell recognition and penetration. In this context, a comparative study of ecto-phosphatase activities between *Trypanosoma cruzi* sylvatic isolates was carried out. In addition, we evaluated *in vitro* epimastigotes proliferation and differentiation to trypomastigotes. Regarding the phosphatase activity, our results showed some biochemical differences among the *T. cruzi*isolates. The increasing order of phosphatase activity was $SMM53 < SMM98$¡ SMM10 $< SMM88$. Studying the kinetic of p-nitrophenol production, we observed that although the curve profile was the same, the substrate affinities presented minor differences. The effect of divalent metals was also analyzed and *T. cruzi* isolates phosphatase activities presented different susceptibilities to these ions. We are now investigating parasites proliferation and differentiation *in vitro*. Preliminary data show that SMM98 grow more efficiently in culture medium, with stationary phase maintained until twenty days after inoculation, while SMM53 begins to die at day 10. For SMM98, trypomastigotes begin to appear at the first day of culture and for SMM53, only after ten days of inoculation. As SMM98 presented high levels of ecto-phosphatase activity, we suggest a relationship between surface phosphatase expression and cell growth and differentiation.

BM016 - Low vanadate concentrations stimulate cell proliferation but not differentiation in *Trypanosoma rangeli* maintained at Pi-starved medium.

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Trypanosoma rangeli is a South American trypanosoma, considered harmless to humans and animals but able to infect triatomines. After the ingestion of trypomastigote forms during the blood meal, *T. rangeli* differentiates into short epimastigotes, proliferates in digestive tract, crosses the intestinal barrier and achieves haemolymph, where occurs the differentiation to long forms. Parasites complete their development in the lumen of salivary gland of the insect, where metacyclogenesis takes place. In this work, we investigate the effects of sodium orthovanadate, a phosphotyrosine phosphatase inhibitor, on cell division and differentiation of *T. rangeli*. We evaluate the influence of different concentrations of this drug on the percentage of short and long forms of *T. rangeli* during the cell proliferation at low- or high-phosphate culture medium. Our results reveal a strong dependence on inorganic phosphate to the growth and differentiation of these parasites. Sodium orthovanadate (1mM) inhibited both events in Pi-supplemented as well as Pi-starved medium. Studying the effects of low vanadate concentrations (0.1mM), we observed that cells maintained at Pi-supplemented medium exhibited the same proliferation profile of the cells kept in medium without vanadate but no long forms could be detected in this culture medium. The cell growth in Pi-starved medium was stimulated by 0.1mM vanadate, presenting the same profile of the cells maintained in Pi-supplemented control medium. However, the differentiation process did not occur. At high-phosphate culture medium, the initial phosphate concentration was reduced to 20% at day 15 of the cell growth curve while no significant change was observed in phosphate-starved cells. Taken together, these results suggest that the inorganic phosphate is an important nutrient required to cell division and differentiation of *T. rangeli*.

BM017 - Polymorphism in the *Trypanosoma cruzi msh2* gene and the role of Mismatch Repair in generating parasite genetic diversity

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Genomic integrity is critical to the cell and depends on the fidelity of the replication system and DNA repair efficiency. MSH2 is a major component of the Mismatch Repair (MMR) machinery, which corrects mismatches and insertion/deletion loops that may arise after DNA replication. Polymorphisms in the *Trypanosoma cruzi msh2* gene correlate with the division of the parasite population in three main lineages and encode 3 MSH2 isoforms we denominated MSH2A, B and C. ATP-hydrolysis assays using recombinant proteins suggest that parasites expressing MSH2A may present a more efficient MMR as compared to parasites expressing other isoforms. We hypothesize that the differences in the MSH2 protein may result in differences in MMR efficiency in the *T. cruzi* population. To test that, we treated parasites belonging to MSH2 haplogroups A and C with cisplatin and H_2O_2. Treatment with both genotoxic agents suggests that MSH2 haplogroup A parasites present a more efficient MMR. The higher H_2O_2 susceptibility of the MSH2 haplogroup C parasites is not related to a differential reducing power, as demonstrated by MTT assays. We are currently generating *T. cruzi msh2-/-* knockouts which will be used in transfection assays to compare the MMR phenotype in cells re-expressing each one of the MSH2 isoforms. *Msh2* single knockout parasites derived from the CL Brener strain (a hybrid strain presenting MSH2 B/C) showed reduced survival rates to H_2O_2 treatment. Meanwhile, we were able to express *T. cruzi msh2* genes in *T. brucei msh2-/-* which also showed decreased H_2O_2 tolerance. However, expression of two distinct *T. cruzi* MSH2 isoforms in *T. brucei msh2-/-* did not showed significant differences in the H_2O_2 survival phenotype.

Support by: FAPEMIG, CNPq and Howard Hughes Medical Institute

BM018 - Patterns of mRNA processing and UTR sizes in *Trypanosoma cruzi* : is bigger necessarily the best?

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The mRNA maturation in Trypanosomatids presents unique aspects. Unlike most eukaryotic genes, trypanosome genes are intronless and are transcribed as polycistronic units, which are processed in the nucleus to produce monocistronic mRNAs. The processing step requires two cleavage events that result in the addition of a 39nt cap-containing sequence known as spliced leader(SL) at the 5 end and poly(A) tail at the 3end of all mRNAs. Both cleavage events are coordinated by polypyrimidine tracts located within the intergenic regions.While no canonical polyadelynation additional signal has been identified, a conserved AG dinucleotide is the unequivocal spliced-leader acceptor site. Despite these studies, little is know about the sequence requirements in-

volved in an efficient mRNA processing. To investigate this more thoroughly, we have initiated a whole genome analysis of the *T.cruzi* processing sites. All available *T.cruzi* ESTs and cDNAs Genebank entries containing the poly(A) tail and/or the spliced-leader sequence were mapped on the *T.cruzi* genome. We generated *T.cruzi* 5 and 3 untranslated databases. Our analysis indicated that the 5UTR median length was 33 nt (mean=39) and the 3UTR median size was 136 nt (mean=304). The *T.cruzi* UTRs are smaller than the corresponding *T.brucei* sequences, in agreement with the greater genome compaction of the former compared to the latter. Alternative trans-splicing sites were identified in 40 cases (2.2 percent of all mapped SL acceptor sites). Analyses of the length and nucleotide composition of the polypyrimidine tracts as well as their distance to the SL and polyadenylation sites are underway. The polypyrimidine content will be compared with the abundance of the mRNA predicted by the ESTs frequency derived from non-normalized libraries and published DNA microarray studies. In addition, the identification of the signals involved in an efficient processing is crucial to the improvement of expression vectors that are used to transfect this parasite.

Support:FAPEMIG,CNPq-Howard H.Medical Institute

BM019 - The envelope composition of a symbiotic bacterium reveals news aspects of the endosymbiosis in trypanosomatid protozoa

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Symbiosis in trypanosomatids is characterized by the presence of a obligatory intracellular bacterium, which has coevolved with the host protozoa through a mutualist relationship. The origin of symbiont envelope is controversial; for some authors it is derived from the host plasma membrane, while others believe that it has prokaryotic origin. Previously, we showed that phosphatidylcholine (PC), which is unusual in prokaryotes, is present in the endosymbiont envelope, as described to bacteria that are closely associated to eukaryotes. The biosynthesis of PC has two pathways: one predominant in mammalian cells, where free choline is directly converted to PC via the intermediates choline-phosphate and CDP-choline (Kennedy pathway); and other predominant in prokaryotes, where PC is formed by three successive methylations of Phosphatidylethanolamine (PE) (Greenberg pathway). In this study we investigated metabolic pathways involved in PC production in endosymbiont-bearing trypanosomatids. Our studies with *Crithidia deanei* grown in ^{32}Pi culture medium showed that, PC is the main phospholipid produced. Isolated endosymbionts obtained from protozoa cultivated in ^{32}Pi containing medium, also presented great amounts of PC. Interestingly, isolated endosymbionts incubated in Warren medium containing ^{32}Pi for 1 h, were able to uptake this tracer and to synthesize phospholipids, especially PE. However, when isolated symbionts were incubated for 3 hs in ^{32}Pi culture medium, part of ^{32}P-PE was converted to ^{32}P-PC. Taken together, these results suggest that the endosymbiont in trypanosomatids has authonomy to synthesize part of its PC, probably through the Greenberg pathway. New studies are necessary to better characterize the phospholipid pathways involved in this mutualist relationship. In this moment we are also investigating the protein composition of the endosymbiont envelope in order to identify the outer membrane origin. This work was supported by: CNPq and FAPERJ

BM020 - Molecular and biochemical characterization of the acyl-CoA-dependent ceramide synthase gene in *Trypanosoma cruzi*

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Ceramide is the key intermediate in the pathway of sphingolipid (SL) biosynthesis, an important intracellular signaling molecule, and is synthesized mainly from the reaction of a fatty acyl-coenzyme A (CoA) with a sphingoid base, a reaction catalysed by an acyl-CoA-dependent ceramide synthase. In order to detect and characterize the ceramide synthase activity in *Trypanosoma cruzi* we assayed epimastigote microsomal fractions with [^{3}H]-DHS, fatty acyl-CoA derivatives, free fatty acids, or an *in situ* acyl-CoA generation system in the absence or presence of free fatty acid, BSA or detergents. The radiolabelled products were extracted, separated on TLC and visualized after autoradiography. *T. cruzi* ceramide synthase can only use fatty acyl-CoA derivatives as substrates, but not free fatty acids. The activity was blocked by Fumonisin B1, a known inhibitor of this activity in fungi, plants and mammals. Three genes, *LAG1*, *LAC1* and *LIP1* have been shown to be required for ceramide synthase activity in *Saccharomyces cerevisiae*. Lag1p and Lac1p are homologues and functionally redundant ER transmembrane proteins. Double deletion mutant cells, *lag1*Δ*lac1*Δ, have strongly reduced level of sphingolipids due to loss of the acyl-CoA-dependent ceramide synthase reaction. Lip1p forms a heteromeric complex with Lag1p and Lac1p and no homologs of Lip1p were found in animals, plants and *T. cruzi* by BLAST on the GeneDB. The *T. cruzi* GeneDB was screened for putative *LAG1* homologues using BLAST and the yeast Lag1p-motif. Two highly homologous sequences (GeneDB No. Tc00.1047053507395.10 and Tc00.1047053510087.30) were found. gDNA from *T. cruzi* (Dm28c strain) was used to amplify a 1.2 kb fragment which, after cloning into pCR4-TOPO and sequencing, showed that the *TcLAG1* presented

all the expected features of *LAG1* homologues from other organisms. The heterologous expression of the full length gene in order to functionally complement a yeast *lag1*Δ*lac1*Δ defficient mutant is underway. Support: CAPES, CNPq, FAPERJ, IFS.

BM021 - Polymorphic microsatellite loci as a tool for *Trypanosoma cruzi* profiling directly in animal and human infected tissues

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In the light of the Clonal Histotropic Model for the pathogenesis of Chagas disease we can hypothesize that a strict correlation between the parasite genetic and clinical aspects of the disease will be only achieved with direct profiling of *T. cruzi* present in tissue lesions. In this view, the advent of the LSSP-PCR opened new possibilities in the epidemiological studies on Chagas disease demonstrating the existence of differential tissue homing for distinct parasites in both human and experimental infections. However, the LSSP-PCR technique presents high complexity and its multilocal nature does not offer a simple way for determining the number and the composition of parasites present in a given sample. To solve this problem, the microsatellites arise as powerful tool, because they are unilocal markers and render highly individual profiles. Although, this feature makes hard to amplify small amounts of parasite DNA, as observed in chronic infected tissues. To bypass these restrains we took advantage of *T. cruzi* genome and used Tandem Repeats Finder program to locate microsatellite loci composed by tri and tetranucleotide motifs with large flanking regions. For all these loci we designed two pairs of primers for full nested PCR assay. By using this strategy we could detect as little as 200 fentograms of DNA corresponding to a single parasite DNA content. This high sensibility allowed us to detect specific *T. cruzi* amplicons in human and mice infected tissues. These results show that it is possible to identify and characterize parasites in human and mouse tissues using polymorphic unilocal DNA markers. Yet, the application of these studies to a broad range of samples certainly will clarify a possible correlation between the parasite genotypes and the different clinical manifestations of Chagas disease. Financial support: WHO, CAPES, CNPq and FAPEMIG.

BM022 - STRUCTURAL BASIS FOR THE INHIBITORY FUNCTION OF CHAGASIN: DIFFERENT REQUIREMENTS FOR THE INHIBITION OF PARASITE VERSUS HOST CYSTEINE PROTEASES.

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Chagasin is a novel inhibitor of papain-like cysteine proteases (CP) identified in *Trypanosoma cruzi* that shares no sequence conservation with previously known CP inhibitors. Recently, the determination of the solution structure of chagasin by NMR showed that it consists of beta-strands adopting an Ig-like fold and that conserved residues are grouped in three exposed loops L2, L4 and L6. Studies of chemical shift perturbation upon chagasin contact with the parasite protease, cruzipain, suggested the involvement of residues present in these loops in binding to the enzyme. However, the molecular basis for the inhibitory function of chagasin is unknown. In order to map the relative contribution of conserved amino acid residues for chagasin activity, we used site-directed mutagenesis to produce 16 chagasin variants containing substitutions of residues located on loops L2 and L4. The inactivation constants of the recombinant proteins were determined for the inhibition of cruzipain, cathepsin L and papain. Our results showed that substitutions of Thr^{31} to the hydrophobic residues Val, Tyr, Ala, but not to Ser, resulted in 100-200 fold increase in the K_i for cruzipain, while it did not significantly affect inactivation of cathepsin L. Along these lines, the mutants bearing substitution of Thr^{32} to Val or the double-mutation Thr^{31}Ala-Thr^{32}Ala displayed K_i values 10-200 fold higher for cruzipain, while their affinity for cathepsin L was similar to that of wild type chagasin. Taken together, our results indicate that efficient inhibition of the endogenous enzyme by chagasin is strictly dependent on the hydroxyl group of Thr^{31} while the requirements for the inhibition of the host enzyme are not well defined. We propose that chagasin is tailored for differential inhibition of parasite and host enzymes and that its function might be finely tuned by subtle changes in the ionization state of the Thr^{31} residue.

BM023 - Mitochondrial maxicircle NADH dehydrogenase subunit 7 gene deletion in *T. cruzi* isolates from asymptomatic patients

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The majority of individuals in the chronic phase of Chagas disease are asymptomatic. Every year 2-3% of these individuals develop severe clinical manifestations (cardiac and digestive forms). Recently we compared the transcript profiles of three isolates from asymptomatic and three isolates from cardiac patients with a *T. cruzi* DNA microarray. The probe corresponding to the maxicircle gene of *NADH dehydrogenase subunit 7* (*ND7*) showed a 30-fold up-regulation in cardiac strains. Northern blots confirmed absence of mature *ND7* transcripts in asymptomatic strains (Baptista *et al.*, 2006). To investigate differences in *ND7* structure among the strains, two PCR primers were designed flanking the *ND7* from CL Brener. Amplification of a ∼900bp fragment in four cardiac strains and a ∼500bp product in the three asymptomatic strains were observed. Dimorphism in the *ND7* structure was confirmed in additional strains. The *ND7* PCR products were cloned and sequenced. A deletion of 455bp was detected in the middle of the asymptomatic VL10 strain *ND7* sequence. Thus, *ND7* constitutes an appealing target for assays aimed at the differential diagnosis of infecting strains. To verify this proposition additional strains isolated from adult patients are under analysis. Because the *ND7* subunit plays an important role in complex I activity, we suppose that the deletion in the middle of *ND7* from asymptomatic strains most probably impairs the electron transport chain. Accordingly, we measured the generation of reactive oxygen species (ROS) in the mitochondria by flux cytometry. The asymptomatic strain produced 50% and 30% more ROS than the cardiac strain, under basal conditions and in the presence of antimycin A, respectively. So far, it cannot be asserted the relationship between complex I activity and pathogenesis. Presently, we are also investigating the susceptibility of the two classes of strains to the oxidative stress promoted by hydrogen peroxide. Support: FAPESP, CNPq, COLCIENCIAS

BM024 - Identification of proteins associated to T.cruzi polyribosome by proteomic analysis

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Trypanosoma cruzi metacyclogenesis corresponds to the differentiation of non-infective (epimastigotes) into infective forms (metacyclic trypomastigotes) and occurs naturally in the invertebrate host. This process can be mimicked *in vitro* under chemically defined conditions being an important model to study several aspects of the parasite differentiation process. The morphological changes observed during differentiation are a consequence of the modulated expression of several genes. Hence, the identification of genes whose expression is modulated as well as the factors responsible for their regulation is of great interest to understand the mechanisms controlling *T. cruzi* differentiation. It has been extensively shown that gene expression regulation in trypanosomatids occurs mainly at the post-transcriptional level. Thus, our goal is to identify proteins involved in controlling translation during *T. cruzi*metacyclogenesis by analyzing the protein content of polyribosomal fractions using mass spectrometry. For this purpose, we isolated *T.cruzi* polyribosomes before and after the onset of the differentiation process. The proteins were digested with trypsin and the peptides were identified by mass spectrometry (LC-MS/MS). Peptides were identified by comparison with usual databases and the accuracy of peptide identification was determined by using the Peptide Prophet software. Four different biological experiments have been carried out and proteins identified in at least three experiments were selected for further analysis. The next step will be the analysis of non-ribosomal proteins that are differently associated to polyribosome during the onset of metacyclogenesis. This will give us further insights of factors that might be involved in modulating the gene expression that trigger the differentiation of infective forms. Financial support from CNPq, Fundação Araucária (Pronex), Fiocruz, NIH

BM025 - Analysis of genetic polymorphism in *Trypanosoma cruzi* isolated from Chagas disease chronic patients from different regions of Minas Gerais and Goiás state, Brazil

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The structure of the *Trypanosoma cruzi* population is still not completely understood. However, this understanding is essential to verify the role of different parasite populations on epidemiology, pathogenesis and treatment of the disease. We analyzed the genetic profile of 93 *T. cruzi* isolates from chronic chagasic patients from different regions of Minas Gerais (MG) and Goiás (GO) state. At least two isolates were obtained from each patient at different times aiming to understand the parasite population dynamics during disease progression in the chronic phase. *T. cruzi* DNA was submitted to PCR for typing rDNA24Sa and the COII mitochondrial gene. Thirty-two isolates, seven from MG and seven from GO were analyzed for nine microsatellite loci. Results from rDNA24Sa and the COII mitochondrial gene characterization showed a strong correlation of these, since most of the isolates presented rDNA24Sa type 1 and COII haplogroup C, corresponding to the *T. cruzi* II lineage. However, isolates from two patients, one from MG and one from GO, showed rDNA type 2 and 1/2, respectively. Typing of COII mitochondrial gene in these isolates shows that they belong to the haplogroup B, related to *T. cruzi* III or to hybrid strains, suggesting that rDNA24Sa analysis alone is not enough to classify isolates from the parasite. The microsatellite profiles have shown that parasite populations isolated from the same patient are genetically identical and monoclonal, except for three isolates from a patient from GO, where differences were detected for five out of the nine loci analyzed. In this case, the first two isolates are genetically identical and the third one is different, suggesting that at least two populations of the parasite are present in this patient. Our results show the importance of studying different isolates from the same patient for validating the histotropic-clonal model proposed for Chagas' disease. Supported by PRONEX/CNPq/FAPEMIG

BM026 - Characterization of the gene TcAPX encoding the Ascorbate Peroxidase enzyme in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole

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Ascorbate peroxidase (APX) is one of the enzymes involved in the antioxidant defense of trypanosomatids. It is responsible for hydrogen peroxide (H2O2) metabolism, and use the dihydrotrypanthione (T[SH]2) as donator of electrons in the presence of ascorbate. APX is also present in plants with similar function. There is not peroxidase enzyme dependent of ascorbate in mammals, so the pathway redox used by trypanosomatids can be considered good target for Chagas disease chemotherapy. TcAPX is located in endoplasmatic reticulum of *T. cruzi* but few molecular data are known from this enzyme. In this study we have characterized the TcAPX gene encoding the ascorbate peroxidase enzyme in *T. cruzi* populations susceptible, naturally resistant or with in vitro-induced (17LER) or in vivo-selected (BZR) resistance to benznidazole. We cloned the the TcAPX encoding region (987 bp) into pGEX expression vector. The results of expression showed that *E.coli* BL 21 expressed a GST-fusion recombinant TcAPX protein with a relative molecular weight of 62 KDa for the rTcAPX (33 KDa) fused with GST (29 KDa). The protein recombinant was purified using electroelution method and then inoculated in rabbit for anti-TcAPX antibody polyclonal production. Western blot analysis showed that the anti-TcAPX antibody recognized one polypeptide of the 33 KDa in all analyzed *T. cruzi* populations, showing that the reaction of the antibody was specific to TcAPX. Levels of expression analysis of the protein TcAPX and chromosomal localization in the TcAPX gene in the *T. cruzi* populations susceptible and resistant to benznidazole are being realized. Supported by CNPq, FAPEMIG and CPqRR/Fiocruz

BM027 - Decreased expression of alcohol dehydrogenase (TcADH) enzyme in a *Trypanosoma cruzi* population with *in vitro*-induced resistance to benznidazole

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Alcohol dehydrogenases (ADH) belongs to a group of enzymes that catalyze the reversible oxidation of ethanol to acetaldehyde, with consequent reduction of NAD. This enzyme contains iron as prostetic group. In *T. cruzi* the *TcADH* gene has not been characterized yet. Murta et al (2002) using DNA microarray methodology selected some genes differentially expressed in *T. cruzi* benznidazole (BZ)-resistant (R) and susceptible (S) populations.The level of transcription the *TcADH* gene was four-fold lower in the *T. cruzi* population with *in vitro*- induced resistance to BZ (17 LER) than in the wild-type (17 WTS). Here, we investigate the differences in *TcADH* mRNA level, the copy number *TcADH* gene and the level of ADH production in *T.cruzi* populations susceptible, naturally resistant and with *in vitro*- induced resistance (17LER), or *in vivo*- selected resistance (BZR) to BZ. Real time RT-PCR analyses confirmed our finding that *TcADH* transcription levels were 2.5 times lower in 17 LER than in

17 WTS. In contrast, we detected no differences in *TcADH* trancription level among the other *T. cruzi* samples. Using real- time PCR, we found that the number of *TcADH* gene copies was similar in all samples. Western blot analysis of *T.cruzi* protein extracts probed with rabbit anti- TcADH polyclonal serum revealed a 41 kDa polypeptide present in all samples. The level of this polypeptide was similar for all samples except 17LER that was nearly two-fold lower than the wild-type 17 WTS. Our data show that a decreased expression of the TcADH enzyme was found only in a *T. cruzi* population with in vitro-induced resistance to BZ. Supported by: CNPq, CAPES, FAPEMIG, CPqRR/FIOCRUZ

BM028 - Increased expression of tryparedoxin peroxidase citosolic (TcTXNPc) enzyme in *Trypanosoma cruzi* population with *in vitro*-induced resistance to benznidazole.

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The tryparedoxin peroxidase catalyzes the reduction of hydrogen peroxide or small-chain organic hydroperoxides to water or alcohols, respectively. Tripanosomatids maintain the path of the tryparedoxin to regulate the oxidative stress. This enzyme belongs to the family of the peroxiredoxins and it was characterized in several parasites such as *Trypanosoma cruzi;T. brucei; Leishmania* spp. and *Crithidia fasciculata* (Wilkinson et al., 2000). It can be found in the citosol or in the mitochondria of tripanosomatids. In this work, tryparedoxin peroxidase citosolic (TcTXNPc) was characterized in 8 *T. cruzi* populations susceptible, naturally resistant or with *in vitro*-induced resistance (17LER), or *in vivo*-selected (BZR) resistance to Benznidazole (BZ). The northern blot profile of total RNA from *T. cruzi* samples, hybridized with TcTXNPc probe revealed a unique transcript of 0.8 Kb to 17WTS/17LER populations and a transcript of 0.9 Kb to other *T. cruzi* strains analyzed. In the 17LER *T. cruzi* population, the levels of TcTXNPc mRNA were 2-fold higher than its drug-susceptible conterpart 17WTS. This difference of expression was confirmed by real-time RT-PCR (qPCR). The other *T. cruzi* populations and clones expressed the same level of TcTXNPc independent of their drug resistance phenotype. The results of Southern blot and quantification by qPCR showed that this gene is not amplified in the *T. cruzi* genome. Southern blot analysis showed the presence of polymorphisms that were strain specific. They are not associated with zymodeme neither drug resistance phenotype. Western blot analysis of *T. cruzi* protein extracts probed with anti-TcTXNPc polyclonal antibody revealed an unique polypeptide of 23 kDa for all strains analyzed. The intensity of this polypeptide was similar in all samples, except 17LER,which displayed a polypeptide 2-fold more intense. Thus, we observed an increased expression of tryparedoxin peroxidase citosolic (TcTXNPc) only in a *Trypanosoma cruzi* population with *in vitro*-induced resistance to benznidazole.
Support:CNPq,FAPEMIG and CPqRR/FIOCRUZ.

BM029 - Characterization of the gene Tc52 encoding Thiol Transferase protein in *Trypanosoma cruzi* populations susceptible and resistant to Benznidazole

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Thiol transferase (Tc52), released protein of 52kDa, shares functional and structural properties with the family of the tioredoxin and the glutaredoxin. It is involved in the reactions redox thiol-disulphide, and in immunoregulatory activities. In our laboratory, the Tc52 protein was selected through of the proteome analysis of *T. cruzi* populations susceptible and resistant to Benznidazole (BZ). Tc52 was overexpressed in *T. cruzi* population with "in vitro-induced resistance to BZ (17LER), compared to its susceptible pair 17WTS. In this study, we investigate the differences in the levels of transcription of the Tc52 gene using Northern Blot and quantitative Real Time RT-PCR (qPCR). *T. cruzi* populations susceptible and with "in vitro-induced (17WTS/17LER) and "in vivo-selected resistance (BZS/BZR) to BZ were used in this study. Northern Blot profile of total RNA from *T. cruzi* samples hybridized with Tc52 probe revealed one transcript of 2.4 Kb. Quantitative analysis showed that the Tc52 mRNA level was the same for all *T. cruzi* samples analyzed, independent of their drug resistance phenotype. In addition, we cloned the Tc52 encoding region (1221 bp) into pQE-31 expression vector. The results of expression showed that *Escherichia coli* strain M-15 expressed a 6His-fusion recombinant Tc52 protein with a relative molecular weight of 53 KDa. Further studies will focus the Tc52 protein level analysis in the *T. cruzi* strains susceptible and resistant to BZ. In addition, we will investigate the tripanosomicidal effect of the Tamoxifen, an inhibitor of thiol transferase, in these *T. cruzi strains*. Supported by: CNPq, FAPEMIG, CAPES and CPqRR/FIOCRUZ

BM030 - *Trypanosoma cruzi* epimastigotes oxidative stress induced by heme

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Trypanosoma cruzi interacts with heme from the triatomine insect blood meal. Heme is a generator of reactive oxygen species (ROS), which can damage a variety of biomolecules. We investigated the contribution of the antioxidant enzyme superoxide dismutase (SOD) and glutathione (GSH)

to the parasite survival in the presence of different concentrations of heme. In order to assess this hypothesis we added to the medium diethyldithiocarbamate (DETC), a SOD inhibitor, or buthionine sulfoximine (BSO), the inhibitor of gamma-glutamylcysteine synthase (gamma-ECS). Epimastigotes Dm28c strain, at the exponentional phase, was maintained in BHI supplemented with 10 % FCS at 28 ^{o}C without addition of heme for 14 days. Afterwards cells were incubated in the absence or in the presence of different concentrations of heme, 5 μM of DETC or 2 mM of BSO, as well as 5 mM GSH. Growth of epimastigotes was evaluated in the presence of these drugs. A decrease in parasites growth was observed when treated with 2 mM of BSO and heme. The addition of 5 mM GSH in these treated cells was able to partially reverse this effect. The treatment with DETC also decreased epimastigotes proliferation caused by heme addition. ROS formation was assessed by microscopy and flow cytometry analysis of CM-H_2DCFDA fluorescence. We observed a increase of basal ROS formation in the presence of 30 μM of heme, and a increase of ROS with the addition of 300 μM of heme. Pre incubation with 5 μM DETC showed an increment in ROS formation. The incubation of parasites with BSO also increased ROS formation, although the addition of 5 mM GSH did not reversed this effect. Our data suggest that, *T. cruzi* likely developed effective mechanisms to efficiently cope with high concentrations of heme found in its environment and these defenses are involved with GSH and SOD activity.

BM031 - Molecular caracterization of ribonucleoproteic antigens from *Trypanosoma cruzi*

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Sequence analysis of clones isolated after immunoscreening a *T. cruzi* cDNA library indicated that ribonucleoproteins and proteins containing repetitive amino acids sequences are among the most proeminent antigens expressed in amastigotes. We present the caracterization of two cDNAs encoding RNA binding proteins with repetitive amino acids sequences. Sequence analyses of the cDNA denominated TcRBP48, show high homology with two RNA binding proteins from *T. brucei* named p34 and p37. The other cDNA named TcRpL7a presents homology to eukaryotic L7a ribosomal protein. However, sequence alignments with various L7a proteins indicate that TcRpL7a is the only one containing long extension of repetitive amino acids in its N-terminal region. The repetitive sequences of TcRpL7a and TcRBP48 share homology. Both proteins, as well as the truncated N-terminal and C-terminal fragments of TcRpL7a, were cloned and expressed as GST fusion proteins. Western blots assays, using recombinant TcRpL7a, its fragments and human chagasic sera, showed that antibodies are directed exclusively to the region containing the repetitive amino acids. ELISA assays showed that 73% of a panel of chagasic patients sera ($n = 59$) reacted with the repetitive region of TcRpL7a, 71% ($n = 59$) reacted with TcRBP48 and 80% ($n = 30$) reacted with 1:1 mixture of both antigens. In addition, 43% of chagasic sera recognized synthetic peptide covering the repetitive region of TcRpL7a. Both proteins were localizated in the parasite after expression, in transiently transfected cells, of cDNAs cloned in fusion with GFP. The transfected epimastigotes showed that GFP::TcRpL7a localizes in the nucleus/nucleoli whereas GFP::TcRBP48 localization was dispersed in the cytoplasm. Antibodies raised against both recombinant antigens identified the native proteins in cell extracts of all stages of the parasite at similar levels. TcRpL7a was also found in polysomal fractions suggesting that, in spite of the presence of a large repetitive amino acid region, it is part of the ribosome complex.
Support:FAPEMIG/CNPq

BM032 - Characterization of TcTAT gene encoding the tyrosine aminotransferase enzyme in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole

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Tyrosine Aminotransferase (TAT) enzyme is involved in amino acid biosynthesis, degradation and carbohydrate metabolism. Biochemical differences between *T. cruzi* and mamalian TATś suggest this enzyme as a promising target for Chagas disease chemotherapy. Recently, in our laboratory proteomic analysis of *T. cruzi* populations susceptible (S) and resistant (R) to benznidazole (BZ) revealed that TcTAT is overexpressed in an resistant population. In this study TcTAT gene was characterized in 15 *T. cruzi* strains susceptible or naturally resistant to BZ, and *T. cruzi* population in vitro-induced (17LER), or in vivo-selected resistance (BZR) to BZ. Northern blot profile of total RNA from *T. cruzi* samples S and R hibridized with TcTAT probe revealed the presence of one transcript of 2.0 Kb. Quantification of TcTAT mRNA by RT-PCR real time (qPCR) showed that the resistant population 17LER presented 2-fold more mRNA than its drug-susceptible conterpart 17WTS. The other *T. cruzi* populations expressed the same level of TcTAT mRNA independent of their drug resistance phenotype. Southern blot analysis of *T. cruzi* genomic DNA digestion with XhoI showed that the probe of the TcTAT gene recognized fragments of 3, 6 and 12 Kb in all *T. cruzi* populations. Our results showed that TcTAT is organized in a tandem multicopy gene array, confirming literature data. The chromosomal location revealed that TcTAT gene is present in chromosomal bands of 900, 1000 and 2000 Kb. This profile was heterogeneous among

the different *T. cruzi* strain. Western blot analysis of *T. cruzi* protein extracts probed with rabbit anti-TcTAT polyclonal serum revealed a 42kDa polypeptide present in all *T. cruzi* samples. No difference of expression level this polypeptide from *T. cruzi* samples. Our results show no association between TcTAT gene and drug resistance phenotype.
Support: CNPq, CPqRR, FAPEMIG, PDTIS/FIOCRUZ .

BM033 - Characterization and Evaluation of *Trypanosoma cruzi* ecto-nucleotidase activity and its relationship with *in vitro* infectivity.

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Extra-cellular nucleotides have been related as regulatory molecules and seen to be involved in some different biological process (cellular differentiation, adhesion, and immune response modulation). The main objective of this study is to evaluate the role of ecto-nucleotidases in *T. cruzi in vitro* infectivity. To achieve this objective we infected mouses with different T. cruzi strains/clone (Y, CL, Be-62, CL-Brener). Recovered blood trypomastigotes was used to infect VERO cells. After the first infection cycle we collected trypomastigotes and analyzed the *in vivo* ecto-nucleotidase activity and infectivity to VERO cells. In the first cell passage, the highest infection capacity was observed in Y strain and the lowest one in Be-62 and CL-Brener. *In vivo* ecto-ATPase activity was higher in trypomastigotes showing the following decreasing profile ($Y > CL > CLBrener > Be62$). ADP hydrolysis was similar in all parasites except in Y strain that presented lower hydrolysis. The resulting ATP/ADP ratio was quite different in trypomastigotes of different strains (17,6:1; 4:1; 3:1; 2,2:1 for Y, CL, CL-Brener, Be-62 respectively). AMP hydrolysis was only detectable in CL-Brener. In vitro differentiated amastigotes showed preference for ATP in all strains. ADP hydrolysis was undetectable (CL) or very slow (Y) and only Y strain presented detectable ecto-AMPase activity. Furthermore we evaluated the ecto-nucleotidase activities in Y trypomastigotes recovered after more cellular passages. As we suspected the continue cellular-infection (third to fourth passage) generate parasites with higher ecto-ADPase activities leading to decreasing in ATP/ADP hydrolysis. This phenomenon seems to be related with infectivity decreasing (about 80% of total trypomastigotes did not penetrate in VERO cells and differentiated to amastigote-like). These parasites presented very lower ecto-nucleotidase activity when compared with previous passages. These data suggested that the high ATP/ADP hydrolysis ratio could be related with higher infection capacity.
Financial Support:FAPEMIG / UFOP / UFV / CNPq / CAPES

BM034 - DETECTION AND CLASSIFICATION OF Trypanosoma cruzi STRAINS IN CLINICAL AND FIELD SAMPLES USING MULTIPLEX PCR

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In this work, we sequenced and analyzed PCR products of a satellite DNA composed by 195 bp repeats of 12 *T. cruzi* strains previously characterized according to zymodemes Z1, Z2 and ZB. The *T. cruzi* satellite DNA of these strains were amplified for PCR using Diaz 7 and 8 primers. These products were cloned and sequenced. The sequences were compared and a phylogenetic tree stablished. Two major branches were observed associated with the two major groups *T. cruzi* I and *T. cruzi* II. Further we developed a Multiplex PCR with three specific primers which allowed us simultaneously to detect *T. cruzi* DNA and classify *T. cruzi* I and *T. cruzi* II strains. The Multiplex PCR was able to detect 10 fg from *T. cruzi* DNA which corresponds to 1/30 DNA from one parasite. Its sensitivity was not influenced by excess of the hosts DNA. It was specific for *T. cruzi* DNA, non amplifying DNA from other trypanosomatids, neither the vertebrate host. Multiplex PCR was evaluated using genomic DNA from 30 *T. cruzi* strains previously characterized by isoenzymes, rDNA and mini-exon gene; 24 blood samples from experimentally-infected mice and controls not infected; 20 buffy coat samples from patients in the acute phase of Chagas disease and people not infected; and 15 samples of feces from naturally infected *Triatoma infestans*. Our results showed that samples from patients and from Y strain infected mice were classified as *T. cruzi* II and samples from *T. infestans* and Colombian strain infected mice, as *T. cruzi I*. In this work, we developed a methodology, which was able to simultaneously detect and classify *T. cruzi* strains from groups I and II in clinical and field samples. Supported by CNPq, FAPEMIG and CPqRR/FIOCRUZ.

BM035 - P-BODIES IN TRYPANOSOMA CRUZI

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In trypanosomatids regulation of gene expression occurs mainly at the post-transcriptional level by mechanisms involving either changes in stability, localization or ribosome occupancy of mRNAs. The regulation of mRNA turnover

and mRNA storage-availability plays a significant role in controlling gene expression; accordingly, recent experiments in yeast, *C. elegans* and mammals have defined cytoplasmic foci, referred as processing bodies (P-bodies), where mRNA decay and/or storage occur. P-bodies are dependent on mRNA for their formation and include the decapping enzyme, activators of decapping, 3‘ deadenylases, 5‘ - 3‘ exonuclease, and a cap-binding protein. In this study, we identified the putative *Trypanosoma cruzi* genes encoding P-bodies components. Although some P-bodies proteins seem to be absent in *T. cruzi*, most of the different functions inherent to these structures are covered by the identified genes. To characterize P-bodies in *T. cruzi* several putative components were cloned in expression vectors and the recombinant proteins were used for the production of polyclonal antibodies. Western blot was performed using the antibody against the activator of decapping Dhh1, demonstrating that this protein is constitutively expressed during the whole parasite life cycle. Immunofluorescence analysis showed that Dhh1 is localized in distinct cytoplasmic foci in *T. cruzi* epimastigotes. When epimastigotes and nutritionaly stressed epimastigotes lysates were loaded onto a 15-55% sucrose gradient for immunoblot analysis, Dhh1 protein was present in large complexes that sedimenting between the lighter fractions and the disome (polysomes with two ribosomes) fraction. Tap-tag assays are being performed to identify the proteins that participate of these complexes. Taken together, our data show for the first time the existence of these specialized cytoplasmic compartments in trypanosomatids. Financial support, CNPq, FIOCRUZ, Fundação Araucária (PRONEX)

BM036 - IDENTIFICATION OF THE HEAT SHOCK ELEMENT(S) IN THE POST-TRANSCRIPTIONAL REGULATION OF HSP70, HSP60 and HSP10 GENES OF *Trypanosoma cruzi.*

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

BM037 - Molecular characterization of Serine-, Alanine- and Proline- rich protein of *Trypanosoma cruzi*

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In a previous work we presented the functional characterization of a protein gene family termed Serine-, Alanine- and Proline- rich protein (SAP), from *Trypanosoma cruzi* metacyclic trypomastigotes. SAP peptides display several repeats (P2-4, S2-3, A2-3, AS, SA, PA, AP, SP, PS, TP) that are partially homologous to the serine-, alanine-, and proline-containing motifs of *Leishmania major* and *Leishmania mexicana* proteophosphoglycans. Recombinant SAP protein was able to inhibit parasite internalization and also triggered host cell Ca^{2+} response required for parasite invasion, suggesting that SAP could be involved in invasion of mammalian cell by metacyclic trypomastigotes. Here, we present the characterization of SAP gene family and the analysis of two transcripts isolated from metacyclic trypomastigotes of G and CL strains. Aided by the availability of the completed genome sequence of *T. cruzi*, we have identified 39 full-length sequences of SAP distributed in 33 contigs, six pseudogenes and four partial genes. These contigs appeared to be enriched in surface antigen genes (MASP, TS, GP63, mucin, DGF-1), RHS (retro-transposon hot spot protein), and retrotransposon-like elements (VIPER, L1Tc, SIRE, DIRE). SAPs share a central domain of about 55 amino acids and can be divided into four groups based on their amino and carboxy terminal sequences. Some SAPs have conserved N- and C-terminal domains encoding a signal peptide and a glycosylphosphatidylinositol anchor addition site, respectively. To analyze the transcripts of members of the SAP family in metacyclic trypomastigotes, we sequenced cDNA clones obtained by reverse transcriptase PCR from strains G and CL. The cDNA clones from the two strains were very similar, displaying 89% identity with SAP 1 at nucleotide and amino acid levels. Our results show that SAPs are products of a multigene family, like many surface antigenes from *T. cruzi*, and were found near retrotransposon-like elements and genes encoding surface antigens. Supported by: FAPESP.

BM038 - Heme metabolism in *T. cruzi*: evidence of a functional heme oxygenase in epimastigotes

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Pathogenic protozoa take up heme from the environment to supply its nutritional needs. To avoid the harmful effects of free heme different strategies were developed, such as the formation of hemozoin (heme polymer) found in Plasmodium. We started to investigate the heme detoxification by *T.cruzi* epimastigotes (EPI), looking for an eventual hemozoin formation. We followed the procedure of Marcus F. Oliveira et al. (*Nature*, 400:517,1999) and found no hemozoin in EPI. Other organisms resource on the enzyme heme oxygenase for heme catabolism. The enzymatic heme cleavage by heme oxygenase yields biliverdin, carbon monoxide and iron and the biliverdin is reduced to form bilirubin. Therefore we investigated the products of this enzyme in EPI grown in brain/heart infusion medium supplemented with hemin and serum. Culture supernatants were vacuun-dryed before use, as well as fresh medium (control). The material was acidified and extracted with 2 volumes of chloroform. After centrifugation, the chloroform layer was washed with distilled water and dried under Nitrogen. The samples were dissolved in 400 μl DMSO, and 100 μl aliquots were analyzed in a Shimadzu HPLC system, ACE 5 C18 250 x 4.6 mm column, utilizing a 70-98% methanol containing 2% acetic acid gradient, at a constant flux of 1,5 ml/min and UV detection at 398 nm. We have used heme, biliverdin and bilirubin solutions as standards for the HPLC runs. Surprisingly, the results showed billiverdin present in the culture supernatants extracts (not in control) and only traces in the cells extracts. These findings suggest that an ezymatic system (possibly heme oxygenase) present in EPI is able to oxydize heme to its product biliverdin, which is excreted in the medium. No traces of bilirubin were found in the samples. Supported by: *PIBIC/UERJ, CNPq, FAPERJ, **CAPES

BM039 - Partial maxicircle sequence of a clone derived from Colombiana strain of *Trypanosoma cruzi* major lineage I

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Knowledge about maxicircle sequence composition of *Trypanosoma cruzi* is a fundamental tool for comprehension of its population′s structure and evolutionary history. It was proposed the existence of three classes of maxicircle DNA sequences (clade A, B and C) which analyses can provide evidences of sexual events that occured in the past producing hybrid strains from parental ancestral lineages of *T. cruzi*. Especially intriguing was the observation that all hybrids so far analyzed presented the same clade B mitochondrial DNA, suggesting the existence of a kind of female or mitochondrial donor in *T. cruzi* (Freitas *et al.* 2006). The complete sequence and annotation of maxicircles from CL Brener (a hybrid strain from mitochondrial clade B) and Esmeraldo (a *T. cruzi* II strain from mitochondrial clade C) were recently achieved by Westenberger *et al.* (2006). In the present work we present the partial maxicircle DNA sequence of Col1.7G2, a clone derived from Colombiana, which is a strain belonging to *T. cruzi* I major lineage and mitochondrial clade A. We have sequenced a 2988 base pair region comprised between NADH dehydrogenase subunit 1 and cytochrome oxidase III genes. The sequenced region contains four putative genes encoding mitochondrial proteins including cytochrome b (*CYb*) and intergenic regions. Comparisons with other *CYb* gene sequences available in GenBank were done. These analyses corroborated the phylogenetic partion of the parasite populations into three identifiable clusters. To investigate further genetic relationships among the strains, comparative studies of other sequences of the mitochondrial genome are being conducted. Finnantial support: CNPq, FAPEMIG, WHO and the Howard Hughes Medical Institute.

BM040 - CaM Kinase and Heme oxygenase in Cell Signalling mediated by Heme (Fe-protoporphyrin IX) in *Trypanosoma cruzi*

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Trypanosoma cruzi, the ethiologic agent of Chagas disease, is transmitted through triatomine vectors during their bloodmeal on vertebrate host. One important intracellular mechanism to control heme homeostasis is its enzymatic degradation by heme oxygenase (HO). HO catalyzes the degradation of heme to biliverdin (Bv), carbon monoxide (CO) and iron (Fe^{+2}). We have investigated the role of heme at cell proliferation of *T. cruzi* epimastigotes Dm28c strain, and observed that the addition of heme increased significatively the parasite proliferation in a dose-dependent manner. In order to investigate a possible contribution of heme and its products we evaluated the effect of cobaltic protoporphyrin IX (CoPP, a HO inducer), Sn protoporphyrin IX (SnPP, a HO inhibitor), and Bv. The addition of SnPP decreased the parasite proliferation in a dose-dependent manner, also decreasing T. cruzi

growth in the presence of heme. When Bv was added to the culture this effect was reversed, leading to an increase of proliferation. CoPP did not interfered on proliferation in the absence or in the presence of 30 μM of heme. To investigate whether the proliferative effect of heme was determinated by protein kinases (PK) mediated signaling pathway, we evaluated the effect of several PK inhibitors. The parasites were incubated in the absence or in presence of 30 μM heme and PK inhibitors. Among all inhibitors tested, only KN93, a classical inhibitor of CaM kinases, had a signifficative effect at cell proliferation mediated by heme. In order to test the specificity of KN-93 effects, we used KN-92, inactive analog of KN-93 and no effect on epimastigotes proliferation was observed. Our results shows that heme increases epimastigotes proliferation, biliverdin is important to *T. cruzi* proliferation and CaM kinase pathway is involved on the signalling mediated by heme. Supported by PIBIC/UERJ, FAPERJ, CNPq and Fiocruz.

BM041 - The Role of Phosphoglycerate kinase A (PGKA) in glycolytic flux regulation in *Trypanosoma cruzi*

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In *Trypanosoma cruzi*, glycolysis is not regulated in the two classic points of control HK and PFK, and it is compartmentalized up to phosphoglycerate kinase (PGK) in a peroxisome-like organelle known as glycosome. PGK catalyzes the conversion of ADP and 1,3-bisphosphoglycerate to ATP and 3-phosphoglycerate in the glycolytic pathway. In *T. cruzi*, PGK is present in glycosome (PGKA and PGKC) as well as in cytosol (PGKB). It has been proposed that PGKA should take part in the glycolytic flux control and be involved in the maintenance of ATP balance inside glycosome; however, so far, the PGKA role in the intermediate metabolism of *T. cruzi* is not known with certainty. In this study, along a growth curve of a culture of *T. cruzi* epimastigotes, the expression of PGKs isoenzymes was determined. A constitutive expression for both PGKA and PGKC isoenzymes was found, while a differential expression was observed for PGKB, whose levels increased during the stationary phase when the reduction of the medium glucose occurs. The subcellular localization of PGKA in the glycosome of *T. cruzi* and its association with the organelle membrane were confirmed (by partial permeabilization of epimastigotes with digitonin, osmotic shock, and treatment with Triton X-114 of intact glycosomes). It is suggested that part of PGKA N-terminal domain is located towards the cytosolic side of glycosomal membrane (by experiences of labeling with biotin and protection against proteases of intact glycosomes). Furthermore, it was found that, when incubating epimastigotes, with permeabilized plasmatic membrane and intact glycosomes, using a specific serum developed against a PGKA N-terminal region of 80 amino acids, the glucose consumption by the glycosome is blocked, suggesting a role for PGKA in the glycolytic flux control, and supporting the idea that part of the protein N-terminal could be placed toward the organelle cytosolic side.

BM042 - TcOcrl1, the GTPase-activating protein (GAP) of T. cruzi is an ortholog of the human Ocrl1 (Oculocerebrorenal syndrome of Lowe), and possibly a linker between phosphoinositides metabolism and GTPase activation.

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GTPases of the Rho family regulate cell signaling pathways mainly through their ability to cycle between an inactive GDP bound and an active GTP bound state. GTPase-activating proteins (GAPs) are main regulators of GTPase activities, increasing the intrinsic rates of GTP hydrolysis, thereby inactivating the GTPases. In this work we report the indentification of an ortolog of GAP Ocrl1 (Oculocerebrorenal Lowe syndrome) in *Trypanosoma cruzi*. In humans, mutation in Gap Ocrl1 leads to syndrome of Lowe, rare X-linked disorder characterized by congenital cataracts, mental retardation, renal Fanconi syndrome, and deficient growth. The enzyme Ocrl1 of *Trypanosoma cruzi*, TcOcrl1, presents a conserved structural domains: a RhoGAP domain in N-terminal region, and in the C-terminal region an IPPC domain (Inositol polyphosphate phosphatase) responsible by the removal of the phosphate from the 5-position of the inositol ring from both soluble (PtdIns(4,5)P2, PtdIns(3,4,5)P3) and lipid substrates (Ins(1,4,5)P3, Ins(1,3,4,5)P4), although being phosphatidylinositol 4,5-bisphosphate (PIP2) the preferred substrate. Phylogenetic analysis reveals that Ocrl1 is conserved also in other trypanosomatids, such as *T. brucei, L. major, T. vivax*, and *T. congolense* which form an ancestral group, demonstrated a genetic duplication with presence of two paralogs from Tetrapoda. Characterization of the transcripts of TcOcrl1 reveals an alternative *trans* splicing with two AG dinucleotide acceptor sites on 5 UTR region. Furthermore, production of antibody for TcOcrl1 and lineages of T. cruzi Dm28c expressing the fusion proteins GFP-TcOcrl1-wild type, GFP-TcOcrl1-ΔIPPC, and GFP-TcOcrl1-ΔGAP are in course and will be used in functional assays to elucidate the role of this protein in signaling pathways of phosphoinositides metabolism and GTPase activation.

BM043 - The actin family and actin-binding proteins of Trypanosoma cruzi: unusual subcellular localization of actin homologue and phylogenetic studies

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The actin cytoskeleton controls pivotal cellular processes such as motility, cytokinesis and cell-cell and cell-substrate interactions. Assembly and spatial organization of actin filaments are dynamic events regulated by a large repertoire of actin-binding proteins. In *T. cruzi*, the actin protein has been unsuccessfully described by several groups and the exact subcellular localization was uncertain up to this moment. Furthermore actin-binding proteins have not been characterized. In this work, we report the extensive analysis of trypanosomatids genome to discover actin-binding proteins and actin-related conserved proteins. The main proteins responsible for actin nucleation in higher eukaryotes are also present in *T. cruzi* (all the subunits of the Arp2/3 complex, five sequences encoded formins), besides proteins responsible by actin treadmilling such as profiling, ADF/cofilin, CAPZ, CAP/Srv2 are also found in *T. cruzi*. Phylogenetic validation using trypanosomatid genomes of all encoded actin-like proteins entries confirmed the existence of Arp4 in *L. major* and *T. cruzi*, and Arp6 e Arp8 in *T. brucei, L. major*, and *T. cruzi*. The trypanosmatids specific sequences absent in all other clades were grouped as Actin-like proteins (ALPs) and distributed in five groups, from ALP1 to ALP5. Immunofluorescence assays carried out with antibody raised against a *T. cruzi* actin peptide revealed numerous rounded and punctuate structures spread for the entire parasite cytoplasm and no difference could be found between epimastigotes, tripomastigotes or amastigotes. Moreover, in cell fraction analyses, actin localized only in the soluble fraction, being absent in membrane and cytoskeleton fractions. Further studies are in progress to address the role of actin in *T.cruzi* evolutive forms.

BM044 - EST sequences from *Trypanosoma cruzi*: generation of new sequences from metacyclogenesis and the usage of the whole public set for differential expression and gene annotation analyses.

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We have conducted an EST sequencing project of *Trypanosoma cruzi* with samples taken from the metacyclogenesis process to increase the gene coverage of our microarray platform. As a direct consequence of this effort, we have analysed 6756 sequences produced in our laboratory along with the 13971 EST sequences deposited on Genbank (July 2006 release), in a total of 20727 EST sequences. These data should allow getting further insight into the identifycation of differential gene expression based on EST coverage and, mainly, to establish the mRNA determinant sequence on the genome recently released. We have divided the Genbank EST dataset in six major projects, made analysis based on the life cycle phase (epimastigotes, trypomastigotes, amastigotes and differentiating epimastigotes), strain (CL Brener, Dm28c, Tulahuen and Y) and technique used to construct the cDNA. We have accessed the redundancy in a general and specific level by two different approaches: sequence length after clustering and gene coverage, based on the *T. cruzi* genome annotation. The clustering was performed by two distinct methods: CAP3 and TGI Clustering Tools. The redundancy for the different groups varied from 34 to 54% based on the sequence length. This data also demonstrated gene finding errors, mainly in determing the initial ATG, but also to determine UTR regions and to establish putative CDS and/or UTR for genes that are truncated. Furthermore, a differential expression analysis, based on the gene redundancy approach, was performed and compared with microarray and proteomic data, showing a low level of correlation among methods, as expected. Nevertheless, it was possible to select some consistent examples of differential expression, which are probably more reliable, since they were selected by distinct methods. Finally, a web-based interface was designed to facilitate the visualization and analysis of the covered transcripts, providing a useful tool for the *T. cruzi* scientific community.

BM045 - CHARACTERIZATION OF DNA POLYMERASEβ AND β-PAK OF *Trypanosoma cruzi* USING FLUORESCENT PRIMER EXTENSION TECHNIQUE.

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DNA polymerase β is the smallest DNA polymerase in eukaryotes. This enzyme is capable to fill small DNA gaps and is involved in base excision repair (BER) pathway in mammalian cells. In short-path BER, DNA polymerase β removes the terminal 5′-desoxiribosil phosphate from an apurinic-apyrimidinic (AP) site and then inserts one nucleotide in

the resulting gap. In long-path BER, DNA polymerase β is the main enzyme responsible for filling gaps with 2-6 nucleotides long. Even though eukaryotic DNA polymerase β is located in the nucleus of most species, *Crithidia fasciculata* DNA polymerase β was the first one described as being located inside mitochondria. Two DNA polymerases in *Trypanosoma brucei* (TbDNA pol β and TbDNA pol β-PAK) were described, and both have a mitochondrial localization signal. It is believed that TbDNA pol β and TbDNA pol β-PAK participate in mitochondrial DNA replication. TbDNA polβ show 70% identity and is homologous to *Crithidia fasciculata* DNA polβ. TbDNA polβ-PAK shows 30% identity with *Crithidia fasciculata* DNA polβ and has a non-usual structure including a C-terminal tail and a N-terminal region rich in proline, alanine and lysine. This work deals with a partial characterization of DNA polymerase β and DNA polymerase β-PAK, counterpart proteins from *Trypanosoma cruzi*. After isolation, cloning and protein purification, polymerization conditions were determined through fluorescent primer extension technique and the products were analyzed in an automatic sequencer. Both polymerases showed different responses in all tested conditions (NaCl, dNTPs and $MgCl_2$ concentration, pH and temperature). These differences suggest a non-redundant role for TcDNA polβ and TcDNA polβ-PAK. In addition, only TcDNA pol β-PAK was capable to polymerize though 8-oxoG lesions. The next step is to localize both polymerases inside the parasite cell to better understand they biological role in *T. cruzi*. Financial Support: CNPq, PRONEX-FAPEMIG, Howard Huges.

BM046 - Heterogeneous expression of metallo-type proteases in parasites belonging to the Trypanosomatidae family

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Proteolytic enzymes play a central role in the physiology of all living organisms, participating in several metabolic pathways and in different phases of the parasite-host interactions. Various human pathogenic microorganisms as well as opportunistic pathogens produce metalloproteases, in which zinc is an essential metal ion for the catalytic activity, with a wide multiplicity of pathological actions. For instance, in local infections, the metalloproteases can cause necrotic or hemorrhagic tissue damage through digestion of structural components of the ground substance, and also form edematous lesions through generation of inflammatory mediators, while in systemic infections this class of proteases can act as a synergistic virulence factor through disordered proteolysis of many plasma proteins. In the present study, we have identified cell-associated protease activities in thirty-three distinct flagellates, including representatives of almost known trypanosomatid genera parasitizing insects (*Herpetomonas, Crithidia, Leishmania, Trypanosoma, Leptomonas, Phytomonas, Blastocrithidia* and *Endotrypanum*) and *Bodo*, by using SDS-PAGE containing gelatin as copolymerized substrate and proteolytic inhibitors. Under alkaline pH (10.0) conditions employed, all the flagellates presented at least one protease, with the exception of *Crithidia acanthocephali* and *Phytomonas serpens*, which did not produce any detectable proteolytic enzyme. All the proteolytic activities were completely inhibited by 1,10-phenanthroline, a zinc-chelating agent, putatively identifying these activities as metalloproteases. EDTA and EGTA, two other metalloprotease inhibitors, E-64 (a cysteine protease inhibitor), pepstatin A (an aspartyl protease inhibitor) and PMSF (a serine protease inhibitor) did not interfere with the metalloprotease activities detected in the studied trypanosomatids. Conversely, *Bodo*-derived proteases were resistant to 1,10-phenanthroline and only partially inhibited by EDTA, showing a distinct inhibition profile. Together, our data showed a great heterogeneous expression of metalloproteases in a wide range of parasites belonging to the Trypanosomatidae family. Financial support: CNPq, CAPES, FAPERJ and FUJB.

BM047 - Overexpression of amastins provides new insights into their function in Trypanosoma cruzi

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The protein composition of the Trypanosoma cruzi amastigote surface is relatively unknown. However, the study of protein components present in the intracellular stage of this parasite is of great interest due to its importance in the maintenance of the infection and for the host immune response during the Chagas' disease.Among previously described amastigote surface proteins, there are amastins, a group of 174 amino acid proteins, encoded by a multi-copy gene family and presenting four hydrophobic domains and several potential sites for O-glycosilation. Their mRNA is 60 times more abundant in amastigotes than in other stages of the parasite life cycle. Genomic sequence analyses derived from different parasite strains demonstrated that amastin genes present in strains belonging to T. cruzi II lineage are more heterogeneous than those from T. cruzi I lineages. These analyses also showed that the variability concentrates in the extracellular hydrophilic regions. In an attempt to elucidate the function of amastins, we decided to generate T. cruzi cells overexpressing this protein in fusion with a His tag. Western blot analyses of several clones stably expressing mRNA showed that the protein in fusion with six histidines (AmaHis) is present at the parasite surface. Clones of AmaHis expressing parasites present a higher cell division rate compared to wild type parasites. In vitro infections experiments of showed that AmaHis expressing parasites are

capable of infecting Vero cells at similar levels compared to non-transfected parasites, thus suggesting that amastins mat not be directly involved with the infection process. However, AmaHis expressing parasites presents four-time higher percentage of rounded forms (spheromastigotes) than those wild type cultures of epimastigotes. A larger number of amastigotes was also found in the supernatant of infected Vero cell cultures.Taken together, our data suggest that amastins may be involved with the differentiation process that results in amastigotes.
Support:FAPEMIG/CNPq/Howard Huges Med.Institute

BM048 - Pre-SSU rRNA processing by SL addition trans-splicing in trypanosomatids

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The capped miniexon addition by*trans*splicing is an essential premRNA processing step in the generation of all translatable mRNAs in trypanosomatids. However, we have evidenced that RNA pol I promoter of *T. cruzi* or*L.(L.) amazonensis* can drive the expression of the chloramphenicol acetyltransferase (CAT) gene even when no heterologous *trans*splice acceptor site was added in reporter construction. Those experiments raised the possibility that the 5ÉTS rRNA region could encompass a native 3ácceptor site, which in turn suggests that prerRNA can be *trans*spliced. To verify the SL addition *trans*-at the 5ÉTS region of prerRNAs, we applied a common seminested RTPCR strategy in four different trypanosomatids, *T. brucei*,*T. cruzi*, *L. (L.) amazonensis* and *C. fasciculate*. cDNAs were produced by reverse transcription of total RNA preparations of each organism with a SSU rRNA based primer. Then two successive PCRs were performed using a miniexon derived forward primer in combination with nested SSU rRNA reverse primers. Agarose gel analyses revealed different patterns of putative transspliced products for each organism and no products in mock reactions. Sequence analysis of the RTPCR products showed that the generation of hybrid SL5ÉTS molecules obey the same basic rules as for the premRNA *trans*splicing splicing that generally takes place at the first AG dinucleotide following a T rich polypyrimidine. Here, for the first time, we provide evidences that the RNA pol I transcribed pre-SSU rRNA of trypanosomatids is also processed by SL addition *trans*-splicing, suggesting a an ancient behavior of RNA molecules to join each other even after the partial specialization of the RNA polymerases.

BM049 - Ancestral genomes, sex and the population structure of *Trypanosoma cruzi*

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Acquisition of detailed structure and evolution of *Trypanosoma cruzi* populations is essential for control of Chagas disease. We have profiled 75 strains of the parasite with five nuclear microsatellite loci, 24Sα RNA gene, and sequence polymorphisms in the mitochondrial cytochrome oxidase subunit II gene. We also used sequences available in GenBank for the mitochondrial genes cytochrome B and NADH dehydrogenase subunit 1. A multidimensional scaling plot (MDS) based in microsatellite data divided the parasites into four clusters corresponding to *T. cruzi* I (MDS-cluster A), *T. cruzi* II (MDS-cluster C), a third group of *T. cruzi* strains named by us as *T. cruzi* III (MDS-cluster B), and hybrid strains (MDS-cluster BH). The 24Sα rDNA and microsatellite profiling data were combined into multilocus genotypes that were analyzed by the haplotype reconstruction program PHASE. We identified 141 haplotypes that were clearly distributed into three haplogroups (X, Y, and Z). All strains belonging to *T. cruzi* I (MDS-cluster A) were Z/Z, the *T. cruzi* II strains (MDS-cluster C) were Y/Y, and those belonging to MDS-cluster B (*T. cruzi* III) had X/X haplogroup genotypes. The strains grouped in the MDS-cluster BH were X/Y, confirming their hybrid character. Based on these results we propose the following minimal scenario for *T. cruzi* evolution. In a distant past there were at a minimum three ancestral lineages that we may call, respectively, *T. cruzi* I, *T. cruzi* II, and *T. cruzi* III. At least two hybridization events involving *T. cruzi* II and *T. cruzi* III produced evolutionarily viable progeny. In both events, the mitochondrial recipient (as identified by the mitochondrial clade of the hybrid strains) was *T. cruzi* II and the mitochondrial donor was *T. cruzi* III.
Financial Support:Pronex - FAPEMIG

BM050 - FUNCTIONAL CHARACTERIZATION OF THE DNA POLYMERASE KAPPA FROM *T. cruzi*

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The genetic information is continuously subject to the attack by endogenous and exogenous chemical and physical agents that damage DNA, thus compromising its biochemical functions. Replication of lesion containing DNA, a process termed translesion synthesis (TLS), is a major source of

point mutations. Recently this process has gained much understanding since the specialized DNA polymerases involved in TLS have been characterized. DNA Polymerase Kappa (PolK), a low-fidelity DNA polymerase which is able to perform DNA synthesis across several damaged bases, is one of the enzymes involved in this process. In this work, we started the functional characterization of DNA Polymerase Kappa from the protozoan *Trypanosoma cruzi* (TcPolK). To determine its cellular localization, we inserted the TcPolK gene into the pTREX-GFP vector, to produce the TcPolK-GFP fusion protein. After transfection of an epimastigote culture, cells were examined by immunofluorescence microscopy. This indicated a mitochondrial localization, confirming the in silico cellular localization prediction. Because there is also a putative Nuclear Localization Signal in the peptide sequence, we suggest that there could be a biochemical mechanism regulating the nuclear import of this polymerase. To examine if a Polymerase Kappa overexpression could modify the parasite′s resistance to different stress conditions, we cloned the TcPolK gene into the pROCK-NEO expression vector and analysed the survival of this culture exposed to UV and gamma radiation. The overexpression of TcPolK did not increase the parasite′s survival to UV. However, parasites overexpressing this polymerase are more resistant to gamma radiation, what suggests that TcPolK is involved in Double-Strand Break Repair - possibly playing a role at the recombination process - as well as it supports the hypothesis of nuclear translocation of TcPolK. At this point, we are cloning the TcPolK gene into the pMAL-C2G vector in order to express and purify the protein in *Escherichia coli*.
Financial Support:Pronex-FAPEMIG

BM051 - Is histone H4 acetylation variable in *Trypanosoma cruzi* forms and cell cycle stages?

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Histone tails provide sites for a variety of post-translational modifications that implicates in the control of gene expression and chromatin assembly. As both histones and control of gene expression in trypanosomes are highly divergent compared to most eukaryotes, post-translational modifications of *Trypanosoma cruzi* histone H4 were investigated. Tandem mass spectrometry analysis demonstrated that the N-terminal alanine is methylated, and lysine residues at positions 4, 10, 14 and 57 are acetylated; lysine at position 18 is mono-methylated, while arginine at position 53 is dimethylated. Western blotting analysis of total histone extracts using antibodies against acetylated histone H4 peptides revealed the presence of slow migrating bands in Triton-acid acetic urea gel electrophoresis, corresponding to acetylated forms of the protein and indicates that lysine 4 is acetylated in the majority of histone H4, while the other N-terminus acetylations are less abundant, independently of the parasite stage. The acetylations of K4, K10 and K14 are found in nuclear foci as seen by immunofluorescence and are not directly related to different rates of transcription found in distinct parasite stages. Studies to determine if the histone H4 acetylation is related to the cell cycle progression are underway. Supported by FAPESP

BM052 - Kinetoplast associated proteins in the parasite protozoa *Trypanosoma cruzi*

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Trypanosomatidae family is constituted by early branching eukaryotes and some of them cause tropical diseases. Such protozoa present unique features, like the kinetoplast, a specialized region of the mitochondrion that contains the mitochondrial DNA (kDNA). The kDNA is composed by circular molecules which are interlocked forming a single network. When isolated, this network has a diameter comparable to that of the whole cell. However, within the protozoa mitochondrion, the kDNA is highly condensed into a disk-shaped structure. The mechanism by which kDNA is compacted is poorly understood and little is known about molecules involved in this process. Several kinetoplast associated proteins (KAPs) have been described in *Trypanosoma cruzi* and *Crithidia fasciculata*. In this latter species, four histone H1-like proteins were identified: KAP1, KAP2, KAP3 and KAP4. These basic proteins present low molecular weight and are able to compact kDNA networks *in vitro*. Based on *T. cruzi* genome annotation data, we have identified contigs which share homology with genes that encode *kap3* and *kap4* in *C. fasciculata*. The *T. cruzi kap3 (Tckap3)* is a single copy gene in the parasite genome, while the *T. cruzi kap4 (Tckap4)* present five copies. In this work, *Tckap3* and *Tckap4* were expressed in *E. coli* in order to produce antisera against recombinant proteins. Western blot analysis revealed that the antiserum TcKAP3 recognize a polypeptide of 25 kDa in epimastigotes whereas the TcKAP4 recognizes a polypeptide of 16 kDa in amastigotes, epimastigotes and trypomastigotes. Interestingly, the TcKAP4 is immunolocalized at two antipodal sites that flank the kinetoplast disc, which correspond to multiprotein kDNA replication complexes. The over expression of KAPs, which will provide insights for possible roles of KAPs in kDNA organization has also been performed, as well as two hybrid assays to study the interactions of KAPs with others proteins. Supported by CNPq and Faperj

BM053 - Trypanosomes of the subgenus *Megatrypanum*: parity between spliced leader and ribosomal sequences supported lineages divergence according to host restriction with artiodactyl species

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Trypanosoma theileri and related species of the subgenus *T. (Megatrypanum)* from cattle and water buffalo isolated from distant Brazilian geographic regions (North, Northeast, Central and Southeast) were used to evaluate genetic relatedness based on polymorphisms of the spliced leader (SL) gene. SL repeats showing high similarity of length and sequences were shared for all *T. theileri* trypanosomes, a clade including only trypanosomes from artiodactyls. In contrast, length and sequence of SL were very variable among *Megatrypanum* species of other mammalian order than Artiodactyla. Dendrogram constructed based on SL transcript (exon and intron) sequences were analyzed aiming to evaluate the degree of genetic relatedness among these organisms. Trypanosomes of European cattle, deer and sheep used for comparison were not clustered with Brazilian isolates. Phylogenies using SSU rRNA gene presented genetic divergences that suggested an ancestral origin for bovids and cervids trypanosomes. Moreover, results supported parity between SL and ribosomal (SSU and ITS1) sequences, with total congruency of lineages. Taken together, *T. theileri* trypanosomes from Brazil and Europe were segregated into six lineages, with cattle isolates distributed into three lineages (B, C, D) separated from lineages of isolates from buffalo (A), deer (E) and sheep (F). Thus, segregation pattern of *T. theileri* trypanosomes supported the hypothesis of lineages divergence according to their artiodactyl hosts. Moreover, parity between lineages defined by SL and ribosomal gene markers suggested clonal structure of parasite populations, with lineages associated to geographical origin of isolates. In conclusion, isolates from different artiodactyl hosts, as well as isolates from cattle living separated by a large geographical distance, were not clustered together.
Financial Support:FAPEMIG / PAPES IV - Fiocruz

BM054 - Analysis of DNA polymorphisms in translesion synthesis DNA polymerases from T. cruzi.

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The population structure of *Trypanosoma cruzi* suggests that this organism presents two main phylogenetics lineages: *T. cruzi* I and II. *T. cruzi* I is more associated to the sylvatic cycle of the parasite and shows lower genetic variability. In the other hand, *T. cruzi* II is closely related to the domestic cycle, and presents higher genetic variability. The existence of a third group has recently been suggested. In order to verify the importance of the events of DNA repair and replication for the generation of genetic variability in this organism, we initiated a project that intends to infer the phylogeny of each one of the translesion synthesis DNA polymerases found in T. cruzi (DNA polymerases β, β-Pak, η and κ). Initially, we used protein sequences in NCBI databases from different organisms to construct phylogenetics trees of DNA polymerase kappa, eta, beta and beta-Pak. In all three trees, the polymerases sequences are closer to lower organisms. We constructed primers to amplify a stretch of 400 pb of each polymerase to compare polymorphic regions in different strains of *T. cruzi* I and II. These primers encompass both variable and conserved regions when the *T. cruzi* sequence is compared with homologous sequences from other organisms. We used genomic DNA from strains P209, Colombiana and Silvio (*T. cruzi* I); Esmeraldo, 577, 578 and JG (T. cruzi II) and SO3 (hybrid). After that, these amplification products were cloned and sequenced. The results presented here indicate that nucleotide sequences of all polymerases allowed the division of *T. cruzi* in three groups, however at the protein level only the DNA polymerase Beta and Beta-PAK showed polymorphisms typical of each *T. cruzi* group.
Financial Support: CNPq, PRONEX-FAPEMIG, Howard Hughes Medical Institute.

BM055 - Parity between phylogenetic relationships among *Trypanosoma rangeli* isolates based on spliced leader and ribosomal sequences and congruency with vector phylogenies supported lineages associated to ecogeographical structure of Rhodniini species

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Trypanosoma rangeli infects humans, domestic and sylvatic mammals from Central to Southern South America sharing with *T. cruzi* mammalian hosts and triatomine vectors in overlapping geographical regions. Previous phylogenetic analysis based on ribosomal sequences disclosed four *T.*

rangeli lineages and suggested association of lineages with geographic origin, transmission cycles and complexes of Rhodnius spp. In this study we have analyzed polymorphism on SL gene of *T. rangeli* by genotyping 61 isolates and inferring phylogenetic analysis among 33 isolates of mammals and triatomines from distinct geographical regions with the following purposes: a) to assess the consistency and the structure of *T. rangeli* lineages through analysis of a large collection of isolates and parity analysis between spliced leader and ribosomal markers; b) to estimate the degree of genetic relatedness among isolates of *T. rangeli* from distinct lineages; c) to evaluate phylogeographical events by confronting vector and *T. rangeli* phylogenies; d) to define SL sequences suitable for simultaneous diagnosis and genotyping of *T. rangeli*. Analysis of genetic relationships among isolates of overall phylogenetic diversity and geographical range of *T. rangeli* based on SL intergenic sequences supported four lineages of isolates in agreement to phylogenetic tree based on SSU rRNA sequences suggesting clonal propagation of *T. rangeli* populations. These lineages show no relationships with the mammalian host-species, whereas appear to be related to triatomine complex of Rhodniini, presenting patterns of segregation compatible with ecogeographical vector structure. A PCR developed based on SL intergenic sequences showed to be suitable for simultaneous identification and genotyping of *T. rangeli* lineages. Supported by CNPq, FAPESP and CAPES

BM056 - Diversity and complexity of deubiquitinating enzymes in *Trypanosoma cruzi*

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Modification of proteins by the covalent attachment of ubiquitin is a key regulatory mechanism of many cellular processes including protein degradation by the 26S proteasome. Deubiquitination, reversal of this modification, must also regulate the fate and function of ubiquitin-conjugated proteins. Deubiquitinating enzymes (DUBs) catalyze the removal of ubiquitin from ubiquitin-conjugated substrate proteins as well as from its precursor proteins. DUBs occupy the largest family of enzymes in the ubiquitin system, implying their diverse function in regulation of ubiquitin-mediated pathways. We used the genome sequences of *T. cruzi* (TcruziDB http://tcruzidb.org/) in an *in silico* analysis to identify novel members of the DUBs family of cysteine proteases, using the BLAST algorithm to screen the genome databases looking for DNA sequences encoding putative orthologues proteins. According to our analysis of the *T. cruzi* genome, we have at least 25 members that contain both the conserved cysteine and histidine boxes essential for catalysis. Most of these proteins have also been annotated in the protease database MEROPS (http://www.merops.ac.uk/) as members of the C19 family of cysteine proteases. As a preliminary step to study the physiological role of DUBs in *T. cruzi*, we examined by semi-quantitative RT-PCR the expression pattern of DUB-10, -12, -14 and -15 in epimastigote from *T. cruzi* I (Colombian) and *T. cruzi* II (CL, CL-Brenner, Berenice-62, Berenice-78 and Y) strains. Our results demonstrate that whereas level of DUB-12 and -14 remained constant in both *T. cruzi* I and II strains, DUB-10 is down-regulated in Colombian strain and transcripts for DUB-15 are up-regulated in Colombian and down-regulated in Berenice-62 and -78 strains. These results provide evidence of the extreme complexity and diversity of the DUBs in *T. cruzi* and open the possibility to explore the relevance of their multiple components in the regulation of ubiquitin pathway in this parasite. Supported by FAPEMIG,CNPq,UFOP.

BM057 - *In vivo* chromosome targeting of TcNUP-1, a nuclear lamina protein of *Trypanosoma cruzi*

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The nuclear lamina is a structure that lines the inner nuclear membrane. In metazoans, lamins are the primary structural components of the nuclear lamina and are involved in several processes. Eukaryotes that lack lamins have distinct proteins with homologous functions. Recently, a coiled-coil protein in *Trypanosoma brucei*, NUP-1, was identified as the major filamentous component of its nuclear lamina. However, its precise role has not been determined. We characterised a homologous protein in *Trypanosoma cruzi*, TcNUP-1, and identified its in vivo DNA binding sites using a chromatin immunoprecipitation assay (ChIP). We found a clear association of the TcNUP-1 with fragments containing (pseudo)genes from the ts (trans-sialidase)-like family, members of the *gp*85 gene family and sequences related to SIRE (short interspersed repetitive element), which are commonly associated with the subtelomeric region of the *T. cruzi* chromosomes. We suggest that TcNUP-1 is a structural protein that plays an essential role in nuclear organisation by anchoring the ends of the *T. cruzi* chromosomes to the nuclear envelope.

BM058 - COHESIN AND CONDENSIN: MOLECULAR CHARACTERIZATION OF THE STRUCTURAL MAINTENANCE OF THE CHROMOSOME COMPLEXES IN *Trypanosoma cruzi*

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Cohesin is a protein complex necessary to maintain sister chromatid cohesion from chromosome duplication until the onset of anaphase. This complex is formed by two SMC proteins (SMC1 and SMC3) and two non-SMC proteins (SCC1and SCC3). On the other hand, the condensin complex is required for the establishment and maintenance of chromosome condensation. The complex of condensin is composed of two SMC proteins (SMC2 and SMC4) and three non-SMC subunits (capD2, CapG and CapH). These proteins are highly conserved from yeast to humans. In *T. cruzi*, the mechanisms involved in the structural maintenance of the chromosome and in the distribution and condensation of chromatin along the parasite life cycle are poorly understood. In order to shed light into these issues we have characterized the *T. cruzi* genes encoding for cohesin and condensin complexes. We have characterized four *smc* genes of *T. cruzi* (*smc1*, *scc1*, *smc4* and *capD2*), which were collectively named *Tcsmc* genes. Recombinant proteins were produced and antisera raised in mice showed that SMC proteins are localized in the nucleus of epimastigotes and metacyclics trypomastigote stage. The nuclear localization was confirmed by transfecting *T. cruzi* with derivatives of pTEX vector containing the *GFP* gene fusioned with *Tcsmc* genes . Two-hybrid assay is in progress in order to detect interactions between TcSMCs and other proteins. In addition, ablation of SMC by interference RNA in *T. brucei* is being performed to investigate the role of the *smc* gene expression to the viability of the parasite.

BM059 - Characterization of protein kinase CK2 activities in the protozoan *Trypanosoma cruzi*

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T. cruzi undergoes complex morphological changes through its life cycle in both insect vector and vertebrate host. This cell differentiation is highly regulated and includes significant changes in signaling pathways. Therefore, studies related to enzymes responsible for the phosphorylation and dephosphorylation of proteins present on the external surface of these parasites are extremely important. Protein kinase CK2 activities have been described on the cell surface and as secreted enzymes of *Leishmania major*, *L. braziliensis*, *L. tropica* and *L. amazonensis*, where these enzymes seem to be involved in cell growth, morphology and infectivity. In the present study, we demonstrate CK2 activities in *T. cruzi* (Colombian strain CTC-IOC 004), present on the surface of this parasite, in the cytoplasmic content and as a secreted form. The addition of dephosphorylated casein promoted an increase of 53% only in the secreted CK2 activity. Protein extract from mouse peritoneal macrophages, as well as inactivated human serum were able to promote an enhancement (67% and 36%, respectively) on the secreted CK2 activity. The secreted form of CK2 showed a specific activity of 1.68 nmoles Pi/mg.min after purification by HPLC. Polyclonal antibodies raised against the mammalian CK2 alfa catalytic subunit were able to recognize, through immunoblotting, a protein from supernatant of *T. cruzi*, with a molecular mass of 55 kDa, which is compatible with the catalytic subunit of the mammalian CK2. We have also incubated the parasites with the midgut contents of *Rhodnius prolixus*, which promoted an increase of 75% on the secreted CK2 activity. CK2 inhibitors (heparin and TBB) abolished the growth of the parasites, while CK2 activators (spermine, spermidine and putrescine) stimulated their growth, which confirmed the great importance CK2 present for the life cycle of these protozoans. Supported by: CNPq, FAPERJ, CNPq/PIBIC-UERJ, CAPES

BM060 - Expression of ubiquitilation enzymes during Trypanosoma cruzi metacyclogenesis

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Metacyclogenesis is driven mainly by changes in gene expression. Ubiquitin-mediated proteolysis should play a role in the remarkable adptative protein turnover which leads to new morphology, physiology and behavioral features. Three enzymes are required for ubiquitilation: an E1 (ubiquitin-activating enzyme), an E2 (ubiquitin-conjugating protein) and an E3 (ubiquitin ligase). The E1 transfers ubiquitin to an E2 in the cell, and this E2 directly transfers ubiquitin to either an E3 or to a lysine residue on the substrate. Accumulating evidence indicates that additional factors modulate the

proteolysis of polyubiquitilated substrates, E4. Polyubiquitilation is antagonized by deubiquitilating enzymes (UBP and UCH) that can proof-read substrate selection, in addition to performing various other functions. We have study the expression of some of these genes during the cellular differentiation of Trypanosoma cruzi using microarray analysis. The results indicated complex gene expression regulation for genes included in this pathway. Some of the ubiquitin-proteasome genes share the same expression pattern during metacyclogenesis. In T. cruzi 15 E1, 26 E2, 10 E3, 4 E4, 4 UBP e 31 UCH are predicted by genome annotation. To gain further insight into the mechanisms involved in T. cruzi ubiquitilation and to investigate its possible role during metacyclogenesis, one E1, three E2 and three E3 were selected for the production of recombinant proteins for antisera production. These are being used to immunolocalize the proteins in the parasite and to investigate their expression profile during the metacyclogenesis. The results are in agreement with those obtained using microarray competitive hybridizations. The expression of the selected E1 was decreased in the metacyciclic trypomastigotes and one of the E3 has an increase in its expression at 12 hours of differentiation from epimastigotes to metacyclic trypomastigotes. We are presently identifying the interactions between these enzymes during T.cruzi differentiation. Financial support from CNPq, PRONEX (Fundação Araucária).

BM061 - FUNCTIONAL CHARACTERIZATION OF RECOMBINANT Trypanosoma cruzi DNA POLYMERASE ETA

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Cells are constantly exposed to endogenous factors, such hydrolysis and oxidation, or exogenous, like UV light and $\gamma rays$, that cause injuries in the DNA. A variety of repair pathways act to keep DNA integrity, but many lesions escape this repair and can block the replication machinery. DNA polymerase η, in contrast the majority of DNA polymerases, is capable of translesion synthesis and can efficiently replicates through DNA single strand with a variety of lesions, such thymine dimmers, apurynic/apyrimidinic (AP) sites and 8-oxoguanine. In *Trypanosoma cruzi*, the generation of genetic variability can be related to recombination, repair and replication of DNA. The fact that DNA polymerase *eta* present low fidelity, compared to DNA polymerases "replicases"acting on DNA without lesions, justify a better characterization of its mode of action. The gene of DNA polymerase η was amplified from genomic DNA of CL Brener strain and cloned in pMAL c-2 expression vector. Preliminary assays showed that the recombinant protein was capable to synthesize from different DNA templates *in vitro*. For in vivo experiments, parasites from CL Brener strain was transfected with an integrative plasmid that promotes overexpression of DNA polymerase η. Both wild and transfected strains showed similar growth curves, contrary of what is seen in humans, where an overexpression of DNA polymerase η is lethal. In addition, we will test the behavior of both strains under treatment with UV light, H_2O_2 and γ radiation, as well the capacity of the recombinant protein in bypassing lesions in DNA templates. Financial Support: CNP_q, PRONEX-FAPEMIG, Howard Huges Medical Institute.

BM062 - *Trypanosoma cruzi* DNA mismatch repair: analyses *in vitro* and *in vivo*.

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It has been recently suggested that the *T. cruzi* population should be divided into three distinct groups, I, II and III, based on gene polymorphisms among others. Interestingly, group II seems to be more heterogeneous than group I according to studies involving *T. cruzi* surface proteins and a multi-copy antigen. However, few studies have investigated mechanisms involved in the generation of such genetic variability in this protozoan. Mismatch repair (MMR) is a factor normally associated with mutation rate increase and therefore higher genetic variability. Recent data from our group suggest strains from groups II and III present lower MMR efficiency when treated with genotoxic agents if compared to *T. cruzi* I strains. In the present work we propose to further characterize *T. cruzi* MMR, focusing on MSH2, a fundamental gene in this pathway. In order to do this, recombinant MSH2 from Colombiana (group I) and CL Brener (hibrid strain, group II/III) was produced and purified. Aminoacid substitutions were detected by DNA sequencing and the ATPase activity of these proteins is currently being investigated under different conditions such as temperature, salt and pH. Preliminary data indicate that Colombiana′s MSH2 presents a higher ATPase activity than CL Brener′s. As another approach to investigate *T. cruzi′s* MMR we have made use of a *Trypanossoma brucei* MSH2 -/- cell as a model for in vivo studies. The MSH2 from both Colombiana and CL Brener was expressed in this context. Preliminary results indicate that we were unable to obtain heterologous complementation of MMR deficiency phenotypes, such as microssatelite instability and MNNG sensitivity, using this approach. However, we have obtained evidences indicating for the first time the involvement of MSH2 from trypanosomatids in the response to oxidative stress.This phenotype was complemented by *T. cruzi* MSH2 and this role of MSH2 is apparently independent

of MMR.
Support:Pronex-FAPEMIG

BM063 - In silico analysis of the putative pre-replication complex component Orc/Cdc6 of *Trypanosoma cruzi*

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The pre-replication complex formation is conserved among eukaryotes. Orc (origin replication complex) a hexameric complex formed by Orc1-6 first binds origin DNA and then recruits Cdc6 and Cdt1. Together, these proteins act to load the Mcm2-7 helicase onto origin. In spite of DNA duplication is well established in eukaryotes, the molecular bases of replication in trypanosomes remain to be determined. *T. cruzi* genome sequencing revealed genes possibly involved in DNA duplication in this parasite. Analyzing *T. cruzi* genome database we found that it does not present genes coding to Orc1-6. Unlike, this parasite contains one open reading frame homologous to Orc1 and Cdc6. This fact suggests that Orc and Cdc6 proteins would be expressed in fusion as one protein. Interestingly, this Orc/Cdc6 protein is also found in Archaea, where its functional role in replication is described. ATP binding and hydrolysis are essential for pre-RC formation. Many proteins that participate in pre-RC assembly are members of the AAA+ family of ATP binding proteins. ATP binding by the Orc1 subunit is required for origin-specific DNA binding. In this Orc1-ATP bound state, Orc can direct an initial round of Mcm2-7 loading, but ATP hydrolysis is required to allow ORC to participate in repeated rounds of Mcm2-7 loading. Phosphorylation of Cdc6 is essential to target this protein to degradation or to nuclear export, blocking a new round of replication in the same cell cycle. In fact, the predict structure of *T. cruzi* Orc1/Cdc6 putative protein showed an ATP/GTP binding domain, and phosphorylation sites, suggesting a functional role for this protein. We are expressing this protein in order to verify in vitro activities such as binding and hydrolysis of nucleotides, self-phosphorylation and DNA-binding. This work points that the replication process in trypanosomes is in part similar to Archaea and different from other eukaryotes. Supported by FAPESP

BM064 - Analysis of mRNA-protein interactions in the $3^{p}rime$ UTR of SSU processome co-regulated genes

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During metacyclogenesis a drastic reduction in gene expression is observed. Ribosomal proteins are down regulated and the nucleolus disassembles accompanied by a 10 fold decay of the RNA polymerase I activity, suggesting a general repression of ribosome synthesis. Quantitative PCR analysis of the expression of SSU processome specific proteins strongly suggests a stage specific co-regulation at the post-transcriptional level of the 18S rRNA processing pathway. In eukaryotes post-transcriptional regulation is achieved through specific interactions of *trans* acting factors with the $3^{p}rime$UTR sequences of the target mRNAs. We analyzed *in silico* the possible existence of common sequence elements in the mRNA $3^{p}rime$UTR of five co-regulated SSU processome genes. The MEME and PRATT algorithms were used to identify shared patterns between the $3^{p}rime$UTRs. Results from both methods were compared and patterns shared by at least four genes were selected for further analysis. EMSA using riboprobes with the selected sequence patterns formed stage specific complexes in epimastigote and metacyclcic trypomastigote protein extracts. In addition the role of the intergenic and $3^{p}rime$UTR sequences of *TcSof1* in stage specific expression was studied by transfection assays using the CAT gene as a reporter. The relevance of the selected patterns in gene expression regulation is currently being assessed by mutational analysis.

BM065 - Microarray identification of mRNAs associated with the Trypanosoma cruzi TcPUF6 protein.

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Kinetoplastid parasites regulate gene expression mainly at the post-transcriptional level. Control of mRNA stability, localization or translation is achieved through interaction of trans acting factors with sequences or structural elements present in the mRNAs 3′UTR. In eukaryotes members of the PUF (Pumilio/FBF) family of RNA-binding proteins regulate the expression of mRNAs by binding to conserved elements in their 3′UTR sequence. PUF proteins reduce target mRNA expression by repressing translation or inducing mRNA deadenylation and subsequent degradation. In T. cruzi the PUF protein family consists of ten proteins.

TcPUF6 is localized in the parasite cytoplasm showing a characteristic pattern of cellular distribution also described in other eukaryotes. The cytoplasmic signal is not uniform but concentrated in discrete foci in all parasite′s life cycle forms. A TcPUF6 tandem-affinity purification (TAP) tagged protein was over expressed in T. cruzi Dm28c parasites. No obvious phenotype was observed in transfected epimastigote forms nor significant differences in the ability to differentiate in vitro into metacyclcic trypomastigotes or amastigotes. Vero cells were infected with the transfected parasites showing a 50% increase of infectivity when compared with controls. Microarray analysis of TcPUF6-tagged over expressing parasites showed altered expression patterns of functionally related genes. Affinity purification of TcPUF6 containing mRNPs and microarray hybridization with the purified mRNAs identified the putative target transcripts of the TcPUF6 protein. Financial support: CNPq, FIOCRUZ, PRONEX/Fundação Araucaria

BM066 - Molecular characterization of *Trypanosoma cruzi* isolated from humans and triatomines from potiguar dry region, RN, Brazil

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Trypanosoma cruzi, the etiologic agent of Chagas disease, is a complex zoonotic whose life cycle involves multiple vectors, reservoir hosts and several transmission cycles in different geographic regions. In this work we evaluated genetic polymorphism of *T. cruzi* populations isolated from human and triatomines from potiguar dry region of the Rio Grande do Norte state. Two *T. cruzi* isolates have been obtained from humans through hemoculture and xenodiagnosis and eleven triatomines have been captured intra and peridomicile and in wild environments. DNA from these isolates have been analyzed by (i) Ribosomal 24Sa RNA gene amplification (Souto& Zingales, 1993); (ii)Multiplex PCR analysis for simultaneous detection and identification of *T. rangeli* and *T. cruzi* (Souto et al., 1999); Typing by microsatellite (Oliveira et al., 1998) and Cytochrome oxidase mitochondrial gene - subunit II RFLP (Machado& Ayala, 2001). Results have shown that from the eleven triatomines isolates, eigth presented *T. cruzi II* profile (Freitas et al., 2006) and three presented *T. cruzi* III profile. *T. cruzi* I isolates have not been identified in the region yet. The isolates RN01TbN and RN03TbA from triatomines, both of *T. cruzi* II strain, presented the same monoclonal and heterozygous profile for different microsatellite loci. Interestingly, RN01TbN was isolated from a *Triatoma brasiliensis* nymph instar captured at peridomiciliary area and RN03TbA was isolated from an adult insect that was found in an ecological reserve 40 km apart. Moreover, two isolates from humans and one from *T. brasiliensis* nymph are from *T. cruzi* II strain and have shown polyclonal profile in the microsatellite analysis, yet the profile seems to be identical among the populations. These results indicate a large distribution of *T. cruzi* populations in the studied area. Supported by CAPES and PRONEX/CNPq/FAPEMIG

BM067 - Effect of calcium and inhibitors on phosphatase activity in two strains of *Trypanosoma cruzi*

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Trypanosoma cruzi displays a remarkably high degree of both structural and functional intraspecific heterogeneity, which could modulate its pathogenicity, survival and adaptability. Phosphorylation and dephosphorylation are fundamental pathways that regulate a wide variety of cellular events. A better understanding of phosphatase activity in *T. cruzi* could be useful to identify potential targets for the development of a more specific therapy. We have previously shown differences in phosphatase activity in two strains of *T. cruzi*; Tulahuen 2 and Y. Tulahuen 2 strain has a higher phosphatase activity than the other strain, which is even more explicitly on pHs 4 and 7. Considering these facts, the purpose of this project was to evaluate phosphatase activity in these strains, using pNPP as a substrate, in the presence of calcium and phosphatase inhibitors. The effects of calcium in a pH range (3-8) showed no significant effect on the Tulahuen 2 strain, but showed a decrease in phosphatase activity in the Y strain in pHs 6, 7 and 8. The results using phosphatase inhibitors in pH 4 showed that p- hydroxymercuriobenzoate (pHMB) (1mM) and EDTA (1mM) had no significant effect on both strains. Sodium Fluoride (10mM) inhibited 91% of phosphatase activity in Tulahuen 2 strain and 76% in the Y strain. O-vanadate (1mM) inhibited 88% of the Tulahuen 2 strain phosphatases and only 50% in the other strain. Tartarate (10mM) inhibited 50% and 63% of the Y and Tulahuen strain, respectively. The differential profile promoted by these inhibitors and calcium suggests the presence of distinct phosphatase activities on both strains. *Supported by*: FAPESP

BM068 - Partial analysis of an ecto-ATPase gene from *Trypanosoma rangeli*

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ATPases are enzymes that hydrolyse nucleoside triphosphates, being related with protection from cytolytic effects of extracellular ATP, regulation of ectokinase substrate concentrations, involvement in signal transduction and cellular adhesion. Previous biochemical studies on *T. rangeli* described an ecto-ATPase activity implicated in the crucial attachment/invasion of the parasite in *Rhodnius prolixus* salivary glands. Also, cDNA fragments with high similarity with *T. cruzi* ATPase genes were detected during the *T. rangeli* Transcriptome Project carried out by our group. Based on these findings, the aim of this work was to study and characterize *T. rangeli* ATPase genes. For that, cDNA clones from *T. rangeli* Choachi and SC-58 strains presenting blastx hits with any ATPase type were selected and had both ends sequenced. After quality analysis by Phred ($\geq$ 20) a total of nine clusters were obtained, all of them showing similarity with distinct *T. cruzi* ATPases. Among such sequences, a 1,028bp cluster represented 37% of a predicted *T. cruzi* plasma membrane proton efflux ATPase gene sequence. These proteins are membrane-bound enzyme complexes/ion transporters that combine ATP synthesis and/or hydrolysis with the transport of protons across a membrane. Inter-specific comparison of this cluster with the TriTryps homologous sequences revealed 90% identity with *T. cruzi*, 81% with *T. brucei* and 82% with *Leishmania major*. The search for conserved domains within this cluster with rpsBLAST showed a protein with 329aa, completely covering the E1-E2 ATPase-associated region domain (aa 102-323) and N-terminus of a cation transporter/domain (aa 40-96). Further studies are in progress to obtain the complete gene sequence for intra/inter-specific comparative studies. Supported by CNPq and UFSC.

BM069 - Hydrogen Peroxide Modulates Ecto-Phosphatase Activity in *Trypanosoma rangeli*

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*Trypanosoma rangeli*is a protozoa parasite of the Trypanosomatidae family that alternates its life cycle between two kinds of hosts: vertebrates and invertebrates. Although in vertebrates this life cycle remains to be determined, in invertebrates hosts, such as the insect of the genus *Rhodnius*, it is well established and begins with the ingestion of the metacyclic trypomastigote forms during food ingestion. Parasites proliferate as short epimastigotes in the digestive tract, pass through intestinal barrier and achieve the hemolymph where they differentiate to long forms. From hemolymph the cells migrate to salivary glands to perform metacyclogenesis. Phosphatases represent the hallmark enzymes able to control the phosphorylation level of proteins. Regulated reversible phosphorylation of proteins and other cellular molecules plays a ubiquitous role in the control of cellular behavior. In this context, it appears that several groups of this enzymatic activity are important targets of reactive oxygen species (ROS) produced physiologically in cell signalling and/or pathologically during oxidative stress. Moreover, activation of certain receptors induces both the stimulation of downstream kinases and the oxidative inactivation of inhibitory phosphatases, and both components are required for efficient signaling. The present study is designed to address the influence of hydrogen peroxide (H_2O_2), a very common ROS molecule produced by aerobic organisms, on ecto-phosphatase activity of *T. rangeli*. Our data showed that H_2O_2, 500μM, inhibited the ecto-phosphatase activity of short and long epimastigotes forms in 94% and 80%, respectively. Studying the effects of increasing concentrations of this reagent, we observed that the inhibition occurred in a dose dependent manner, with IC_{50} values of 0,025μM and 0,020μM for short and long forms respectively. We are now evaluating whether this mechanism is involved in cell proliferation and differentiation. This work was supported by grants of CNPq and Faperj.

BM070 - Proteomic analysis of the GPI-enriched extracts of metacyclic trypomastigotes of *Trypanosoma cruzi*.

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The surface of trypanosomes is coated by glycosylphosphatidylinositol (GPI)-anchored proteins that have been implicated in parasite-host cell interactions. Metacyclic forms of *T. cruzi* express on its surface several GPI-anchored glycoproteins, such as the stage-specific antigens GP82 and GP90, the metalloprotease GP63. Here, we report further characterization of metacyclic GPI-anchored proteins using a combination of Triton X-114 partition and electrospray ionization time-of-flight tandem mass spectrometry (ESI-TOF-MS/MS). Parasite proteins were extracted with Triton X-114, digested with trypsin, fractionated by strong cation-exchange chromatography, and submitted to ESI-TOF-MS/MS. The peak list files were uploaded on the Phenyx database search platform (http://phenyx.vital-

it.ch/pwi) and screened against the NCBInr database. The proteins were additionally analyzed at the Expasy server (http://www.expasy.org) employing the DGPI, TMHMM, and NMT softwares to detect possible GPI-anchor addition sites and predict putative transmembrane domains and N-terminal myristoylation sites, respectively. We identified 64 proteins, which included several members from the group II of the trans-sialidase (TS)/gp85 superfamily (GP90, GP82, TSA-1, GP85, Tc-85/35, ASP-1, and ASP-2), GP63, calpain-like proteins, MASP, and flagellar calcium-binding protein (FCaBP). We obtained high quality peptides encompassing 45% and 32% of the GP90 and GP82 sequences, respectively. The finding of calpain and FCaBP in the detergent-rich extracts is possibly due to the presence of N-myristoylation in these proteins. Taken together these data suggest that the Triton X-114 extraction is a reliable approach to study GPI-anchored and hydrophobic components of the plasma membrane of infective forms of *T. cruzi*. Therefore, it could be employed for the identification of potential molecular targets for rational development of vaccines and drugs against the parasite. Note: Cordero E.M., and Nakayasu E.S., contributed equally to this work. Support: FAPESP, BBRC/Biology/UTEP (NIH grant # 5G12RR008124), CNPq, CAPES, and The Wellcome Trust

BM071 - Analysis of sequences of the Group II Trans-sialidase superfamily in Trypanosoma rangeli.

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Trypanosoma rangeli, an American nonpathogenic parasite for humans is not very well-known in many of its facets, for example, in its biochemical, immunologic and cellular biology aspects. However, this protozoan infects human in many areas of Center and South-America, in those which T. cruzi is endemic, and where share a wide range of vertebrate hosts, and vectorial triatomine. Additionally, both parasites present similar morphologies. The lack of appropriate specific diagnostic procedures and the absence of clinical manifestations have been responsible for the underestimate of the infection for T. rangeli. Recently we have described that T. rangeli possesses genes of the group II of the Trans-sialidase superfamily, collectively known in T. cruzi as gp85. In T. cruzi these genes have been implicated in the invasion and infectivity of the host cells. In contrast, since T. rangeli does not enter into mammalian cells, gp85-like proteins may play a different role. In this work we present the sequence analysis of the corresponding N-terminal sequence of several members of gp85-like genes obtained by PCR and RT-PCR from genomic DNA and total RNA of epimastigotes of a Venezuelan T. rangeli isolated, as well as, two complete copies from de ATG to hydrophobic tail, including the GPI anchor site. We also found the transcription of pseudogene members by cloning one of these by RT-PCR. These data confirm that these sequences are part of a multigene family as in T. cruzi. The evidence of high homology with representatives of the group II of the Trans-sialidasas confirms the presence and expression of these genes in T. rangeli. These results force us to continue studying the role of these proteins in the biology of this parasite. Keywords: Trypanosoma rangeli, Trans-sialidasas, family of genes. This work was supported by FONACIT grant N° S1-2002000542 and CDCHT-UCLA 025-ME-2002.

BM072 - Effect of plant inhibitors on Trypanosoma cruzi infection

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Bauhinia bauhinioides Cruzipain Inhibitor BbCI, *Bauhinia bauhinioides* Kallikrein Inhibitor BbKI and *Enterolobium contortisiliquum* Trypsin Inhibitor EcTI are cysteine and serine peptidase plant inhibitors Kunits type, but BbCI and BbKI lack disulfide bridges. BbCI inhibits cruzain, cruzipain and cathepsin L with Ki_a_p_p 0.5 nM, 1.2M and 0.22 nM, respectively, BbKI is a potent human plasma kallikrein inhibitor with Ki_a_p_p 2.40 nM and EcTI inhibits trypsin, human plasma kallikrein Ki_a_p_p 0.88 nM and 6.15 nM, respectively and metallopeptidase 2 and 9 activation. Our recently studies showed that BbCI mobilizes Ca^{2+} but it does not promote smooth muscle contraction. BbKI, like BbCI, also mobilizes Ca^{2+}, however in contrast to BbCI it promotes smooth muscle contraction and desensitizes BK2 receptor in a similar effect to bradykinin BK. EcTI does not interfere with Ca^{2+} mobilization and muscle contraction. In cell invasion experiments using metacyclic tripomastigote form of *T. cruzi* CL strain on HeLa cell line, both BbCI and EcTI inhibited *T. cruzi* invasion but BbKI did not have a significant effect. On the other hand, using *T. cruzi* Y strain BbKI showed a potent inhibition of cell invasion, in contrast to BbCI which no significant effect was observed. BbCI, BbKI and EcTI may be considered as potential drugs against *T. cruzi* infection.
Supported by FAPESP, CNPq, CAPES e DAAD.

BM073 - Different Effects of Heat-Shock on Ecto-enzyme activities in *Trypanosoma sp.*

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Ecto-nucleotidases are surface enzymes able to hydrolyze extracellular nucleotides. Some functions are suggested for these proteins: cell adhesion, purine acquisition, protection against cytotoxic effects of extracelular ATP and, recently, MDR phenomenon. A well-known family of enzymes, called heat-shock proteins, is stimulated in response to stress such as heat and can present ATPase activity. In this work, we verified whether the ecto-nucleotidase activities from *Trypanosoma sp* are modulated by heat-shock stress. For this purpose, cells were submitted to heat-stress (37 degrees). After pre-incubation at 37 degrees, cells were used to determine Mg^{2+}-dependent ecto-ATPase and ecto-phosphatase activities by measuring ^{32}Pi release from the substrate [γ-^{32}Pi]ATP and p-nitrophenol release from the substrate p-nitrophenylphosphate at wavelength of 405nm, respectively. Our results show that ecto-ATPase from *T. cruzi* was increased in a time-dependent manner. This effect can be observed in the first hour of incubation at 37 degrees, 50%, increasing gradually until three hours of incubation achieving a maximum stimulation of 300%. In addition, two strains of *T. cruzi* showed different stimulation pattern by the heat-stress. At three hours of heat incubation, the percentage of stimuli was, approximately, 6 and 2 fold higher for Y or Dm28c strains, respectively. On the other hand, the ecto-phosphatase activity was not influenced by the heat treatment, suggesting a specific effect of heat-shock stress on ecto-nucleotidases that can act in concert in purine salvage pathway. Studying the effects of heat-stress on the ecto-ATPase activity of another parasite, *T. rangeli*, we showed that the pre-incubation of the cells at 37 degrees for 2 hours inhibited approximately 60% the total activity. Taken together, these data indicates different strategies to cell survival in the mammals organisms.

BM074 - Culture medium phosphate content modulates protein expression and morphology in *Trypanosoma rangeli*

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Trypanosoma rangeli is a South American trypanosoma, considered harmless to humans and animals but able to infect triatomines. After the ingestion of trypomastigote forms during the blood meal, *T. rangeli* differentiates into short epimastigotes, proliferates in digestive tract, crosses the intestinal barrier and achieves haemolymph, where occurs the differentiation to long forms. Ecto-phosphatases are enzymes with catalytic site faced to the external medium and have been detected in different microorganisms. Several reports have shown that surface phosphatase activities can be modulated by the environmental phosphate content, which makes this fact relevant to study these enzymes in response to phosphate starvation. In this study, we show the effect of inorganic phosphate starvation on morphology, cell growth and ecto-phosphatase activities of *T. rangeli*. Cells maintained at Pi-starved medium presented as spherical forms, showing low motility, citosol vacuolization and inefficient cell proliferation. SDS-PAGE analysis of total protein profile revealed that cells maintained at Pi-starved medium expressed low amount of proteins in comparison to the cells kept at Pi-supplemented one. In addition, it was also observed a unique profile of protein mobility for the Pi-starved cells, being concentrated at low molecular weight region. The addition of exogenous phosphate modulated the ecto-phosphatase activity of *T. rangeli*, since cells maintained at the high-phosphate culture medium presented hydrolytic activity of 5,98 nmols p-NP x h^{-1} x 10^{-7} cells while epimastigotes maintained at low-phosphate showed an increase of 5 fold on the phosphatase activity. We also observed the presence of an acid phosphatase activity when the cells were incubated in low-phosphate culture medium while the extracellular pH did not influence the activity of the cells incubated in high-phosphate culture medium. These results support the hypothesis that *T. rangeli* ecto-phosphatase activities can be modulated by the exogenous phosphate content and these activities are a result of different enzymes.

BM075 - The repetitive cytoskeletal protein H49 of Trypanosoma cruzi is a calpain-like protein

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We have previously isolated a T. cruzi recombinant clone, termed H49, that encodes tandemly arranged repeats of 68-amino acids (aa). The repeats are found in an immunodominant antigen (>300 kDa) which is involved in the attachment of the flagellum to the cell body of the parasite. Here we present further characterization of the structure of H49 gene protein. Blast search of T. cruzi databases showed that the 68-aa repeats can be found in the central domain of 8 calpain-like cysteine peptidases. In two of them, the H49 repeats are highly conserved and arranged in tandem arrays, whereas in other calpains the repeats are degenerated and can be separated by short nonrepeat sequences. These proteins show the domains CysPC and calpain_III, characteristics of calcium-dependent cysteine proteinases. The association between H49 and calpain sequences was further con-

firmed by PCR amplification and analysis of YAC clones using primers designed to amplify specific regions of calpain and H49 repeat. We mapped the H49 locus in clone CL Brener using YAC overlapping clones. The association of H49 and calpain in these YACs was demonstrated by PCR and restriction analysis. Consistent with this, chromoblot analysis revealed that H49 and calpain probes hybridized with the same chromosomal bands. Our data indicate that the H49 repeats are part of some calpain genes. Recently, a novel family of cytoskeleton-associated proteins was described in Trypanosoma brucei and Leishmania. These proteins are characterized by their similarity to the catalytic region of calpain, and also by the presence of tandemly arranged amino acid repeats that share 30−40% similarity with H49.These results suggest that the acquisition by trypanosomatids of calpain containing repeats (maybe by gene fusion) was a relatively late event in the evolution of this family of proteins. Support: Beca Presidente de la Republica-Chile, FAPESP, CNPq, CAPES

BM076 - Role of DNA polymerase β in DNA metabolism of *Trypanosoma cruzi*.

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The DNA polymerase β is involved in DNA repair in eukaryotic cells. This enzyme plays a central role in base excision repair, being responsible for filling gaps during repair of lesions caused mainly by oxidative stress and spontaneous DNA base loss. However, its function remains controversial in trypanosomatids. *Trypanosoma brucei* DNA polymerase β has mitochondrial localization and it is associated to kinetoplast DNA replication. In the other hand, *Leishmania infantum* DNA polymerase β is found in the nucleus as determined by immunolocalization. Furthermore, the *L. infantum* protein has desoxiribosil phosphatase (dRPase) activity, which is in agreement with a function in DNA repair. In other to characterize the protein encoded by DNA polymerase β gene from *Trypanosoma cruzi*, we cloned this gene in pMAL plasmid. The protein was expressed in fusion with Maltose Binding Protein (MBP) in *E. coli*. This protein retains the ability to synthesize DNA when incubated with fluorescent primer annealed to a template substrate and desoxiribonucleotides (dNTPs). The samples were analyzed in a DNA sequencer. We tested its capability to incorporate didesoxiadenine triphosphate (ddATP) in DNA under optimal conditions. The TcDNA polymerase β was able to insert ddATP opposite to thymine *in vitro*. However, this protein shows a high level of distinction between ddATP and dATP once this last one is preferentially used to DNA synthesis even at low concentration. Interestingly, this protein is also able to incorporate dGTP opposite to thymine and it may be associated to the low fidelity of this enzyme during DNA synthesis. To better understand the incorporation of ddNTPs *in vivo*, we are producing a parasite strain that over express the DNA polymerase β gene. It may be a useful tool to comprehend the role of this enzyme in DNA metabolism of *T. cruzi*. Financial Support: CNPq, PRONEX-FAPEMIG, Howard Hughes Medical Institute.

BM077 - Studies of recombination in *Trypanosoma cruzi* through overexpression of TcRad51.

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In *Trypanosoma cruzi*, only a few genes involved in DNA metabolism have been described in this species. Recently, our group characterized a gene encoding one of the key proteins involved in homologous recombination in *T. cruzi*, TcRad51. To better understand this process, we overexpressed TcRad51 in the CL Brener strain of *T. cruzi*. Northern blot assays showed an increase in mRNA levels of TcRad51 in the population of transfected cells as well as in cloned transfected cell lines. When we submitted these cells to agents that cause double strand DNA breaks, such as gamma radiation and Zeocin, we observed that cells over-expressing TcRad51 show an increased resistance to both agents. In addition, using pulse field gel electroforesis (PFGE), we observed a difference in the level of fragmentation of genomic DNA after exposition to gamma radiation, with the kinetics of chromosomal reconstitution being faster in transfected cells than in wild type cells. These results indicate TcRad51 has a role in the maintenance of genomic stability in this parasite, and participates in the process of double strand break repair after exposure to genotoxic agents. We also obtained clones derived from transfected cultures over-expressing TcRad51. The clones show similar growth when compared with the transfected population and wild type cells but show variable levels of TcRad51 mRNA expression. Further analysis using these clones will allow us to better understand the role of the TcRad51 gene in the *T. cruzi* DNA metabolism. Financial support: CNPq, FAPEMIG, CAPES e Howard Hughes Medical Institute.

BM078 - Incrimination of Eratyrus cuspidatus (Stal) in the transmission of Chagas′ disease in a geographically restricted area in the North of Colombia.

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Following the report of two cases of acute Chaga′s disease and the appearance of several triatomine species in human dwellings in an area considered non endemic for domestic transmission of T. cruzi; a epidemiological, entomological and T. cruzi molecular epidemiology analysis was performed in order to establish the transmission dynamic of the parasite in the studied area. 2 T. cruzi isolates from human patients, 5 from Eratyrus cuspidatus, 4 from Rhodnius pallescens, 4 from Pastrongylus geniculatus and 7 reference stocks were analyzed by mini-exon gene, random amplified polymorphic DNA (RAPD) and multilocus enzyme electrophoresis (MLEE). All isolates from vectors and human resulted T. cruzi group I by mini-exon, RAPD and MLEE. While mini-exon and MLEE did not showed any differences between the studied isolates, RAPD analysis identified a common T. cruzi genotype for the E. cuspidatus isolates and human isolates and distinguished different strains from R. pallescens and P. geniculatus isolates. The presence of the same T. cruzi genotype in isolates from patients and E. cuspidatus suggests that this specie can be responsible for the transmission of Chagas disease in the study area. RAPD analysis showed better resolution and discrimination of T. cruzi strains than mini-exon and MLEE and can be considered a useful tool for molecular epidemiology studies. Incrimination of Sylvatic triatomine species in the transmission of Chagas′ disease indicates that more knowledge about the ecology of these vectors is necessary to improve control strategies. Supported by Colciencias 11150414387 and Proyecto de sostenibilidad Chagas, CODI, U de A, 2006.

BM079 - Biological and genetic characterization of two Colombian clones of the groups Trypanosoma cruzi I and II

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Trypanosoma cruzi in Colombia presents high genetic variability and it has been suggested that both *T. cruzi* I and II are circulating in the country. The objective of the present study was to characterize biological and genetically two Colombian clones of the groups *T. cruzi* I and II in the murine model. For this, stocks Cas15 and AF1 belonging to the groups *T. cruzi* I and II, respectively, were cloned in semisolid medium. A clone of each strain and a mix of both were used to infect mice, which were subsequently sacrificed at different post-infection times. In order to analyze the parasite presence in blood and different organs, microhematocrit and polymerase chain reaction (PCR) were used with satellite DNA (sat-DNA) and the intergenic region of mini-exon gene markers. The *T. cruzi* I clone was more infectious, with a preferential tropism observed in heart, rectum and skeletal muscle, whereas clone *T. cruzi* II exhibited a preferential tropism for spleen and liver. During the infection with the clone mixture a preference of the *T. cruzi* I clone for blood as well as for organs was observed. The results corroborate that the genetic differences between the *T. cruzi* groups could determine the tissue tropism, and in this way, play an essential role in understanding the clinical manifestations the Chagas disease in Colombia. Financiado por: Proyecto de sostenibilidad Chagas, CODI, U de A, 2005-2006.

BM080 - Exploring the transcriptome of *Trypanosoma vivax* by ESTs and comparative analysis

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Trypanosoma Duttonella vivax (Kinetoplastida: Trypanosomatidae) is a pathogenic protozoan parasite and the causative agent of bovine trypanosomosis that affects the agriculture development in Africa and Latin America. Because in vivo and in vitro cultivation is a limiting factor to obtain parasites and DNA, there has been few studies about its biology and molecular characterization. Randomly DNA sequencing using Genome Sequence Survey (GSS) and Expressed Sequence Tags (EST) approaches are ideal for gene discovery and genome exploration. In the present study 1.328 ESTs from *T. vivax* VTA01 strain Pantanal, Brazil were generated, sequenced and analyzed. After analysis using the GARSA (Genomic Analysis Resources for Sequence Annotation) system, the sequences were clustered with CAP3 resulting in 463 non-redundant ESTs (EST-nr), showing an average length of 485bp. The mean G+C content of EST-nr was 43%. BLAST similarity searches were done with 10 databases (Uniref90, RefSeq, etc) and Conserved Domains searched by RPSBlast and CDD database. From EST-nr, 16.6% (77/463) had at least one BLAST hit, the remaining 386 EST-nr (83.4%) are potential species-specific or orphan genes that will need further analyses to obtain some annotation. Among significant BLAST hits are: ribosomal protein L21, superoxide dimutase, histone H4, protein kinase C, proline-rich protein, 14-3-3 protein-like-protein that is involved in the growth factor signaling and interacts with kinases. Significant hits with the CDD database are (a) DrfFH1 (formin homology region 1) is found in some of the Diaphanous related formins (Drfs), it consists of low complexity repeats of around 12 residues; (b) extensin-like domain, (c) and p23, homolog to co-chaperone p23. These

data will be useful for the development of new species-specific assays for diagnosis and typing systems. Supported by FAPERJ-Fiocruz, IOC-Fiocruz, PAPES IV, IFS and IAEA.

BM081 - Cruzipain-like of *Trypanosoma cruzi* sylvatic isolates from State of Rio de Janeiro.

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*Trypanosoma cruzi*Chagas 1909, ethiologic agent of Chagas′ disease, is represented by a set of wild parasites which circulate between men, vectors, reservoirs and domestic animals. Cysteine-proteinases from parasitic protozoa have been recently characterized as factors of virulence and pathogenicity in several human and veterinary diseases. The lysosomal cysteine protease designated as cruzipain or cruzain, is the archetype of a multigenic family. The aim of this study is to characterize the proteases profile of four wild samples of *T. cruzi* isolates from Triunfo, district of Santa Maria Madalena, State of Rio de Janeiro. All the samples have shown a similar protein profile with 35 polypeptides presenting apparent molecular weights from 118 to 20 kDa, excepting SMM10 sample, which has shown a differenciated total proteins profile. Proteolitic activity was performed by zymograms analysis with different samples using SDS-polyacrylamide gel electrophoresis containing gelatin as substrate. Our results demonstrated a major band 45kDa in all the samples, which seems to be the main cysteine protease of *T. cruz*i, the cruzipain. In order to confirm this data, a western blotting was done using anti-cruzipain antibody. These findings indicate one band between 40 and 50 kDa in all the different isolates: SMM10-xenodiagnostic; SMM88- hemoculture; SMM53 and SMM98 - wild triatomines. The implications of these results will be relation with the level cruzipain expression and parasite infectivity compared with *T. cruzi* strains already studied.

BM082 - *Trypanosoma cruzi* Z3A and Z3B are associated with terrestrial and arboreal ecotopes, respectively, according to phylogenetic relationships based on SSUrRNA

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Trypanosoma cruzi is highly polymorphic as demonstrated by several molecular markers used to genotype isolates in three major groups: TCI, TCII and Z3. Z3 is considered a sylvatic group of isolates segregated into two lineages, Z3A and Z3B, apparently sharing a hybrid (TC1-TC2) origin. Aiming to better understand the evolutionary relationships within Z3 isolates, we selected 28 isolates among more than 290 new *T. cruzi* isolates from several mammalian species using classical methods based on ribosomal and mini-exon gene. The 28 selected isolates were from human and non-human primates of several species, coati, triatomine bugs, armadillos and marsupial. New isolates are most from Brazilian Amazon region, excepting those from armadillos that are from Northeastern region. Phylogenetic relationships among *T. cruzi* isolates from all lineages inferred using the polymorphic region of V7-V8SSUrRNA always segregated Z3 in two subgroups: Z3A, consisting of new isolates from armadillos and marsupial of *Monodelphis* plus reference-strains from humans and *P. geniculatus*; Z3B, a high homogeneous cluster constituted by isolates from non-human primates, coati, *Rhodnius robustus* II and *R. brethesi* that were tightly clustered together and with reference-strains JJ and CANIII. Analysis by different methods always positioned Z3A, a hybrid lineage, closer to TC2 and $\frac{1}{2}$ lineages than to Z3B isolates. Together, phylogenetic relationships, host-origin and ecological data corroborated association of Z3A, in Amazonia and Northeastern Brazil, with terrestrial ecotopes of armadillos, *Monodelphis* and *Panstrongylus*, as previously defined for Z3. In contrast, Z3B was associated with an arboreal ecotope of the Amazonian Region, with non-human primates, coati and *Rhodnius* spp. Genotyping of several isolates of *Didelphis* from the same geographic regions always disclosed TC1 isolates. Therefore, results corroborated a high complexity of *T. cruzi* sylvatic cycle, with determined vertebrate and invertebrate hosts, in their respective ecotopes, involved in transmission of different *T. cruzi* lineages. Supported: CNPq and FAPESP

BM083 - Proteasome inhibition leads to growth changes in *Trypanosoma cruzi* strains.

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26S Proteasome is a multicatalytic complex that controls the protein degradation, from archaebacteria to mammalian, being formed by a nucleus, the 20S proteasome composed of 4 rings super ranks, with 7 subunits each one (α_{1-7} β_{1-7} β_{1-7} α_{1-7}). Subunits β1, β2 and β5 possess caspase, tripsin and quimiotripsin like activity, respectively. 20S proteasome is activated by a protein complex, the PA700 or 19S, forming the 26S proteasome. Results in our laboratory showed differences between the enzymatic activities of proteasome obtained from Y, Berenice-62 and Berenice-78 *Trypanosoma cruzi* strains, suggesting that the protease can be differently

regulated in these strains. Thus, the objective of this work was to evaluate the role of the proteasome in the parasite growth. For *in vitro* analysis, $3x10^6$ parasites each were incubated with different concentrations of the classic inhibitor of 26S proteasome, PSI. The number of parasites was quantified in Newbauer chamber on ten days for Y and Berenice-62 and 12 days for Berenice-78 after culture starting. Experiments performed *in vitro* showed 90% of parasite growth inhibition after 1μM of PSI addition in the LIT culture medium. Similar results were observed in experiments *in vivo* performed with the same strains and 70% of parasitemia reduction was detected in Swiss mice infected with blood trypomastigotes previously incubated with PSI in relation to the control group. The results demonstrated that the inhibition of the *T. cruzi* proteasome by PSI induced an important reduction in *T. cruzi* growth, both *in vitro* and *in vivo*. Supported by FAPEMIG, CAPES and CNPq.

BM084 - Potent synergistic effect of two novels Hydrazones coordinated to Pt on *Leishmania (L) mexicana*

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Leishmaniasis constitutes a severe world health public problem classified as not controlled emergent disease (category I, WHO, 2005). The use of transition metal complexes as potent chemotherapeutic agents against leishmaniasis is a new alternative since the drugs available are not totally safe and active. Moreover, the appearance of drug-resistant strain of *Leishmania spp.* justifies the screening of new compounds. In this sense 20-hydrazone-imidazol-2-yl-5α-pregnan-3β-ol (Hydra1) and 22-hydrazone-imidazol-2-yl-5-colen-3β-ol (Hydra3) were coordinated with Pt to obtain two novel organometallic complexes: Trans-[dichlorodi(20-hydrazone-imidazol-2-yl-5α-pregnan-3β-ol)platinum(II)] or (Trans-[$PtCl_2$(Hydra1)$_2$]) and Trans-[dichlorodi(22-hydrazone-imidazol-2-yl-5-colen-3β-ol)platinum(II)] or (Trans-[$PtCl_2$(Hydra3)$_2$]). Drugs dissolved in DMSO at 10 μM final concentration were added to cultures of *Leishmania (L) mexicana* (NR strain) promastigotes maintained at 26^{o}C in Schneider medium (Sigma) supplemented with 5% of fetal calf serum (Gibco). Cell density was measured in Neubauer chamber including viability by trypan blue exclusion. After 1h of incubation Trans-[$PtCl_2$(Hydra3)$_2$] induced 100 % of cell lysis (LD_{100}) whilst Hydra3 required 4h to induced similar effect. Trans-[$PtCl_2$(Hydra1)$_2$] after 4h of treatment were totally leishmanicidal (LD_{100}). In contrast Hydra1 produced only a growth inhibition of 35% (IC_{35}) under the same conditions and LD_{100} only was reached after 48h. By direct observations parasites showed mobility loss, swelling and cytoplasm vacuolization previous to cell lysis. These findings pointed out a potent synergistic effect derived by enhancement of the activity of the parental organic drugs due to binding to the transition metal. Although the design of metal complexes with good therapeutic index at the present is in development, the metal-drug synergism is a powerful tool to be used in efforts to find the definitive cure for leishmaniasis.

BM085 - Dipeptidyl Peptidase and X-Prolyl Dipeptidyl Aminopeptidase of *Trypanosoma cruzi*

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The serine peptidases with α/β hidrolase fold are grouped in the SC clan, which includes enzymes such as prolyl oligopeptidases (POP, S9A family), dipeptidyl peptidases (DPP, S9B family) and X-prolyl dipeptidyl aminopeptidases (X-PDAP, S15 family). These specialized peptidases are capable of cleavage of proline bond in polypeptide chain. A member of the SC clan, POP of *Trypanosoma cruzi* (POPTc80), is involved in the process of the parasite entry into mammalian cells. In this study, we present the identification of DPP (DPPTc) and X-PDAP (X-PDAPTc) of *T. cruzi*, other potential drug targets for Chagas′ disease chemotherapy. Their genes were isolated by PCR using primers according to sequences of the parasite genome. DPPTc displays similarity mainly to human DPP8 and DPP9. In contrast, it does not share any similarity to X-PDAPTc, although both enzymes hydrolyze similar substrates. It is worth to notice that the distribution of X-PDAP is restricting to bacteria, protozoa and archea. This feature turns X-PDAP a very interesting target in development of selective drugs to Chagas′ disease. Supported by Capes and CNPq.

BM086 - TcRRMs are differentially expressed in Trypanosoma cruzi life cycle

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Trypanosomes are a group of eukaryotic organisms with many unusual characteristics in their molecular biology; so the identification and characterization of RNA binding proteins in T. cruzi is particularly relevant as they play key roles in the regulatory mechanisms of gene expression. In this work, we have identified coding sequences for the proteins, named TcRRM1 and TcRRM2, in the EST database generated by the T. cruzi Genomic Initiative. TcRRM1 and TcRRM2 contain two RNA binding domains (RRM) and are very similar to two T. brucei RNA binding proteins previ-

ously reported, Tbp34 and Tbp37. Western blot analysis using heterologous antibodies raised against Tbp34 and Tbp37 revealed that the RBP proteins are present not only in T. cruzi but also in Leishmania braziliensis and Crithidia fasciculata. The T. cruzi RRM genes are organized in a tandem of at least 8 copies, alternating with copies of Tcp28, a gene of unknown function. However TcRRM transcripts accumulation is higher in the spheromastigote stage, while Tcp28 transcripts accumulate more in the trypomastigote stage suggesting developmental regulation. Western blot analysis revealed that only TcRRM1 is present in the amastigote stage, while in the epimastigote stage both proteins are present and in the trypomastigote stage, none of them are. Both TcRRM and Tcp28 genes have been cloned in pGEX vectors and the recombinant proteins have been purified. Preliminary imunofluorescence assays localize the RBP genes in the cytoplasm of the parasite cells suggesting a function distinct from the one presented by the T.brucei genes which are thought to participate in the ribosome biogenesis. Electrophoretic mobility shift assays are being performed in order to verify the specificity of the ligation to ribonucleotides.

BM087 - Liposome System Constituted by DPPC:DPPS:cholesterol to Carry Multiple Antigenic Proteins from *Trypanosoma cruzi*

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The reconstitution of membrane proteins into liposomes is an useful tool to enhance the antigenic properties of these molecules. In the present study, we generated proteoliposomes carrying *T. cruzi* proteins of trypomastigotes and amastigotes for use as immunogens in mice. Parasite forms were firstly sonicated and mixed with detergent at a ratio of 0.5 mg/ml of protein to 0.5% (w/v) SDS, achieving a protein recovery of 94%. To prepare proteoliposomes, a protocol in which DPPC:DPPS:cholesterol (5:1:4 w/w) were incubated 0.5 mg of solubilized parasite proteins. The SDS-PAGE analysis of these proteoliposomes revealed proteins with a distribution different from that observed with the SDS-solubilized proteins extract of T. cruzi, indicating a certain degree of selective incorporation. BALB/c mice were immunized with 20 μg of the proteoliposome. A week later, intraperitoneal macrophages were recoverd and infected with trypomastigotes of the Y strain, in vitro. After 24 h, the percentage of parasites in infected immunized macrophages were similar to the control. However, after 72 h, the number of intracellular parasites in immunized macrophages decreased significantly as compared to controls. These results indicate that exposure of mice to trypomastigotes plus amastigotes incorporated into proteoliposomes generate immune cells that are able to kill intracellular *T. cruzi* more effectively than unchallenged macrophages. Supported by: CNPq, CAPES and FAPESP.

BM088 - *Trypanosoma cruzi*: evaluation of cubebin derivatives in their mRNA processing by trans-splicing reaction

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The use of the permeable cells system for study trans-splicing reaction in trypanosomes, as described for Ullu & Tschudi (1990) and standardized for *T. cruzi* by Ambrósio et al. (2004), it helps in the inquiry of the synthesis, processing and regulation of mRNAs in trypanosomes. In this work, the activities of two substances derived from the cubebin were evaluated, hinokynin (HQ) and 6,6'-dinitrohinokynin (DNH), using Y strain of T. cruzi epimastigote permeable cells. Initially, to screen the activity of these derivatives, the non-radioactive trans-splicing reaction was done and submitted to the polyacrilamide gels and silver stain. To confirm the results and evaluate the activity of substances in the RNA maturation process, an anti-sense probe using the SL RNA was transcribed and used with permeable cells radioactive reaction to make the RNase protection. The evaluation by densitometry showed that the both substances had modified the total amount of parasite RNA and the results of RNase protection reaction showed that RNA bands just synthesized were more evident after the addition of substances, allowing to conclude that HQ and DNH would be modifying the RNA metabolism in these parasites, promoting an increase of the transcription and, consequently, the mRNA synthesis. These substances also seem to affect the posterior stage of mRNAs maturation, the trans-splicing reaction itself. Because of the analysis of the trans-splicing products occurred at 20 minutes after drug incubation with the cells, these results suggest that their trypanocidal activity would promote accumulation in the cell of any kind of mRNA, in the attempt to supply some vital enzymatic blockage in the metabolism of the parasite. Further studies are in progress in our laboratory to better understand these findings. Project Financial Support: PADC-FCF-UNESP ESO is a fellowship from PIBIC/CNPq

BM089 - FURTHER CHARACTERIZATION OF FOUR HOMOLOGUES TO THE TRANSLATION INITIATION FACTOR eIF4E IN Trypanosoma brucei

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The Trypanosomatidae family is known to possess unique mechanisms for regulation of its gene expression. These are performed mainly at the posttranscriptional level, possibly through the control of mRNA stability and translation. Despite its importance, the study of translation and its control in these protozoans has been so far neglected by researchers and little is know about how it occurs or the implications for possible targets for chemotherapy. In other eukaryotes, translation is mainly controlled through the ribosome recruitment to the mRNAs, a process helped by the translation initiation factors (eIFs). In this context, binding of the ribosome is mediated by the eIF4F complex, which is composed by the subunits eIF4E, the cap binding protein; eIF4A, a RNA helicase; and eIF4G, a scaffolding protein. In order to study translation initiation in these parasites, and its role in regulating gene expression, we proposed to characterize four eIF4E homologues previously identified in *Trypanosoma brucei*, called TbEIF4E1-4, by assays of cytolocalization and RNAi. The four genes were amplified and cloned in the p2215 and p2T7-177 transfection vectors, respectively for the expression of fusions with the enhanced yellow fluorescent protein (EYFP) and for RNAi. These constructs were transfected in procyclic cells, through electroporation, and stable transfectants obtained. Sub-cellular localization assays with EYFP showed that TbEIF4E1, E3 and E4 are located in the cytoplasm, like bona fide class eIF4Es, but TbEIF4E2 showed both nuclear and citoplasmatic localization. This may indicate functions beyond translation, perhaps a role in splicing and/or mRNA decay. The RNAi assays indicated that only the TbEIF4E3 is essential for parasite viability, although some minor growth effects were detected in the parasites after TbEIF4E2 and E4 RNAi. Our results are consistent with different functions for these homologues in T. brucei and indicate that TbEIF4E3, at least, may be a true translation initiation factor

BM090 - Characterization of =*Trypanosoma vivax* minicircles

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The minicircles are the smaller and most abundant kinetoplast DNA (kDNA) component (Simpson, 1986). They present one to four conserved regions (CR) and nonconserved regions, depending on the species (Simpson, 1997). Each CR contains three conserved sequence blocks (CSB) identified in different trypanosomatid species: CSB-1 (ACGGGCGTTC), CSB-2 (ATACGTAG) and CSB-3 (GGGGTTGGTGTA). The minicircles of *Trypanosoma vivax* were previously investigated by Borst et al., (1985), however their sequences were not determined and are not available in the databases. Therefore, pair of primers of CSB3 region was utilized in PCR to obtain minicircles sequences in different *T. vivax* strains. We obtained 36 minicircles, and found only one conserved region (CR) of about 110 bp in each of them, *T. equiperdum* (Barrois et al., 1981) and *T. brucei* (Jasmer & Stuart, 1986) also presented 1 CR. The estimated size of the minicircles was 480 bp which would be in accordance with the 465 bp reported by Borst et al. (1985), through electron microscopy. CSB-1 and CSB-3 are highly conserved in all species studied to date, whereas CSB-2 is less universal (Ray, 1989) *T. vivax* minicircle CSB-2 was less conserved among other trypanosomatids since from eight nucleotides that compose this sequence, only three nucleotides are identical. We observed that the distance from CSB-1 to CSB-2 was 21 nucleotides, the same distance observed between these CSBs in the T. equiperdum minicircles (Barrois et al., 1981). While the distance of CSB-2 sequence to CSB-3 was 56 nucleotides, however, according to the literature the distance between this CSBs range from 38 nucleotides in *T. equiperdum* to 49 nucleotides in T. cruzi. The studies with repeats in tandem will allow to investigate the filogenetic relation between different *T. vivax* strains, we observed inverted repeats *palindromes* and tandem repeats in almost all *T. vivax* of minicircles analysed.

BM091 - MAPPING OF THE INTERGENIC REGION OF TcP28, THE INTERCALATED GENE OF CODING GENE OF RNA BINDING PROTEINS (TcRRM) IN TRYPANOSOMA CRUZI.

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The function of Tcp28 is unknown in *Trypanosoma cruzi* genome located at the TcRRM locus as a multicopy gene. Tcp28 is intercalated between genes of TcRRM1 and TcRRM2 (Gomes, *et al.* Biochem. Bio. Res. Comm. 2004, 322 985-992). The Tcp28 transcripts analyzed by Northern Blot assays, accumulate in trypomastigotes when compared with other forms. These results suggest that Tcp28 may be regulated through different cell forms and may play a specific function in these cells. The control of gene expression in trypanosomatids usually is exerted posttranscriptionally. The objective of this work is to analyze the intergenic re-

gions of the gene Tcp28 of *Trypanosoma cruzi* in order to find sequences that regulate the Tcp28 gene expression in different cell forms of *T. cruzi*. Total RNA from epimastigote forms were extracted by the GuSCN/GUHCI, to map the polyadenylation and trans-splicing sites in 3′UTR and 5′UTR region. Specific initiators were used to produce the cDNA using the 3′RACE kit (*Gibco BRL*). The 3′ UTR amplification was done with initiators homologue to the polyA tail and to the carboxyl-terminal region of Tcp28. The product of this amplification was cloned into plasmid, transformed into bacteria and sequenced. The same procedure was realized for the 5′ UTR region, however for the PCR step we have used initiators homologue to the miniexon and to the N-terminal region of Tcp28. The sequences obtained for the 3′UTR region showed only one site of polyadenylation and one type of sequence of 3′UTR region. For the 5′UTR region, we have obtained two fragments of 500bp and 450bp from PCR reaction. This suggests that the 5′UTR region of Tcp28 have two trans-splice sites. We are cloning these PCR products for mapping the site of trans-splicing.

BM092 - GENE CHARACTERIZATION OF MOLECULAR CHAPERONE HSP100 OF TRYPANOSOMA CRUZI

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The heat-shock protein HSP100 is a molecular chaperone that is mainly responsible for disassembling higher order protein structures and aggregates. It comprises a family of HSPs with diverse functions such as thermotolerance, proteolysis of specific substrates and regulation of transcription. HSP100 has been shown to be present in the *Trypanosoma cruzi* parasite, the causal agent of Chagas′ disease. HSPs are inducible proteins and are, therefore, good models for studying kinetoplastid gene regulation. Our objective is to study the HSP100 gene structure, expression and regulation in *T. cruzi*. The predicted amino acid sequence of HSP100 of *Trypanosoma brucei* was used to search for orthologous sequences in the draft genome of *T. cruzi* clone *CL Brener* (available at *http://TcruziDB.org*) with the computational search tools *BLASTX* and *TBLASTX*. A HSP100 coding region of 1810 pb (*GenBank ref. XM_798230*) was identified, and an internal segment of 980 pb was selected for PCR-based amplification. The amplified fragment was cloned and is being used as a probe in Southern blots containing restriction enzyme-digested genomic DNA. We are also performing northern blot analysis to determine HSP100 mRNA levels in normal and elevated temperatures, as well as the mRNA half-life. The complete coding region will be obtained, cloned into expression vectors and the recombinant protein used to generate HSP100-specific antibodies in order to characterize the subcellular localization of the protein. *Supported by CNPq and FAPERJ.*

BM093 - KNOCK DOWN OF TWO eIF4G HOMOLOGUES IDENTIFIED IN TRYPANOSOMATIDS

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In higher eukaryotes, protein synthesis starts with the binding of the translation initiation complex eIF4F to the mRNA. This complex (formed by the eIF4A, eIF4E and eIF4G subunits) allows the recognition of the mRNA by the 40S ribosomal subunit and the initiation of translation. The eIF4G factor is a large scaffolding protein that is responsible for the correct assembly of the eIF4F complex. So far little is known about translation initiation in trypanosomatids. To investigate this process, we have identified multiple homologues for each eIF4F subunit within the *Leishmania major* genome. Five homologues for the eIF4G factor were identified and their sequences showed different degrees of similarity to vertebrate factors. Nevertheless they were all conserved in *Trypanosoma brucei* and *T. cruzi*. So far, these homologues, called *Lm*EIF4G1-5, have been studied by various approaches, including expression analyses during the *L. major* life cycle and pull down assays. The five proteins seem to vary in different aspects, but at least two of them (*Lm*EIF4G3-4) showed consistent results and possibly fulfill an important role in translation initiation. In order to supplement in vitro data, we used *T. brucei* orthologues (*Tb*EIF4G3-4) to perform in vivo analysis. Both genes were amplified and cloned into different plasmids vectors, allowing protein expression in *Escherichia coli*, antibody production and interference of RNA (RNAi). Strikingly, cells expressing TbEIF4G3-dsRNAs (double stranded RNAs) began to die before 24h of RNAi induction. In contrast, cells expressing TbEIF4G4-dsRNAs just showed growth retardation after 72h of induction and cell death after the 5th day of maintenance under RNAi conditions. They also exhibited reduction of mobility and change in morphology. The *Tb*EIF4G3-4 antibodies are being used in western blots to verify protein depletion after RNAi. We are currently performing cellular localization of these proteins to obtain more data to confirm their roles in trypanosomatids metabolism.

BM094 - Cloning and characterization of asparagine synthetase and L-asparaginase of Trypanosoma cruzi

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Trypanosoma cruzi has a metabolism largely based on the consumption of amino acids, which can constitute carbon and energy sources. Some amino acids also participate in

the differentiation process of the non-infective to the infective form. Asparagine is synthesized from aspartate in a reaction catalyzed by asparagine synthetase. On the other hand L-asparaginase hydrolyse L-asparagine to yield aspartate. Both enzymes, has been characterized in bacteria, fungi, yeasts, plants and mammals, but has not yet been study in Trypanosomatids. The *Escherichia coli* L-asparaginase has been used as an antineoplasic agent. The mechanism of action of asparaginase in vivo has usually been related to blocking protein synthesis caused by asparagine starvation in cell with a low intracellular activity of asparagine synthetase. In the present work we aim to characterize some aspects of the genes involved on asparagine metabolism in *T. cruzi*, focusing on L-asparaginase and asparagine synthetase. Bioinformatics analyses shows that there are five ORFs coding for putative asparaginases, and two ORFs coding for putative asparagine synthetases in the *T. cruzi* genome. Comparative studies on the sequences of this genes shows that there are 49% identity between the *T. cruzi* and *Leishmania major* asparaginases, while no gene coding for that enzyme were found in *T. brucei*. The *T. cruzi* asparagine synthetase has 64% identity with *T. brucei* and 61% with *L. major*. We designed primers for a cytoplasmic L-asparaginase like protein and for a putative asparagine synthetase from *T. cruzi* .We cloned it in PTEX and tranfected into the epimastigotes form and now we are characterizing the phenotype of the overexpressed parasites. We are interested in analyze if there is a up-regulation of asparagine synthetase upon the overexpression of asparaginase in *T. cruzi* to understand the role of these enzymes on parasite metabolism. Supported by CAPES, FIOCRUZ and CNPq.

BM095 - Biological characterization of Trypanosoma cruzi class I and II strains.

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Trypanosoma cruzi presents a heteroxenous life cycle including invertebrate and vertebrate hosts. The taxon is known as a heterogeneous species consisting of several sub-populations of the parasite. *T. cruzi* I is associated to sylvatic transmission cycle infecting marsupial as well as placental mammals, while *T. cruzi* II parasites are mostly associated to the domestic transmission cycle, infecting mainly placental mammals. Our group is interested in *T. cruzi* species diversity. Assays comparing class I and II resistance to complement system mediated-lysis have been performed (Cestari et al., Chagas 2005). In order to study the role of the parasite diversity in the pathogenesis of Chagas disease, a comprehensive characterization of parasite populations found in nature is crucial. At the moment we have 17 *T. cruzi* field-isolated available strains, that have already been typed as class I or II according to molecular markers. At the present work our goal is to compare biological characteristics among these strains, such as: (i) growth curve and metacyclogenesis, infectivity to Vero cells and mice; (ii) activation and resistance to normal human serum complement mediated lysis; and (iii) proteases expression profile, including cruzipain, a known parasite virulence factor. The different strains presented heterogeneous patterns among the biological aspects described above. We expect to have an overall view of biological features linked to differences in parasitism comparing T. cruzi field-isolated strains in order to identify new pathogenicity markers that contribute to the definition of *T. cruzi* I and II phylogenetic groups. This may contribute to *T. cruzi*-hosts relationship understanding and to the development of novel chemotherapic alternatives against Chagas disease. Supported by: CNPq, FIOCRUZ& FAPERJ.

BM096 - Proteomic analysis of *Trypanosoma cruzi* epimastigotes cultivated in different carbon sources

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Chagas disease (American trypanosomiasis) continues to be an important public health problem in Central and South America. The etiological agent of the disease is the hemoflagellate protozoan *Trypanosoma cruzi*, and the occurrence of the protozoan and the respective insect vectors encloses an area from the south of the United States until the north of Argentina. The objective of this work is to analyze the proteomic expression of *T. cruzi* when using different carbon sources and to characterize keys enzymes in the metabolism of the parasite. Axenic cultures of epimastigote forms of *T. cruzi* (strain CL Brener) were used for the assays of bidimensional electrophoresis. The epimastigotes were cultivated in defined medium (AR103) supplemented with either L-proline, D-proline or glucose. Cells were lysed by freezing and thawing and proteins were precipitated with 10 % trichloroacetic acid /90% acetone. The samples were submitted to bidimensional electrophoresis, which separates proteins by isoelectric focussing (different pH ranges were tested) and molecular weight. Gels were stained by Coomassie colloidal and the images were analyzed using the PDQuest/Bio-Rad program. Our preliminary results show few qualitative and quantitative differences at the protein expression. Some spots were sent for mass spectrometry analysis and we identified several important proteins in the metabolism of the parasite, not yet identified in another studies. This work is of considerable relevance for the study of the parasite metabolism, and subsequently for the development of new strategies of immune therapy and drug design against this pathogen. Supported by: CNPq, FAPERJ, FIOCRUZ-PDTIS

BM097 - Expression of Universal Minicircle Sequence Binding Protein (TcUMSBP) during the growth of epimastigotes forms of *Trypanosoma cruzi* in axenic cultures in the presence and absence of acriflavine

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Trypanosoma cruzi, the causative agent of Chagas′ disease, belongs to the Order Kinetoplastida and it is characterized by presenting kinetoplast, a structure where the mitochondrial DNA is allocated. This kinetoplast DNA (kDNA) consists of a network of concatenated DNA molecules known as the minicircles and maxicircles. The process of minicircles replication begins at the Universal Minicircles Sequence (UMS), a dodecamer (5′-GGGGTTGGTGTA-3′) under (theta) structure form. The Universal Minicircle Sequence Binding Protein, UMSBP - characterized in *Crithidia fasciculata* binds the UMS and causes the replication besides recruiting protein factors. We characterized in our laboratory the TcUMSBP (Coelho, 2003), which contains 134 amino acids and molecular weight of 14,5 kDa. In the present work, we have analyzed the expression of TcUMSBP in the presence and absence of acriflavine, a DNA intercalating agent, in the development phases of *T. cruzi*. Epimastigotes cells from *T. cruzi* clone CL Brener were collected at 24, 48 and 72 h after treatment with acriflavine. Electrophoretic analysis by SDS-PAGE of total protein indicated a decrease in total protein in cells treated with 5 μg/ml of acriflavine, after 72h, as noted for molecular weights of 20 and 30 kDa. The acriflavine induces the inhibition of cellular growth of epimastigotes forms. Moreover, assays obtained by western blot with 1:2.000 anti - *C. fasciculata* UMSBP, indicated similar abundance of TcUMSBP in control and treated cells after 24h with 5μg/ml or 25 μg/ml of acriflavine. These results suggest that the level of UMSBP protein are maintained in cells treated with acriflavine, nevertheless, previous results shown a correspondent accumulation of mRNA in these cells. These results suggest a possible cell response mechanism after 24h of drug treatment, which can be important for the maintenance and cellular viability. Supported by FAPERJ, CNPQ

BM098 - Thermodynamic characterization of Methylthioadenosne Phosphorylase (MTAP) from *Trypanosoma cruzi.*

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The rational drug design is based on biochemical and physiological differences between pathogens and their hosts. The purine metabolism has been identified as an excellent potential target in trypanosomatids since these organisms lack the molecular machinery to synthesize the purinc ring de novo, obtaining purines through purine salvage pathway or specific transporters. The methylthioadenosine phosphorylase (MTAP) of *T. cruzi* plays a crucial role in this pathway cleaving MTA in adenine and methyl-thioribose-1-phosphate (MTR1P). This pathway is potentially exploitable for chemotherapy target in protozoan parasites because of the needs of pre-formed purines and the high cost of methionine synthesis. We report on the obtaining of fully active recombinant MTAP that allowed us to study its pH dependence unfolding process, thermal and chemical stability through circular dicroism and fluorescence emission. The secondary structure content was predicted using the data from the circular dicroism. The *T. cruzi* MTAP showed a high thermal stability, over the pH range from 4 to 8.6, with a transitional temperature (Tm) between 75 and 80 ^{o}C The protein was also very resistant to chemical denaturation since its unfolding with guanidinium chloride occurred with more than 3 M. In addition the protein kept folded when treated with a concentration as high as 8 M of urea. The thermal denaturation data were also used to calculate the ΔG and the ΔH. The physicochemical characterization of MTAP can help establish a better condition to perform biological assays, to understand its interactions with the environment and to facilitate its structural analysis. Supported by CNPq.

BM099 - Thermodynamic properties of prolyl oligopeptidase from *Trypanosoma brucei*

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Prolyl oligopeptidase is a ubiquitous serine protease of S9 family that hydrolyses substrates at the carboxyl side of proline and alanine residues. We have demonstrated that the irreversible inhibitors against *Trypanosoma cruzi* prolyl oligopeptidase arrest trypomastigotes entry into nonphagocytic host cells, suggesting a role for this enzyme in the parasite life cycle. In this study we present the biophysical characterization of prolyl oligopeptidase from *Trypanosoma brucei* (POPTb) that present similar molecular properties with its *T. cruzi* counterpart. Aiming at the thermodynamic characterization of this enzyme, we carried on studies of chemical denaturation and quenching of the intrinsic fluorescence. Gdn-HCl and Urea cause alteration on the secondary structure of the protein with a change in wavelength. POPTb is not very stable with deltaG equal 3.6 and 3.7 for Gdn-HCl and Urea, respectively. The presence of CTAB induces a structural alteration that results in loss of activity, whereas that of Sorbitol induces quenching of fluorescence with smaller loss of activity. As for quenching of fluorescence,

Stern-Volmer constants show pH-dependence and CsCl more powerful a quencher than Acrilamide. The transition from the native to the denatured state is a two-state one with C equal 3.8 for Gdn-HCl and 5.1 for Urea.

BM100 - The role of *Trypanosoma cruzi* classes I e II GPI anchored hypothetical proteins in the interaction of the parasite with host cells

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The recently finished **Trypanosoma cruzi** genome project has shown that 50% genes code for hypothetical proteins These proteins have unknown function and may be involved in parasite life mechanisms. T. cruzi is a heterogeneous species, being divided in two classes of strains. While class I strains are mainly found in marsupial mammals, class II strains are involved in placental mammals infections, preferentially. This host preference differences may be consequence of divergent surface molecules expression between classes I and II strains. The majority surface proteins with important roles at the interaction between T. cruzi and its hosts are linked to the membrane by a glicosyl phosphatidyl inositol (GPI) anchor, like gp82 and gp90, on metacyclic trypomastigotes. Aiming to understand the role of hypothetical proteins our group have already done a fine bioinformatic analysis from the initial 1810 hypothetical proteins data base, where were selected 5 hypothetical proteins by: transmembrane domain (-), transamidation site at C terminal end (+) and signal peptide (+) (Fampa et al., Chagas 2005). It was named Hip2, Hip3, Hip4, Hip6, Hip16. Primers have been drawn to these 5 sequences ORFs and the PCR products presented high similarity with data base sequences. Southern-blotting analysis suggest that these genes are present in the parasite genome in a low copy number in both classI and classII strains. After cloning Hip2 and Hip4 in pET28, we are currently analysing expression of these hypothetical proteins, and trying to obtain recombinant proteins. Hip4 and Hip2 will be cloned in pTEX vector and overexpressed in epimastigotes forms. To determine the function of these hypothetical proteins at the host cell-parasite interaction comparative biological assays between wild-type and transgenic **T.cruzi** will be performed.

BM101 - Isolation and characterization of the gene encoding NO-synthase from the digestive tract of the blood-sucking, Triatoma brasiliensis

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Innate immunity is a conserved survival mechanism in eukaryotes, being an ancient line of defense against foreign organisms. In insects such as Drosophila sp, the resistance to microorganisms is mediated by two different pathways: Toll-receptors and imd, icluding the synthesis of a battery of antimicrobial peptides (lysozyme, defensin, attacin and cecropin), which are induced in the fat body and released to the hemolymph of the insects. Immune function is also mediated through the activation of the Nitric Oxide (NO) pathway. Nitric Oxide (NO) is a highly reactive molecule, which is produced in mammalian macrophages by nitric oxide synthase isoform (NOS), strongly up-regulated following infection. In the fat body of Drosophila sp, NO plays a signalling role in the induction of immune responses to gram-negative bacteria, mediating the induction of the antimicrobial peptide, Diptericin. Additionally, NO is an important cytotoxic and cytostatic effector molecule for several intra- and extracellular parasites, e.g. Plasmodium, Leishmania, Trypanosoma, Toxoplasma and Schistosoma, being able to inhibit the parasitic cystein-like proteases. In the present work, we have isolated and characterized a fragment of a gene encoding NO-synthase from the digestive tract of Triatoma brasiliensis. The nucleotide sequence of this fragment is constituted of 1022 bp, which was amplified by PCR using degenerated and specific oligonucleotides derived from Rhodnius prolixus NO-synthase sequences. RACE was used to amplify the 5′- and 3′-end of the NO-synthase encoding cDNA. The overall amino acid identity between T. brasiliensis and R. prolixus NO-synthase was ca. 90%. Expression of the gene encoding NO-synthase, PCR amplifications and RACE will be under performance, until the complete sequence is obtained. Key words: Triatoma brasiliensis, NO-synthase, antimicrobial peptides, Toll-receptors and imd pathways Financial Support: FAPERJ, CNPq and FIOCRUZ.

BM102 - The presence of surface gp63 homologues alters the interaction rates of *Crithidia deanei* wild and aposymbiotic strains to *Aedes aegypti* midgut cells

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In this study, the expression of surface gp63-like proteins in the endosymbiont-bearing and aposymbiotic strains of *Crithidia deanei* was compared through flow cytometry analysis using anti-gp63 antibodies raised against recombinant gp63 from *Leishmania mexicana*. The absence of the endosymbiont reduced the binding of anti-gp63 to the cell surface, suggesting a regulation of this metallo-type enzyme by the presence of the endosymbiont. For FACS analyses cells were incubated with either rabbit anti-gp63 antiserum or rabbit pre-immune serum and then incubated with FITC-labeled goat anti-rabbit IgG. Immunoblots analysis was performed with the supernatants from the reaction mixtures of either *Bacillus thuringiensis* phospholipase C (PLC)-treated or control cells. The primary antibodies used were a rab-

bit antiserum raised against recombinant gp63 (H50) from *L. mexicana* or anti-cross-reacting determinant (CRD) and the secondary antibody used was horseradish peroxidase-conjugated goat anti-rabbit IgG. Flow cytometry and Western blotting analyses indicated that these molecules were glycosylphosphatidylinositol (GPI)-anchored to the surface. Live parasites treated or not with PLC were added to dissected guts of adult female mosquitoes (*Aedes aegypti*), which were sliced open longitudinally and incubated for 1 h at room temperature in PBS. The aposymbiotic strain of *C. deanei* presented interaction rates about 2-fold lower with guts. It has been established that gp63 homologues are relevant to the adhesion of several lower trypanosomatids, including *C. deanei*, to the insect gut. The treatment of the wild strain with PLC reduced the exposition of surface gp63 and the adhesion rate to *A. aegypti* guts to similar levels to those found in the untreated cured strain. Collectively, these results suggest that the cell surface gp63 homologues may account for the better interaction of the wild strain of *C. deanei* to the insect gut epithelial cells. Supported by: MCT/CNPq, CEPG/UFRJ, FAPERJ

BM103 - Analysis of a Fatty Acid Binding Protein (FABP) expression in the Midgut of the Blood-sucking Bug *Rhodnius prolixus* Midgut

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Fatty acid-binding proteins (FABP) are low molecular mass proteins (14-17 kDa), which are members of a superfamily of cytoplasmic hydrophobic ligand-binding proteins. FABps are expressed in a wide variety of species ranging from yeast to mammals as well as insects. The best studied protein of this superfamily is the FABP for which a number of roles have been proposed . Fatty acids are important components in lipid uptake and transport in insects. The insect gut lumen absorbs free fatty acids, converting them to diacylglycerols inside absortive cells. During the blood digestion diacylglycerols, are transferred to the major hemolymphatic lipoprotein called lipophorin. We have previously demonstrated that *Rhodnius* midgut is the main organ in fatty acid absorption. This organ uses the fatty acids to synthesize phospholipids (60%) and diacylglycerol (40%). In *Rhodnius*, FABP can be involved in intracellular transport of fatty acid to phospholipids and diacylglycerols synthesis and posterior transfer to lipophorin. FABP gene has been sequenced from a follicular epithelium of *Rhodnius prolixus* cDNA library. BLAST analysis show that it has a great homology and identity with other FABP sequences already described. In order to investigate the presence of FABP and its relationship with blood digestion, five midguts of adult females three days after blood meal were dissected and the RNA was extracted. RT-PCR analysis revealed that FABP is expressed in *Rhodnius* midgut. To further investigate the relation between blood meal and FABP expression, we analyzed FABP expression in midgut in different days after blood meal using Real Time-PCR. The results showed a significant variation in FABP mRNA levels. One day after feeding this expression was maximum; after that, a gradual decrease was observed from day 3 to 10 and, on day 15, mRNA levels were comparable to the non-fed sample.

BM104 - Identification and metabolic characterization of carotenoids in intraerythrocytic stages of Plasmodium falciparum

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Lethal forms of the malaria disease are caused by P. falciparum and the spreading resistance of this parasite against virtually all drugs calls for the identification of new therapeutic targets and the development of new drugs. In order to avoid the rapid emergence of resistance, combinations of antimalarial drugs acting on different points of the same metabolic pathway are believed to increase therapeutic success. An important target for the development of new antimalarial drugs is the isoprenoid biosynthesis, which occurs in P. falciparum via the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. Carotenoids, a major class of natural pigments, are naturally occurring pigments found in a wide variety of plants and microorganisms, which make for the secondary metabolites from the MEP pathway. They are potent antioxidants and free radical scavengers, can modulate the pathogenesis of cancers and as immune system enhancers. Derivates play crucial biological roles in humans and animals for nutrition (vitamin A), for the visual system (retinal), and as cellular growth regulators (retinoic acid), or in plants as hormones (abscisic acid). All photosynthetic prokaryotes and eukaryotes, as well as certain fungi, yeasts, and nonphotosynthetic bacteria, synthesize carotenoids. The early reactions of general isoprenoid metabolism specific to carotenoid biosynthesis proceed through common intermediates to phytoene, the first C40 carotenoid. Herein we described the biosynthesis, isolation and characterization of carotenoids in intraerythrocytic stages of Plasmodium falciparum. For the first time, it is demonstrated that the biosynthesis of carotenoids occurred in a protozoan parasite. The carotenoids were purified by RP-HPLC and structurally characterized by biochemical and electrospray mass spectrometric analyses. We suggest that the identified the carotenoids in P. falciparum could be exploited in the screening of novel drugs. Key words: Malaria, Plasmodium falciparum, Carotenoids, HPLC, MS/MS Supported by CNPq and FAPESP

BM105 - STRATEGIES TO GENERATE PARASITE BLOCKING MOSQUITOES WITHOUT COMPROMISING THEIR FITNESS

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The genetic manipulation of mosquito vectors has been explored as an alternative malaria control strategy. The bee venom phospholipase A2 (PLA2) has been expressed in *Anopheles stephensi* mosquitoes, and was successful in blocking the development of *Plasmodium berghei*, although those mosquitoes had significant fitness reduction (Moreira *et al.*, 2002; 2004). As the parasite blocking mechanism was independent of the PLA2 enzymatic activity (Zieler *et al.*, 2001), we developed a mutated version of that molecule in order to inactivate the enzyme and, consequently reduce the probability of fitness impairment. The PLA2m sequence was cloned into the pET32a expression vector and the recombinant protein has been produced in *E. coli* BL21DE3pLysS by induction with 1 mM of IPTG. The protein was detected in inclusion bodies, being solubilized with a guanidine buffer. The purification was performed through affinity chromatography on a nickel column, showing a 38 kDa protein band on a SDS-PAGE gel, which corresponds to the 18 kDa PLA2m and the 20 kDa vector protein. Polyclonal antibodies were raised in rabbits and were able to recognize the recombinant protein at up to 1:5,000 dilution. These antibodies will be used to localize the PLA2m protein in transgenic mosquitoes. For that we construct a hybrid gene, using the *An. gambiae* peritrophic protein 1 promoter (AgPer1) to secrete into the mosquito lumen the PLA2m protein. The final cassette (AgPer1 + PLA2m) was inserted into the *piggyBac* transposable element vector. By using the microinjection technique we were able to obtain four genetically modified *Aedes fluviatilis* lines expressing the PLA2m protein. We are now in the process of comparing the effect of the recombinant PLA2m towards the development of *P. gallinaceum* (in vitro assays), and the blocking ability of the transgenic mosquitoes expressing the effector molecule.

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BM106 - Lipid Metabolism during *Aedes aegypti* development

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he mosquito *Aedes aegypti* is a vector for many pathogens that cause several diseases, as dengue and yellow fever, and affects human health. The blood feeding is the key to the transmission of these diseases, because, during this process, human beings are infected through mosquito saliva which contains the pathogens. Furthermore, from the bloodstream, the mosquitoes can obtain important energy sources such as lipids to provide the requirements to growth and oogenesis. In this work, the lipid dynamic between absorption, storage and utilization sites was investigated. The lipid metabolism was studied using insects in different metabolic and development phases. Larvae from different stages, pupae and male and female adults were separately homogenized in a mixture of protease inhibitors and subjected to lipid extraction. The lipids were separated by thin-layer chromatography (TLC) and analyzed by densitometry. The results shown that diacylglycerol was the main lipid detected throughout the larval stages. Besides, a decrease of the triacylglicerol content from the first larvae stage to the second one was verified. It can mean that the larvae is able to utilize the maternal lipid reserves in the initial stages of development. Moreover, we observe an increase of the triacylglycerol content in the fourth larvae stage indicating that the last larval stage can accumulate lipids in order to make reserves to be used during the whole pupae development. After pupae-mosquito moult, TAG and DAG are catabolized at a greater rate during food deprivation until the next meal. The lipid distribution through the mosquito tissues and the role of these resources during the adulthood is now under investigation.

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BM107 - AN INVESTIGATION INTO THE MECHANISM OF LIMONENE'S ACTION ON *Leishmania amazonensis*

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Limonene is an isoprenoid found in a variety of vegetables, herbs and fruits, particularly in oils of lemon, orange, dill and bergamot. This monoterpene has insecticide and antimicrobial properties that act as natural defense mechanisms in plants. Limonene presents antifungal, antibacterial, anti-inflammatory and antitumoral activities. This terpene has been shown to possess antiprotozoal activities against *Trypanosoma cruzi* and *Plasmodium falciparum*. In *P. falciparum*, limonene inhibits the biosynthesis of isoprenoids as dolichol and ubiquinone and protein isoprenylation (RODRIGUES GOULART et al, Antimicrob. Agents. Chemother., 48:2502-9, 2004). In a previous work, we showed that limonene inhibited the growth of *Leishmania amazonensis* promastigotes (IC50 $\sim 252.8 \pm 4.9\ \mu$M) and amastigotes purified from lesions (IC50 $\sim 147.3 \pm 4.6\ \mu$M). In the present study we investigated if limonene intervenes in the isoprenoid biosynthetic pathway in *Leishmania*. Promastigotes or amastigotes of *L. amazonensis* were treated with 85 or 150 μM limonene and labelled with precursors of

this pathway. The analysis of the hexane extract of these parasites showed that limonene inhibits the incorporation of [^{14}C]-acetic acid into dolichol, ergosterol and ubiquinone. However, the labelling of these products is not altered when [^{14}C]-mevalonate, [^{3}H]-FPP or [^{14}C]-leucine were used as precursors. These results indicate that limonene is probably an inhibitor of hydroxymethylglutaryl-CoA reductase in *L. amazonensis*. To investigate whether limonene interferes in protein prenylation, promastigotes were treated with 85 or 150 μM of limonene and labelled with [^{14}C]-mevalonate. Total protein extracts analysed by SDS-PAGE showed increased labelling in proteins of apparent molecular weights of approximately 70 and 90, kDa. In conclusion, we show in this study that, in *Leishmania*, limonene interferes in the isoprenoid pathway. However, this is probably not sufficient to explain the observed antileishmanial activity. Supported by FAPESP .

BM108 - CHARACTERIZATION OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* FRACTIONS ENRICHED OF CYSTEINE-PROTEINASES

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Currently, there are few studies concerning cysteine-proteinases (CPs) activity from *Leishmania (V.) braziliensis*. In this work, we have examined CPs from infective (MCAN/BR/1998/619) and non-infective (MHOM/BR/1975/M2903) promastigotes of these microorganisms. Our strategy consisted of an association of Triton X-114 method with chromatography in Concanavalin A-Sepharose column, followed by DEAE-Sephacell column. Both strains presented a peak of enzymatic activity over pEFLpNan substrate coincident with the highest protein peak eluted from this column: infective strain = 165 x 10^{-2} mM of pNan minute $^{-1}$ and non-infective = 180 x 10^{-2} mM of pNan minute^{-1}. SDS-PAGE analysis of the material eluted from the ionic exchange column showed four main protein bands (63kDa, 43kDa, 30kDa and 27kDa), for both strains. Enzymography assays indicated that the 43kDa and the 63kDa bands, from both strains, can hydrolyze gelatin and pEFLpNan at neutral pH and are sensitive to E-64 reagent presence; and these bands were recognized in immunological assays by two CP specific antibodies. Additionally, enzymatic activity studies with these proteins, immobilized in polyacrylamide gel fragments indicated that, for the non-infective strain, activity was relevant only for the 63kDa enzyme (0.8 $\pm$ 0.1mM of pNan minute^{-1}), which was strongly inhibited (90%) by E-64. While for the infective strain, both enzymes exhibited high activity: 63kDa (2.2 $\pm$ 0.3mM of pNan minute^{-1}) and 43 kDa (3.0 $\pm$ 0.2mM of pNan minute^{-1}); also, these enzymes were differentially inhibited by E-64 (47% and 36% inhibition, respectively). Studies of ultra-structural organization and RT-PCR assays for CPA, CPB and CPC enzymes isoforms are underway to assess localization and expression of these enzymes in these parasites.

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BM109 - The glutamate uptake in Leishmania (Leishmania) amazonensis

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Leishmania spp. is a group of protozoan parasites which are the causative agents of leishmaniasis, a complex of diseases with a broad spectrum of clinical manifestations. *Leishmania (Leishmania) amazonensis* is the main etiological agent of the diffuse cutaneous leishmaniasis. The organisms of the genus *Leishmania*, as other trypanosomatids, have a metabolism strongly based on the consumption of amino acids, particularly proline. However, the transport of amino acids in these organisms remains poorly understood with the only exception of proline, which was studied with some detail. In the present work, the transport of glutamate, an amino acid which is metabolically related to proline, is characterized. This process is performed by a single kinetic system $Km = 0.59 \pm 0.04mM$, $V_{max} = 0.123 \pm 0.003$ nmoles x min^{-1} per 20 x 10^6 cells). This system was shown to be partially inhibited by analogues such as glutamine, α ketoglutarate and oxaloacetate, methionine and alanine, but not by aspartate. The transport activity was not sensitive to the extracellular concentration of ions Na^+ and K^+ but it was shown to be sensitive to H^+. The participation of these ions in the transport process was confirmed by the diminished transport of glutamate in the presence of valinomycin, monensin and FCCP. Support: CAPES and FAPESP

BM110 - COMPARATIVE STUDY OF METACASPASE GENE (MCA5) OF TWELVE DIFFERENTS SPECIES OF LEISHMANIA

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Apoptosis is a PCD phenotype well characterized in metazoan, as well as in some unicellular eukaryotes. Trypanosomatids display several of the apoptotic hallmarks, such as caspase-like activation. The role of caspase-like activation in the apoptotic death of trypanosomatids remains to be char-

acterized. No homologous of caspase genes have been found in the Leishmania (L) major genome. However Uren and collaborators (2000) have identified genes of the caspase-like family in some unicellular organisms, not present in mammalian genomes, and called them metacaspase genes. In order to investigate the underlying mechanisms of apoptosis in Leishmania spp we cloned, sequenced and performed a comparative study of metacaspase gene (MCA5) of twelve differents species of Leishmania: 1- L. (L) amazonensis, 2- L. (L) mexicana, 3- L. (V) braziliensis, 4- L. (V) guyanensis, 5- L. (L) chagasi, 6- L. (L) donovani, 7- L. tarentolae, 8- L. gymnodactyli, 9- L. adleri, 10- L. hoogstraali, 11- Leishmania (L) major, and 12- Leishmania (L) infantum. From the sequence of the metacaspase gene (MCA5) of Leishmania (L) major and Leishmania (L) infantum,deposited in GeneDB, we designed primers which were used to screen the metacaspase genes in the other species of Leishmania spp. The alignment and analysis of the sequences obtained from each one of the studied species allowed us to deduce the primary structure of the coded molecules and to define that metacaspases of Leishmania spp are highly similar. The primary structure of the region coding for the catalytic site of the molecule, in the amino-terminal portion (P20), displays maximum similarity within the different species while the carboxi-terminal portion is highly variable and rich in proline residues.

BM111 - Molecular analysis of *Leishmania* apyrases, cloning into expression vectors and characterization of a putative GDPase from *L. major* as a genuine NTPDase

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Leishmania major has two mapped apyrase genes in its genome (putatives NTPDase and GDPase). Apyrase function, characterized as tri and di-nucleotide hydrolysis, were previously demonstrated in intact *L. amazonensis* and *L. braziliensis* cells. The very distinct ecto-nucleotidase capacity between Leishmania species suggests its involvement with virulence and control of host-immune responses. In order to evaluate if a molecular difference could explain these data we amplified, cloned and partially sequenced both genes from these 3 species. Analysis of partial genes and deduced proteins from L amazonensis and *L. braziliensis* showed 100 % identity at DNA and protein levels with *L. major* isoforms, suggesting that the different ecto-nucleotidase capacity could not be explained by only this molecular approach. *In silico* molecular analysis between *L. major* paralog genes showed only one possible transmembrane region in the amino-terminal domain of both putative proteins. Possible GDPase has a signal peptide at amino acid position 45 (SIGNAL P program), suggesting a possible soluble excreted protein. On the other hand, the putative N-terminal transmembrane domain in NTPDase and the presence of ecto-apyrase activity on live cells could be suggestive of an ecto-membrane localization. Despite both predicted proteins have all Apyrase Conserved Regions, alignment of their sequences revealed a very low identity only 19,9 %. To perform further evaluations isolated full-apyrase genes were cloned into expression vectors (pET21b and pYES-CT). Furthermore, the *L. major* GDPase and NTPDase heterologous expression and purification had been performed and putative GDPase showed preference for di- and tri-nucleotides, but did not hydrolyze GDP, because of this the putative GDPase would be named as *L. major* NTPDase, a genuine apyrase. Complete characterization of these purified proteins could help in the elucidation of the real role of these apyrases in *Leishmania* infection and virulence. Supported by: UFOP, FAPEMIG. CNPq and MEC

BM112 - *Leishmania braziliensis*: differential expression of proteinases in avirulent and virulent promastigotes

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The genus *Leishmania* includes parasitic protozoa responsible for a species-dependent spectrum of diseases extending from localized, self-healing cutaneous lesions to more severe visceral infections. *Leishmania braziliensis* is the etiological agent of the muco-cutaneous form of leishmaniasis in the New World, which causes facial disfigurement with high morbidity. Proteases have been implicated to play crucial roles in parasitic cytoadherence, tissue invasion activities, survival and proliferation in host cells and virulence. The present study reports the differential expression of proteolytic enzyme profiles in virulent and avirulent promastigote forms of *Leishmania braziliensis*. The presence of E-64 (cysteine peptidase inhibitor), EGTA and phenanthroline (metallopeptidase inhibitors) drastically inhibited the cell growth of virulent form, while in the avirulent form these inhibitors had no effect. These inhibitors also promoted a decrease on the association index between *Leishmania*virulent form and macrophage. EGTA, phenantroline and E-64 inhibited 31.05%, 35.40% and 42.57% this process, respectively. After extraction, the peptidases founded in cytoplasmic content were separated in gelatin-SDS-PAGE. The profile presented by virulent and avirulent promastigote forms were distinct. The virulent parasites presented a pronounced peptidase activity (40, 49, 60 and 80 kDa) in acid pH (5,5), while in avirulent form, this activity was lower (60 kDa). EGTA and phenantroline inhibited peptidase activity presented by 60 and 80 kDa, while E-64 inhibited peptidase activity presented by 40 and 49 kDa. Theses results suggest

that enzymes might play an important role in the virulence of *Leishmania braziliensis*.

BM113 - *Leishmania major Hus1-like* gene confers resistance to the radiomimetic drug phleomycin.

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The H region of *Leishmania spp.* encodes various genes involved in drug resistance and virulence modulation. This 45-Kb locus is found amplified in cell lines selected in unrelated drugs. The H locus also contains genes of unknown function, such as the 996-bps ORF *L.mjF23.0290*. This gene contains a possible Hus1-like protein domain. Hus1 is a conserved checkpoint protein that forms a trimeric complex with Rad1 and Rad9 (the 9-1-1 complex) to encircle damaged DNA. In other organisms, the complex activates checkpoint signaling pathways that block cell cycle progression, regulate DNA repair, and trigger apoptosis. The precise mechanisms controlling this process remain unclear. The *L.mjF23.0290* gene (*LmHus1*) was transfected into the parasite and northern analysis revealed that selected mutant had an increase in its transcript levels. The cell line was also shown to be resistant to phleomycin, a radiomimetic drug. The mutant presented an EC_{50} value for phleomycin that was 3,5-fold higher when compared to wild type cells and was not resistant to terbinafine, methothrexate or antimonials. The possible involvement of *LmHus1* in DNA damage checkpoint control was initially investigated in unsynchronized cells of the mutant. Flow cytometry analysis suggested that *LmHus1* transfectant had a slightly higher proportion of cells in the G1 phase of the cell cycle. The integrity of chromosomal DNA of phleomycin-treated wild type and mutant cell lines is being investigated in pulse-field gel electrophoresis analysis. We have also constructed targeting fragments and our current efforts are focused in the generation of *LmHus1*-null mutants. Supported by FAPESP, CNPq and CAPES.

BM114 - Binding of leishporin to liposomes membranes: an approach to pull down the cytolysin from *L. amazonensis* extracts and new insights on the cytolysin identity and the mechanism of lysis.

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Leishporin is a pore forming protein present in protozoan of the Genus *Leishmania*, which was partially characterized by our group in *Leishmania amazonensis*. Since leishporin is optimally active at pH 5.5 and 37^oC, it migh act inside the macrophage phagolisosome and be involved in the rupture of the fagolisosome and of the macrophage itself. To lyse cells, leishporin must be activated by proteolytic digestion or dissociation of an inhibitor oligopeptide, which seems to redder the molecule able to bind to lipids. In this work we have used these features in an attempt to purify the cytolysin. We found that liposomes with or without cholesterol remove all the hemolytic activity while bind 5 major proteins from promastigots extracts, as distinguished in SDS-PAGE. Using liposomes with trapped calcein we verified that the vesicles that had bound these proteins were lysed, showing that they are sufficient to cause their lysis and thus candidates to be leishporin. Like hemolysis, lysis of liposomes is a temperature-dependent phenomenon and does not require cholesterol. Preliminary mass spectrometry results (MALDI-Tof-Tof) of 4 of the proteins led us to identify 3 of them as gp63, GAPDH and β-tubulin. A fourth of 48 kDa is an yet unknown protein of *Leishmania amazonensis*, which is a good candidate to be leishporin.
Financial Support:Pronex - FAPEMIG / CNPq

BM115 - Proteolytic activation of leishporin and its resistance to proteolytic digestion.

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Previous results from our group had shown that, in order to cause lysis, leishporin, a pore-forming protein from *L. amazonensis*, must be activated by proteolytic digestion or dissociation of an inhibitor oligopeptide. In this work, we have shown that leishporin can be activated by a membrane-associated serine protease of *L. amazonensis* promatigotes without being destroyed. To determine the extent of protease-resistance of leishporin, we have investigated the hemolytic activity of promastigotes membrane extracts after treatment with increasing amounts of proteinase K, monitoring the digestion products by SDS-PAGE and native PAGE. We verified that the hemolytic activity of parasite extract is not altered after treatment with up to 200 ug/ml, indicating that the cytolysin is quite resistant to proteolysis. On SDS-PAGE, the digestion products did not contain proteins with MW higher than 20 kDa. We also verified that the hemolytic activity colocalized with a restricted number of proteins after incubation of the native gel containing the digestion products with red blood cells embedded in agar . This work shows that the smaller active leishporin molecule is probably no larger than 20kDa protein.

BM116 - MOLECULAR CHARACTERIZATION OF *LEISHMANIA* SPECIES IN PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS FROM BIRITICUPU, MARANHÃO STATE

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Molecular diagnostic techniques are important and necessary to understand the epidemiology of American Cutaneous Leishmaniasis (ACL) caused by subgenus *Viannia*, its different patterns of lesions and choice of adequate treatment. The use of molecular techniques makes possible the detection of parasites in lesions with low parasitism and negative parasitological diagnosis by conventional techniques. The main purpose of this study was to characterize, by semi-nested PCR, a strain of *Leishmania* in biopsy of patients with ATL. Twenty-two skin biopsies of patients with ACL from Buriticupú, Maranhão state, in the Brazilian Amazon region were tested through the Semi-Nested PCR assay, using the LVF and $ISVC_2$, primers which are specific for subgenus *Viannia*. The positive samples by this assay were submitted to Semi-Nested PCR using the $ISVC_2$ and ISVB primers specific for amplifying exclusive sequences of *Leishmania(Viannia) braziliensis*. The results showed that biopsies from eight patients (36%) were PCR-positive and classified in the subgenus *Viannia*, while five of them were classified as *Leishmania (Viannia) braziliensis*, corresponding to 62% of the cases. The Semi-Nested PCR assay is an important diagnostic tool because it has high sensibility and specificity, making possible the identification of *L. (V.) braziliensis*, contributing to epidemiological studies in areas where there exist more than one species of *Leishmania*.
Supported by: LIM-50/FMUSP, FAPESP

BM117 - EDIBLE VACCINES: A PROMISING ALTERNATIVE FOR LEISHMANIASIS?

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The induction of oral tolerance against disease-inducing antigens has emerged as a feasible strategy to prevent immunopathologies. Previously, we described that the disease-promoting whole antigens of *Leishmania amazonensis* promastigotes (LaAg) could be rendered protective if administered by the oral route (Pinto E.F.*et al.*Vaccine.21:3534-, 2003). We later showed that DNA codifying the LACK protein, a subunit component of LaAg that knowingly activates TH2 response, can also induce protection against murine cutaneous leishmaniasis when given by the intranasal route (Pinto E. F. *et al.* Infect Immun.72:4521-, 2004). Bacterial recombinant LACK were ineffective by the i.n. route possibly due to its high solubility hampering mucosal uptake and increasing degradation, but the absence of glycosylation may also be a drawback. In the present work we envisaged to use plant biotechnology to develop glycosylated LACK-expressing plants to be used for oral immunization against leishmaniasis. As a first trial, tobacco plant was used for its high transfectability. Thus, several leaf discs of *Nicotiana tabacum* L tobacco were transformed by the plant pathogen *Agrobacterium tumefaciens* containing the LACK gene plasmid. After culturing in appropriate plant culture medium containing selective antibiotic, the calluses were excised and grown in agar containing antibiotic and nutrients under sterile conditions. The presence of the transgene was confirmed by PCR and the production of LACK protein was confirmed by dot-blot. One out of 11 clones expressed high levels of recombinant LACK protein (0.18% of total dry leaf tissue), as quantified by densitometry. Clones of this highly- LACK expressing plant are presently growing on the soil. They will be used for the preparation of fresh leaf extracts that will be given orally to mice to assess the feasibility of using plants as bioreactors for the production of edible antileishmanial vaccines.

BM118 - *Leishmania amazonensis*: Solubilization and incorporation of antigenic proteins of amastigotes into liposomes to generate immunogens with adjuvant activity

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Liposomes have been known to express adjuvant activity in vaccines against protozoan or bacterial organisms. The objective of the present study was to obtain a crude extract of detergent-solubilized proteins of *L. amazonensis* amastigotes and reconstitute them into liposomes. Neutral and zwiterionic detergents were less efficient than an ionic detergent. In order to obtain efficient solubilization using only SDS, the effects of detergent and protein concentration and incubation time were studied. The maximum solubilized proteins were obtained instantaneously using a ratio of 0.5 mg/ml of protein to 0.1% (w/v) detergent at 4°C. With the objective of preparing proteoliposomes, different mixtures of lipids and an SDS-solubilized proteins extract were used and an optimization of the incorporation of protein was obtained using

the mixtures of DPPC, DPPS and cholesterol, at weight ratios of 5:1:4 and 0.5 mg/ml of protein. The incorporation of proteins results in a proteoliposome presenting a final lipid weight ratio for DPPC, DPPS and cholesterol of 1:1:5. SDS-PAGE of proteoliposomes revealed a variety of proteins with a distribution similar to that observed in SDS-solubilized proteins extract, indicating that the lipids chosen were efficient to incorporate the antigenic proteins of *L. amazonensis* amastigotes. In a preliminary experiment, BALB/c mice inoculated with the proteoliposomes were able to produce antibodies against the reconstituted proteins, as evaluated by Western blotting, indicating that these immunogens may be used to induce protective immunity against *L. amazonensis*. Supported by: CNPq, CAPES and FAPESP.

BM119 - The 3' UTR of phosphoglycerate kinase gene is involved in the regulation of transcript level in the extra-chromosomal environment in *Leishmania major*

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Phosphoglycerate kinase B *(PGKB)* and C *(PGKB)* genes of *Leishmania major* code for the cytosolic and glycosomal isoforms of the enzyme, respectively. Genomic fragments bearing either *PGKB* or *PGKC* were subcloned into pX63Neo and the recombinants, pX63Neo*PGKB* and pX63Neo*PGKC*, were transfected into *Leishmania*. Phenotypic changes of the overexpressor cells were investigated and Southern analysis showed that the *PGKC* episome is kept at low levels, almost five times lower than *PGKB* and control transfectants. Northern blotting experiments demonstrated an analogous difference between transcript levels of *PGKC* and *PGKB* genes. To investigate the role of untranslated regions (UTR) in the observed differences, we engineered chimeric constructs, in which the 5ánd 3ÚTR of *PGKC* and *PGKB* were swapped. Our data suggest the involvement of the 3ÚTR of *PGKC* in the control of transcript level of the gene present in the episome. To extend our analysis we replaced *PGKB* and *PGKC* genes by the hygromycin phosphotransferase gene into the original recombinants (pX63Neo*PGKB* and pX63Neo*PGKC*), the flanking regions of both genes were kept intact. Leishmania transfectants overexpressing the *pgkc*::HPT gene presents a behaviour similar to that observed for the *PGKC* overexpressor clone. Therefore, our results indicate that the *PGKC* 3ÚTR is involved in the control of transcript levels and that this control is not linked to the gene function or subcellular localization. We are currently generating mutants to evaluate whether a similar control is observed in the genomic context. Supported by FAPESP

BM120 - Phenotypic differences between a virulent strain and its attenuated mutant overexpressing SL RNA in *Leishmania*.

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The miniexon gene has an important role in the mRNA metabolism of Kinetoplastids. The product of this gene (the spliced leader sequence-SL) is the common substratum for the trans-splicing reaction. In this reaction, the 39 nucleotide-long SL is spliced to the 5′-end of virtually all *Leishmania* mRNAs. Our group has previously shown that the artificial induction of the miniexon overexpression in *Leishmania major* reduced the virulence pattern of a lineage originally virulent (LV39) in BALB/c mice in vivo (Antoniazi, S. et al., Mol. Biochem. Parasitol., 107(1), 2000). This study was extended to *Leishmania braziliensis* and a more drastic effect of attenuation of virulence was observed in the New World species (Toledo, J.S. *et al.*, in preparation). The proposal of this work is to investigate molecular mechanisms involved in the observed attenuation process. Differentially expressed mRNAs and proteins were investigated by comparative analyses: proteins were identified by 2DGE followed by mass spectrometry and mRNAs by DDRT-PCR followed by cloning and sequencing of the candidates. We are currently validating DDRT-PCR results by Northern blotting experiments. Comparative analyses of protein profiles revealed several differences which were not reproducible in all biological triplicates evaluated. Nevertheless, we observed a consistent difference in mRNAs and proteins related to stress response, proteolysis, and transcription and translation control. Furthermore, we observed that the ultra-structure of the mutant promastigotes is affected. Our results indicate that the excess of miniexon transcript could be leading to the translation of some transcripts that should be down-regulated for appropriate differentiation from pro- to amastigotes or for amastigote multiplication within macrophages. Supported by FAPESP

BM121 - NF-κB ACTIVATION IN HUMAN MACROPHAGES INFECTED BY *Leishmania amazonensis* AND TREATED WITH HIV-1 TAT PROTEIN

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Leishmaniasis is emerging as a frequent opportunistic infection in AIDS patients, particularly in countries such as Brazil and India where both infections are becoming more prevalent. Previous results have demonstrated that the treatment of human macrophages with HIV-1 Tat protein increases *Leishmania amazonensis* growth. Tat regulates a number of signal transduction pathways in host cells and some of these effects are dependent on the activation of the transcription factor NF-κB by this protein. As NF-κB regulates the expression of immunomodulatory genes, we aimed in this work to investigate the modulation of this activation factor in the context of macrophages infected with *L. amazonensis*, treated or not with Tat. Eletrophoretic Shift Assays (EMSA) with nuclear extracts of human macrophages from healthy donors infected by different periods of time with *L. amazonensis* promastigotes and treated or not with Tat recombinant protein were carried out. Time curves of *Leishmania* infection revealed a strong NF-κB activation in 18 hours of infection. We could verify through supershift assays that this complex is comprised by NF-κB subunits p50 and RelB, different from the complex previously described to be activated by Tat protein, composed by p65 and p50. Assays with human macrophages infected by *L. amazonensis* for 18 hours and than exposed to Tat showed that this protein stabilizes NF-κB complex activated by *Leishmania*. Ours results of NF-κB activation in human monocytic cell line THP-1 differentiated with PMA corroborate the pattern observed in human primary macrophages. These results suggest that the treatment of human macrophages with Tat favors NF-κB activation by *Leishmania*, what can be related with the regulation of genes whose products determine the susceptibility to *L. amazonensis* infection.

BM122 - ACQUISITION OF HUMAN LOW DENSITY LIPOPROTEIN (LDL) IN *Leishmania amazonensis* AND *Leishmania chagasi.*

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Visceral leishmaniasis caused by *Leishmania chagasi* and cutaneous leishmaniasis caused by *Leishmania amazonensis* are serious health problems in tropical and subtropical countries. During the life cycle, they alternate between gut vector as a promastigote form and macrophage as an intracellular amastigote form. Trypanosomatids have incomplete *de novo* lipids synthesis. Therefore they avidly take up lipids from vertebrate blood stream presumably to provide the requirements for growth and differentiation. The objective of this work is the study of LDL endocytosis by *Leishmania*. To observe the LDL endocytosis by *L.amazonensis* and *L.chagasi*, cells were incubated in the presence of LDL-I^{125} for different times. After the incubation, cells were collected and the radioactivity was estimated by gamma counting. The time-course of LDL-I^{125} incorporation by *L. amazonensis* was linear up to 22 hours whereas the incorporation by *L. chagasi* was linear up to 30 hours. The endocytosis was significantly inhibited by an excess of LDL, BSA or Transferrin. In order to verify if LDL endocytosis is a lipid raft-dependent process, the LDL was labeled in the protein moiety with FITC and in the lipid moiety with phosphatidylethanolamine-TEXAS RED. *L.amazonensis* cells were pre-treated with MBCD (Methil-β-cyclodextrin) for 60 min and then incubated in the presence of fluorescent LDL for different times. After 10 hours, cells were collected and the fluorescence was analyzed by microscopy. It was observed that LDL endocytosis was significantly inhibited by MBCD suggesting that, in *L.amazonensis* cells, this process is dependent on the presence of lipid raft. Supported by CNPq, FAPERJ, IFS

BM123 - *Leishmania amazonensis*: Efficient and rapid extraction procedure for GPI-anchored Proteins in a single step by incubation with Triton X-114.

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Glycosylphosphatidylinositol (GPI)-anchored surface proteins of *Leishmania* contribute to the parasite resistance within the host macrophage. The main objective of the present study was to obtain GPI-anchored proteins of *L. amazonensis* and to reconstitute them into liposomes. Triton X-114 phase partitioning has frequently been used to obtain preparations enriched with GPI-anchored proteins from crude cellular homogenates. Here we describe a rapid and efficient method with this detergent that allows the recovery of proteins with intact GPI anchor. Membrane fractions of *L. amazonensis* promastigotes were solubilized (0.5 mg/mL of total protein) in 1% (w/v) Triton X-114 at 4^{o}C. The maximum recovery of solubilized GPI-anchored protein was of

8% in the detergent-rich phase. To confirm that we were recovering GPI-anchored proteins from the parasite extract, after phase separation at 20°C, the detergent rich phase was treated (0.2 mg/mL of total protein) with 0.2 U/mL of PI-PLC of the *Bacillus thuringiensis*, and analyzed by SDS-PAGE and Western blotting. The PAGE analysis of membrane fractions from promastigotes revealed three major protein bands of 65-60, 52 and 50-47kDa, which in Western blotting were very reactive to an anti-serum against whole *L. amazonensis* promastigotes. This standardized method using Triton X-114 is now being used for the reconstitution of antigenic parasite GPI-proteins into liposomes. The resultant proteoliposomes will be applied as carriers with adjuvant activity of these antigenic GPI-anchored proteins to induce immunity against several protozoan organisms in mice. Supported by: CAPES, CNPq and FAPESP.

BM124 - Biochemical characterization of the serine transport in Leishmania (Leishmania) amazonensis

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The role of amino acids in trypanossomatids goes beyond protein synthesis, involving processes such as differentiation, osmoregulation, energy metabolism and precursors of other metobolites (Silber *et al.* 2005 and Zilberstein *et al.* 1993). The availability of the amino acids involved in those functions depends, among other things, on their transport into the cell. Besides protein composition, serine is a precursor for the synthesis of phosphatidylserine, a membrane molecule involved in macrophage invasion without its activation (Balanco *et al.* 2001). Here we characterize the serine transport in *Leishmania (Leishmania) amazonensis* by measuring the incorporation of radioactively labeled serine in promastigotes. Kinetic data show a single saturable transport system, with a $Km = 0,82598 \pm 0,18325mM$ and a maximum velocity of $355,37 \pm 19,41$ pmol x min^{-1} x 2 x 10^7 promastigotes. These values are greater than those described for the arginine transport in *Leishmania (Leishmania) donovani* ($kM = 0,01428mM$ and $Vmax = 33,2$ pmol x min^{-1} x 2 x 10^7 cells)(Kandpal *et al.* 1995), but are of the same order than proline transport described for *Trypanosoma cruzi* ($Km = 0,3$ and $Vmax = 98,34$ pmol x min^{-1} x 2 x 10^7 cells) (Silber *et al.* 2005). The serine transport increased linearly with temperature in a range from 20 to 45°C, allowing the calculation of an activation energy of $40,82 \pm 0,17kJ/mol$. To further characterize the energy dependance and source, inhibitors of respiratory chain, ATP synthesis and ionophores are being used.

BM125 - Expression in *E. coli* and purification of the Nucleoside Diphosphate Kinase b from *Leishmania major* and site-directed mutagenesis of the active site region

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Leishmania is unable to synthesize purines *de novo* and nucleoside diphosphate kinases are involved in the salvage pathway by which free purines are converted to nucleosides and subsequentely to nucleotides. The nucleoside diphosphate kinase (EC 2.7.4.6; NDK) catalyses the transfer of the γ-phosphoryl group from a nucleoside triphosphate to a nucleoside diphosphate by a ping-pong mechanism involving a phosphohistidine intermediate state. Subproteomic analysis of the microsomal fraction of *L. major* promastigotes has identified the NDKb as an abundant component in three spots with different isoelectric points, indicating a possible post-translational modification of protein. The region containing the NDK coding sequence was amplified from *L. major* genomic DNA by PCR and cloned into the pT7T3 and pET28a vectors. The pET28aNDK construct were used for protein expression in *E. coli* BL21(DE3)pLysS and the recombinant NDK (rNDK) containing a His6-tag was expressed as soluble form and subsequently purified from the cell lysate by affinity chromatography using a Ni-NTA resin. The secondary and tertiary structure of the rNDK was evaluated by circular dichroism and intrinsic tryptophan fluorescence spectroscopy respectively. The presence of rNDK phosphotransferase activity was detected by the chromatographic separation of donor (ATP), acceptor (GDP) and produced (GTP) nucleotides. The activity of the rNDK was further evaluated using a pyruvate kinase/lactate dehydrogenase-coupled method that detects ADP as product of the phosphotransfer reaction between ATP to dTDP nucleotides. The purification yield showed that 47,2% of total activity (4558U) was retained by the Ni-NTA column, yielding a final activity purification of 82 fold. The site-directed mutagenesis of the NDKb coding sequence produced a mutant protein presenting a H117Q substituition in the active site of the enzyme. Polyclonal antibodies raised against the corresponing recombinant protein recognized the native protein from the promastigote extracts and the H117Q mutant protein. Supported by FAPESP, CNPq, PRP-USP and PRONEX.

BM126 - Analysis of minicircles sequences of kDNA obtained from clinical samples (active and scars) of patients with American cutaneous leishmaniasis in Pernambuco State, Brazil

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American cutaneous leishmaniasis (ACL), caused by *Leishmania (Viannia) braziliensis*, presents cutaneous lesions that heal spontaneously or after specific treatment. In this study, in vitro and in silico approaches were performed to analyse the mitochondrial genome. Two hundreds and ninety complete *Leishmania sp.* kDNA minicircles obtained from clinical samples (lesions and scars) of patients with ACL were studied. This work demonstrate multiple alignment of minicircle sequences that size polymorphisms (ranging from 518-797 bp) were present, as well as sequence polymorphisms, indicating significant heterogeneity of classes, particularly in minicircles amplified from active cutaneous lesions. The minicircle sequences obtained from scars were grouped in one cluster, indicating some degree of homogeneity, possibly due to clonal selection. The compositional analysis showed A + T 70%. Fifty five polimorphic microsatellites were mapped in both the conserved and variable regions of the minicircles. Specific motifs were identified and mapped in the conserved and variable regions of kDNA minicircles, and classified according to frequency as pertaining to scar minicircles or lesion minicircles. For the same motif, the frequency was higher in the scar minicircles. In conclusion, some molecular features seems to be common to all minicircles. On the other hand, the classes of minicircles obtained from scars are more similar, as judged by the multiple alignment and cladogram analyses, possibly due to the predominance of specific motifs. In future work, it would be interesting to precisely identify the regions responsible for the genetic differences between minicircles obtained from scars and cutaneous lesions. The regions encoding gRNA deserve a particular interes, as different types of gRNA may be essential to the survival of *Leishmania* in the biological context of healed tissue or cutaneous lesions. However, the possible biological consequences of our findings for *Leishmania* persistence need further investigations aiming at a better understanding of this complex issue.

BM127 - Compartive analyses of three Poly(A) binding protein (PABP) homologues in *Leishmania major*

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The Poly(A) binding protein (PABP) is a highly conserved eukaryotic protein that binds the mRNA poly(A) tails and functions in the regulation of translation efficiency and mRNA stability. PABP facilitates the formation of a closed loop structure of the mRNA that is crucial for some of its activities, such as translation initiation and termination and recycling of ribosomes. Association with PABP is also a requirement for some mRNAs to be exported from the nucleus. Little is known about protein synthesis in trypanosomatid protozoans but single PABP homologues have been described from *Leishmania major*, *Trypanosoma cruzi* and *T. brucei*. Our group is interested in characterizing homologues to relevant proteins required in translation initiation in trypanosomatids. To study PABP we began by searching *L. major* genome sequences for possible homologues. We found not only the previously described protein (*Lm*PABP1) but also a second and a third homologue; the latter has no *Trypanosoma* counterpart. All three genes were amplified, cloned, expressed in *Escherichia coli* and used to immunize rabbits. *Lm*PABP1-3 antisera recognize respectively all three proteins in total parasite extract as well as the recombinant proteins. *Lm*PABP1, but not the others, seems to be phosphorylated. Quantitation analyses of the intracellular levels of these proteins were performed in promastigotes and showed that *Lm*PABP2 is the most abundant of the three, *Lm*PABP3 is present in intermediary levels and *Lm*PABP1 is the least abundant. Subcellular localization experiments indicate that *Lm*PABP1 is found only in the cytoplasm whereas *Lm*PABP2-3 are found both in the nucleus and cytoplasm. RNAi analyses using *Lm*PABP1-2 orthologues in *T. brucei* (*Tb*PABP1-2) showed that the cells stopped growing 72 hours after induction of RNA silencing of either protein. Additional studies must be done to understand how these three proteins differ functionally and what are their roles in protein synthesis and mRNA metabolism.

BM128 - CHARACTERIZATION OF THE INTERGENIC SEQUENCES WHICH CONTROLS RNA PROCESSING OF THE UNIVERSAL MINICIRCLE SEQUENCE BIDING PROTEIN (TcUMSBP) LOCUS IN *Trypanosoma cruzi.*

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The Universal Minicircle Sequence (UMS) is a conserved dodecameter, present in mitocondrial DNA minicircles of Try-

panosomatids studied so far. The Universal Minicircle Sequence Biding Protein (UMSBP) is the protein that recognizes this sequence, binds to it and apparently directs the minicircles to its site of replication. This protein, described in *Trypanosoma cruzi* (TcUMSBP) by Coelho *et al.* , in our laboratory, presents five zinc-fingers motifs and 60% of identity with the Universal Minicircle Biding Protein found in *Crithidia fasciculata* . TcUMSBP gene is present as a single copy on the XX chromosomal band of the protozoa′s genome, between the coding sequences of proteasome beta-five subunit and the poly-zincfinger protein 1 (PZFP1) genes. Our laboratory reported that the TcUMSBP locus presents two polymorphic alleles characterized by the insertion / deletion of 62bp in the 5′ intergenic region of the TcUMSBP gene. This 62bp polymorphism affects the efficiency of the pre-mRNA processing and the polyadenylation sites generating a differential accumulation of the transcripts originated from each allele. Our objective is to determine the intergenic sequences involved in the differential mRNA processing of the TcUMSBP gene of *Trypanosoma cruzi* CLBrener clone. We have cloned the 5′ and 3′ untranslated region of the HSP70 and TcUMSBP genes in plasmids containing the Clorafenicol acetyl transferase (CAT) reporter gene. The large-scale preparation of these plasmids is used for transfection in Trypanosoma cruzi cells. Messenger RNA extracted from different cell forms will be used to establish the polyadenylation and trans-splicing sites of the TcUMSBP gene. We are also establishing the extraction of epimastigotes kDNA cells for future studies with minicircles. Supported by FAPERJ, CNPQ.

BM129 - Expression analysis of proteins involved in translation initiation during life cycle of representative species of *Leishmania*.

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The initiation stage is a critical point in protein synthesis, which requires a large number of the so-called translation initiation factors (eIFs). Among them is found the eIF4F complex, composed of three subunits: eIF4A, eIF4E and eIF4G. The Poli-A binding protein, PABP, is also critical to translation initiation and interacts directly with eIF4G. In previous work, through genomic database searching, various homologues of these proteins were found in Leishmania major (*Lm*EIF4A1-2, *Lm*EIF4E1-4, *Lm*EIF4G1-5 and *Lm*PABP1-3) and antibodies against most of them were made available. Through western blots, their expression was then investigated and quantification of their intracellular levels achieved for promastigote forms of *Leishmania major*. Here we describe preliminary data about the expression of some of these proteins during different forms of the life cycle of *L. chagasi* and *L. amazonensis*, species more amenable to differentiation studies. Expression of selected homologues of eIF4E (*Lm*EIF4E3), eIF4G (*Lm*EIF4G1 and 3) and PABP (*Lm*PABP1-3) was investigated in exponentially growing promastigotes, metacyclic promastigotes and axenic amastigotes. *Lm*EIF4G3, *Lm*EIF4E3 and *Lm*PABP2-3 were found in the three different life forms tested, with *Lm*EIF4E3 and *Lm*PABP3 showing a marked decrease in levels in the amastigote stage. *Lm*PABP1 is found in two different forms, one of which is phosphorylated and shows a reduction in levels in metacyclic promastigotes. *Lm*EIF4G1 was found only in exponentially growing promastigotes and in amastigotes. To compare the expression of these factors with those of a protein known to be essential for translation we further analyzed the expression of the *L. major* homologue of the ribosomal protein P0. As expected this protein is present in very high levels throughout the various forms investigated of both species of *Leishmania*. Next we plan to finish similar analysis with the remaining eIF4F homologues so that their role in the *Leishmania* protein synthesis can be better understood.

BM130 - Identification of new proteins from *Leishmania chagasi* as potential components for a new serological test for Visceral Leishmaniasis.

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The Visceral Leishmaniasis (VL) is an important public health problem in Brazil, where this disease is endemic and potentially fatal. Its control is hindered, however, by the lack of an efficient method for the precocious diagnosis of the disease. Currently available serological tests, using *Leishmania chagasi* extracts, have low accuracy and reproducibility, and those using recombinant antigens have variable sensitivities in field tests in Brazil. A systematic selection of *Leishmania* recombinant antigens, therefore, is crucial for the development of an accurate test. In the study described herein, a *L. chagasi* genomic DNA expression library was screened using a pool of sera from VL patients, resulting in 60 different clones. Sequencing of the various inserts and homology analysis using the *L. infantum* and *L. major* genome databases allowed the identification of 7 distinct protein coding fragments. The sequences of the corresponding proteins, some of which are novel and have no homologues outside the family Trypanosomatidae, were designated as Lcg7, Lcg15, Lcg22, Lcg27, Lcg36, Lcg53 and Lcg56. So far all of them were subcloned in the pRSET expression plasmid. The Lcg7, Lcg15, Lcg22, Lcg36 and Lcg56 proteins were successfully expressed in *Escherichia coli*, as His fusions, and purified by affinity chromatography. Preliminary ELISA analyses were

then performed to investigate the potential use of some of the recombinant proteins for the diagnosis of VL. Sera from 46 naturally infected dogs were tested against total parasite extract as well as proteins Lcg7, Lcg22 and Lcg36. Our results shows that 100% of the sera yielded a positive result for Lcg7, with 95% recognizing the parasite extract and only 80% recognizing Lcg22 and Lcg36. These results confirm the identification of relevant new antigens with potential use for the diagnostic of human and canine VL. Further studies must be carried out, nevertheless, to confirm the preliminary data.

BM131 - *Leishmania (Leishmania) amazonensis* promastigote stage-specific lipids defined by monoclonal antibodies

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We describe the production and characterization of mAbs LST-1 and LST-2 (both IgM) directed to lipid antigens of *L. (L.) amazonensis* promastigotes. By high performance thin layer chromatography (HPTLC) immunostaining it was demonstrated that mAb LST-1 recognized an acidic lipid component chemically characterized as inositol phosphorylceramide (IPC). On the other hand, the mAb LST-2 was reactive with 4 glycolipid components visualized on HPTLC by orcinol staining. By indirect immunofluorescence, both antibodies presented high reactivity with *L. (L.) amazonensis promastigotes*. Conversely, no reactivity was observed with amastigotes isolated from infected hamsters. MAb LST-1 did not react with live parasites, suggesting that IPC is cryptic in the membrane, and maybe localized only in the inner leaflet of plasma membrane. Contrasting, mAb LST-2, showed a strong reactivity with live promastigotes. By HPTLC, after staining with Dittmer-Lester reagent and orcinol, it was verified that promastigotes and amastigotes presented distinct (glyco)phospholipid profiles. Amastigotes are rich in glycosphingolipids, not recognized by LST-1 or LST-2, whereas promastigotes present glycoinositolphospholipids (GIPLs) and IPC recognized by LST-2 and LST-1, respectively. The LST-1 antibody recognized IPC from promastigotes of all species analyzed, and it was established that the inositol residue and the ceramide are key structural features for IPC recognition by mAb LST-1. On the other hand, mAb LST-2 is specific for GIPLs of promastigotes of *L. (L.) amazonensis* and no cross-reactivity was detected with GIPLs from *L. (L.) major* and *L. (L.) chagasi*. Supported by: CAPES, CNPq and FAPESP

BM132 - Involvement of *Leishmania (Viannia) brasiliensis* lipid microdomains in parasite-macrophage interaction

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Lipid microdomains of mammalian cells are involved in different functions such as adhesion, signal transduction and polarized trafficking of proteins. These microdomains are equivalent to detergent-resistant membranes (DRMs) and are usually enriched in cholesterol and sphingolipids. In our lab it was demonstrated that DRMs in *Leishmania (Viannia) brasiliensis* presented glycoinositolphospholipids (GIPLs), inositol phosphorylceramide and cholesterol/ergosterol. In order to understand the role of DRMs in parasite-macrophage interaction, promastigotes from *L. (V.) brasiliensis* were submitted to disruption of lipid microdomains by treatment with 20mM and 40mM of methyl-β-cyclodextrin (MβCD) at room temperature for 1 hour and for control parasites the incubations were carried out in the absence of MβCD. No difference in the parasite viability was observed. By treatment of parasite with 20mM and 40mM of MβCD about 40% and 70% of cholesterol content were removed from parasites, respectively. By radioimmunoassay using monoclonal antibody SST-1, specific to *L. (V.) brasiliensis* GIPLs, it was observed that only 19% of GIPLs remain in non-ionic detergent insoluble fractions after MβCD treatment, indicating that microdomains containing GIPLs were also disrupted by MβCD. The parasites preincubated or not with MβCD were placed in plates with peritoneal macrophages for 1 hour and the non-adherent parasites were removed by washing with medium. Infected macrophages were maintained in RPMI with 10% fetal calf serum in CO_2 incubator for 24 hours. The phagocytic index was 44.9 ± 6.4 for control parasites, 22.5 ± 2.9 and 21.3 ± 0.3 for treated parasites with 20mM and 40mM of MβCD, respectively. The disruption of lipid microdomains present in promastigotes decreased the infectivity in macrophage by 50% for parasites treated with 20mM of MβCD and 53% for parasites treated with 40mM of MβCD, indicating that membrane microdomains enriched in sterol and GIPLs are important to *Leishmania*-macrophage interaction. Supported by CAPES, FAPESP and CNPq.

BM133 - Investigation of the role of vesicle trafficking in *Leishmania* terbinafine resistance.

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The *LmYPT* gene of *Leishmania* encodes for a Rab GTPase possibly involved in vesicle trafficking between endoplasmatic reticulum and Golgi complex. The gene is 603-pbs long and

is encoded in the chromosome 27 of the parasite. To investigate the role of LmYPT in mechanisms of drugs resistance the gene was cloned into the pXG1 vector and co-transfected in terbinafine-resistant cell lines overexpressing *HTBF*. The *HTBF* gene mediates resistance to terbinafine and has significant homology to the YIP1 protein of *Saccharomyces cerevisiae*, which is known to interacts with YPT and participate in vesicle trafficking (Marchini et al., 2003. MBP, 131: 77-81). Transfection of *LmYPT* led to the abolition of HTBF-mediated terbinafine resistance. Therefore, it is possible that the interaction between LmYPT and HTBF suppresses HTBF-mediated drug resistance. *LmYPT* was cloned into the pDsRed1.C1 vector and transfected into melanoma cell lines to express the parasite gene in a N-terminus fusion with the red protein. The subcellular localization of the fusion product demonstrated that LmYPT is distributed in the perinuclear region, with some extensions throughout the cell. This localization suggested that the fusion protein was close to the RE and Golgi. These results indicated not only the functional conservation of LmYPT but also its possible involvement of the vesicle trafficking machinery in terbinafine resistance. Others reagents were generated to test the interaction between LmYPT and HTBF. Our current efforts are focused in the use of a HTBF-myc fusion in the subcellular localization of HTBF. This fusion product is also being used in a immunoprecipitation assay in order to confirm the interaction with LmYPT. Supported by FAPESP, CNPq and CAPES

BM134 - Long SAGE (Serial Analysis of Gene Expression) in *Eimeria tenella*

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Eimeria tenella is one of the most relevant causing agents of poultry coccidiosis and is the model species for coccidiosis research. A central topic to better understand the biology of the parasite is the transcriptome expression level and, especially, the differential gene expression. To address this point, we carried out serial analysis of gene expression (SAGE) on sporozoites and second-generation merozoites, both representing invasive stages of the parasite. We employed the long SAGE method, a modified protocol that generates 21-bp tags, thus allowing a better tag mapping. We have generated a total of 19,095 tags (8,773 unique tags) from two different merozoite libraries and 13,650 tags (6,491 unique tags) from a sporozoite library. Analysis of individual tag abundance in both developmental stages showed that 72% of the tags occurred only once, 25% from 2 to 10 times, 2.4% from 11 to 49 times, and less than 0.2% of the tags occured more than 50 times. Only 17% of the unique tags are shared between sporozoites and merozoites. These results suggest that only a very small set of genes is highly expressed in any developmental stage. The unique tags were mapped onto the *E. tenella* genome sequence. From this set, 50% of the merozoite, and 44% of the sporozoite tags, presented hits. When these tags were mapped onto the EST/ORESTES clusters of *E. tenella* (∼42,000 reads), both libraries presented circa 22% of positive hits. The frequency distribution of these positive tags, mapped onto the genome and EST/ORESTES clusters, revealed similar results, with approximately 84% mapping to a single site, 11% to two sites, and 5% mapping to multiple sites. Another SAGE library of sporozoites under construction to dilute possible sample biases. Statistical analysis will be used to evaluate the differential gene expression of these stages. Financial support: FAPESP, CNPq and PRP/USP.

BM135 - The use of microsatelites and tandem repeats in genetic population analysis of field *Plasmodium vivax* isolates from Brazilian endemic areas

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In Brazil, 600.000 malaria cases were reported last year and around 80% of these were caused by *Plasmodium vivax*. The strategies for parasite control depend on the understanding of their genetic variability and population structure. The molecular markers are important tools that can help in this understanding. Only a few markers have been described for *P. vivax*, including microsatelites and tandem repeats (TRs). The aim of this work is to verify whether these molecular markers are polymorphic in the Brazilian isolates. We selected the most polymorphic molecular markers, being 5 TRs and 1 microsatelite. We analyzed the variability of these molecular markers in 33 *P. vivax*-infected individuals from three different Brazilian regions (Manaus/AM, Cuiabá/MT and Macapá/AP). Each locus was amplified using specific primers and DNA extracted from whole blood of the patients. The amplified products were visualized in agarose gel and some fragments were sequenced. We observed that the locus MN25 was the most variable with an average of 3.33 alleles and the locus MN23 showed the higher number of alleles (5) being the second most variable. The locus MN7 was the least variable one, showing only two alleles in the studied populations. The *P. vivax* subpopulation from Manaus were the most polymorphic ones, due to the higher average of alleles. The next step will be the selection of the most polymorphic loci for the standardization a PCR multiplex to identify *P. vivax* subpopulations. Some samples amplified were selected for DNA sequencing. Samples showing size

polymorphism on agarose gel exhibited deletion or insertion of some nucleotides, including the repeat units. Moreover, amplified fragments of the same size on agarose gels have showed single nucleotide polymorphisms, inclusive within of the repeat units. The analysis of several sequences will help to better understand the mechanisms generating the variability in TRs.
Financial support:PAPES IV-Fiocruz.

BM136 - Polymorphism of Plamodium vivax Apical Membrane Antigen 1 (AMA-1) in Brazilian Isolates.

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Studies of the population diversity of the malaria parasites have great practical significance for the development for strategies of disease control, including vaccine development. The gene encoding AMA-1 is highly conserved among the Plasmodium species suggesting that this protein is essential for parasite′s biology and survival, although exhibits allelic diversity. Considering that Plasmodium vivax is widely distributed geographically, being the prevalent specie in Brazil, the aim of this study was to characterize the genetic diversity of PvAMA-1 polymorphic domain (domain I) in P. vivax populations from different endemic areas with distinct epidemiological sets in the Brazilian Amazon. Eight-one samples from six different Amazonian Brazilian states (AC, AM, MT, PA, RO and RR) were sequenced and analyzed. Twenty-eight polymorphic sites between the amino acids 92 and 248 of the 555 present in the protein were found (against 23 previously described), including ten new polymorphic ones. The following analyses were performed between amino acids 103 and 236. The synonymous (ds) and nonsynonymous (dn) substitution frequencies were, respectively, 0,0103 $\pm$ 0,0062 and 0,019$\pm$ 0,0052. Twenty sites were informative for parsimony, allowing the discrimination of 19 haplotypes. The nucleotide variability and the haplotype diversity were π = 0,01687 $\pm$ 0,00083 and Hd = 0,908 $\pm$ 0,015, respectively. The average number of nucleotide differences was k = 6,77. Our results point to a high haplotype diversity among the sequences analyzed since there was a few nucleotide changes that generated amino acids changes. The neutrality tests (Tajima and Fu and Li‘s tests) were not significant, showing that there are evolutionary forces under this protein (probably immune responses). The mean diversity interstates were d = 0,007 indicating that there are no geographic barriers impending genetic flow between P. vivax populations among those states.
Financial support: FAPEMIG

BM137 - Retinoids biosynthesized by intraerythrocytic stages of *Plasmodium falciparum.*

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This work is part of the studies on the biosynthesis pathway of isoprenic compounds in the intraerythrocytic stages of *Plasmodium falciparum*- which is a target for the development of antimalarials. An important target for the development of new antimalarial drugs is the isoprenoid biosynthesis, which occurs in *P. falciparum* via the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. Isoprenoids play important roles in all living organisms as components of structural cholesterol, steroid hormones in mammals, carotenoids in plants and ubiquinones. The spreading virtually resistance all drugs of the *P. falciparum* calls for the identification of new therapeutic targets and the development of new drugs. Herein we described the biosynthesis, isolation and characterization of retinoids in intraerythrocytic stages of *Plasmodium falciparum.* For the first time, it is reported that the biosynthesis of retinoids in a protozoan parasite. Retinoic acid and retinol were identificated by TLC, purified by RP-HPLC and structurally characterized by biochemical and electrospray mass spectrometric analyses. We suggest that the identified the retinoids in *P. falciparum* could be exploited in the screening of novel drugs, because their absence in the human host, retinoids are excellent molecular and very attractive as potential targets for the development of new antimalarial drugs.

BM138 - A proteomic approach to unravel the secreted antigens of the Apicomplexan *Babesia bovis*

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Babesia bovis is a tick-transmitted parasite of bovines and closely related to malaria parasites. It induces severe pathology during the asexual cycle consisting of repeated steps of erythrocyte invasion and intracellular multiplication. Animals that have recovered from initial infection become a carrier for life and remain fully susceptible to reinfection but are protected from severe disease. When *B.bovis* is cultured in vitro it secretes a complex mixture of soluble protein antigens (SPA) into the medium. SPA can be used to immunize animals against severe disease but the protective components could not be identified by a trial and error approach

of fractionation and immunization. We aim to unravel the composition of SPA by 2D-PAGE and mass spectrometry to support rational vaccine development. To obtain functional insight it is crucial to reveal the origin of SPA components (e.g. secreted from invading free parasites; shed from parasite or infected erythrocyte membranes; secreted into the erythrocyte cytoplasm; non specific leakage from damaged parasites). Comparative 2D analyses were performed to compare different extracts, namely (1) SPA (culture supernatant), (2) the supernatant obtained during in vitro invasion, (3) cytoplasmic proteins of merozoites as a control for leakage from damaged or decaying cells and (4) proteins from the cytoplasm of infected erythrocytes All fractions have been produced and analysed on bidimensional gels at least 3 times in a reproducible way. LC-MS have so far identified around 10 SPA proteins and their potential origin and applicability will be discussed.

BM139 - *PLASMODIUM VIVAX*: EXPRESSION OF MEROZOITE PROTEINS ON THE SURFACE OF COS-7 CELLS TO DEVELOP AN ASSAY FOR ERYTHROCYTE-BINDING ACTIVITY.

Barbedo, M.B. *(Universidade de São Paulo)*; Rosa, D.S. *(Universidade Federal de São Paulo)*; Scopell, K. *(Universidade Federal de Minas Gerais)*; Braga, E.M. *(Universidade Federal de Minas Gerais)*; Rodrigues, M.M. *(Universidade Federal de São Paulo)*; Soares, I.S. *(Universidade de São Paulo)*

Recently, we have studied several aspects of the antibody response against recombinant merozoite antigens in individuals from malaria endemic areas of Brazil, where *P. vivax* is endemic.We found that a high frequency of individuals had IgG antibodies to recombinant proteins based on *P. vivax* Apical Membrane Antigen-1 (PvAMA-1) ectodomain. However, the role of these human antibodies is still unknown.PvAMA-1 ectodomain can be divided into three subdomains (I, II and III). Someone evidences supports the idea that subdomains I and II play important roles on erythrocyte binding activity. The aim of this study is establish an in vitro functional assay for erythrocyte binding using transfected COS-7 cells with recombinant plasmids containing selected portions of the PvAMA-1 ectodomain. The genes encoding subdomains I-II or III of PvAMA-1 were inserted into pDisplay - EGFP vector that allows expression of recombinant proteins fused to the N - terminus of enhanced Green Fluorescent Protein. Also, we used as control, constructs containing the 19 kDa C-terminal of Merozoite Surface Protein-1 ($PvMSP1_{19}$) and Duffy Binding Protein (PvDBP-RII) genes. These four constructs were used to transfect COS-7 cells using lipofectamin. Transient transfections were analyzed by immunofluorescence assay using polyclonal antibodies to PvAMA-1, monoclonal antibody anti-$PvMSP1_{19}$ or a human serum pool (anti-PvDBP-RII). Using these antibodies we confirmed the cell surface localization for all proteins. Currently, we are working in the establishment of the erythrocyte binding assay to study the role of PvAMA-1 in erythrocyte binding and to analyze the capacity of human antibodies against PvAMA-1 in blocking this possible binding.

BM140 - Protein dolichylation in *Plasmodium falciparum*

D'Alexandri, F. L. *(Universidade de São Paulo)*; Kimura, E. A. *(Universidade de São Paulo)*; Peres, V. J. *(Universidade de São Paulo)*; Katzin, A. M. *(Universidade de São Paulo)*

The post-translational modification of protein by the addition of isoprenoids has been recognized as a key physiological process and isoprenylated proteins act in different cellular events such as cell cycle control, protein-protein interactions and membrane-associated protein trafficking. Previous work from our laboratory has shown that [1-(n)-^{3}H] farnesyl pyrophosphate ([^{3}H]FPP) and [1-(n)-^{3}H] geranylgeranyl pyrophosphate ([^{3}H]GGPP) are incorporated in a number of specific proteins in *Plasmodium falciparum*. The major radioactive materials released by treatment of delipidated proteins with methyl iodide were farnesyl and geranylgeranyl but other unknown compounds were also found. In this study, these unknown compounds released from 21-28 kDa *P. falciparum* proteins were analyzed by RP-HPTLC, NP and RP-HPLC and mass spectrometry (ESI-MS). The R_f in thin layer chromatography, the retention time in liquid chromatography and the MS and MS/MS spectrum profile of one of these compounds were completely coincident with a dolichol of eleven isoprene units standard, revealing that the dolichyl group can be attached to the *P. falciparum* proteins of 21-28 kDa. Further, treatment with cycloheximide and chloramphenicol, inhibitors of *de novo* protein synthesis, did not decrease the radioactive labeling of the proteins, demonstrating that this is a post-translational modification. Extensive digestion of these proteins labeled with L-[^{35}S]cysteine or [^{3}H]FPP followed by RP-HPLC analysis of butanol-extractable proteolytic products suggested that dolichyl group is attached to cysteine residues as it occurs also in protein isoprenylation. Once that protein dolichylation was only described in tumor cell lines and probably is involved in cell replication, this study presents the first characterization of protein dolichylation in parasites and perhaps could be of use as a new target for anti-malarial drugs. Supported by FAPESP and CNPq.

BM141 - DIFFERENTIAL EXPRESSION OF SPLEEN MACROPHAGE RECEPTORS IN EXPERIMENTAL INFECTIONS OF BALB-c MICE WITH RODENT MALARIA PARASITES

Rosanas-Urgell, A. *(Universidade Sao Paulo)*; Marques, J *(Universidade Sao Paulo)*; Yamauti, F. *(Universidade Sao Paulo)*; Rodríguez, S. *(Universidade Sao Paulo)*; Kimura, E. *(Universidade Sao Paulo)*; del Portillo, H.A. *(Universidade Sao Paulo)*

In spite of major efforts, malaria continues to be the most devastating parasitic disease worldwide. Our group identified a subtelomeric multigene family, named *vir*, in the human malaria parasite, *Plasmodium vivax*, implicated in immune escape (del Portillo, HA *et al.*, 2001). Results from single-cell RT-PCR and laser confocal microscopy demonstrated that expression of *vir* genes and their encoding variant proteins is not clonal, excluding a role in the strict sense of antigenic variation (Fernandez-Becerra, C *et al.*, 2005). Our present working hypothesis is that *P. vivax* escapes spleen macrophage clearance by citoadhering to barrier cells in the spleen (del Portillo, HA *et al.*, 2004). To advance this hypothesis, we are using the lethal *P. yoleii* 17X strain, as well as the non-lethal *P. yoelii* 17XL and *P. chabaudi* AS strains in BALB-c mice to identify spleen macrophage receptors specifically activated or suppressed in experimental infections. To this end, we are performing real time PCR analysis with material obtained from spleens at different times of infection. Spleen macrophage receptor expression patterns of BALB-c mice infected with *Toxoplama gondii* and *Leishmania amazonensis* are being used as controls of specificity of receptor expression. During *P. chabaudi* AS malaria infection at 10% parasitemia, Marco, EmR1 and SignR1 receptors showed significantly higher transcript abundance than non infected mouse. As infection progressed to 50% parasitemia, the SignR1 receptor showed a 7-fold increase in transcript levels relative to controls of non-infected animals. In contrast, Tlr2 and Msr1 receptors showed down regulation of transcript levels. Presently, we are performing similar analysis with the *P. yoelii* lethal and non-lethal strains to get a comprehensive view of macrophage-receptor expression in these contrasting murine malaria models. The implications of these findings with regard to our working hypothesis will be discussed.

BM142 - Analysis of *antisense* transcripts in a central *var* gene cluster of *Plasmodium falciparum*: correlation with locus activation/inactivation?

Gölnitz, U. *(Universidade de Sao Paulo - ICBII)*; Wunderlich, G. *(Universidade de Sao Paulo - ICBII)*

Var gene transcription in *Plasmodium falciparum* is controlled by a poorly understood, epigenetically controlled allelic exclusion mechanism and apparently only one *var* gene is transcribed in trophozoites. We selected a parasite adhesion phenotype by multiple panning procedures on E-selectin where only 1 *var* gene (PFD1000c) in a centromeric cluster of 4 *var* genes and 1 *rif* gene is transcribed. To investigate if small RNA species (heterochromatin-associated RNAs, *antisense*/double strand RNAs) are involved in the transcriptional control mechanism, we used Real time PCR analysis of the selected parasite culture 3D7^{Selectin} and the unselected 3D7 wildtype. We hypothesized that upregulation of a *var* gene would coincide with the decreased abundance of complementary *antisense* RNA, while downregulated *var* genes would be associated with an increase in corresponding *antisense* RNAs. Recent microarray data demonstrated by Ralph et al., 2005, did not show a global relationship between *sense* and *antisense* production of *var* genes in *Plasmodium*. Our preliminary results are inconsistent with both hypotheses: First, *var* gene PFD0995c, which is inactive in both parasite cultures, did not show the same *antisense* RNA quantity as PFD1005c, which is also inactive in the *var* gene cluster on chromosome 4. Intriguingly, PFD1000c, which is the dominantly transcribed *var* gene in the selected parasite culture 3D7^{Selectin} but not 3D7 parasites, only showed *antisense* RNA in 3D7^{Selectin} ring stage parasites and no considerable amounts of *antisense* transcript were detected in the 3D7 wildtype culture. To confirm these results further experiments are in progress and results will be presented. Supported by FAPESP

BM143 - Symptomatic *Plasmodium falciparum* infections are predominantly caused by genetically different parasites with highly similar variant gene repertoires

Castiñeiras, C *(USP)*; Hoffmann, EH *(USP)*; Ferreira, MU *(USP)*; Albrecht, L *(USP)*; Wunderlich, G *(USP)*

In the Western Amazon, infecting *Plasmodium falciparum* isolates always show a number of genetically different parasite strains. In this area, parasites apparently also contain less variant antigen encoding *(var)* genes than parasites from other endemic regions in the world. In this study, we asked how different distinct strains, cloned from single infections, were in terms of variant antigen repertoire. After PCR amplification, cloning and sequencing of 50 partial *var* genes per strain (and 5 genetically different strains per isolate, three isolates) we observed that the number of shared *var* genes (repertoire overlap) was very high between strains (up to 22 shared genes) from each isolate. The *var* gene repertoires between strains from different isolates also showed significant repertoire overlaps (around 8 shared sequences), however, always significantly smaller than intra-isolate overlaps. Together with the finding that mosquitoes are often infected by genetically similar parasites, we conclude that a big part of symptomatic infections in the Western Amazon is caused by antigenically very similar strains which may stem from

single infective mosquito bites. Supported by CAPES and FAPESP

BM144 - Rapid genotyping of the Duffy antigen receptor for chemokines by Real-Time multiplex allele-specific polymerase chain reaction

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Plasmodium vivax is the most widely distributed human malaria species in the world causing an estimated 70-80 million cases each year. Erythrocyte invasion by *P. vivax* merozoites is absolutely dependent on binding to the Duffy antigen/receptor for chemokines (DARC), since Duffy-negative individuals are completely resistant to *P. vivax* infection. The single copy *FY* gene coding for human Duffy antigen have three mainly alleles: *FY*A*, *FY*B* and $FY*B^{null}$. Recently, the molecular basis of these alleles have been elucidated, being a single polymorphism 125G>A responsible for *FY*A* and *FY*B* alleles, and the mutation -46T>C on the promoter responsible for $FY*B^{null}$ allele, resulting in the lack of Duffy expression only in the erythroid lineage. Here, we developed and validated a less expensive assay based on the use of SYBR Green fluorescent dye to identify the three major Duffy alleles by Real-Time multiplex allele-specific PCR. This high throughput Duffy genotyping assay is extremely useful for malaria epidemiology, considering the large *P. vivax* infected populations. Deliberated mismatch and C/G tail introduced on primers allowed the Duffy genotyping based on different melting curves. DNA was extracted from peripheral blood samples of non-infected volunteers (n=57) and *P. vivax* infected individuals (n=117) from Brazilian malaria endemic area and the target sequences were amplified using real-time polymerase chain reaction. Our results showed that the frequency of individuals carrying one Duffy antigen negativity allele ($FY*B^{null}$) was significantly lower among infected *P. vivax* individuals (P=0.006). Furthermore, the frequency of genotype *FY*B/ FY*B* was significantly higher in infected individuals (P=0.031), while the frequency of *FY*B/* $FY*B^{null}$was lower in the same group (P=0.036). By using the present approach for Duffy genotyping we obtained the same results as the conventional PCR with allele-specific primers (PCR-ASP), however in a high throughput assay.

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BM145 - Structures of the Pteridine Reductase (PTR1) from *Trypanosoma brucei* Complexed with Folate Analog Inhibitors

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The enzymes dihydrofolate reductase-thymidylate synthase and pteridine reductase (PTR), are involved in pterin/folate dependent metabolism, and together represent an important target for chemotherapy of parasitic leishmanias and trypanosomes (Nare, B., Hardy, L.W. & Beverley, S.M, 1997; Hyde J. E, 1990). X-ray crystallography was used to elucidate the structure of PTR1 from *Trypanosoma brucei* in complex with folate analogues. Several ligands were assayed, amongst them: the substrate folate, melamine, 6-thioguanine, cyromazin, triamterene, and new molecules synthesised in the laboratory of Prof. C. Suckling: WSG1012 (WSG - synthesis codification), WSG1034, WSG3065, WSG3066, WSG3067. Diffraction patterns from some dozen crystals were measured; a few were selected for a full data-set collection, later processed with the computational programs Mosflm/Scala and Xds/Xscale. Of these, five structures were further refined at UEPG, the ones complexed with the ligands WSG3065 (later revealed to be absent), WSG3066, WSG3067, triamterene and cyromazin, using the programs CNS, Refmac5 and Coot. The structures were validated by the programs Procheck, Whatcheck, Sfcheck and ValidationPDB. All five structures belong to the space group $P2_1$ with unit cells around a=79Å, b=90Å, c=82Å, β=115 °, and present 4 monomers each of 268 residues per asymmetric unit and complex active sites. Besides the expected folate analog ligands (except WSG3065), several other ligands derived from the crystal preparation were modeled either near or outside the active site: dithiothreitol, glycerol, sodium and acetate ions. Analyses on the ligand positions and corresponding interactions with the protein are being carried out to understand modes of inhibition and to guide design of improved inhibitors. Acknowledgements: CAPES, University of Dundee (UD).

BM146 - Molecular identification of opportunistically pathogenic free-living amoebae in the Furnas UHE Reservoir (Alfenas, MG)

Silva, M. V. G. *(Universidade Federal de Alfenas)*; Orlando, T. C. *(Universidade Federal de Alfenas)*

The free-living amoebae *Naegleria fowleri* and *Acanthamoeba* spp. are now recognized as potential pathogens of humans and animals. *Naegleria fowleri* causes primary amoebic meningitis and *Acanthamoeba* spp. causes granulomatous amoebic encephalomyelitis and are also agents of keratitis, a severe chronic infection of the cornea. Pathogenic strains of these two genera have already been isolated from diverse habitats as thermal waters, polluted lakes and rivers, swimming pools, marine waters and soil. In our study, water samples were collected and amoebae were isolated and cultivated at room temperature on non-nutrient agar plates seeded with

living *Escherichia coli*. DNA was extracted using SDS lysis, phenol-chloroform extraction and isopropanol precipitation. For the molecular identification of the isolates primers for the 5 line and 3 line ends of the 18S rDNA gene from *Acanthamoeba* spp. (Alves, J. M. 2001) were used in order to specifically amplify *Acanthamoeba* isolates. Primers for the internal transcribed spacer (ITS) in the rDNA gene (Pelandakis & Pernin, 2002) were used for genera identification of *Naegleria* isolates. The PCR analyses identified three isolates (13%) belonging to the genus *Acanthamoeba* and two isolates (8,6%) belonging to the genus *Naegleria*. For the *Acanthamoeba* species identification, other approaches as RFLP analyses of the 18S rDNA PCR product and/or sequencing could be applied. The next step for *Naegleria* identification includes a multiplex PCR to identify the *Naegleria fowleri* and also to test RFLP analyses for the 18S rDNA gene. Since *Acanthamoeba* and *Naegleria* can act as opportunistic pathogens of human, the presence of these two opportunistically pathogenic genera of amoebas in Furnas UHE Reservoir is of extreme importance for public health as the reservoir is largely used for economical purpose as tourism and agriculture. Supported by: Unifal-MG, FINEP, FAPEMIG.

BM147 - Ca^{2+} transport in permeabilized *Phytomonas francai*

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Most of trypanosomatids are known to posses PMCA-type Ca^{2+}-ATPases located both in the plasma membrane and acidocalcisomes, and a SERCA-type Ca^{2+}-ATPase located in the endoplasmic reticulum. Experiments using promastigotes of *Phytomonas francai*, a plant parasite, permeabilized by digitonin (40 μM) allowed the identification of a Ca^{2+} uptake promoted by ATP. This activity was completely inhibited by 100 μM of vanadate, a specific inhibitor of P-type ATPases. The addition of nigericin and FCCP, had no effect in this Ca^{2+} transport. Antimycin A1 and rotenone, inhibitors of the mitochondrial respiration, on the contrary to the described in *Phytomonas serpens*, had no effect in the Ca^{2+} uptake, indicating that the Ca^{2+} transport is not promoted by this organelle. Furthermore, SHAM, inhibitor of the mitochondrial alternative oxidase present in the Phytomonas genus, was not able to inhibit the transport. The addition of Na^{+} was not capable to promote an efflux of the Ca^{2+} actively uptaked, suggesting the absence of a Ca^{2+}/Na^{+} antiport in endoplasmic reticulum. When ATP was replaced by others nucleotides, like UTP, ITP and GTP, the rate of Ca^{2+} uptake was in the range of 50% from that observed with saturating ATP, showing the low specificity of this enzyme by its substrate. Taken together, these results indicate that the plant parasite *Phytomonas francai* have a Ca^{2+} transport promoted by a SERCA-type ATPase, highly sensitive to vanadate, poorly specific to ATP.

BM148 - CHARACTERIZATION OF *Entamoeba histolytica* STRAINS ISOLATED FROM PATIENTS WITH DIFFERENT CLINICAL MANIFESTATIONS AND ANALYSES OF GENIC EXPRESSION BY MICROARRAY

Freitas, MAR *(Universidade Federal de Minas Gerais)*; Tillack, M *(Bernhard Nocht Institute for Tropical Medicine)*; Pesquero, JL *(Universidade Federal de Minas Gerais)*; Tannich, E *(Bernhard Nocht Institute for Tropical Medicine)*; Gomes, MA *(Universidade Federal de Minas Gerais)*

Entamoeba histolytica, the etiologic agent of amoebiasis, is characterized by its great capacity to destroy host tissue, leading to potentially life-threatening diseases such as ulcerative colitis or liver abscess. Data from the literature suggest that cysteine proteinases (CP) play a key role in the tissue invasion and disruption of host tissues by the *E. histolytica*. Trying to verify and elucidate the CP association with the amoebiasis pathogenesis, we propose evaluate the differential expression of 48 CP genes in *E. histolytica*strains obtained from different clinical cases. It was selected to perform this study 2 *E. histolytica*strains isolated from asymptomatic patients (452 and RPS) and 3 strains from symptomatic ones (EGG, CSP and DRP). The EGG strain was isolated from hepatic amoebiasis, DRP from anal amoebiasis and CSP from dysenteric colitis. These strains were maintained in axenic culture and characterized in respect to their capability to induce hamster hepatic abscess, cytopathic effect upon CHO cells, and CP activity. The CP gene expression in these strains was evaluated by the microarray technique using the HM1 strain as control. The strain virulence characterization *in vivo* and *in vitro* showed agreement with the patients clinical symptoms, from whom the isolates were obtained. It was observed a great variation in the virulence evaluation of the five studied strains. Nevertheless, our results did not show any statistical differences about the expression of the 48 CP analysed genes or about the CP activity. These data suggest that the functions of virulence evaluations realized in this study did not have any relation with the CP expression. Support by CAPES.

BM149 - IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN Entamoeba histolytica STRAINS BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION.

Freitas, MAR *(Universidade Federal de Minas Gerais)*; Pesquero, JL *(Universidade Federal de Minas Gerais)*; Gomes, MA *(Universidade Federal de Minas Gerais)*

The amoebiasis is a human infection caused by the *Entameoba histolytica*, an invasive and virulent pathogen that kill approximately 100.000 people each year, mainly because fulminant colitis. One of the most remarkable *E. histolytica* biological aspect is the great variability on the virulency potential, and this still yet well understood. The *E. histolytica* has the notable ability of tissue destruction that can be considered its main pathogenic behave. In spite of this, it does not extend to all strains, and the molecular bases of the cytotoxicity process have been investigated. In the present work, we analysed the differential expression of genes from virulent and non-virulent *E. histolytica* strains, using the technique of suppression by subtractive hybridization (SSH). This methodology combines the cDNA hybridization with the PCR techniques, and allows the selection of low expression genes in relation with the high expression ones. The EGG *E. histolytica* strain, isolated from a symptomatic patient and 452, isolated from asymptomatic patient, were classified by their virulence through inoculation in hamster liver and cytopathic effect in monolayers cells. Our results showed that the EGG strain destroyed 70% of the monolayers cells against 17% destroyed by the 452 strain. Furthermore the EGG strain was the only one that produced hamsters liver abscess, demonstrating a higher virulence in relation with the 452 strain. The analyses of SSH data showed that 5 genes are differentially express by the EGG strain and 5 by the 452 strain. The sequence analyse of these differentially express genes can expand the comprehension about the amoebiasis pathogenesis, meaning a possibility to define the specific inhibitors that can be used like chemotherapy or in the new molecules description that can be designated as efficient vaccine.

Support by CAPES.

BM150 - HORIZONTAL GENE TRANSFER IN THE PLANT TRYPANOSOMATID *Phytomonas* spp?

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The genus *Phytomonas* has been associated to devastating diseases in commercially important crops. Conversely, these trypanosomatids do not show apparent damage in other plant species. Recently, to unveil new features of *Phytomonas* metabolism and host interaction, we reported the cloning, sequencing and analysis of 2,190 ESTs of *P. serpens* (Pappas *et al.*, 2005). We observed that the consensus sequence (698nt) of one contig showed high similarity (BLASTX) with indolepyruvate decarboxylases (IPDCs) of phytobacteria, which, in turn, can be pathogenic and non-pathogenic to plants. This observation suggests that the putative IPDC gene of *Phytomonas* was acquired by horizontal gene transfer from microorganisms that co-existed in plants. The goals of the present study were: (i) to clone and sequence the complete IPDC gene of *P. serpens*; (ii) to perform phylogenetic analyses to explore the IPDC origin; (iii) to verify the presence of IPDC in *Phytomonas* isolates and other trypanosomatids; (iv) to investigate the genomic localization of IPDC. Results: The complete IPDC gene was cloned, showing a 1,657bp ORF that encodes 550 amino acids. BLASTX similarity searches against NCBI′s non-redundant database confirmed high similarity with IPDCs from bacteria and fungi. Preliminar phylogenetic analyses showed two clades corresponding, respectively, to IPDCs and pyruvate decarboxylases. *Phytomonas* gene clustered with the IPDC clade. Two primers were designed based on *P. serpens* IPDC sequence. PCR conditions were standardized. A detection sensitivity up to 10^{-3} ng *P. serpens* DNA suggests multiple copies of IPDC. Amplification of a ∼400bp fragment was produced in 14 *Phytomonas* isolates. PCR in 17 trypanosomatids from human and vectors showed faint amplification products in the genera *Crithidia*, *Herpetomonas* and *Leptomonas*. Southern blots confirmed IPDC is integrated in *P. serpens* genome and localized in a 3.3Mbp chromosomal band. IPDC activity and the genomic region where IPDC is inserted will be determined. Support: FAPESP and CNPq.

BM151 - Investigation, isolation and preliminary molecular characterization of trypanosomatid in plants and insects.

Peloso, E.F. *(Universidade Federal de Alfenas)*; Da Costa, M.P. *(Universidade Federal de Alfenas)*; Silva, T.M. *(Universidade Federal de Alfenas)*; Souza, L.B. *(Universidade Federal de Alfenas)*; Franco, M.C. *(Universidade Federal de Alfenas)*; Marques, M.J. *(Universidade Federal de Alfenas)*; Faria e Silva, P.M. *(Universidade Federal de Alfenas)*

Several studies demonstrate the occurrence of *Phytomonas* (Trypanosomatidae) in various plants and insects, however, few species has been isolated or are available as reference samples. The work evaluated the presence of *Phytomonas* in plants and insects in Alfenas, MG, Brazil. Forty samples of the plant *Euphorbia pillulifera*, 25 of *E. Prostrata* and 84 of *Allamanda cathartica* were evaluated. We also analyzed samples of the insects *Nezara viridula* and *Lincus* sp, found in the referred plants. The samples were inoculated in biphasic medium (blood agar plus Roitman¡¯s complex medium amended with penicillin, streptomycin, gentamycin and nys-

tatin) and incubated at 28 degrees C for 92 hours. The isolates obtained were incubated at 28 degrees C in Roitman¡¯s complex medium for 48 hours and DNA was extracted by the phenol/chloroform. For RAPD, the primers OPA 3 and OPA 17 were used under annealing at 36 degrees C in a total of 45 cycles. The band pattern generated of the isolates obtained was compared with a reference sample of *Phytomonas serpens*. The frequency of occurrence of flagellates was 88.0% in samples of *A. cathartica*, 62.5% in *E. pillulifera* and 60.0% in *E. prostrata*; 80.0% in *Nezara viridula* and 20.0% in *Lincus* sp. Flagellated were isolated from one sample of the plant *A. Cathartica* and from one of *N. Viridula*. Find strong similarity in the band pattern among the samples obtained from plants and insects but not among the isolates and the reference sample. Concluded that isolates obtained from plants and insects might be the same organism that belongs to the *Phytomonas* genus. This observation added molecular evidences to the hypothesis that *Nezara viridula* acts as a vector of *Phytomonas* to infect plants. Further studies are necessary to better characterization of these isolates. Supported by Capes, CNPq, Fapemig, Finep, Unifal-MG.

BM152 - Differential gene expression analysis of primary culture of skeletal muscle cells infected with *Toxoplasma gondii* tachyzoites

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Microarray analysis is a powerful technique to investigate changes in gene expression occurring during an infectious process. This work aims to study the interaction between a host cell and an intracellular parasite at the transcriptional level, using a high density microarray (Genechip). Samples were collected at 30 minutes, 4 hours and 24 hours of interaction, using skeletal muscle cells from primary culture infected with tachyzoites of Toxoplasma gondii, strain RH in three independent experiments. We have selected genes modulated in the infectious process using two different analysis methods (Li-Wong and gcRMA). In addition, a comparative analysis with previously published work (BLADER et al. 2001, JBC 276:24223-31) showed a good correlation between both datasets. We have observed 42 differentially expressed genes (DEG) at 30 minutes, 95 (DEG) at 4 hours and 631 (DEG) at 24 hours of infection, after a selection based in statistical (5% FDR) and fold change (1.75x) criteria. The DEG were grouped according to Gene Ontology (GO) and some important functions were selected: 120 genes (27.6%) are involved on nucleotide and amino acid metabolism, 78 genes (17.9%) on transcriptional regulation, 77 genes (17.7%) implicated on cellular signaling, 44 genes (10.1%) related to lipids and carbohydrates metabolism, 29 genes (6.7%) implicated on cell cycle, 28 genes (6.4%) on cellular adhesion and cytoskeleton, 24 genes (5.5%) involved on protein synthesis and degradation and 22 genes (5.1%) on apoptosis. The comparison between the selected genes and gene interaction information were used to select several protein networks modulated during the infection, including vesicles and vesicle mediated transport, inflammatory response and apoptosis genes. These data provide new insights into the Toxoplasma infection, in particular with cells where the cystogenesis in vivo occur. Financial support from NIH, CNPq, FAPERJ, IOC and PAPES-Fiocruz.

BM153 - Cystogenesis induced by temperature variation in two *Toxoplasma gondii* strains with diverse genetic profiles

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During *Toxoplasma gondii* infection, inter-conversion between tachyzoite and bradyzoites are one crucial step in the cell biology of this agent. Tachyzoites are fast-growing forms found in the acute stage of infection, and bradyzoites are intra-cystic slow growing forms, remaining in organs of the host in the chronic stage of the infection. The conversion tachyzoite-bradyzoite could be produced *in vitro*, by chemical (pH) or physical (heat) stress and each form presented specific antigens, that could be used as a markers of each stage. Each strain of *T.gondii* presented specific characteristics of virulence and cyst production. In this study, we studied the cystogenesis in two *T.gondii* strains, using gradient models of temperature stress, looking for thresholds and stage markers. Free cell tachyzoites were incubated in a temperature gradient by 6 h, with ultrastructural analysis, protein synthesis, oxidative mitochondrial metabolism and expression of BAG-1 mRNA and protein, a marker of bradyzoite stage. Short period culture of free cell tachyzoites was used to avoid inter-conversion induced by cellular penetration and cell host apoptosis by heat shock. There are clear temperature threshold of cyst induction around 38 °C for the RH strain and 36,5 °C for ME-49 strain, determined by a low steady state protein synthesis and oxidative metabolism, without loss of cellular viability or structure, confirmed by the expression of BAG-1 m-RNA after heat shock. This model allows clear-cut studies in the early steps of cyst formation, becoming a useful tool for targeting cyst formation for therapeutic or pathogenesis studies in toxoplasmosis. The different temperature threshold of each strain could represent selection for intermediate host temperature, as mammals had lower mean body temperature as compared to birds.

BM154 - Sialoglycoconjugates in choanomastigote-shaped trypanosomatids: taxonomic implications

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The genus *Crithidia* comprises trypanosomatids usually found inhabiting the digestive tract of insects. Several biochemical and/or molecular approaches have been used to show that this genus is highly heterogeneous. However, choanomastigote form is considered the characteristic stage for the genus *Crithidia*, and is characterized by its barleycorn form. Lectins have become valuable tools to study the insertion, fate, distribution and function of glycoconjugates on and in parasites. Here, the glycoprotein profiles of seven choanomastigote-shaped trypanosomatids (six *Crithidia* spp. and one *Herpetomonas* sp.), which have been suggested to form three distinct taxonomical groups (*Crithidia, Angomonas,Strigomonas*), were analyzed by Western blotting using the lectins *Limax flavus* (LFA), *Sambucus nigra* (SNA) and *Maackia amurensis* (MAA), which specifically recognize sialic acid residues, and Concanavalin A (ConA) that recognizes mannose-like residues in glycoconjugates. All lectins showed a sugar-inhibited recognition with the parasite extracts, with the exception of LFA, which did not show any reactivity with the studied species. The SNA agglutinin presented a characteristic and specific pattern for each taxonomical group. The MAA lectin showed an identical profile for all species analyzed, while ConA grouped the choanomastigote-shaped species in two different patterns, one specific for the *Angomonas* group, and the other comprehending both *Strigomonas* and *Crithidia* groups. These results corroborate the heterogeneity of the genus *Crithidia*. The dendrogram analysis indicates the existence of three main groups of choanomastigote-shaped trypanosomatids. One group (*Angomonas*) included some choanomastigote-shaped parasites isolated from insects and harboring a bacterium-like endosymbiont in its cytoplasm (*C. deanei, C. desouzai,H. roitmani*). Another group (*Strigomonas*) is represented by *C. oncopelti*, an endosymbiont-bearing trypanosomatid isolated from a plant. The third group (*Crithidia*) composed of choanomastigote-shaped insect trypanosomatids without endosymbiont (*C. fasciculate, C. lucilae, C. guilhermei*). Therefore, we suggest a reevaluation of the taxonomic status of the heterogeneous *Crithidia* genus. Financial support: CNPq, CAPES, FUJB.

BM155 - Exploring the *Phytomonas serpens* genome

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Phytomonads are flagellates found in phloem, fruits/seeds and flowers in a wide variety of plants species and very little is known about the genus *Phytomonas*. The goal of this work is to explore the *Phytomonas serpens* genome by generating and comparatively analyzing sequences of its genome. Genomic DNA of *P. serpens* was partially digested with the restriction enzyme *Sau*3A. DNA fragments with an average size of 1.0-3.0 kb were cloned into pUC18 cloning vector. 829 clones of the *Phytomonas serpens* genomic DNA library were sequenced obtaining a total of 379 high quality sequences GSS (Genome Sequence Survey). Sequences from the EST (Expressed Sequence Tag) project of *P. serpens* available at GenBank were used to complement this study. 221 GSS-nr (non-redundant GSS) and 697 EST-nr (non-redundant EST) were compared with different databases by BLAST, obtaining 599 (65,25%) clusters with positive hits and 303 (33%) clusters without hit, indicating potential species-specific genes. 357 (38.9%) clusters were partially annotated using Gene Ontology (GO), representing the first analysis of *P. serpens* genome using GO. Phylogeny (individual and concatenated genes) and similarity studies with Taxonomy database from NCBI were carried out corroborating that *P. Serpens* is most similar to the *Leishmania major* genome. 20 hypothetical genes in the GSS sequences were identified by GLIMMER (Version 2.15). We found genes in GSS sequences that were not present in EST before, among them: Pyroglutamil-peptidase I, proliferating cell nuclear antigen (PCNA) and Peptidase_M8. More sequencing is being done from *P. serpens*, in order to have a better picture of its genome. Supported by PAPES IV, Fiocruz, CNPq

BM156 - Homologues of leishmanolysin expressed by *Crithidia deanei* persuade the interaction with mammalian fibroblasts: influence of the endosymbiont

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Crithidia deanei is an insect trypanosomatid that harbor a bacterial symbiont in its cytoplasm, which can be removed by antibiotic treatment (cure). It is well known that the intracellular symbiont is considerably integrated into the physiology of the host cell. The possibility of comparison of endosymbiont-containing (wild strain) and endosymbiont-free populations (aposymbiont strain) of the same species has made trypanosomatids an interesting model to study symbiotic relationships. In this context, a correlation between differential surface molecules expression and the advantage of the endosymbiont-harboring strains on the interaction step

has been attempted for different cell lineages and invertebrate vectors. In the present study, we demonstrated the influence of endosymbiont and the surface leishmanolysin-like molecules of *C. deanei* in the interaction with fibroblasts (3T3 lineage) in vitro. Initially, we confirmed that the *C. deanei* wild strain expressed a higher amount (2-fold) of leishmanolysin-like molecules in comparison with cured strain by flow cytometry analysis. Interaction with fibroblasts was performed up to 4 h. The aposymbiotic strain of *C. deanei* presented interaction rates about 2- and 3-fold lower with fibroblasts when compared with the endosymbiont-bearing counterpart, respectively, after 1 and 2 h of interaction. However, after 3 and 4 h, we did not observe any difference in the association indexes between both strains of *C. deanei* and fibroblasts. In order to ascertain the possible function of homologues surface leishmanolysin molecules of *C. deanei* during the interaction process, we pre-treated the parasites with two distinct anti-leishmanolysin (H50 and H52) antibodies (at 1:1000 dilution) as well as the fibroblast cells with purified leishmanolysin-like from *C. deanei*. Collectively, our results showed that both treatments significantly reduced the association index between *C. deanei* and fibroblasts, suggesting a possible role for the leishmanolysin-like molecules in the interaction process. Financial support: CNPq, FUJB and FAPERJ.

BM157 - Detection of cell-associated leishmanolysin-like molecules in three distinct *Leptomonas* species: *L. collosoma, L. samueli, L. wallacei*

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The *Leptomonas* genus comprehends parasites of the gut and associated organs of a wide range of insects, which are classically nonpathogenic to humans. However, a case of leishmaniasis-like syndrome was reported by a parasite belonging to the *Leptomonas* genus. Recently, our group reported the presence of leishmanolysin-like molecules in a wide range of monoxenous trypanosomatids belonging to the *Herpetomonas, Crithidia* and *Blastocrithidia* genera, playing essential roles in the parasite nutrition and in the interaction with the invertebrate. Leishmanolysin is a zinc-dependent metallopeptidase mainly located in the surface of *Leishmania*. This metallopeptidase is a well-known virulence factor expressed by these digenetic protozoa. The present study aims to characterize the proteolytic profile and to detect the presence of homologues of leishmanolysin molecules in three *Leptomonas* species *L. collosoma, L. samueli, L. wallacei*. Parasites were grown in brain heart infusion supplemented with 10% fetal bovine serum at 26°C for 72 h. The proteolytic profiles were analyzed on gelatin-SDS-PAGE containing gelatin as a substrate. The results demonstrated that *L. collosoma* did not produce any peptidase under our experimental conditions, while *L. samueli* and *L. wallacei* produced distinct proteolytic profiles. EDTA and 1,10-phenanthroline, two metallopeptidase inhibitors, inhibited all proteolytic activities. However, EGTA, leupeptin and E-64 did not alter the peptidases behavior. The best hydrolyses were observed at acidic pH 5.5. Leishmanolysin-like molecules were detected in the three *Leptomonas* studied by flow cytometry using anti-leishmanolysin antibodies. Additionally, immunoblotting analysis showed the presence of a reactive polypeptide of 63 kDa in the cellular extract in these *Leptomonas* species. Our results suggested an immunological cross-reactivity between *Leptomonas* and *Leishmania* species. Finally, the ability of *Leptomonas* to express homologues of *Leishmania* virulence factors confirms its relevance as a model for the biochemical studies among the Trypanosomatidae family. Financial support: CNPq, FUJB, CAPES.

BM158 - Partial purification and characterization of digestive proteinases involved in hemoglobin processing in the gut of the tick *Boophilus microplus*

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Hb 33-61 is an antimicrobial peptide derived from the α chain of bovine hemoglobin. It was purified from the gut contents of the tick *Boophilus microplus* (*J. Biol. Chem.* 274: 25330-25334, 1999). Previous studies suggested the presence of aspartic- and cysteine proteinases involved in the generation of this peptide. Enzyme activities were determined at pH 4 and 7 using chemically synthesized fluorogenic substrates containing the aminoacid sequences 29-35 (named SF 29-35) and 57-67 (named SF 57-67) of the α chain of bovine hemoglobin. Firstly we determined enzyme enrichment in different midgut compartments: gut contents, digest cells and total gut homogenates. Gut contents showed low aspartic- and cysteine proteinase activity compared to the other compartments. In total gut homogenates, aspartic proteinase activity was detected at pH 4, using the substrate SF 29-35. Cysteine proteinase activity was detected at both pH using SF 57-67. Initial purification was performed with total gut homogenates in FPLC system. These homogenates were loaded onto a Mono Q column previously equilibrated with 50 mM Tris-HCl pH 7.4 and eluted with a 0-0.5 M NaCl gradient in the same buffer. This chromatography yielded one aspartic proteinase peak that cleaved SF 29-35 at pH 4.0 and was inhibited by pepstatin, and a major cysteine proteinase activity peak that cleaved SF 57-67 at pH 4 and 7, increased with cysteine and was inhibited by E-64. The sites of aminoacid cleavage of SF 29-35 and SF 57-67 were analyzed through liquid chromatography coupled to mass spectrometry (LC/MS). Cleavage was detected at the aminoacids M32-F33 and L34-S35 in SF 29-35, and at A63-A64 and T67-Q68 in SF 57-67. Thus the expected cleav-

age site was detected only in SF 29-35 (M32-F33), but not in SF 57-67 (K61-V62). Aspartic and cysteine proteinases are being further purified through hydrophobic interaction chromatography. Supported by FAPESP and CNPq.

BM159 - Morphology, molecular characterization and phylogenetic relationships among Brazilian trypanosomes from lizards.

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Trypanosomes infect several lizards species in all continents. Although controversial, traditional taxonomy is based on morphology of blood trypomastigote and host origin, most species lack molecular studies. There are few cultures of trypanosomes from lizards and only 3 were included in phylogenies of *Trypanosoma*. Despite descriptions of trypanosomes in South America lizards, cultures were not obtained so far and the genetic diversity was not investigated. This study was focused on isolation and characterization of Brazilian lizard trypanosomes. Despite examination of more than 250 hemocultures, we isolated only 8 trypanosomes from lizards: 4 of *Tropidurus plica* from the Amazonian Region (RO State) and 4 of *Mabuya frenata* from Central Brazil, 2 from MS and 2 from MT. Morphological analysis disclosed high pleomorphism between cultured epimastigotes from both different lizard species and within the same culture. Molecular characterization was done by polymorphisms on kDNA minicircles, SSU and ITSrDNA. The isolates from *T.plica* showed minicircles from ∼2,3 to 2,5kb, whereas isolates from *M.frenata* presented larger minicircles. Length polymorphism of PCR-amplified ITSrDNA also distinguished between isolates of *T.plica* and *M.frenata*. RFLP of ITSrDNA distinguished two isolates of *T.plica*. Thus, KDNA and ITSrDNA markers suggested that isolates of the same species are more closely related each other than to isolates from distinct species. Phylogenies based on SSUrRNA clustered all Brazilian trypanosomes in a highly supported clade, which were segregated into two branches according to lizards-species. This clade was closest to clade containing *T. thereziene* (African chameleon) and anuran trypanosomes (*T. mega* and *T. rotatorium*), which is positioned nearest to fish and chelonian trypanosomes, clearly separated from the other clade of lizards trypanosomes constituted by *T. varani* (African lizard) and *T. scelopori* (USA lizard). Thus, phylogenetic relationships among lizard trypanosomes indicated high complexity and polyphyletic origin of species.
Supported CNPq, FAPESP.

BM160 - Isolation, morphological and molecular characterization of free-living protozoa of the phylum Euglenozoa

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The phylum Euglenozoa comprises flagellate protists of Euglenida, Kinetoplastida and Diplonemida with a broad diversity of lifestyles, ranging from free-living phototrophs, osmothrophs and phagotrophs, to symbionts, commensals and obligate parasites of plants, invertebrates and vertebrates. The kinetoplastids comprise trypanosomatids and bodonids and are the most extensively studied euglenozoans, whereas euglenids and diplonemids were poorly studied, making the phylogeny of the Euglenozoa unclear and controversial. There is no data on the diversity of Brazilian free-living euglenozoans. To evaluate the diversity of these flagellates in this study we examined soil and freshwater samples from important Brazilian biomes: Amazonia, Pantanal and Atlantic Forest. Initially, samples showed a high diversity of protozoa and were divided for both culture and DNA extraction to assess the diversity before culture-selection. Several culture media and growth conditions were tested, and different approaches have been used to obtain axenic and cloned cultures. The majority of samples contain two or more flagellates, alongside ciliates and amoebas. So far, we have isolated over 40 flagellates, either green or colourless, most presenting one flagellum, few are biflagellates. Despite high variability of shape and size, they are morphologically compatible with euglenids and bodonids. SSUrRNA of new isolates was PCR-amplified using specific primers for euglenids or kinetoplastids and results corroborated the morphological findings. Length polymorphism of PCR-SSUrRNA disclosed a high diversity among the new isolates. Sequences of SSUrRNA of new isolates were aligned with other euglenozoans (GenBank) to assess: taxonomy of the new isolates; relationships among Brazilian euglenozoans from distinct biomes; relationships between isolates from distant countries. These analyses disclosed high genetic diversity among new and old isolates, even when recovered from the same sample, thus confirming the complexity of free-living euglenozoans. Inclusion of new isolates in the phylogenies of Euglenozoa is essential to understand the evolutionary relationships within this taxon.
Supported by CNPq.

BM161 - The histone chaperone Anti-Silencing Factor 1 (ASF1) in *Leishmania major* and *Trypanosoma brucei*

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Anti-Silencing Factor 1 (ASF1) has been shown in distinct organisms to be a histone chaperone that contributes for the histone deposition during the nucleosomes assembly in newly replicated DNA. It has been shown that ASF1 is required for chromatin disassembly for transcriptional activation. The activation of ASF1 seems to be involved in the cellular response to DNA damage. In *L.major* the ASF1 gene (LmASF1) is located at one of the ends of chromosome 20, its sequence shows 75% of identity with the *Trypanosoma brucei* homologue. In order to study the function of ASF1 in trypanosomatids we used a combination of approaches: (i) overexpression of ASF1 in *L. major*, (ii) ASF1 knockdown in *T. brucei* via RNA interference and (iii) heterologous expression of LmASF1 in *E. coli* for ASF1 antibody generation. *L.major*, CC-1, has been transfected with a copy of LmASF1 cloned into pX63NEO to overexpress this gene in an extrachromosomal environment. Increasing concentration of drug of selection (G418) was used to induce overexpression of the LmASF1 in transfectants. As expected, we observed increased number of episomal copies and a corresponding increased amount of LmASF1 transcript in the over-expressors. Nevertheless, western blots revealed no difference in the level of the protein between transfectant and wild type. Immunolocalization revealed that the protein is spread over the cytoplasm and not noticeable into the nucleus. We investigated a possible phenotypic difference regarding sensibility of mutants to DNA damage agents and we observed an increased sensibility of mutants in the presence of Phleomycin. Knockdown of ASF1 transcript in Trypanosoma brucei by RNA interference caused a decreased rate of growth of *T. brucei* procyclic forms in axenic culture. Suscetibility of *T. brucei* mutants to DNA damage and cell cycle disturbances in both mutants, *L. major* and *T. brucei*, are under investigation. Financial support: CAPES; CNPq, FAPESP

BM162 - Bat trypanosomes of the subgenus *Schizotrypanum*: Biological characterization and genealogy of Cathepsin L-like (cruzipain) genes.

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Chiroptera are the only hosts of most *Schizotrypanum* trypanosomes, excepting *T. cruzi*, which infects several mammalian orders. *T. cruzi* and *T. cruzi marinkellei* (*T. cruzi*-like) are restricted to America whereas *T. dionisii* is widespread in the World. The biological cycles and host-restriction of these bat trypanosomes are still unclear. Comparative studies on *T. cruzi* and *T. cruzi*-like can help to improve our understanding of host-interactions of pathogenic and non-pathogenic trypanosomes. *Schizotrypanum* species isolated from Brazilian bats were classified by morphology and SSUrRNA phylogeny. Isolates of *T. c. marinkellei* and *T. dionisii* were compared with *T. cruzi* showing in culture features that are shared by all *Schizotrypanum* spp: morphology; metacyclogenesis; ability to invade mammalian cells; intracellular multiplication as amastigotes and differentiation to tripomastigotes. Different from *T. cruzi*, but in agreement with their classification, *T. c. marinkellei* and *T. dionisii* isolates did not developed in triatomines. Cathepsin L-like are cysteine-proteases developmentally regulated with important roles in the cell-invasion, differentiation and pathogenesis of *T. cruzi*. Cruzipain (cruzain) is a cysteine-protease extensively characterized in *T. cruzi*. All *Schizotrypanum* spp. presented high proteolytic activity and profiles similar to *T. cruzi*. Sequences corresponding to catalytic-domain of cruzipain genes of *T. c. marinkellei* and *T. dionisii* were aligned with sequences from *T. cruzi* and *T. rangeli*. Genealogies using nucleotide or aminoacid sequences positioned *T. cruzi* ($\tilde{2}$% aminoacid divergence among lineages) closer to *T. c. marinkellei* ($\tilde{6}$%) than to *T. dionisii* ($\tilde{2}$0%). Despite these polymorphisms, sequences encoding cruzipain-like from these species always formed a clade separated from *T. rangeli*. Genealogy of cruzipain-like sequences is in agreement with SSUrRNA-phylogeny. We also obtained sequences from bat-restricted trypanosomes that clustered with Cruzipain 2. Understanding the evolutionary relationships among cysteine-proteases of trypanosomes closely genetic related to *T. cruzi* can help to identify their function in life cycles and pathogenic processes. Support: CNPq/Fapesp

BM163 - Structural and functional genomics of a endosymbiotic process: *Crithidia deanei* as a model

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Some protozooan organisms (**Tripanosomatidae** family) contain a symbiotic bacteria in their cytoplasm. Through genetic, biochemical and ultra-structural studies of the association between a symbiotic bacteria and a host protozoa, it is possible to gain a better understanding of how a eukaryotic cell works. Besides that, the study of a symbiotic relationship between two primitive organisms provides important clues about the evolution of endosymbiosis itself. Both cells evolve in a very tight manner and, as a consequence, several biochemical pathways are built from both genomes and several ultra-structural changes are evident. A fast and reliable approach to obtain a broad and profound characterization of the biochemic, genetic, evolutive and physiological aspects of symbiosis is genome sequencing. In this sense, we have conducted a DNA sequencing project of the *Crithidia deanei* endosymbiont, through a whole genome shotgun approach. It is estimated that the genome spans 850 kb and we have produced a 4-fold initial coverage, with 80 contigs (largest, 70 kb) contained into 16 scaffolds (largest, 125 kb). The available data has already been analysed in order to obtain further insights of genome adaptation to endosymbiosis. By now, we have approximately 600 CDS identified, which are being catalogued using Genbank (BLAST), PFAM (HMMer), KEGG and COG, among other tools/databases, to identify missing genes and essential biochemical pathways, present in the endosymbiont genome. We are currently working in the genome closure phase, as well as implementing a microarray platform to study the differential expression of endosymbiont genes, which will be further enhanced by a LC-MS/MS proteomic approach.

BM164 - Comparing pathogenic protozoan genomes by orthology and distant homologies approaches

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Five of the most important and causing-diseases human parasites had their genomes sequenced in the recent past. Three are pathogenic members of Family Trypanosomatidae that cause sleeping sickness (*Trypanosoma brucei*), Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania spp*). The fourth species is *Plasmodium falciparum*, causative agent of human malaria, and the latter is *Entamoeba histolytica*, causative agent of amebiasis. Availability of pathogenic protozoa genome sequences allows to perform comparative analyses towards a better understanding of the genetic and evolutionary bases of the parasitic models and lifestyles of these pathogens. Identification of homologs became an important tool to transfer annotation from experimentally or better characterized genes, allowing (re-)annotation of genes and genomes. This study aims to extend one of the most used principles in ortholog identification, the bi-directional best hit using Blast, by using HMMs (Hidden Markov Models). The methodology proposed uses the traditional approach to identify homolog genes by using the OrthoMCL program, then uses the ortholog groups to build HMM models, to look after distant homologies among the models built. OrthoMCL found 13.615 homolog groups, where 7.137 of those were composed only by orthologs and 6.478 of recent paralogs; composed by 12.566 proteins from *T. cruzi*, 827 from *T. brucei*, 815 from *L. major*, 14.418 from *P. falciparum*, and 4.414 from *E. histolytica*. OrthoMCL also identified 569 orthologs groups common to all five organisms, comprising 7.380 genes. Preliminary results show a promise from this approach for comparative genomics, where models corresponding to subgroups of multigenic families (mucins, transsialidades, *PFEMPI*, *MASP*) were capable of finding each other (corroborating previous annotation), and other groups containing only hypothetical proteins, with highly significant e-values. Preliminary analyses using InterProScan have corroborated the results achieved using our strategy, by finding several domains and signatures, typical of the families in the queries and their corresponding hits.

BM165 - A *Trypanosoma cruzi* Genome Study by Sequence-Structure HighThroughput Comparative Modelling and Enzymatic/Evolutionary Approach: Steps to the Identification of Potential Molecular Targets for Chagas Disease

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In this work, we investigated the *Trypanosoma cruzi* genome, by highthroughput comparative modelling, and identified/classified the subset (9.13% of the total genome) of *T. cruzi* enzymes which 3D-structures could be theoretically

determined. The 1876 models with, at least, the sub-subclass enzyme classification number identified, were investigated by the Analogue Enzyme Pipeline (AnEnπ) to recognize which of these have or not similarity with human enzymes. Aproximately 94.7% were considered similars and 5.3% as putative analogues to *Homo sapiens* enzymes. These results are valuable ones to guide future investigations to identify potential molecular targets for structure based rational drug design researches for Chagas disease. **Key words:** *Trypanosoma cruzi*, Chagas Disease, Structural Comparative Modelling and Molecular Targets

BM166 - The massive nuclear transfer of mitochondrial sequences of *Toxoplasma gondii* has not precluded the maintenance of an organellar genome

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Toxoplasma gondii is an important intracellular protozoan parasite that infects a wide range of warm-blooded vertebrates. The whole genome sequence has been recently derived and made publicly available (http://www.toxodb.org/). The 35 kb apicoplast genome was also sequenced and annotated, but the mitochondrial genome still remains to be characterized. Apicomplexan parasites harbor the smallest mtDNA of all living organisms, constituted by either 6 kb linear monomeric (*Theileria*) or concatameric (*Plasmodium* and *Eimeria*) molecules. The genome is composed by three cytochrome genes (*cyt*b, *cox*I and *cox*III) and some stretches of actively transcribed rRNA genes, but no tRNA genes are found. *T. gondii* presents a massive amount of interspersed truncated copies of mitochondrial sequences in the nuclear genome. This characteristic hampers the distinction between mtDNA- and nuclear-derived sequences when a shotgun sequencing approach is used. Since the mitochondrion represents a potential target for some classes of drugs, we decided to unravel the nature of the mtDNA of *T. gondii*. In this regard, using some bioinformatics tools, we derived the complete sequence of the three individual cytochrome genes. Using a combinatorial PCR approach, followed by DNA sequencing of the amplified products, we were unable to obtain the complete mtDNA sequence due to the many nuclear/mitochondrial chimeric sequences. Thus, we decided to check if the parasite in fact contains a mitochondrial genome inside the organelle. In this direction, we used *cyt*b and *cox*I fluorescent probes in a FISH assay. Using a dye (MitoTracker) that specifically stains the mitochondrion, we were able to show a co-localization of the dye and the probes, strongly suggesting that a complete nuclear transfer of the mtDNA has not occurred. We intend now to purify and characterize the mtDNA from either cesium chloride gradients stained with DAPI or from isolated organelles obtained by cell fractionation.

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BM167 - The Analogous Enzyme Pipeline (AnEnPi) - searching enzymatic functions with independent evolutive origins

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Enzymes are proteins capable of catalyzing chemical reactions. Analogous enzymes are the result of independent evolutionary events. Although they are able to catalyze the same reactions, they present no detectable sequence similarity at the primary level, and possibly different tertiary structures as well. A detailed study of analogous enzymes may reveal new catalytic mechanisms, add new information about the origin and evolution of biochemical pathways and disclose potential targets for drug development. The criteria normally utilized in the search of new targets for the development of new drugs are normally based in the specificity of enzymatic functions and not in the evolutionary origin of the different forms of a determined enzyme. In this work, we implemented a tool (AnEnPi) capable of clustering protein sequences and visualizing the results; it also carries out similarity searches via BLAST and/or HMMER and reconstructs metabolic maps. With this tool it was possible to analyze data about the presence/absence of enzymatic functions in the genomes. The data obtained with AnEnPi can be used in the discovery of possible cases of lateral gene transfer and in the search of new drug targets, based in the structural differences of enzymatic activities shared by the human and its parasites. In addition, the variability of the metabolic pathways can be studied, taking into account the existence of analogy events between different organisms. In the future, studies to determine and compare the tridimensional structures of the proteins involved with these putative events of analogy will be executed in order to produce a list of candidates for further screening as potential drug targets.

BM168 - *In silico* Identification and Characterization of Candidates to Tripanosomatides Translation Initiation Factors

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Protein synthesis or translation is a basic and essential process for the survival of all living beings. One of its key points is its initiation stage, which is regulated by the action of at least twelve protein factors called eIFs (eukaryotic Initiation Factor), summing about 30 polypeptides in mammals. The trypanosomatids display unique cellular characteristics such as its regulation of gene expression, which occurs mainly at the post-transcriptional level. In this context the protein synthesis is a potential target for regulatory mechanisms, however little is known about this process in trypanosomatids.

In previous studies, the eIF4F complex was investigated in these parasites and multiples homologues for each one of its three subunits were observed. In this work, bioinformatic tools were used to identify and characterize proteins homologous to the others eIFs in *Leishmania major*, *Trypanosoma brucei* and *T. cruzi*. Homologues to the factors eIF1, eIF1A, eIF5, eIF5A, eIF5B, eIF6 and the seven sub unities of the eIF3 complex (b, c, d, e, f, i, k) have been identified. In contrast to what was observed for the subunits of eIF4F, and with the exception of the eIF3b subunit (with two identified homologues), only one homologue was identified for each factor studied. The analysis of the amino acid sequences showed a variation in the degree of conservation of these homologues when compared to that of other eukariotes (from 22% identity for eIF3k up to 58% for eIF6). Also, in some cases it was possible to map mutations unique to the trypanosomatids. Overall, the results obtained indicate that the initiation of protein synthesis is conserved between trypanosomatids and other eukariotes. However, significant differences seem to occur and deserve to be studied in these parasites.

BM169 - A *Trypanosoma cruzi* differentially expressed gene clone collection (*T.cruzi* DEG - ORFeome)

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Our group has been working in a functional genomics study of *Trypanosoma cruzi* including the cellular differentiation from epimastigotes to metacyclic trypomastigotes (metacyclogenesis), the comparison among different life cycle forms, stress responses and others biological conditions, selecting more than 1000 genes as differentially expressed (DEG). For the elucidation of the gene function, protein localization, structure and interactions of the selected genes we began to generate a *T.cruzi* clone collection of protein-encoding open reading frames (ORF) from the differentially expressed genes (*T.cruzi* DEG - ORFeome). For this we use DNA cloning tools based on recombination for mass cloning to the generation of flexible ORFs collections. The clone collection is based on Gateway technology (Invitrogen) and by using this technology, it can provide multiple expression and functional analysis methods using different destination vectors. An important set of the *T.cruzi* selected DEG are hypothetical proteins and one of the major impact of this clone collection can be an considerable help for the improvement of genome annotation by the high-throughput expression of proteins in many different experimental settings. For this we are using some commercial vectors (for heterologus expression in bacteria, yeast two hybrid system), and adapt some *T.cruzi* reverse genetic tools to this technology (regulated over expression in *T.cruzi*, pull-down, protein tagging and others). Until the moment we have more than 400 *T. cruzi* DEG cloned and the first results in terms of structure characterization, antibodies production and protein-protein interaction for several clones from *T.cruzi* DEG - ORFeome. This library of protein-encoding open reading frames will allow us to perform functional studies of the corresponding DEGs with high-throughput protein analyses in order to obtain better understanding of the biological functions of the selected genes.

BM170 - Characterization of putative chromosomal rearrangements between three *Leishmania* species: An in silico approach

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Nowadays the large number of complete and ongoing genome projects related to pathogenic organisms together with the advances of bioinformatics, permit not only a comparison between species on the basis of homologous proteins sequence differences but with respect to the rearrangements of groups of genes and large fragments of chromosomes. Protozoan parasites of the genus *Leishmania* are the causative agents of leishmaniasis, a spectrum of diseases with different clinical manifestations ranging from mild cutaneous lesions to severe fatal visceral form. Leishmaniasis is widely distributed in Tropical and Sub-Tropical areas of the world and one of the major parasitic diseases targeted by the World Health Organization (WHO). In this study, we are focusing on characterization of putative chromosomal rearrangements between three *Leishmania* species: *L.major*, *L.infantum* and *L.braziliensis*. In trypanosomatids the study of genomic rearrangements can contribute to the understanding not only of the genetic diversity among these organisms, but also to the knowledge of evolutionary aspects of the mechanisms involved in the control of the changes in genic order and gene distribution among the chromosomes. Genomic rearrangements can also play a key role in molecular evolution. A comparative analyses pipeline using global and local alignment search tools (FASTA and BLAST), specific PERL scripts for the parsing and analyses of the similarities results together with graphical representations of putative recombination events will be presented. Preliminary results of mapped rearrangements and the genes families involved will also be presented.

BM171 - Characterization of a *Trypanosoma cruzi* antigen with homology to intracellular mammalian lectins

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We are characterizing two cDNAs isolated from a *T.cruzi* library of amastigote forms screened with sera from chagasic patients. Sequence analyses of these cDNAs, denominated TcAGL1 and TcAGL2 *TrypanosomeCruziAntiGenLectin*, show that they present significant homology with mammals lectins VIP36 and ERGIC 53, which are eukaryotic proteins involved with cellular sorting or recycling machinery. These proteins present an N-terminal lectin domain and a C-terminal region containing repetitive amino acids and a poly-glutamine sequence codified by a microssatelite element. The sequences of the two cDNAs are high homologue and they represent two polymorphic alleles of gene which is encoded by a single copy in the genome of the parasite, in accordance with southern blot and sequence analyses from the *T.cruzi* genome database. Northern blot analysis revealed that TcAGL is constitutively transcribed into a 2,4kb mRNA, which is slightly more abundant in amastigote than epimastigote and trypomastigote forms. The antibody, obtained from immunize mice with recombinant GST::TcAGL2 fusion protein, recognizes the two isoforms of this protein with apparent molecular weight ranging from 95 and 120 kDa in cell extracts from all three life stages and in various strains of the parasite. The antigenicity of complete protein TcAGL was confirmed by ELISA and immunoprecipitation assays using sera from chagasic patients, however, the truncated form, lacking the poly-Glu and repeat regions, did not present any reactivity in both assays. The localization of this antigen in the parasite was also investigated using the anti-TcAGL2 antibodies in immunofluorescence assay as well as by transfecting epimastigote with a vector encoding the protein tagged with GFP. In both experiments, a distribution near the flagellar pocket, co-localized with the kinetoplast and Golgi region, was determined. Now we are investigating the lectin property of this protein, studying their capacity to interact with components of early secretory pathway machinery.

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BM172 - CHARACTERIZATION OF TRANSGENIC *Aedes fluviatilis* MOSQUITOES EXPRESSING A *Plasmodium* BLOCKING MOLECULE

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The *Plasmodium* resistance to antimalarial drugs and the mosquitoes' resistance to insecticides have contributed to increasing the number of malaria cases. Thus, it is necessary to seek for new control strategies. By using the microinjection technique it is possible to insert genes into the mosquito's embryonic cells to block the parasite cycle within the insect. The *Aedes fluviatilis* mosquito is a suitable vector model for avian malaria, being easy to maintain in laboratorial conditions and safe as does not offer any risk of dengue or yellow fever transmission. The objective of this work was to generate transgenic *Ae. fluviatilis*, expressing an antimalarial protein to block *Plasmodium gallinaceum*. We have used a hybrid gene construct carrying the *Anopheles gambiae* peritrophic matrix protein 1 promoter (AgPer1) linked to an effector molecule, the bee venom phospholipase A2 bearing point mutations (mPLA2) to inactivate the enzyme. This construct was inserted into the *piggyBac* transposable element that contains, as a marker, the green fluorescent protein (EGFP). By microinjecting 770 embryos,15 families were obtained by crossing microinjected mosquitoes with the wild type counterparts. About 22.000 F1 larvae were analyzed on a fluorescence microscope to identify the fluorescent marker. Four transgenic families were obtained, representing 27% of transformation rate. Expression of the EGFP was observed on the larvae neural tube and on eyes of pupae and adults. By using specific primers, we confirmed, by PCR, the presence of the EGFP (700bp), as well as the mPLA2 (500bp) genes. We are now characterizing the transgenic lines by Southern and Western blots and RT-PCR. By feeding mosquitoes on an infected chick, we expect that the expression of mPLA2 shall be able to block the *Plasmodium* cycle, without modifying the female mosquito fitness. This is the first report of a mosquito's stable transformation, generated in Latin America.

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