BM001 - **NEW INSIGHTS INTO THE GENOME STRUCTURE AND DIVERSITY OF *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi has a significant degree of evolutionary diversification, being at present classified in 6 distinct DTUs. Its genome is largely repetitive, precluding a good draft of its sequence. Today, we have 5 genomes public available: the original reference genome, CL Brener, sequenced with Sanger methodology; and other genomes that were sequenced using next-generation techniques. These genomes have provided extreme useful information about this parasite, enabling large numbers of studies focused on understanding its molecular biology. However, all of them suffered from the repetitive nature of *T. cruzi* genomes, what is specially relevant for next-generation sequences whose reads are of small size. Recently, a methodology capable of sequence single DNA molecules was released (PacBio), producing very large reads (>1kb) but with low accuracy (~85%), which hampered its usage, until in the past few months a couple of methods were published proving that PacBio reads could be used for microbial genomes. As producing very long reads is of extreme importance for *T. cruzi* genomics, we have sequenced the Dm28c strain (DTU I) using Ion Torrent technology (~50x coverage, 400nt reads) and PacBio (~100x coverage, mean read size 2,700nt, ~10% reads larger than 10kbp). After an initial assembly using PacBioToCA approach, we have obtained a draft version with 4,564 contigs totaling 58.9 MB (N50=16kb, mean=13kb, largest contig=252kb, contigs>100kb=6 (8.6MB), >10kb=1446 (39MB)). Using this draft we were able to identify a larger number of complete coding regions, correctly identify pseudogenes, measure the size of repetitive microsatellite elements, quantify copy number variation and transposable elements. Although it is still a fragmented assembly, it is the best assembled draft genome of *T. cruzi,* using a pipeline that was tailored for smaller-sized microbial genomes. We are currently sequencing other 5 genomes from other DTUs of *T. cruzi* using this hybrid approach. **Supported by:**CNPq, Fiocruz, Fundação Araucária

BM002 - ***TRYPANOSOMA CRUZI* REGULOME PROJECT: LARGE SCALE DATASET ANALYSIS AND IDENTIFICATION OF CO-REGULATED GENE MODULES**

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The regulome project of *Trypanosoma cruzi* aims to identify its network of gene expression regulation. It is comprised of diverse concurrent steps: creating an extensive transcriptome dataset, identifying the protein-protein and protein-mRNA interaction networks, predicting cis-regulatory elements and linking the cis and trans elements. The final goal is to integrate this information into a network representation enabling the understanding of *T. cruzi* gene expression regulation under a Systems Biology view. Here, we present the analysis of the current transcriptome dataset, with the identification of co-regulated gene modules and the prediction of cis elements. Our transcriptome dataset comprises more than 100 biological situations, analyzed by RNA-Seq in the SOLiD plataform, abranging differentiation processes (metacyclogenesis, amastigogenesis, epimastigogenesis), environmental responses (nutrition, pH, temperature, oxidation), drug response, gene knock-outs etc. This dataset was submitted to analysis using SOM, Click and SAMBA bi-clustering algorithms as well as the FIRE software for clustering and mRNA motif prediction. We were able to fine-tuning the definition of co-expressed (and probably co-regulated) gene groups, an extreme important step to increase signal-to-noise in mRNA motif prediction. We were able to identify classical modules of co-regulation, as ribosome, proteasome, DNA and RNA polymerase, but also other interesting cases of co-expression, mainly with proteins of unknown function. We will keep increasing the number of evaluated situations present in our transcriptome dataset, improving our ability in defining the co-expressed module. In parallel, the other concurrent steps are also being improved and will be integrated in the near future. Finally, the mRNA motifs identified in the present work needs to be validated, and we are planning to develop a high-throughput approach for this evaluation. **Supported by:**CNPq, Fiocruz, Fundação Araucária

BM003 - **ANALYSIS OF SUBTELOMERIC AND TELOMERIC REGION IN DIFFERENT ISOLATES OF *TRYPANOSOMA CRUZI***

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A remarkable feature of T. cruzi is the occurrence of chromosomal polymorphism among the different isolates of this specie. Recombination mechanisms may be responsible for this variability, generating different karyotypes observed in the isolates. Recently, we performed a detailed in silico analysis of the telomeric and subtelomeric regions of T. cruzi and observed that these regions are highly polymorphic, mainly as a result of large changes in the relative abundance and organization of these genes. The lack of synteny observed in the subtelomeric regions suggests the occurrence of homologous recombination between subtelomeric regions of different chromosomes. In order to investigate the role of subtelomeric region in recombination events, we identified telomere specific markers and map them by hybridization on the chromosomal bands separated by PFGE. We identified 32 telomere-specific markers that were mapped on the chromosomal bands of different isolates of T. cruzi (clones CL Brener, Esmeraldo, Dm28 and G strain). Interstitial markers of each chromosome were also hybridized as a reference. Some markers were mapped on homologous chromosomes with the same size whereas others were mapped on sized-different homologous chromosomes. Identical hybridization pattern was observed with the telomere-specific and chromosome-specific markers. Only exception was observed in the Esmeraldo clone using the 3-oxoacyl-ACP reductase marker. PCR analysis confirms the deletion of this maker in this strain. The 3-oxoacyl-ACP-reductase gene is located near subtelomeric region in clone CL Brener. This deletion may have occurred in the subtelomeric region of clone Esmeraldo as a result of an unbalanced rearrangement. We observed that isolates from different T. cruzi lineage exhibit conservation of the chromosome structure despite the polymorphism found in the chromosomal ends. **Supported by:**Fapesp

BM004 - **TELOMERES MAINTENANCE IN *TRYPANOSOMA CRUZI*: ROLES FOR ORC1/CDC6 AND RPA-1.**

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Trypanosoma cruzi is the etiologic agent of Chagas' disease, the second highest illness burden among neglected tropical diseases. Improving the knowledge about the molecular biology of this parasite may facilitate the discovery and the development of antiparasitic drugs. Telomeres are formed by the interaction of DNA with protein complexes which are responsible for maintaining the chromosome terminals and play a crucial role in genomic integrity. Trypanosomes lack homologues of known telomere end binding proteins, thus the molecular biology of this region is poorly understood. Replication Protein A (RPA) comprises a trimeric complex formed by three subunits, that performs, alone or together with other proteins, various vital functions in DNA metabolism, being a fundamental player during replication and also in telomere maintenance. In *T. cruzi*, RPA has not yet been characterized. In *Leishmania amazonensis*, only subunit 1 of the RPA is found associated to telomeric DNA. We have cloned, purified rTcRPA-1 and produced antibody against this protein. *In vitro*, we show that rTcRPA-1 can interact with telomeric G and C rich single strand. *In vivo,*TcRPA-1 is a nuclear protein and imunoprecipitates G strand in ChIP assays, in both epimastigote and trypomastigote forms. Trypanosomas Orc1/Cdc6 is part of the pre-replication complex, which licenses replication origins allowing the establishment of replication machinery onto DNA. TcOrc1/Cdc6 was cloned and purified, but it doesn´t interact with telomeric sequences *in vitro*. These results suggest that telomeres in *T. cruzi* could be replicated by forks originated in subtelomeric regions. Moreover, we are also wondering what role RPA-1 plays in telomeres of *T. cruzi,* since trypomastigotes are a non replicative form of this parasite and therefore TcRPA-1 is probably not involved in replication of this termini in this lifeform. These questions are now being investigated. **Supported by:** FAPESP 2011/16670-0 2011/21570-4

BM005 - **THE CHARACTERIZATION OF A KINETOPLAST ASSOCIATED PROTEIN (KAP4) IN SYMBIONT CONTAINING TRYPANOSOMATIDS.**

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The Kinetoplast Associated Proteins (KAPs) are basic proteins similar to histone H1 of eukaryotes. They have been characterized in different trypanosomatid species considering their role in the mitochondrial metabolism and kDNA topology. In the family Trypanosomatidae, monoxenic species as *Angomonas deanei* and *Strigomonas culicis*, harbor a symbiotic bacterium which maintains an obligatory association with the host protozoan. The presence of the symbiont has been associated to ultrastructural changes in the host, as the looser arrangement of the kDNA network when compared with other trypanosomatids . In this work, sequences coding for KAP 4 were identified on the genome database of *A. deanei* and *S. culicis* and its chromosomal locus was identified by Pulse Field Gel Eletrophoresis. Furthermore, the *kap4* gene expression was investigated by RT-qPCR and the protein immunolocalization was used KAP4 antibodies in immunofluorescence assay. Our results show in *A. deanei* the KAP4 has 13.6 kDa, pI 11.4 and is localized on chromosomes 3 and 2 of the wild-type and the aposymbiotic strains, respectively. In *S. culicis* ScKAP4 has 14.5 kDa, pI 11.57 and the gene is present on the chromosome 2 of both strains. Data obtained by RT-qPCR the *kap4* transcripts are 2.7 more abundant in the aposymbiotic strain of *S. culicis* than in the wild-type. On the other hand, in *A. deanei* the quantity of *kap4* transcripts are similar among both strains of protozoa. Immunolocalization assays KAP4 is distributed along the mitochondrion in aposymbiotics strains of *A. deanei* and *S. culicis*. However, ultrastructural by transmission electron microscopy did not reveal differences in the kDNA topology in both strains. The differences in the gene expression, karyotype and in the localization of *kap4* between strains, may be influenced by the presence of bacterium. Our next goal is to immunolocalize KAP4 by ultrastructural immunocytochemistry and to verify where this protein is distributed in the kDNA network. **Supported by:**CNPq and FAPERJ

BM006 - ***CHARACTERIZATION OF TRYPANOSOMA CRUZI SIRTUINS AND ITS INVOLVEMENT IN THE CONTROL OF GLOBAL PROTEIN ACETYLATION LEVELS***

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Lysine acetylation has emerged as a major post-translational modification involved in diverse cellular functions. While histone proteins are the founding members of lysine acetylation substrates, it is now clear that hundreds of other proteins can be acetylated in multiple compartments of the cell. The acetylation/deacetylation process is regulated through the activities of two families of proteins, histone acetyltransferases and histone deacetylases. Sirtuins are amongst the histone deacetylase family. They are NAD+-dependent histone deacetylases involved in different functional mechanisms, such as gene silencing, DNA damage repair, ageing and metabolic process. Here we characterized the two sirtuins proteins found in *Trypanosoma cruzi*, TcSir2rp1 and TcSir2rp3. Using a tagged version of TcSir2rp3, we showed its mitochondrial localization in the epimastigote form of the parasite. TcSir2rp3 overexpression leads to an increase in the resistance to oxidative stress. On the other hand, TcSir2rp1 has a cytosolic localization in all parasite forms. Heat-shock, starvation, or ER stress did not modify the protein expression, but TcSir2rp1 relocalized around reservosomes area during starvation, suggesting a role of this protein in the regulation of reservosome degradation. Overexpression of both TcSir2 proteins reduced the acetylation levels of specific proteins, as measured by immunoblotting with antibodies against acetyl-lysine. Interestingly, TcSir2rp1 expression increases in stationary cells. Moreover, by co-immunoprecipitation assay we identified that TcSir2rp1 interacts with eIF5A, a translation factor that is required for survival in stationary cells. Altogether, these data provide the first characterization of the *T. cruzi* Sirtuins and suggest that these proteins regulate non-histone proteins acetylation levels, which could be relevant for functional adaptation mechanisms in the parasite upon environmental changes. **Supported by:**Fapesp

BM007 - **TRANSCRIPTOMICS OF *TRYPANOSOMA CRUZI* AMASTIGOGENESIS**

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Trypanosoma cruzi is the protozoan that causes Chagas disease. This parasite has a biphasic life cycle in which four cellular forms alternate between the insect vector (epimastigotes and metacyclic trypomastigotes) and the mammalian host (amastigotes and bloodstream trypomastigotes). The amastigogenesis is the differentiation process that naturally occurs when trypomastigotes penetrate mammal cells and differentiate to amastigotes. Depending on the source of trypomastigotes, the amastigogenesis can be classified as primary or secondary if started from metacyclic or blood trypomastigotes, respectively. The present work aims to analyze the transcriptome of this parasite during *in vitro* primary and secondary amastigogenesis using RNA-Seq technology (SOLiD 4 platform). Metacyclic trypomastigotes were obtained by *in vitro* metacyclogenesis in TAU3AAG medium whereas cell derived trypomastigotes were recovered from the supernatant of infected Vero cells cultures. Both trypomastigotes forms where purified by ion exchange chromatography in DEAE-cellulose columns and the amastigogenesis were induced by exposing the parasites to high glucose DMEM medium at pH 5. As primary and secondary amastigogenesis have differentially kinetic properties, total RNA samples were obtained after different time points: 0, 12, 24, 36, 48 and 72 hours for primary amastigogenesis and 0, 2, 6, 12, 24 and 48 hours for secondary amastigogenesis. After RNA-Seq, the 36 libraries (triplicate experiments) produced more than 630 million reads; about 53% of them were successfully aligned to *T. cruzi* CL Brener genome. We observed about 1040 and 670 differentially expressed genes (DEGs) in primary and secondary amastigogenesis, respectively. Clusterization analysis showed several patterns of co-expressed genes, including cluster similarly regulated in both amastigogenesis. Currently we are analyzing these clusters of DEGs searching for enriched GO terms and regulatory elements in UTRs regions. **Supported by:**CNPq, CAPES, Fundação Araucária and FIOCRUZ

BM008 - **THE SIALOGLYCOPEPTIDASE-LIKE PROTEIN OF *LEISHMANIA MAJOR*: POSSIBLE ROLE IN PARASITE GROWTH AND DIFFERENTIATION.**

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In bacteria, the metallopeptidase O-sialoglycopeptidase (OSGEP) hydrolyses peptide bonds between O-glycosylated and sialylated aminoacids and processes surface antigens of leukocytes. Other OSGEP-like genes, found in bacteria and eukaryotes have been associated with DNA-binding and the maintenance of the mitochondrial genome. Among those, Kae-1 is an essential gene that shares 34% sequence identity with OSGEP, but does not have proteolytic activity. In the *Leishmania major* genome there is a single copy gene with similarity to bacterial OSGEP, but its putative role has never been studied. We set out to characterize the *L. major* OSGEP gene, which shares 60% identity to bacterial Kae-1. The sub-cellular localization of the protein was assessed by the generation of a *L. major* line expressing OSGEP fused to GFP. Confocal microscopy revealed co-localization of the fusion protein with the mitochondrion-selective probe, Mitotracker red. *L. major* lines overexpressing OSGEP from an episomal vector were generated and display accelerated growth as promastigotes. Overexpressing parasites (OVX) reach stationary phase faster than WT parasites but a proportion of the cells display significant morphological alterations in the cell body and shorter flagella. In addition, we observed secretion of amorphous material emerging from the flagellar tip. We generated single knock-out parasite lines, but were unable to select double knock-out clones after several independent rounds of transfection. Stationary phase OVX promastigotes differentiate poorly to metacyclics as assessed by binding to FITC-coupled PNA-lectin, as compared to WT or lines carrying empty plasmid. In addition, OVX parasites display low infectivity to murine macrophages in vitro and induce negligible lesions in BALB/c mice. In contrast, heterozygous lines differentiate to metacyclics more efficiently and are 2-fold more infective to murine macrophages. The mechanisms underlying the OSGEP function are under investigation. **Supported by:**FAPERJ; Cnpq

BM009 - **IDENTIFICATION AND CHARACTERIZATION OF SMALL NON-CODING RNAS IN *TRYPANOSOMA CRUZI***

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RNA interference (RNAi) utilizes small non-coding RNAs (ncRNAs) associated with Argonaute (Ago) proteins to regulate gene expression. The ncRNA within the Ago complex serves as guide to find complementary sequences within RNA targets. Different RNAi pathways can regulate diverse biological processes such as mRNA stability, translation, heterochromatin formation, genome stability and stress responses. RNAse III proteins, such as Dicer, are involved in the biogenesis of ncRNAs although some RNAi pathways do not depend on these proteins. An Ago family protein is present in *Trypanosoma cruzi* suggesting the existence of, at least, one RNAi pathway. Indeed, the presence of small ncRNA in *T. cruzi* has also been described. Here, we attempted to characterize this potential new trypanosomatid RNAi pathway by understanding the biogenesis of small ncRNAs and its possible roles during the parasite life cycle.
Total RNA was extracted from *T. cruzi* epimastigotes and used to construct small RNA libraries that were deep sequenced. Analysis of the library prepared from the 15-45nt RNA fraction showed that 84.59% of all reads was successfully mapped to the *T. cruzi* genome, allowing for zero mismatches. The analysis of mapped reads showed a large amount of ncRNAs corresponding to regions annotated as tRNA with average size of 33nt and preferentially arising from the 5' end of the transcript. tRNA-derived small ncRNAs abundance does not correlate with codon usage. Furthermore, specific tRNAs, but not all of them, seem to generate ncRNAs suggesting that the biogenesis of ncRNAs does not depend simply on the precursor abundance. During genotoxic stress the amount of tRNA-derived small RNAs show a significant increase and the mRNA for the sole Argonaute protein also increases in abundance. This suggests this potential RNAi pathway could be involved in stress responses. We are currently investigating the mechanism of biogenesis and possible functions of these ncRNAs in *T. cruzi.* **Supported by:**CAPES, CNPq, FAPEMIG and PRPq-UFMG

BM010 - **THE ROLE OF ER STRESS AND TOLL LIKE RECEPTORS IN PKR ACTIVATION DURING LEISHMANIA AMAZONENSIS INFECTION**

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The protein kinase R (PKR) is a critical component of the innate immune response and modulates the expression of several immune mediators. We have previously shown that *L. amazonensis* was able to activate and increase the PKR expression in infected macrophages. PKR activation promoted parasite intracellular proliferation in an IL-10-dependent fashion. The signaling pathways triggered by toll like receptors induce the production of innate immune mediators, which in turn may rely on PKR activation. Moreover, TLR-activated XBP1 was required for optimal and sustained production of pro-inflammatory cytokines in macrophages. Activation of IRE1α by ER stress acted in synergy with TLR activation for cytokine production through activation of XBP-1. We previously demonstrated in macrophages from TLR2 or TLR4-KO B6 mice, a dramatic reduction of parasite infection, similar to the observed with PKR-KO macrophages. Our data suggested that toll-like receptors pathways do support *L. amazonensis* infection. Additionally, western blotting assays showed that PKR phosphorylation was reduced in infected macrophages from TLR2-KO or TLR4-KO mice, suggesting that PKR phosphorylation seems to be partially dependent on TLR engagement. We also observed that the expression of the suppressor cytokine IL-10 was reduced in infected macrophages from TLR2 and TLR4-KO, suggesting a role for IL-10 in the infection. We also showed that the *L. amazonensis* infection was able to induce a cytosolic mRNA-splicing event that generates a mature (spliced) XBP1 protein with a potent transactivation domain, indicating a possible role of ER stress in this process. Accordingly, we showed an increase in the parasite burden when macrophages were treated with tunicamycin, a potent inducer of ER stress. Moreover, we observed in immunofluorescence and western blot assays an increase in PERK levels in macrophages infected with *L. amazonensis*. Our working hypothesis is that ER stress components are induced during *L. amazonensis* infection and may be activate PKR via toll like receptors, inducing the production of important cytokines in this process. **Supported by:**CNPq

BM011 - **MICROBIOME COMPOSITION IN LOCALIZED CUTANEOUS LEISHMANIASIS LESIONS**

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Cutaneous Leishmaniasis (CL) is a pleomorphic and spectral skin disease, caused by Leishmania protozoans. The common clinical form Localized Cutaneous Leishmaniasis (LCL) is characterized by single or multiple chronic ulcers. Our working hypothesis is that polymicrobial infections at LCL lesions impair wound healing. Previously studies conducted in order to identify these microorganisms were based on traditional culture which is subject to bias regarding the estimate of bacterial composition. Thus, our aim was to characterize the microbiome present in LCL lesions in ten patients by next generation sequencing and, as a control, we used samples from contralateral intact skin (IS). Ulcers and intact skin swabs were collected from each patient. DNA was extracted and subjected to 16S rRNA amplification with primers containing barcodes and adapters specific to Illumina MiSeq. A total of 20.189.531 reads were obtained. After a quality filter check, we obtained 7.823.305 reads associated with intact skin and 5.935.737 reads with LCL lesions. Sequences were analyzed with Qiime, providing bacterial identification and population structure. *Staphylococcus* (27,1% LCL; 19,6% IS), *Corynebacterium* (5,4% LCL; 8,6% IS) *and Arcanobacterium* (4,7% LCL; 3,5% IS) were detected in both sites*. Fusobacterium* (11.4%) and *Bacterioides* (3,9%) were identified almost solely in LCL. *Streptococcus* (16,9%) and *Acinetobacter* (4.1%) were identified in higher levels in LCL lesions and intact skin, respectively. Intact skin showed significantly higher diversity levels compared to LCL wounds ($p<0.01$). This lower diversity in LCL lesions was also reported in diabetic ulcers. However, anaerobic bacteria were observed more frequently in diabetic ulcers rather than in LCL wounds in which the gram positive aerobic bacteria were observed more often. We are presently conducting follow up studies in order to possibly associate microbiome composition and wound healing in LCL patients submitted to chemotherapy. **Supported by:**iii (Instituto de Investigação em Imunologia)/INCT; CNPq

BM012 - **UPDATING THE KNOWLEDGE ON TRANSPOSABLE ELEMENTS IN TRYPANOSOMATIDS.**

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Transposable elements (TEs) comprise distinct groups of DNA sequences sharing the ability of moving to new sites in genomes. They are an important source of genetic variation in eukaryotes and have strongly shaped the structure, function, and evolution of genomes. Our work aims to study the TE content of trypanosomatids genomes. First, we investigated, under an evolutionary perspective, the presence of known trypanosomatids TEs in 13 genomes available in the TritrypDB. We found *CRE*-like TEs, originally described in *Trypanosoma cruzi, T. brucei* and *Crithidia fasciculata* (*Cf*), are also present in SL RNAs genes of *Leishmania braziliensis* (*Lb*)*, L. panamensis* (*Lp*)*, Endotrypanum monterogeii* (*Em*)*, T. congolense* and *T. vivax,* and they were probably lost in the ancestor of the other *Leishmania* species. *Ingi/L1*-like TEs are found in *Em*, *Cf* and *L. tarentolae*, besides the *Trypanosoma* species. *LmSIDER* sequences, initially characterized as mRNA instability elements in *L. major*, are present in all *Leishmania* species and in *Em* and their role as gene expression regulators remains to be investigated. TATE, a telomere-associated TE from *Lb* is also found in the *Lp*, *Em* and *Cf.* VIPER is found in all *Trypanosoma* species. Phylogenetic analyses and their comparison with the host species relationships will provide a better view of the TEs evolution and pattern of loss. Next steps include searches of new TEs and analyses of their abundance, variability, genomic distribution and expression. We are also sequencing different *T. cruzi* strains and other trypanosomatids using next-generation approaches, including PacBio technology, which resolves single molecules, producing long reads that are interesting to study repetitive sequences. Efforts from our and other groups on sequencing trypanosomatids genomes will offer an unprecedented opportunity for comparative genome analysis and improve our understanding of the TEs role and impact on the variability and evolution of these parasites. **Supported by:**CAPES and FIOCRUZ

BM013 - **UNRAVELING THE TRL1-*LIKE* ENZYME FUNCTION IN *TRYPANOSOMA BRUCEI***

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Transfer RNAs (tRNA) play a central role in protein synthesis, being the translators of the genetic code. The biosynthesis of mature and functional tRNAs involves many steps, including a splicing step performed by a tRNA endonuclease and the junction of the two halves by a tRNA ligase. Yeast tRNA ligase (Trl1) homologous were identified in trypanosomatids, which is in agreement with the need to editing of a sole tRNA containing intron ($tRNA^{Tyr}$) in these organisms. The phylogenetic distribution of these enzymes is restricted to fungi and trypanosomatids, making them promising therapeutic targets to the development of drugs against these organisms. In order to understand the importance of the trypanosomatid Trl1-like enzyme, we constructed a stable *T. brucei* RNAi strain for the knockdown of the trl1-*like* gene. The RNAi induction was very successful (around 80 %) and was followed by a severe growth defect, showing that the *trl-like* gene is essential for parasite survival. Furthermore, the complementation of the RNAi strain with a mature $tRNA^{Tyr}$ rescued the growth phenotype in the silenced strain back to the non-silenced strain levels. Surprisingly, although $tRNA^{Tyr}$ halves accumulation was expected after gene silencing, a northern blotting of the total RNA extracted from the trl1-*like* silenced strain showed only the accumulation of the intron-containing $tRNA^{Tyr}$ and a reduced amount of mature $tRNA^{Tyr}$, thus indicating that the trypanosomal Trl1-*like* enzyme has a role on tRNA processing prior to tRNA halves ligation. One possible explanation for this result is the need of the existence of a tRNA ligase/tRNA endonuclease complex for all the steps of tRNA intron editing. More studies are needed to further prove this hypothesis. We are currently working on the functional confirmation of the enzyme activity *in vitro*. **Supported by:**CNPq, OMS, FAPERJ

BM014 - **PHOSPHATIDYLINOSITOL PRODUCTION IN *ANGOMONAS DEANEI*, A SYMBIONT BEARING TRYPANOSOMATID**

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Some trypanosomatids harbour a symbiotic bacterium that co-evolves with the host. This relationship constitutes an excellent model to study organelle origin and cellular evolution. The presence of the symbiont promotes morphological and physic-chemical alterations in the protozoan. Also, the bacterium influences the trypanosomatid metabolism, since intense nutritional exchanges occurs between both partners. The presence of the symbiont modifies the host lipid content and production, since the symbiont containing (wild) strain of *Angomoas deanei* shows an increased phospholipid production when compared to the aposymbiotic strain. In order to understand the participation of the phosphatidylinositol (PI) in signalling pathways of *A. deanei*, we investigate amount and synthesis of PI by supplementing the protozoan culture medium (Warren) with [H^3]myo-inositol in order to observe the importation of this molecule by wild and aposymbiotic cells, and isolated symbionts. Our results showed mainly incorporation of myo-inositol in PI by both cells. Isolated symbionts did not incorporate the radiolabeled myo-inositol, neither in PI, nor in the PI derivates PIP2 and phosphatidylinositol-phosphate (PIP). Genomic analysis of *A. deanei* revealed the presence of sequences coding enzymes involved in PI synthesis from CDP-diacylglycerol pathway and from exogenous myo-inositol pathway. The synthesis of myo-inositol from glucose-6-phosphate was also found in the protozoan genome, suggesting that part of PI production is not associated to incorporation of exogenous myo-inositol. Although previous studies showed an increase in the PI amount in isolated symbionts after 3 hours, the bacterium genome does not contain genes of enzymes involved in PI synthesis, indicating the presence of a recycling process changing this phospholipid amount. **Supported by:** CAPES, FAPERJ, CNPQ, INBEB.

BM015 - ***LEISHMANIA (VIANNIA) BRAZILIENSIS*: NATURAL RESISTANCE TO NITRIC OXIDE IS NOT RELATED TO ROS RESISTANCE NEITHER TO MRNA EXPRESSION LEVELS OF ARGINASE 1 AND PTERIDINE REDUCTASE**

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Leishmania (Viannia) braziliensis, one of the main etiological agents of American Tegumentary Leishmaniasis (ATL) in the American continent, is associated with various clinical outcomes including self-healing localized cutaneous lesions, multiple disseminated lesions and metastasis to oropharyngeal mucosa. Both the infecting parasite and the host immune response contribute for the clinical presentation. The production of cytokines, reactive oxygen species (ROS) and nitric oxide (NO) normally leads to the destruction of phagocytosed microorganisms. The inducible nitric oxide synthase (iNOS) catalyzes the synthesis of NO from arginine, but the enzyme arginase competes with iNOS for the substrate, resulting in the production of polyamines and failing to control the intracellular replication of Leishmania. The *L. (V.) braziliensis* strains used in this study were previously characterized as being naturally resistant or susceptible to NO. In addition, the resistant strain was also related to higher number of lesions and severe injuries than the susceptible strain, as well as to resistance to pentavalent antimony, the first-line drug for ATL. However, the molecules and/or metabolic pathways of the parasite that contribute to such resistance are unknown. The objective of this work is to identify molecules associated with NO resistance that ultimately may be related to the different clinical manifestations caused by these polar strains. Our preliminary results pointed to similar susceptibility of both strains to exogenous H_2O_2, excluding ROS as part of the molecular mechanism involved in the observed phenotype. In addition, qPCR analysis showed similar levels of arginase 1, pteridine reductase and biopterin transporter mRNAs, suggesting that their gene expression are not directly related to the NO resistance differences in promastigotes. **Supported by:**CAPES, Fiocruz

BM016 - ***TRYPANOSOMA RANGELI* GENOME REVEALS INTERFERENCE RNA PATHWAY PSEUDOGENES BUT LACKS A FUNCTIONAL RNAI MACHINERY**

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RNA interference is a mechanism to control gene expression described in a variety of organisms including *T. brucei*, *T. congolense* and *L. braziliensis*, however it is absent in *T. cruzi* and *L. major.* Blast search for orthologous RNAi players was carried out in the *T. rangeli* genome (SC58 strain) using the five *T. brucei* RNAi genes as query, revealing that all of these components are present as pseudogenes presenting stop codons or frame shifts. In order to evaluate if these characteristics are not due a strain-specific phenomena, sequencing of all RNAi genes amplified by PCR from a distinct *T. rangeli* strain was performed. The Dicer2 gene revealed indel events and the absence of internal stop codons or frame shifts in SC58 (KP1-) and Choachí (KP1+). The RIF4 and RIF5 genes showed high similarity between the strains. However, *T. rangeli* Argonaute (AGO) and Dicer1 (DCL1) genes revealed the presence of SNPs and size polymorphisms between *T. rangeli* strains, including large sequence deletions. PCR primers directed to the flanks of the deleted regions in AGO and DCL1 revealed distinct products sizes for *T. rangeli* KP1(-) and KP1(+) strains, allowing lineage identification for 17 different strains. Comparative UPGMA analysis with sequences of the AGO and DCL1 fragments showed trees that clearly branched *T. rangeli* strains into KP1(-) and KP1(+) lineages, clustering separately the KP1(-) strains from Brazil and Colombia. The presence of functional RNAi mechanism in *T. rangeli* was further investigated using parasites transfected with the pTEXeGFP plasmid. Transient transfections of these parasites using siRNA against eGFP or the TUBdsRNARFP plasmid, which express long dsRNA against endogenous b-tubulin, were carried out. The transfection of eGFP expressing cells with siRNA or by processing of long dsRNA, respectively, failed completely to inhibit GFP expression or altering the parasite morphology that indicate the absence of a functional RNAi machinery in *T. rangeli*.**Supported by:**CAPES, CNPq, FINEP and UFSC

BM017 - **ABUNDANCE OF TRANSCRIPTS CODING FOR KINETOPLAST ASSOCIATED PROTEINS (KAPS) AND FOR REPLICATION PROTEINS OF KDNA FROM *ANGOMONAS DEANEI* USING RNA-SEQ.**

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Most protozoa of the Trypanosomatid family are monoxenics that inhabit only invertebrate hosts during all their life cycle. Among them there are six trypanosomatid species that harbor a symbiontic bacterium in the cytoplasm and an obligatory relationship is maintained between both. The kDNA is localized in a structure named kinetoplast that consists of an enlarged portion of the single mitochondrion is arranged in concatened DNA and forms an extensive network. Interestingly, symbiont harboring trypanosomatids present a looser kDNA topology when compared with other species of family. In the present work, our goal is to analyze the *Angomonas deanei* transcriptome in order to verify the abundance of RNA transcripts coding for kinetoplast proteins. Our search is especially focused on KAPs that participate in kDNA organization and kDNA replication proteins in order to better understand how structural features are related to molecular functioning and organization.Thus, RNA-Seq using new generation sequencing IonTorrent platform from exponential growth phase cells of *A. deanei*. Transcriptome was obtained from mRNA purified in a PolyA-column after Trizol extraction. The obtained library was applied in the 314 and 316 semiconductor chips and generated 3 million reads from size up to 150bp. Transcripts were quantified by values of reads per kilo base per million (RPKM) using CLC genomics workbench software. The kap3 and kap4 transcripts showed values of 16.94 and 14.52 RPKM, respectively. The proteins involved in the replication showed RPKM values for ligase Kα, ligase and TOPO I proteins was more highly expressed with 16.55, 11.02 and 9.03,respectively. On the contrary, the values for UMSBP, TOPO II and TOPO III were 1.92, 1.66, 0.64, respectively. Our next step in to use the aposymbiotic strain of *A. deanei* as a comparative model to elucidate if the symbiont influences the transcription of proteins involved in kDNA structure and replication. **Supported by:**CNPq and FAPERJ

BM018 - **MOLECULAR KARYOTYPE AND SYNTENIC ANALYSIS OF ISOLATES FROM T. CRUZI DISCRETE TYPING UNITS AND TRYPANOSOMA CRUZI MARINKELLEI**

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T. cruzi comprises a complex of genetically heterogeneous isolates classified in six subdivisions designated Discrete Typing Units (DTU´s) namely TcI to TcVI. T.c. marinkellei is a bat-associated parasite of the subgenus Schizotrypanum which has been regarded as a T. cruzi subspecies. In a previous work, we compared the hybridization pattern of 120 chromosome-specific markers with the chromosomal bands of CL Brener (CLB) (TcVI) and G strain (TcI) separated by PFGE. We showed that large syntenic blocks are conserved among the isolates, however, break synteny due to duplication and/or deletion was also observed. In this work we analyze the molecular karyotype of isolates from T. cruzi DTUs and T.c. marinkellei by hybridization of the chromosomal bands with chromosome-specific markers. We determine the karyotype of T.c. marinkellei and 3 isolates of T. cruzi [Sylvio X10 (TcI), clone 4.2 TCC2177 (TcIII), TCC2124 clone 1 CanIII (TcIV)] T.c. marinkellei karyotype is composed of 17 bands with sizes ranging from 2.47-0.63 Mb which is generally close from TcI isolates (G, DM28c, Sylvio X10). Nineteen bands ranging from 3.31-0.56 Mb were identified in Sylvio X10, 19 bands (2.83-0.53 Mb) and 17 bands (2.58-0.57 Mb) were identified in the isolates G and DM28c. The karyotypes of TCC2177 (TcIII) and TCC2124 (TcIV) display 21 and 20 bands ranging from 3.39-0.59 and 3.31-0.67 Mb, respectively, and they are very similar to TcII (Y, Esmeraldo), TcV (S03-cl5) and TcVI (CLB) isolates. The karyotypes are homogeneous within TcII, TcIII, TcIV, TcV and TcVI isolates and are generally different from TcI isolates and T.c. marinkellei. Despite the chromosome polymorphism, several syntenic groups are conserved among T. cruzi DTUs isolates and also in T.c. marinkellei. Our results indicate that T. cruzi DTUs and T.c. marinkelei exhibit conservation of chromosome structure. These results are in agreement with the hypothesis of T. cruzi would be evolved from a bat-restricted trypanosome ancestor. **Supported by:**CNPq, CAPES e FAPESP

BM019 - **RNA-SEQ OF *ANGOMONAS DEANEI*: COMPARATIVE ANALYSIS OF THE WILD TYPE AND THE APOSYMBIOTIC STRAIN**

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Angomonas deanei is a trypanosomatid found in insects and has a single obligate intracellular bacterium in their cytoplasm. The transcriptome is the complete set of transcripts in a cell, and their quantity may support more understanding about specific developmental stage or physiological condition. The study of the wild type parasite and the aposymbiotic strain will help to understand the symbiosis in these organisms. The RNA-Seq is an approach to study transcriptome profiling that uses deep-sequencing technologies. In order to comprehend the influence of the symbiont on these protozoa, we have analyzed the transcriptome of the wild type *A. deanei* and the aposymbiotic strain using the Ion Torrent PGM platform. Total RNA was extracted and mRNA integrity was checked using microfluidic analysis in a Bioanalyzer and the contamination of ribosomal RNA was only 3%. We have obtained 1.553.840 reads from wild type *A.deanei* and 3.479.109 reads of the aposymbiotic strain. GMAP and Cufflinks softwares were used to align the sequences to the already sequenced parasite genome, which has 31Mb. The majority of reads (91.65%) of *A.deanei* were aligned against the genome and only 0.7% of reads aligned with the endosymbiont genome. The more expressed genes in the *A. deanei* were ribosomal protein, hexokinase, histone H4, flagellar calcium binding protein, beta-frutofuranosidase and others. Even so, small differences were seen in the abundance of the mRNA, detailed analysis need to be done between the wild type parasite and the aposymbiotic strain. The results of the full set of the transcripts in these two organisms may suggest some evolutionary role of the endosymbiont of *A. deanei.* **Supported by:**CNPq, Capes, Faperj

BM020 - **SPERMIDINE SYNTHASE IS A STAGE-SPECIFIC MARKER FOR *TRYPANOSOMA RANGELI* LIFE STAGES**

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Considered as non-pathogenic and harmless to humans, *Trypanosoma rangeli* is a protozoan parasite that infects several triatomine and mammalian hosts. Due to the sharing of antigens, hosts and vectors with *T. cruzi*, misdiagnosis of Chagas disease might occur in a wide geographical area in Central and South America. *T. rangeli* has a complex life cycle alternating between replicative and non-infective epimastigotes to non-replicative and infective trypomastigotes. Proteomic maps of both *T. rangeli* stages and intermediate forms were obtained by uni- (1D) and two-dimensional (2D) electrophoresis following peptide mass fingerprinting identification by Nano-LC ESI-MS/MS. Among the ~1,000 proteins identified in the parasite proteome by MS, those presenting differential expression levels between life stages are of major interest. One of these proteins is the *T. rangeli* spermidine synthase (*Tr*SS), that revealed an ORF of 906 bp, predicting to a protein of ~33kDa. Comparative sequence analysis showed that *Tr*SS is 85% identical to the *T. cruzi* and 74% identical to the *T. brucei* homologs. Transcription analysis by qPCR showed that *Tr*SS gene is highly transcribed in epimastigotes rather than transition forms (intermediate forms on *in vitro* differentiation) or trypomastigotes ($p<0.05$). Due such differences, *Tr*SS was pointed out as an epimastigote-specific marker and the complete ORF was amplified, sequenced and cloned into pET 14b vector. The recombinant *Tr*SS obtained by heterologous expression in *E. coli* DH5a is being used to generate anti-*Tr*SS antibodies for cellular assays to asses the sites and confirm the expression levels. **Supported by:**CNPq, CAPES, FINEP, CDC/CCEHIP/NCEH and UFSC

BM021 - ***LEISHMANIA* SPP. TELOMERE MEASUREMENT BY QUANTITATIVE PCR**

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Leishmaniasis treatment consists in the administration of pentavalent antimonials, which are highly toxic to the patients and also records considerable failure. Therefore, the success of leishmaniasis treatment requires new therapeutic strategies. Recently, telomeres have been considered a potential target for drug design. Telomeres are protein-DNA complexes maintained by telomerase that protect chromosome ends from degradation and fusion. In a wide range of eukaryotes, inhibition of telomerase activity results in cell cycle arrest, decrease of telomere length, and senescence. On the other hand, the absence of telomerase activity in infected host cells but not in the parasite cells elects this protein as a specific molecular target, suitable for anti-leishmania therapy. One way of measuring the effect of anti-telomerase treatment on the parasite is by estimating telomere length by Southern blot analysis, considered the gold standard method, although it is labor intensive, time consuming, and requires large amounts of DNA. The recent development of quantitative PCR (qPCR) to measure telomere length in contrast, is rapid and requires low quantities of DNA. It has been widely used to study telomeres from a variety of human cell samples. We adapted this methodology to estimate telomere length in both development forms of *Leishmania amazonensis.* For this we used a standardized commercially available chemistry and automated qPCR method. Ct values from the telomere assay were normalized to the single gene reference assay using the T/S ratio to determine telomere length. The telomere length of each sample was based on the telomere to single copy gene ratio (T/S ratio) and was based on the calculation of ΔCt [Ct(telomere)/Ct(single gene)]. Our preliminary results using different forms of *L. amazonensis* treated and non-treated with a genotoxic agent showed that this method is highly reproducible and accurate. **Supported by:**FAPESP

BM022 - **DETECTION OF *LEISHMANIA (LEISHMANIA) I. CHAGASI* IN MUCOSAL SAMPLES OF DOGS FROM ENDEMIC AREA OF VISCERAL LEISHMANIASIS**

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Background and aim: The varied and non-specific clinical manifestations of canine visceral leishmaniasis (canVL) and the lack of external signs in the infected asymptomatic animals make the diagnosis of leishmaniasis one of the most significant problems concerning the disease, which in most cases may render ineffective VL surveillance and control measures. In this study, we employed a noninvasive, simple and fast collection method by using oral (OS) and conjunctival (CS) swabs for the molecular diagnosis of canine infection and compared the results with those obtained with blood cells (BC). Material and methods: Forty-four dogs from an endemic region of canVL in São Paulo state with proven lymph node parasitism were investigated. The animals were classified in symptomatic (SD) (n=26) and asymptomatic (AD) (n=18) dogs, according with the clinical status and serum biochemistry. Real time PCR (qPCR) was used to inspect *Leishmania* in samples, using a target sequence from the kinetoplast DNA. Results: Overall, the frequencies of positive results by qPCR were as follows: conjunctival swab 61% (27/44); oral swab 39% (17/44) and blood 45% (20/44). The combination of mucosal results from ocular and buccal samples provided better diagnostic result when compared with BC, reaching 66% of positivity. Respective with the clinical status, the parasite detection in all samples were higher ($p \leq 0.05$) in the SD group compared with the AD: 77% (20/26) versus 39% (7/18) for CS, 54% (14/26) versus 17% (3/18) for oral swab, and 50% (13/26) versus 33% (6/18) for BC, respectively. Conclusion: The qPCR using conjunctival and/or oral swab samples did not present good performance to detect infection in asymptomatic dogs, but showed potential for the molecular diagnosis of the symptomatic animals, showing better results in relation to blood cells, a sample considered more invasive. **Supported by:**LIM50/HCFMUSP, CAPES and FAPESP processo 09/54533-4

BM023 - **REAL TIME PCR EVALUATION OF MICE INOCULATED WITH *TRYPANOSOMA CRUZI* I, II AND IV AND TREATED WITH BENZNIDAZOLE**

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Trypanosoma cruzi has a great biological heterogeneity regarding pathogenicity and drug susceptibility. Chagas disease (CD) is a systemic infection and intracellular forms of the parasite can be found in several tissues. An important parameter to be evaluated during treatment is the effect of the drug at the tissue level, monitoring of healing and also the possible return of the disease, stimulating the constant monitoring of patients. Our objective was to evaluate the impact of the treatment with benznidazole (BZ) in tissues of mice inoculated with strains of *T. cruzi* I, II and IV through histopathological and molecular analyzes. Groups of 26 Swiss mice, 21-28 days were inoculated with $1x10^4$ blood or $2x10^6$ metacyclic trypomastigotes/animal from TcI (6 strains), TcII (2 strains) and TcIV (5 strains). Thirteen mice were treated orally with BZ (TBZ) and the other 13 were the untreated group (NT).The animals were sacrificed one day after the peak parasitemia, at days 30 and 100 after inoculation. Fragments were obtained from different organs and stained with HE. Additionally, parasite levels were analyzed in these organs by SYBR Green qPCR and the data were quantified by the comparative method Cq ($\Delta\Delta$Cq). For NT groups animals, regardless DTU, from 312 organs analyzed were observed tissue parasitism (TP) in 10 organs analyzed by HE and 161 by qPCR. BZ treatment promoted reduction in the number of organs displaying TP (from initially 161 for 118 organs presenting TP), 9 out of 13 strains studied showed reduction in this parameter. For NT groups, the mean parasite load was 255.3 for mice infected with TcI, 478.1 for TcII and 38.3 for TcIV. The parasite load was significantly reduced ($P<0.001$) by BZ treatment in animals infected with TcI and TcII, and significantly increased ($P<0.001$) for TcIV. Despite the great effect of the treatment, qPCR data shows the need for long term monitoring of patients with CD, since parasites were found in animal tissues considered cured. **Supported by:**CAPES/CNPQ

BM024 - **ANALYSIS OF THE NEW THERAPEUTICAL MOLECULAR TARGET FROM *TRYPANOSOMA CRUZI*: THE ENZYME GLUCONOKINASE FROM ALTERNATIVE PENTOSE PHOSPHATE PATHWAY**

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Chagas' disease is a public health problem in Latin America with a strong impact in Brazil. Around 8 million people in the Americas are infected with *Trypanosoma cruzi* and a rate of 12,000 deaths/year. Despite this situation, the drugs used in treating this disease are active only against the acute phase and have low efficiency and many side effects. Therefore, the search for new therapeutic targets, by studying the metabolism of *T. cruzi*, is an alternative in the development of new drugs. In this context, we used the AnEnPi, a software tool able to identify and classify enzyme activities using genomic data, allowing the reconstruction of different metabolic pathways. It was identified in *T. cruzi* some potential proteins as molecular targets. We selected the enzyme gluconate kinase (GK), involved on alternative branch of pentose phosphate pathway, and catalyses the phosphorylation of D-gluconate to 6-phospho-D-gluconate. Analysis of genomic sequences using various bioinformatics tools allowed the identification of two genes for GK activity in *T. cruzi* and this activity was analyses several structural motifs identification on primary and secondary GK structures. Subsequently, we performed the cloning of the respective *T. cruzi* GK genes, using the vectors pBAD-TOPO and pET28a, following an heterologous expression in *E. coli*, strain BL21 (DE3). Recombinant GK was expressed in the insoluble and soluble fraction in approximate molecular weight expected around 23 kDa. The recombinant GK was purified by affinity chromatography. The polyclonal antisera raised against the GK were effective in recognition of recombinant GK and their native protein present in epimastigotes. The immunofluorescence assay revealed that the GK enzymes were possibly located in the cytoplasm and inside cytoplasmic vesicles along the parasite, not determined yet. Moreover, the structure of *T. cruzi* GK enzyme was deduced by homology modeling, showing differences between parasite and human enzyme.**Supported by:**CNPq, FAPERJ, FIOCRUZ

BM025 - **IDENTIFICATION OF GENES CODING FOR ENZYMES INVOLVED IN THE SYNTHESIS OF BIOACTIVE PHOSPHOLIPIDS IN TRYPANOSOMA CRUZI**

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Phospholipids form an essential group of lipids that possess structural and biological activities in all organisms. In trypanosomatids, the phospholipids are the main group of lipids of these parasites, comprising mainly the ester and ether lipids. Among these lipids, the most known is phosphatidylcholine (PC), which is the main lipid of membranes of trypanosomatids. In general, PC, lysophosphatidylcholine (LPC) and platelet-activating factor (PAF) are the most studied lipid mediators. PAF has two distinct biosynthetic pathways: a “de novo” biosynthesis pathway and the remodeling one. Our laboratory and others combine several pieces of evidence that *Trypanosoma cruzi* presents biochemical responses to both PAF and LPC. These molecules stimulate cell differentiation and infectivity of this parasite both “in vitro” and “in vivo”. The present study aims to identify genes coding for key enzymes involved in the biosynthesis of PAF and LPC in *T. cruzi*, using *in silico* analysis tools. Then, we selected enzymes from the KEGG database. The presence or absence of these selected enzymes in *T. cruzi* was searched in the TrytripDB database. When an enzyme was absent from *T. cruzi* genome, we used blastp to search for its sequence in organisms genetically related to *T. cruzi*. For the fifty enzymes involved in the synthesis of LPC, twenty gene sequences were found in *T. cruzi*, nine sequences were found in genetically related organisms and twenty one sequences were not found either in *T. cruzi* or in any of the genetically related organisms. Ten enzymes are involved in PAF biosynthesis; genes for two of them were found in *T. cruzi* genome, two in genetically related organisms and six of them were not found either in *T. cruzi* or in any of the genetically related organisms. These data enabled us to construct a map showing which biosynthetic pathways are present in *T. cruzi*, for the biosynthesis of LPC and PAF. **Supported by:**CNPq, FAPERJ, CAPES and INCT-Entomologia Molecular

BM026 - **PHENOTYPIC AND GENOTYPIC EVIDENCE OF DIFFERENT PROTECTIVE BEHAVIOR OF DISTINCT *TOXOPLASMA GONDII* STRAINS IN MICE EXPERIMENTAL CEREBRAL TOXOPLASMOSIS**

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The origin of *Toxoplasma gondii* infecting strain in encephalitis of immunosuppressed patients is controversial, since the disease can occur either due to reactivation of original strain that chronically infects the individual, or reinfection with new acquired strains. For evaluate the effect of cross-protection between genotypes in *Toxoplasma* encephalitis, we study several protocols of infection and sequential challenge with genetically distinct *T.gondii* strains (type II ME-49 or type III VEG strain) in normal or dexamethasone suppressed mice. Infection was evaluated by strain specific IgG humoral response and genotype of brain cysts. Specific IgG were determined by ELISA with peptides GRA6II (specific to ME49 strain) and GRA6I/III (specific to VEG strain). Brain cyst quantification was performed by *T.gondii* real-time PCR, with genotyping by nested PCR RFLP. In addition, CNS tissue cysts were reacted by immunohistochemistry with antibodies induced with strain-specific synthetic peptides. Our data show that primary infection by strain ME49 was unable to prevent colonization of CNS with cysts of challenged VEG strain by PCR-RFLP, regardless of the immune state of the host animals. In contrast, the infection with VEG strain prevented CNS colonization with cysts of challenging ME49 strain, despite the aggressive behavior of the last strain. These findings show that in immunodeficiency, especially AIDS, the primary chronic infection could be protective or not, depending of their genotype. This experimental data implies in the controversial findings in *Toxoplasma* encephalitis in immunosuppressed patients usually attributed to reactivation of latent tissue cysts, but frequently with several lesions, difficult to explain without a new infection. Phenotypic or genotypic tools are promising approaches for understanding the complex *T. gondii* host-parasite relationship as demonstrated in our studies in mice infected with different strains. **Supported by:** CNPq and LIMHCFMUSP.

BM027 - **LOOKING FOR GENES CODING FOR CALPAIN-RELATED PROTEINS IN TRYPANOSOMATIDS OF HUMAN MEDICAL IMPORTANCE**

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Once the completed genomes of the kinetoplastid parasites *Trypanosoma brucei*, *T. cruzi*, and *Leishmania* sp. became available, a comparative genome approach is now feasible to identify peptidases with low abundance and tricky to detect biochemically. To date, there are few reports on calpains in trypanosomatids of human medical importance. Calpains comprise a family of calcium-regulated cysteine peptidases implicated in physiological processes, such as regulation of gene expression, apoptosis and proliferation. We became involved in the study of calpain-related genes in the genome of trypanosomatids. Therefore, we identified and classified sequences of calpains in the genome of *T. cruzi*, *T. brucei* and *L. braziliensis.* The sequences obtained have been considered as calpains based on similarity with sequences from other organisms already described. Our *in silico* analysis revealed 40 sequences in the *T. cruzi* genome, 23 in *T. brucei* and 33 in *L. braziliensis*. Through multiple alignments and phylogenetic analysis of conserved domains in these sequences, we have sorted the calpains into four distinct groups characterized by the size of the gene, and by the presence of classical domains. After this *in silico* analysis, we decided to scrutiny the group that has the highest number of conserved domains and presents domain II, which contains the catalytic site (16 genes for *T. cruzi*, 20 in *L. braziliensis* and 11 in *T. brucei*). The comparison of calpain mRNA abundance by real time qPCR in epimastigote, trypomastigote and amastigote forms of *T. cruzi* revealed at least five genes with modulated expression among the life stage forms. Comparing the expression of each gene in relation to one another within each life stage form, we found no significant alterations. The study of calpains expression in trypanosomatids may help to establish the basis for targeted approaches aiming at a functional characterization of these molecules. **Supported by:**MCT/CNPq, FAPERJ, CAPES & FIOCRUZ

BM028 - **TCGPI: A MULTIPLEX PCR FOR *TRYPANOSOMA CRUZI* DTUS CHARACTERIZATION**

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Trypanosoma cruzi is currently subdivided into six DTUs, named TcI to TcVI. From these, TcIII and TcIV remain poorly understood, since they circulated majorly in sylvatic transmission cycle, exhibit very similar molecular profiles and are hard to be differentiated. To allow the correct identification and appropriate resolution of the evolutionary aspects of these lineages, new molecular marker capable to identify and separate TcIV from the others lineages still needed. To achieve that, we performed data mining in GenBank, NCBI, looking for gene sequences available for all six *T. cruzi* lineages. These sequences were initially used for phylogenetic reconstructions by maximum likelihood and distance methods. Glucose phosphate isomerase gene (GPI) was the one of the genes whose trees' topology allowed distinguishing TcIV from the others DTUs. Thereby, GPI sequences were screened for DTUs specific SNPs capable of generating new molecular markers suitable for multiplex PCR assay. A three-primers based PCR were so designed: one primer specific for TcIV, one another aligns with all the others lineages but not with TcIV and another one common for all strains. An in silico test was performed to measure the PCR sensitivity, specificity and accuracy. Were obtained high levels of these three parameters: 0.9231, 1.0000 and 0.9798 respectively. After amplification, the electrophoretic profiles allowed separating TcIV from other DTUs, since amplicons of 310 bp were observed for TcIV strains, while amplicons of 117 bp were detected for the others DTUs. **Supported by:**FAPEMIG, CNPq and CAPES

BM029 - **IS TCIV A TRUTH DTU?**

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The populations of *Trypanosoma cruzi* are currently divided in six DTUs (*Discret Taxonomic Units*): TcI-TcVI. Understanding *T. cruzi*'s population structure, the origin, and evolution of each DTU is crucial to find their correlations with biological properties as geographic distribution, host specificity and the clinical outcome of infection. As the phylogenetic history of the TcIV is still unclear our major goal in this study was to reconstruct the phylogeny of TcIV and estimate its rule in the evolutionary process of *T. cruzi* populations. For this, we recovered gene sequences of all *T. cruzi* DTUs available in the GenBank database for the genes: Cytochrome b (200 sequences), Cytochrome Oxidase subunit II (145 sequences), and Glucose-phosphate Isomerase (256 sequences). Then, we submitted these sequences to Maximum Likelihood and Bayesian phylogenetic reconstruction methods. The obtained trees demonstrate that there is not a monophyletic clade for TcIV populations, instead they are divided in two principal groups according their geographic origin: North America or South America. Our results also suggest that TcIV populations maybe has undergone to hybridization events, since its relations with other DTUs in the phylogenetic trees are different when we used mitochondrial or nuclear gene sequences. In conclusion, our hypothesis is that what we currently call TcIV can be actually a group of populations with different evolutionary histories, which includes hybrid and pure ancestral populations. **Supported by:**FAPEMIG, Capes, CNPq

BM030 - **IDENTIFICATION AND FUNCTIONAL ANALYSIS OF *TRYPANOSOMA CRUZI* GENES THAT ENCODE PROTEINS OF THE GPI BIOSYNTHETIC PATHWAY**

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Trypanosoma cruzi is a protist parasite that causes Chagas disease. Several proteins that are essential for parasite virulence and involved in host immune responses are anchored to the membrane through glycosylphosphatidylinositol (GPI) molecules. In addition, *T. cruzi* GPI anchors have immunostimulatory activities, including the ability to stimulate the synthesis of cytokines by innate immune cells. Therefore, *T. cruzi* genes related to GPI anchor biosynthesis constitute potential new targets for the development of better therapies against Chagas disease. *In silico* analysis of the *T. cruzi* genome resulted in the identification of 18 genes encoding proteins of the GPI biosynthetic pathway as well as the inositolphosphoceramide (IPC) synthase gene. Expression of GFP fusions of some of these proteins in *T. cruzi* epimastigotes showed that they localize in the endoplasmic reticulum (ER). Expression analyses of two genes indicated that they are constitutively expressed in all stages of the parasite life cycle. *T. cruzi* genes *TcDPM1*, *TcGPI10* and *TcGPI12* complement conditional yeast mutants in GPI biosynthesis. Attempts to generate *T. cruzi* knockouts for three genes were unsuccessful suggesting that GPI may be an essential component of the parasite. Regarding *TcGPI8*, which encodes the catalytic subunit of the transamidase complex, although we were able to generate single allele knockout mutants, attempts to disrupt both alleles failed, resulting instead, in parasites that have undergone genomic recombination and maintained at least one active copy of the gene. Analyses of *T. cruzi* sequences encoding components of the GPI biosynthetic pathway indicated that they are essential genes involved in key aspects of host-parasite interactions. Complementation assays of yeast mutants with these *T. cruzi* genes resulted in yeast cell lines that can now be employed in high throughput screenings of drugs against this parasite. **Supported by:**CNPq, INCTV, FAPEMIG

BM031 - **&DELTA; -AMASTINS FUNCTIONAL CHARACTERIZATION IN DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*BY POTENTIAL DOMINANT NEGATIVE**

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The characterization of *T.cruzi* surface components is relevant, since they are the key players of host-parasite interaction. Amastins are membrane glycoproteins found to be ~60 times more expressed in amastigotes compared to other forms. Besides protein localization and expression profile little is known about amastin function. Studies of amastin gene function in T. cruzi are limited due to its multigene structure and the absence of RNAi machinery. To circumvent this limitations, we decided to investigate the possible phenotypic changes caused by ectopic expression of wild-type or mutated δ-amastins fused to GFP in epimastigotes of *T.cruzi*. In this sense, five conserved threonines, potential O-glycosylation sites, at positions 37, 40, 44, 55 and 60 from δ-amastin, or one conserved cysteine residue at position 42 were replaced by alanines, generating Ama-5TM and Ama-1CM mutants. After transfection with plasmid containing GFP, or δ-amastin::GFP (wild or mutations), the transfectants were selected and submitted to confocal microscopy. Epimastigotes overexpressing AF::GFP or 1CM::GFP showed strong fluorescence at cytoplasmic membrane. 5TM::GFP was expressed at cell surface and some dispersed signals, probably at cytoplasmic organelles. The western blots analysis also showed amastins fused to GFP migrate at the expected theoretical sizes. This result suggests that δ-amastinare not heavily modified post-translationally to interfere with SDS-PAGE migration. The phenotypic analysis of mixed populations overexpressing wild type and mutated δ-amastins showed no differences in growth rate. However, the major phenotypic change was detected during differentiation from epimastigotesto metacyclics, where parasites expressing AF::GFP showed higher differentiation rate compared to wild type, or GFP, or mutated forms. These data suggest a possible role for d-amastin in cell differentiation process. Additional experiments using cloned parasites are being carried out. **Supported by:**CNPq, Capes, Fundação Araucária.

BM032 - **LMHUS1 IS INVOLVED IN CELL CYCLE PROGRESSION CONTROL IN *LEISHMANIA MAJOR* AND FORMS A COMPLEX WITH AN UNUSUAL RAD9 HOMOLOGUE**

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To ensure the faithful transmission of the genome to progeny, all cells must carry out mechanisms to efficiently repair any DNA damage. For example, DNA replication stress activates checkpoint-signaling pathways leading to cell cycle arrest and DNA repair. In many eukaryotes, the Rad9-Hus1-Rad1 proteins form a complex with a clamp-like structure (9-1-1 complex), which participates in the early steps of the DNA damage response and is a pivotal contributor to genome stability. Given the remarkable genome plasticity of the protozoan *Leishmania* we have been interested in the mechanisms involved in DNA metabolism and genome stability in these ancient eukaryotes. Therefore, we have investigated the existence of homologues of the 9-1-1 components in*Leishmania* and found that LmRad9, LmHus1 and LmRad1 are phylogenetically related to the 9-1-1 complex subunits from other eukaryotes. We observed that LmHus1 was recruited to the chromatin and co-localized with single-strand DNA binding protein, LmRpa1, upon DNA damage. We observed that reduced levels of LmHus1 affected the parasite ability to arrest cell proliferation when treated with hydroxyurea or camptothecin and cell cycle progression analysis revealed that this defect was due to a defective progression through G2/M phase of cell cycle. Also, co-immunoprecipitation between LmHus1 and LmRad9 suggested that these two proteins interacted to form a DNA damage responsive complex *in vivo*. Altogether, our findings indicate the participation of LmHus1, LmRad9 and LmRpa1 in the *Leishmania* DNA damage response and suggest their involvement in genome maintenance mechanisms. We are currently characterizing a LmRad9 deficient cell line that will be used to better understand the involvement of the possible 9-1-1 complex of *Leishmania* in the genome maintenance of this parasite. **Supported by** : FAPESP and CNPq.

BM033 - **ROLE OF THE ORC1/CDC6 ATP BINDING DOMAIN AND ATPASE ACTIVITY FOR THE STABILITY OF THE PRE-REPLICATION COMPLEX IN *TRYPANOSOMA BRUCEI***

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DNA replication starts with the formation of the pre-replication complex (pre-RC) into DNA regions known as origins of replication. Different from yeast trypanosomes don't have Cdc6 and Cdt1 in its pre-RC, but only a protein homologous of Cdc6 and Orc1 named Orc1/Cdc6, which interacts with the MCM helicase, licensing origins of replication. Our laboratory has demonstrated that the *Trypanosoma cruzi* and *Trypanosoma brucei* recombinant proteins Orc1/Cdc6 bind and hydrolyze ATP *in vitro* and that the ATPase activity increases in the presence of nonspecific DNA. The objective of this study is to evaluate the importance of ATP binding and hydrolysis for the formation and stability of pre-replication machinery in the genome of trypanosomes. We produced recombinant *T. brucei* Orc1/Cdc6 protein mutated at sensor 2 region (TbOrc1/Cdc6R251,252E) and mutated in the ATP binding region (rTbOrc1/Cdc6K79T). Mutation in the sensor 2 region of *T.brucei* (TbOrc1/Cdc6R/251, 252E*)* drastically reduced ATPase activity compared with wild type. rTbOrc1/Cdc6 mutated in the ATP binding lost its ability to bind ATP. TbOrc1/Cdc6 tridimensional structure was obtained by computational modeling and exhibited a conserved architecture with winged-helix domains for interaction with DNA and with sub-domains for ATP binding. Structural analyses of TbOrc1/Cdc6K79T model showed that it alters the ATP binding pocket, explaining our functional results. A "horseshoe-shaped" structure of the protein appears to be highly flexible and potentially stabilized by the binding of ATP. Genes were cloned into transfection vector and *T. brucei* procyclic forms were transfected and selected with Phleomicin. The formation and stability of the pre-RC as well as DNA replication and survival of parasites will be analyzed. **Supported by:**FAPESP

BM034 - ***RNAI KNOCK DOWN OF AMASTIN GENES IN LEISHMANIA BRAZILIENSIS AFFECTS PARASITE VIRULENCE***

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Leishmaniasis constitutes an important global health problem for which there is no vaccine and the available drugs for treatment are inadequate. *L. braziliensis* is a protozoan parasite member of the Trypanosomatid family that causes cutaneous leishmaniasis. Its genome has been sequenced recently and post-genomic studies on *L. braziliensis* can be largely benefited from the discovery of a functional RNAi pathway. Amastins are surface glycoproteins initially described in *Trypanosoma cruzi* and subsequently found to be encoded by large gene families also present in the genomes of several species of the genus *Leishmania*. Although amastins have been found to be highly expressed in the intracellular amastigote stage of several *Leishmania* species and are among the most immunogenic of all leishmanial surface antigens, their function remains unknown. Here we described the characterization of different members of the amastin gene family present in the genome of *L. braziliensis*. *In silico* analyses revealed 47 amastin genes located on eight different chromosomes and belonging to all of the four previously described amastin subfamilies: 1 copie of α-amastin, 2 copies of β-amastins, about 6 copies of γ-amastins and 38 copies of ð-amastins.Northern blot analysis showed that whereas ð-amastins mRNA is up-regulated in *L. braziliensis* amastigotes, β-amastins expression is increased in promatigotes.To investigate the function of ð-amastins, we analysed the phenotype of genetically modified *L. braziliensis* overexpressing this gene and a cell line in which its expression was inhibited by RNAi. In vitro infection assays showed that over expression of Amastin-0300 results in increased number of intracellular parasites 24 hour after infection whereas RNAi knock down results in decreased numbers of intracellular amastigotes in infected mouse macrophages. Furthermore, in vivo infection showed that overexpressing amastin or RNAi knock down parasites do not survive in infected Balb/c mice. **Supported by:**CAPES, FAPEMIG, INCTV

BM035 - **ANALYSIS OF CALPAIN EXPRESSION IN PROCYCLIC AND METACYCLIC FORMS OF *LEISHMANIA BRAZILIENSIS***

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Peptidases play central roles in all biological systems analyzed so far. In parasites, particularly, peptidases are involved with cell invasion, spread by the extracellular matrix, protection against the host immune system and intervention with a cascade of reactions catalyzed by peptidases of the host. Our study is focused on calpains, which comprise a family of cysteine peptidases involved in a variety of crucial cellular processes. Moreover, the existence of calpain in trypanosomatids such as Leishmania braziliensis has been discovered recently, so there is a lack of information about the functions of these molecules. Here, we evaluated the mRNA expression of 20 calpain sequences from procyclic and metacyclic promastigote forms of L. braziliensis by means of real time quantitative PCR. We decided to focus our study on the group that has the highest number of conserved domains, and presents domain II, which contains the catalytic active site (either altered or conserved). Up to now, our analysis of calpain mRNA expression in procyclic and metacyclic forms of L. braziliensis revealed at least five genes with enhanced expression in the procyclic form and one gene with higher expression in the metacyclic form. Comparing the expression of each gene in relation to one another within each life stage form, we found no significant alterations. The study of calpain expression pattern in L. braziliensis life cycle will help to unveil calpain function in trypanosomatids. **Supported by:**MCT/CNPq, FAPERJ, CAPES and FIOCRUZ.

BM036 - **DEVELOPMENT OF A LARGE SCALE SYSTEM FOR PRODUCTION OF ANTIBODIES AGAINST TRYPANOSOMA CRUZI PROTEINS &NDASH; PART I: A BIOINFORMATICS TOOL FOR SELECTION OF ANTIGENS BASED ON IMMUNOGENICITY AND SPECIFICITY**

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Several studies of *Trypanosoma cruzi*, the causative agent of Chagas disease, rely on the use of antibodies to characterize protein function, elucidate peculiar aspects of the parasite biology and develop diagnostic strategies. However, such studies may be hindered by the difficulty in obtaining these reagents at required amounts and with appropriate reactivity. Thereby, this work aims to establish a feasible and efficient methodology for large scale production of immunoglobulins with high affinity and specificity for proteins of *T. cruzi*. The proposed system starts with the prediction of immunogenic protein regions (50 to 200 aa), which will then be expressed, purified and inoculated in mice. Lymphocytes that present antibodies against the antigen will be isolated from the immunized animals by fluorescence-activated cell sorting, and the genes encoding the variable regions of antibodies will be amplified from these single cells, fused and expressed as single-chain fragments. Once purified and characterized with respect to their reactivity, these recombinant molecules can eventually be destined for several applications. We are currently leading a bioinformatic analysis to search for potentially antigenic stretches in polypeptides, based on the evaluation of physicochemical and structural properties and on comparative alignment in order to avoid regions that may lead to cross-reactive antibodies. The latter subject is extremely important as it deals with the *T. cruzi* supergene families, large clusters of very similar sequences resulted from genome redundancy or sequencing issues. In summary, we present a bioinformatics tool to select polypeptide stretches which allow the generation of immunoglobulins directed to an entire supergene group, as well as specifically to one or some proteins of a cluster. This is the first step to obtain antibodies in a high throughput approach that will bring significant contributions to the research of *T. cruzi*. **Supported by:**CAPES

BM037 - **DEVELOPMENT OF A LENTIVIRAL SYSTEM TO INVESTIGATE THE ACTIVATION OF G2A AND TOLL-LIKE RECEPTORS IN RESPONSE TO LYSOPHOSPHATIDYLCHOLINE DURING VECTOR-BORNE TRANSMISSION OF CHAGAS DISEASE**

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The innate immune response has an important role in the elimination of multiple pathogens in mammals by the recognition of pathogen-associated molecular patterns. *Membrane receptors such* as Toll-like receptors TLR4 and TLR2 (*that recognize* lipoproteins, *lysophospholipids and lipopolysaccharide)*, or receptor *G2A (that recognizes* lysophosphatidylcholine -LPC as its probable ligand) play pivotal roles on host innate immune response. Previous data of the group shows that LPC plays important roles in the pathogenesis of the Chagas disease. This molecule is present in the saliva of *Rhodinius prolixus*, and is capable of decrease the production of iNOS and so the NO produced by macrophages stimulated with *Trypanosoma cruzi* or LPS, increases the association with macrophages and increases the levels of the parasite in the blood of mice. To investigate these receptors we are developing a system of *lentivirus*-mediated gene *transduction* to express different sets of receptors and co-receptors in mammals cells. The lentivirus vector pHIV was modified by replacing the eGFP coding region for genes conferring different drug-resistance: Zeocin, Puromycin, Hygromicin or Neomycin. Previously obtained cDNAs of TLR4, its co-receptors (MD2, CD14) and the G2A receptor are being subcloned in distinct pHIV vectors with the different *drug resistance genes.* Thus, generate bicistronic expression of TLR4-puromycin, G2A-puromycin, G2A-neomycin, MD2-zeocin, or CD14-hygromicin. Overexpression will be carried out after transduction of HEK293T cells, via viral particles containing different combinations of receptor and co-receptors. Finally, the transcriptional activation by specific pathways will be observed after treatment with immunomodulators as LPC. The pJET1.2/Blunt vector was used as entry vector to all cDNAs. The coding regions of Zeocin (zeo+) and Hygromicin (hyg+) were amplified of pBABE-Zeo and pBABE-Hygro, respectively. Neomycin (neo+) and Puromycin (puro+) were amplified from pcDNA3.1 and pLKO.1-puro, respectively. The murines sequences of TLR4, MD2 and CD14 amplified from vectors pDisplay-TLR4, pcDNA3.1-CD14, and pEFBOS-MD2 were subcloned in pJET1.2/Blunt vector and sequencing, while a full-length human G2A cDNA was amplified from pcDNA3.1-G2A. Actually, the selections of the lentiviral bicistronic expression vectors are in course. Production of cell lineages stable expressing the different lentiviral constructs *will soon be achieved to carry out functional assays on presence of* immunomodulators. **Supported by:**IFRJ e FAPERJ

BM038 - **THE USE OF OMIC APPROACHES TO INVESTIGATE THE *TRYPANOSOMA CRUZI* RESPONSE TO IONIZING RADIATION**

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Trypanosoma cruzi is an organism highly resistant to ionizing radiation. Following a dose of 500 Gy of gamma radiation, the fragmented genomic DNA is gradually reconstructed and the pattern of chromosomal bands is restored in less than 48 hours. Although the cell growth is arrested after irradiation and DNA is completely fragmented, the RNA pool seems to maintain its integrity and protein quantities do not alter. In this work we analyzed the transcriptomic and proteomic profiles of irradiated and non-irradiated epimastigotes at different times after irradiation using microarray and 2D DIGE followed by MS/MS. The use of these complementary approaches accounts for a more comprehensive understanding of gene expression alterations induced by this stress. The microarray study revealed a total of 273 genes differentially expressed, being 160 up-regulated and 113 down-regulated. From these, genes with predicted functions are the most prevalent among the down-regulated category. Translation and protein metabolic processes, as well as generation of precursor of metabolites and energy pathways were affected. In contrast, the up-regulated category was mainly composed of obsolete sequences (which included some genes of the kinetoplast DNA), genes coding for hypothetical proteins, and Retrotransposon Hot Spot genes. The proteomic approach has revealed a total of 582 differentially expressed spots after irradiation considering all time points, and 53 proteins were further successfully identified. The majority of the spots were down-regulated. We have observed a strong tendency to the expression of molecules of low molecular weight. A significant negative correlation accessed by linear models between molecular weight and fold-change values was observed in all time points. This study is therefore revealing the peculiar response of *T. cruzi* to ionizing radiation, raising questions about how can this organism change its gene and protein expression to survive such a harmful stress. **Supported by:**CNPq, CAPES, Fapemig

BM039 - **IONIZING RADIATION EFFECTS ON MOLECULAR KARYOTYPE AND GENE ORGANIZATION OF *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi displays a remarkable resistance to ionizing radiation probably due toits and a high DNA repair ability. Gamma radiation induces cell growth arrest and chromosomal fragmentation in epimastigotes, which can be associated with DNA double-strand breaks (DSBs). However, 48h after irradiation, normal sized chromosomal bands can be detected by pulsed field gel electrophoresis (PFGE). We exposed epimastigotes of clone CL Brener to gamma-radiation doses of 500 to 2000 Gy and observed that doses above 500 Gy are lethal for the parasite. After 10 days, the parasites submitted to 500 Gy of gamma radiation were capable to restore the molecular karyotype and synteny in the chromosomal band XX. We evaluated the DNA repair and the gene organization under sub-lethal doses of gamma irradiation. Exponentially growing epimastigotes (clone CL Brener) were exposed to gamma-radiation doses of 100, 250 and 500 Gy. Cell growth, DNA damage and chromosomal recovering were evaluated for 10 days after irradiation. The parasites were cloned by serial dilution and a total of 18 clones were recovered. The chromosomal bands from these 18 clones were separated by PGFE and hybridized with specific chromosome-markers. We observed differences in the chromosomal pattern of 12 clones suggesting the occurrence of chromosomal rearrangement. The hybridization with the repetitive sequences DNA satellite and splice leader showed chromosome alterations in the majority of clones. The gene organization in irradiated clones was investigated comparing the level of synteny using 3 linkage groups from chromosomal bands XX, XVI and XIII. We observed a striking conservation of gene order. Therefore, *T. cruzi* displays a remarkable capacity to repair DSBs, being capable to restore the molecular karyotype with synteny conservation. **Supported by:**Fapesp

BM040 - **THE CENTRAL DOMAIN OF GP82, THE MAIN ADHESIN OF *TRYPANOSOMA CRUZI* METACYCLIC TRYPOMASTIGOTES, IS UNDER NEGATIVE SELECTION**

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Mammalian cell infection by metacyclic forms of *T. cruzi* relies on several receptor mediated pathways. The metacyclic surface glycoprotein GP82 is an 82 KDa GPI-anchored stage-specific adhesin which is directly involved in the host-cell invasion by these forms. This protein, recognized by the monoclonal antibody 3F6 (MAb 3F6), belongs to the transsialidase multigene family with more than 1,400 members per *T. cruzi* haploid genome. By gene survey on CL-Brener genomic database, we identified 19 sequences with more than 60% identity with the GP82 protein encoded by the cDNA J18 (L14824). Screening of a metacyclic cDNA library with a J18 radioactive-labeled probe or MAb 3F6, renders a repertoire of 14 clones which were thoroughly sequenced. A database retrieval of GP82 related sequences available at GenBank (excluding pseudogenes) allow us to perform a detailed analysis on the genetic diversity of the GP82 proteins, putatively, expressed by metacyclic trypomastigote in several *T. cruzi* strains. Analysis of GP82 central domain, which contains the cell-binding site, MAb 3F6 epitope and mucin binding stretch, showed a high degree of conservation of this domain among the *T. cruzi* isolates. The results on positive/negative selection events showed a strong conservative selection process in this region. Nine negative selection sites were detected at the cell-binding site and gastric mucin-binding region without any positive selection event, reinforcing the conservation of this region for proper biological function of GP82 as adhesin. Although the high degree of conservation in the central domain, several amino acid substitutions were found at the Mab 3F6 epitope. Expression of GP82 variants in heterologous systems is currently under way to determine their implications in cell invasion. **Supported by:** FAPESP, CNPq and CAPES.

BM041 - ***HAEMOPROTEUS* SP AND *PLASMODIUM* SP (SPOROZOA: HAEMOSPORIDA) IN SAO PAULO ZOO, BRAZIL: HOST SHARING**

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Sao Paulo Zoo is located in an area of Atlantic forest, where mosquitoes of the genera *Aedes* and *Culex*, the vectors of avian malaria, are common. In the Zoo, the space destined for local birds is shared by migratory species, as well as opportunistic species that live freely in the area of the park, favoring many infections. In captivity, these infections can develop as asymptomatic disease allowing the maintenance of lineage circulating for long periods and further spreading the parasite. *Plasmodium* sp. infections were detected, by light microscopy, in two Black Swans (*Cygnus atratus*) and one King Vulture (*Sarcoramphus papa*). One Black Swan was also infected by *Haemoproteus sp*. *Plasmodium* morphological characteristics provided evidence of the subgenus *Haemamoeba*. Erythrocytic meronts contained plentiful cytoplasm and fully grown gametocytes were roundish, oval or irregular. Size of both markedly exceeded the erythrocytic nuclei size. *Haemoproteus* morphological characteristics were similar to *H. greineri*, since fully grown gametocytes completely encircle the nucleus of infected erythrocytes and occupy all available cytoplasmic space in the host cells. Final microscopic identification is being carried out. PCR confirmed the *Plasmodium* infections and amplified the same ~1kb *cytb* sequence in all three birds. A phylogenetic tree is in agreement with our morphological identification since the sequence clustered with *Haemamoeba* and *Novyella* species, forming a well-supported clade with high posterior probability. This *cytb* sequence may assist in identification (barcoding) of this *Plasmodium* species in other birds. According the MalAvi database, the lineage PESA01, with 99% of identity with our sequence, was recorded in two birds: Pectoral Sandpiper (*Calidris melanotos*) and White-tipped Dove (*Leptotila verreauxi*) from Alaska and Uruguay, respectively. These findings provide evidence for possible broad host specificity of this parasite species.

BM042 - **GENETIC POLYMORPHISMS IN GENES RELATED TO INNATE IMMUNITY AND ASSOCIATION WITH MALARIA SUSCEPTIBILITY OR RESISTANCE IN A POPULATION WITH LOW EXPOSURE TO MALARIA**

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Malaria transmission is sporadic outside the Amazon region, but annually a number of cases are reported in the Atlantic Forest. These patients, with *P. vivax* or *P. malariae*, are usually asymptomatic. Many factors may be related to disease development but innate immunity is the first line of host defense. Toll-like receptors (TLRs) are one of the key factors involved in recognizing and defending against invading pathogens. Genetic variations in genes encoding TLRs are associated with increased susceptibility or resistance to a variety of infectious diseases, including malaria. In this context, we examined the association of TLR polymorphisms with the risk of malaria in residents of a low endemic area for malaria. We measured the frequencies of SNPs detected by PCR-RFLP in 5 genes. Microsatellite polymorphisms in intron II of TLR2 gene (GTn) were also analyzed. Data were analyzed considering the presence of *Plasmodium* detected by PCR and the presence of antiplasmodial antibodies detected by ELISA (CSP and *Pv*MSP1$_{19}$). Regarding TLR1 (I602S), our analysis showed a tendency of a malaria risk factor associated with I allele. In TLR6 (S249P), our data suggested a possible risk factor associated with the S allele. We failed to detect polymorphism in TLR4 (D299G) and TIRAP (S180L). Although the frequency for the mutant allele in the latter is very low in other, similar populations, this allele has been associated with the development of malaria symptoms, which would explain our findings, since our patients were all asymptomatic. In TLR9 (T-1237C), a significant association was found between the mutant allele and the presence of antibodies against *Pv*MSP1$_{19}$. For TLR2, our results demonstrate an absence of any genotype association with malaria. Results of the Hardy-Weinberg exact test are also presented. These data contribute to knowledge of the exact role of TLRs in malaria susceptibility/resistance in an epidemiological setting different from that has been studied worldwide. **Supported by:**FAPESP (2011/50492-1) and CAPES.

BM043 - **CHARACTERIZATION OF CYSTEINE BIOSYNTHETIC PATHWAY IN THE PROTOZOAN HEMOFLAGELLATE *TRYPANOSOMA RANGELI***

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Sulfur-containing amino acids play an important role in a variety of cellular functions such as protein biosynthesis, methylation as well as in polyamine and glutathione synthesis. We cloned and characterized a gene encoding for a cystathionine β-synthase (CBS), a key enzyme of transsulfuration pathway, from the hemoflagellate protozoan parasite *Trypanosoma rangeli*. Our results show that *T. rangeli* CBS, unlike it's mammalian homologs, lacks the regulatory carboxyl terminus and does not contain the N-terminal haem-binding motif. *In vitro* enzymatic assays reveals that *T. rangeli* CBS does not present serine sulphydrylase (SS) and cystein synthase (CS) activities. Also, a gene encoding for a CS, a key enzyme in the sulfate assimilatory cysteine biosynthetic pathway, was cloned and characterized for this taxon. Dissimilar to bacterial, plant and other parasites, the *T. rangeli* CS lacks two of the four lysine residues (Lys^{67} and Lys^{199}) required for activity. Enzymatic studies on *T. rangeli* extracts confirmed the absence of CS activity, but confirmed the expression of an active CBS. These results demonstrate that *T. rangeli*, differently from *T. cruzi* and *Leishmania* sp., exclusively possess the transsulfuration pathway for cysteine metabolisms, instead of transsulfuration and assimilatory pathways present in these other trypanosomatids. **Supported by:**CAPES, CNPq, FINEP and UFSC

BM044 - **CONFLICT BETWEEN REPLICATION AND TRANSCRIPTION: ANALYSIS OF THE REPLICATION FORK IN *TRYPANOSOMA BRUCEI* CELLS THAT PRESENT THE TRANSCRIPTION MACHINERY STAGNANT IN THE GENOME.**

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Several studies suggest that there are conflicts between the complexes that replicate DNA and those that transcribe the DNA template. These conflicts can lead to blockage of replication and genomic instability. To circumvent these conflicts, cells protect themselves in two distinct ways. The first is preventing the replication and transcription machinery of taking place in the same DNA template. The second is by counting on proteins capable of removing from genome the stagnant transcription. This work aims to check if *T. brucei* replissome activity is compromised when the RNA polymerase is stalled in the genome. Thus, it was used the SMARD technique (Single Molecule Analysis of Replicated DNA). In this technique, DNA molecules that are being replicated are marked by two successive pulses with different analogs of thymidine, allowing visualization of replication origins and also the extension of sister forks. When DNA is equally replicated in both directions from the origin of replication, sister forks present equal extension (symmetrical molecules). Thus, the bloodstream form of *T. brucei*, which presents the CSB gene silenced by RNAi and therefore is not capable of removing the stagnant transcription machinery from the genome, will be compared with the control cell (normal expression of the gene CSB) for (i) average speed of the forks replication, and (ii) the number of symmetrical replication forks and asymmetrical replication forks (corresponding to compromised replissomes). We have standardized SMARD technique for bloodstream form of *T brucei,* but the pulses we used was too long, covering the entire DNA molecules fragments and not allowing to know if the end of sister forks extension is due to the end of replication or cleavage of DNA molecule. The pulses now will be reduced in order to allow the visualization of symmetrical and asymmetrical molecules in analyzed cells. **Supported by:** grant 2011/21570-4 and grant 2012/24554-2, São Paulo Research Foundation (FAPESP)

BM045 - **IDENTIFICATION OF PHOSPHOLIPIDS TRANSLOCATORS IN *LEISHMANIA AMAZONENSIS***

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The parasites of the genus Leishmania havea strategy called apoptotic mimicry and when inoculated by the insect in the host mammal the promastigote form can change its lipid composition of the external leaflet in the plasma membrane, as a result from the translocation of some phospholipids to the external face of the lipid bilayer. The macrophages can recognize this signal and without launching an inflammatory process, they perform the phagocytosis of the parasites. In this study we describe a strategy to identify the mechanism responsible for the exposure of phospholipid in *Leishmania (L.) amazonensis* using the binding of annexin V to the exposed phospholipid. We use a genomic library of this organism cloned in a shuttle cosmid containing the hygromicin resistance marker for the transfection with wild-type promastigotes. The transfected mutants will carry then extra copies of segments of the Leishmania genome and who carrying the information for the lipid exposure should be able to bind annexin V in the external membrane in an earlier development stage than normal (mid-log phase). The transfectants were labeled with annexin V-FITC and the cells with increased fluorescence were selected by fluorescence-activated cell sorting (FACS) and cloned in semi-solid medium. The analysis of the obtained clones showed that 10.7 % (± 0.26) of the population was able to bind annexin V in the mid-log phase, and the wild type 2.11 % (±1.63). To start the search of the responsible of phospholipid exposure we will sequencing the DNA and compare to genomic data-bases and appoint possible candidates. To confirm the role in the observed phenotype, the DNA fragments coding for the candidate genes will be cloned in a Leishmania expression vector and transfected into WT cells and they are expected to bind annexin V in the same phase of the transfected mutants (mid-log phase) as observed in the cells bearing the cosmids. **Supported by:**FAPESP and CNPq

BM046 - **LACALA2, A POSSIBLE NEW COMPONENT OF THE *L. AMAZONENSIS* TELOMERIC COMPLEX**

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Leishmaniasis is a spectrum of diseases caused by parasites of the genus *Leishmania*. The telomeric region of *Leishmania amazonensis*, the main causative agent of cutaneous leishmaniasis in Brazil, is composed by 5'-TTAGGG-3' repeats which form *in vitro* and *in vivo* DNA:protein complexes, named LaGT1-3. The protein components of LaGT2 LaGT3 were respectively identified as LaRbp38 and LaRPA-1. LaGT1 complex is the most abundant and specific for the G-rich telomeric single-strand, since it remains associated with telomeres even under high salt concentrations and temperature variations. LaGT1 protein component was purified from a promastigote nuclear extract using affinity chromatography with the G-rich telomeric single-strand DNA as the binding agent. The protein was fractionated and isolated from a Coomassie blue-stained SDS-PAGE gel and was sequenced *de novo* using mass spectrometry analysis. *In silico* analysis of digested peptide sequences using the *Leishmania* genome databases identified a sequence annotated as a putative calmodulin, CALA2-like, which has EF-hand domains. The gene that encodes LaCALA2 is present in three copies organized in *tandem* in the genome of *Leishmania* spp. LaCALA2, the ortholog of LmjCALA2-like, was cloned using a PCR-based strategy from genomic DNA of *L. amazonensis* promastigotes. The gene was subsequently cloned into a cloning vector, characterized by restriction digestion and automated sequencing. Further, *LaCALA2* was subcloned in a bacterial expression vector and we are currently starting the expression tests in order to obtain the optimal conditions for the recombinant protein expression and purification.We propose that LaCALA2 is a new component of *L. amazonensis* telomere/ telomerase complex since it shares high similarity with the human KIP1 protein (DNA-dependent protein kinase), which also presents EF-hand domains and was described as part of the human telomeric complex by its interaction with telomerase. **Supported by:**FAPESP

BM047 - **BIOCHEMICAL CHARACTERIZATION OF THE L- LEUCINE UPTAKE IN EPIMASTIGOTES OF *TRYPANOSOMA CRUZI***

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T. cruzi, has the ability to use amino acids as carbon and energy sources. Leu as other amino acids like Asp, Glu, Asn, Gln, Pro and Ile can be metabolized by epimastigotes of *T. cruzi*. It is well established that amino acids play several roles in the trypanosomatids biology, i.e., Pro, Leu, Glu and Asp are involved in the metacyclogenesis. Furthermore, Leu was reported as precursor for sterols biosynthesis and is necessary for the translation process being important on the globular and membrane proteins structure. Due to the absence of putative genes for branched chain amino acids biosynthesis in *T. cruzi* genome, the availability of Leu should depend on its uptake into the cell or protein degradation. In spite of the metabolic relevance of amino acids, their transport and recycling are poorly known. In the present work, we study the Leu uptake system in *T. cruzi*; it was measured in epimastigote forms using L-[4,5-^{3}H]-Leucine. The assay was initiated by the addition of 100 µl of the desired dilution of Leu in PBS (0.05 µCi of L-[4,5-^{3}H]-Leucine), to 20x10^{6} parasites. The range in which Leu uptake is linearly proportional to time was up 2 min of incubation. V_0 was measured at 28 ºC for 1 min by incorporation of radiolabelled amino acid. A Leu concentration of 3 mM was chosen for time progression study, data showed a saturable transport system with a Km of 1.076 mM and a V_{max} of 0.47 nmoles min $^{-1}$ per 20x10^{6} cells. The Leu transport increased linearly with temperatures from 10 to 45 ºC, allowing the activation energy calculation (51.30 kJ/mol). The Leu uptake was not dependent on Na^{+} or K^{+} but presents a pH dependence (optimal range between 6 and 7). Competition assays using other amino acids, showed that Ile inhibited the Leu uptake by 68%, possibly suggesting that Ile is internalized by the same system. These data prove the presence of a specific transport system for Leu and Ile in the parasite through a single kinetic system. **Supported by:**CNPq

BM048 - **PRODUCTION OF A PROTEIN WITH 53 IMMUNOGENIC SUBUNITS BASED ON *PLASMODIUM VIVAX* SURFACE ANTIGENS**

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Malaria control is still based on prophylaxis and treatment with antimalarial drugs. There is no effective vaccine for this disease and parasite-host mechanisms are not well understood. This study aims the production of a recombinant protein containing subunits based on epitopes of 11 antigens of *Plasmodium vivax*. Furthermore, the ability of serum samples from individuals exposed to Malaria in the Amazon region to recognize this protein was evaluated. Linear epitopes of *P. vivax* surface proteins were mapped by Spot-Synthesis technique and grouped in a protein with 53 immunogenic subunits. A synthetic gene was commercially obtained and cloned into an expression vector (pET-28a+). *E. coli* BL21 (DE3) competent cells were transformed and protein expression was induced. A protein of aproximately 100 kDa was expressed and purified. Enzyme-Linked Immunosorbent Assays (ELISA) showed that Malaria-exposed sera are able to recognize this protein. The knowledge generated in this study involves innovative information about naturally acquired immunity and has potential applications in differential diagnostic approaches as well as in development of subunit vaccines against Malaria. **Supported by:**PIBIC

BM049 - **CHARACTERIZATION OF LAPINX1, A PUTATIVE NATURAL INHIBITOR OF *LEISHMANIA AMAZONENSIS* TELOMERASE.**

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Protozoan parasites of the genus Leishmania cause leishmaniasis, a disease that can cause several clinical forms. The disease affects about 12 million people worldwide, most of which are located in underdeveloped countries. The drugs used to treat leishmaniasis are highly toxic and present low efficiency and high cost. The study of the telomeric region of the parasite is an important way to obtain molecular information that can be used for the development of new anti-parasitic drugs. There are several proteins associated with telomeric regions that are responsible for ensuring their stability and also for regulate the access of telomerase holoenzyme (responsible for the maintenance of telomere length). PinX1 telomeric protein, described in humans and other eukaryotes, is reported as a direct inhibitor of telomerase through a small domain TID (telomerase inhibitory domain) that directly interacts with the catalytic subunit (TERT) of telomerase. A search in the *Leishmania major* genome database done using the polypeptide sequence of human PinX1, retrieved a candidate for a putative *L. major* PinX1, which shares similarities with hPINX1 in both G-patch and TID domains. The LmPinX1 gene candidate was used to design oligonucleotides to amplify *L. amazonensis* PinX1 (LaPinX1) ortholog, since the genomes of *L. major* and *L. amazonensis* share high similarity. Genomic DNA of *L. amazonensis* was used as template for the amplification of the LaPinX1 gene ortholog using PCR. The amplicons (LaPinX1) were cloned in a cloning vector, and subsequently used to transform *E. coli* DH5 α and for plasmid extraction (mini-prep). We are currently performing the subcloning of LaPinX1 in a bacterial expression vector. Our goal is to express and purify the recombinant protein (LaPinX1) to further characterize its structural domains and the interactions with its possible partners such as telomerase and other *L. amazonensis* telomeric proteins. **Supported by:**PIBIC-CNPQ

BM050 - **INTERACTION HOST CELL-*TRYPANOSOMA CRUZI*: THE ROLE OF IMMUNOPROTEASOME.**

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Over the course of evolution, the *Trypanosoma cruzi* (etiologic agent of Chagas disease) developed efficient strategies to evade the immune system of the mammal host. In consequence, the infection by this parasite tends to be chronic, indicating that the *T. cruzi* escapes the immune system by down regulating the antigen processing routes. Within the antigen presentation pathway of MHC class I, the majority of intracellular antigenic peptides are generated by the proteasome, a multicatalytic protease complex consisting of constitutive subunits, three of which can be replaced by enzymatically active IFN-γ-inducible subunits, β1i, β2i, and β5i, forming the immunoproteasome. The immunoproteasome has its protease activity modified promoting the generation of suitable peptides presented by MHC class I. In this scenario, we analyzed if the expression and activity of the immunoproteasome and MHC I pathway components were altered upon infection of HeLa cells by *T. cruzi*. Perfoming RT-qPCR experiments comparing non-infected or infected cells and untreated or treated cells with IFN-γ we observed that the mRNA levels of the immunoproteasome subunits β1i, β2i, β5i and PA28, as well as TAP I and β2-microglobulin did not show differences in expression between the treatments. In contrast, western blot analysis and catalytic assays showed a dramatic reduction in the protein levels and activity of the immunoproteasome IFN-γ-induced subunits, TAP-I and MHC class I molecule on the infected-treated cultures. These results suggest that the parasite developed a mechanism that inhibits the immunoproteasome biosynthesis as well as other components of the intracellular pathway of antigen presentation, which could be related with the *T. cruzi* persistency, favoring the escape of the immune response of its host. **Supported by:**CNPq

BM051 - **MOLECULAR DIAGNOSTIC AND IDENTIFICATION OF LEISHMANIA SPECIES IN CANINE VISCERAL LEISHMANIASIS IN FEDERAL DISTRICT-DF-BRAZIL**

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Leishmaniasis is one of the most relevant parasitic diseases, being in expansion on urban and periurban areas in Brazil´s Center East. Actually, serologycal diagnostic is employed in Brazil determining euthanasia of positive dogs. In this study we examined the relevance of the molecular diagnostic in the control of canine visceral leishmaniasis. On the other hand, a positive serology is not directly correlated to an active disease. In this way, we evaluated forty (40) dogs from different regions of the Federal District from may 2012 to january 2013 concerning Leishmania infection. For this purpose, animals were provided by the Center for Control of Zoonoses, after epidemiological investigation. We analyzed biopsies from liver, spleen, lymph nodes and bone marrow through parasitological (HE imprints and culture parasite isolation) and molecular (PCR from DNA ITS 1) tests. Our sample had more than 70% of male dogs and most of these animals had short hair and dropping ears. Biopsy PCR analysis showed positive results in 32 (80%) animals. Positive samples were from 21 (62,5%) symptomatic and 11(37,5%) asymptomatic dogs. PCR-RFLP revealed *L. infantum/chagasi* in 31 (77,5%) samples. One sample showed a different pattern related to *L .major.* In this way, we concluded that the molecular diagnosis is more sensible and specific to identify visceral leishmaniasis in dogs. **Supported by:**FAP-DF e REUNI/CAPES

BM052 - **CHARACTERIZATION OF TRYPANOSOMA CRUZI HYPOTHETICAL PROTEINS, DIFFERENTIALLY EXPRESSED IN METACYCLIC TRYPOMASTIGOTES.**

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Chagas disease is a sickness caused by the protozoan parasite *Trypanosoma cruzi*, and is estimated that 10 million people are infected worldwide, with nearly 300 thousands new cases detected annually. *T. cruzi* was discovered in the early 20th century by Carlos Chagas, and has been studied by several research institutes around the world. The *T. cruzi* genome shows around 50% of it genes coding hypothetical proteins, that is, without a known function. Due the lack of knowledge about this group of proteins, the fact that they represent a large portion of the *T. cruzi* genome, and more important, that they are modulated during the life cycle of the parasite, this work aims to molecularly characterize a group of 10 *T. cruzi* proteins annotated as hypothetical, differentially expressed in the metacyclic trypomastigote cell form. All selected genes were cloned in the appropriate entry and destination vectors of the Gateway® platform. From this group, 8 were heterologously expressed in bacteria, 5 were inoculated in mice, and the polyclonal antibodies were successfully obtained. The antibodies are now being used in immunological assays. It was also initiated the analysis of the protein expression and cellular localization in the *T. cruzi*. The selected genes were recombined in *T. cruzi* expression vectors, which fuse a GFP label in the protein, and were transfected in epimastigotes parasites. As the studied proteins are over expressed in metacyclic trypomastigotes, *in vitro* metacyclogenesis was performed in the transfected parasites, and the metacyclics were analyzed by fluorescence microscopy. At the same time, was started the construction of the knock-out cassettes, using the fusion PCR methodology developed by our group. Of the 10 proteins selected, knock-out cassettes have been built to 5 genes, and are now being amplified to be transfected in the parasites. This work is expected to add knowledge about the studied proteins, increasing the understanding about the *T. cruzi*. **Supported by:**CAPES

BM053 - **FUNCTIONAL CHARACTERIZATION OF HYPOTHETICAL PROTEINS DIFFERENTIALLY EXPRESSED IN *TRYPANOSOMA CRUZI***

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The protozoan *Trypanosoma cruzi* has four different forms in its life cycle: metacyclic trypomastigote, blood trypomastigote, amastigote and epimastigote. We have analyzed the transcriptome of these forms using RNA-Seq, but a large fraction of the modulated genes have unknown function. It is of crucial importance for the deep understanding of this parasite as well as interpreting high throughput datasets that we minimize this problem. We have selected 384 differentially expressed genes whose proteins have no known function for further characterization. In this work, we will present the results for 12 proteins, mainly overexpressed in the amastigote form (n=7), as well in both trypomastigotes (n=5). The general guideline of this project is to clone the complete CDS of the selected proteins with (close) and without (open) stop codon in a Gateway® vector; after that, the entry clone goes through two distinct pipelines: expression in *E. coli* (close configuration) or in *T. cruzi* (open and close configuration). The expressed protein in bacteria is used for the generation of polyclonal antibody in mouse, enabling differential expression corroboration, immunolocalization and protein-protein interaction (PPI) identification based on immunoprecipitation; the protein expressed in *T. cruzi* is fused to GFP and used for localization and PPI identification by immunoprecipitation. Besides the Gateway cloning pipeline, we also attempt to knock-out the genes using a PCR fusion procedure developed in our group. We were able to generate new data for all proteins in our selected set; obviously, the rate of success for each step is distinct for each protein, but overall it has provided useful information increasing our understanding of *T. cruzi* biology and the functional annotation in our omics database. We will keep deepening the functional knowledge about these proteins through completion of the generic pipeline and by specific analyses driven by the initial results. **Supported by:**Fundação Araucária

BM054 - **STUDY OF THE HEAT SHOCK TRANSCRIPTOME OF *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the causative agent of Chagas disease, is a parasitic protozoan of the family Trypanosomatidae, order Kinetoplastida, which exhibits a complex life cycle characterized by several cellular forms and the ability to infect insect and mammalian hosts. The heat shock proteins (HSPs) are mainly molecular chaperones, which represent the model group and most studied cellular response to heat stress. Since *T. cruzi* naturally undergoes temperature variation during its life cycle, when it transitions between the insect vector and the mammalian host, it is believed that the parasite synthesizes HSPs to prepare for the infection. Although the induction of HSPs is observed during differentiation of these parasites, it is not clear whether this program is part of the induction of differentiation, or merely a response to environmental changes. We aim to characterize the *T. cruzi* transcriptome during heat shock in an attempt to identify genes involved in the cellular response to heat stress. The transcriptome of epimastigotes from clone CL Brener incubated at different temperatures was obtained by deep sequencing (RNA-seq). Total RNA was extracted, enriched for poly (A)+ RNA, fragmented, and used for whole transcriptome sequencing with the Ion Total RNA-seq kit (Life Technologies). The RNA fragments were converted to cDNA and amplified by emulsion PCR in an Ion OneTouch System (Life Technologies), and then subjected to DNA sequencing in an Ion Torrent Personal Genome Machine (Life Technologies). Between 2-3 x 10^6 reads per sample were generated. In a reference-based strategy, reads were mapped to the publicly available genomic sequence of *T. cruzi* (clone CL Brener) using the CLC Genomics Workbench software package (CLC Bio). Gene expression analysis of transcriptomes from cells incubated at 29ºC, 37ºC and 40ºC is currently underway. **Supported by:** CNPq, FAPERJ and CAPES.

BM055 - **COMPARATIVE GENOMICS OF VIRULENT AND AVIRULENT CLONES OF THE CL STRAIN OF TRYPANOSOMA CRUZI REVEALS MAJOR DIFFERENCES IN THE TRANS-SIALIDASE SAPA REPEATS**

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Trypanosoma cruzi has a peculiar genome with a massive expansion of surface protein gene families, part of it is due to repetitive sequences. The completion of the CL Brener genome, in 2005, reveals new features related to the parasite virulence. The CL-14 is an avirulent clone derived from the same *T. cruzi* CL strain, however, in contrast to CL Brener, CL-14 is neither infective nor pathogenic. To investigate the molecular determinants of *T. cruzi* virulence, we performed a direct comparison of the CL Brener and CL-14 genomes, based on the available CL Brener sequences and sequences we generated from CL-14 using the 454 FLX platform. We found that they have highly similar nuclear genome organization, almost identical mitochondrial kDNA, similar numbers of predicted coding sequences as well as number of copies of members of multi-gene families. *In vitro* and *in silico* analysis showed that CL-14 is a hybrid strain that belongs to the same DTU as CL Brener (TcVI). Southern blot analyses indicate a similar karyotype and, for most multigenic families, sequence identity among the two clones is higher than 99%. One major difference we detected between these two genomes is related to a sub-group of the large Trans-Sialidase gene family (TcTS), known to have a C-terminal domain with 12-amino-acid repeats called 'shed acute phase antigen' or SAPA repeats. At least three copies of TcTS containing a repetitive domain varying from 19 to 41 repeats, which are highly immunogenic, are present in the CL Brener genome, whereas in CL-14 only one copy containing 2 SAPA repeats was identified. Those results were confirmed by southern and western blot analyses using probes containing SAPA sequences and anti-SAPA antibodies, respectively. As SAPA repeats allow the parasite to evade the host immune response, we found that the lack of a large repetitive domain in the TcTS of CL-14 may thus be one of the factors that could explain the differences in virulence between these two strains. **Supported by:**CAPES, CNPq, INCTV

BM056 - **FUNCTIONAL CHARACTERIZATION OF HYPOTHETICAL PROTEINS DIFFERENTIALLY EXPRESSED IN *TRYPANOSOMA CRUZI***

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The protozoan *Trypanosoma cruzi* has four different forms in its life cycle: metacyclic trypomastigote, blood trypomastigote, amastigote and epimastigote. We have analyzed the transcriptome of these forms using RNA-Seq, but a large fraction of the modulated genes have unknown function. It is of crucial importance for the deep understanding of this parasite as well as interpreting high throughput datasets that we minimize this problem. We have selected 384 differentially expressed genes whose proteins have no known function for further characterization. In this work, we will present the results for 12 proteins, overexpressed in the amastigote (n=9) or blood trypomastigotes (n=3). The general guideline of this project is to clone the complete CDS of the selected proteins with (close) and without (open) stop codon in a Gateway® vector; after that, the entry clone goes through two distinct pipelines: expression in *E. coli* (close configuration) or in *T. cruzi* (open and close configuration). The expressed protein in bacteria is used for the generation of polyclonal antibody in mouse, enabling differential expression corroboration, immunolocalization and protein-protein interaction (PPI) identification based on immunoprecipitation; the protein expressed in *T. cruzi* is fused to GFP and used for localization and PPI identification by immunoprecipitation. Besides the Gateway cloning pipeline, we also attempt to knock-out the genes using a PCR fusion procedure developed in our group. We were able to generate new data for all proteins in our selected set; obviously, the rate of success for each step is distinct for each protein, but overall it has provided useful information increasing our understanding of *T. cruzi* biology and the functional annotation in our omics database. We will keep deepening the functional knowledge about these proteins through completion of the generic pipeline and by specific analyses driven by the initial results **Supported by:**IBMP

BM057 - **NAVIGATING THE DARK MATTER OF PROTEIN CODING SEQUENCES IN *TRYPANOSOMA CRUZI***

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A large number of protein coding sequences (CDS) in *Trypanosoma cruzi* have no known function; besides, for CDS with functional annotation a significant proportion is extreme generic, adding little value to understanding the molecular organization of this parasite. This is specially important when dealing with high throughput omics analyses, as genomics, transcriptomics or proteomics, because having extensive meta-data for the candidate genes enriches at a large extent the applicability of these studies. Aiming to diminish this lack of information, several groups are trying to impute function to these proteins, mainly in other trypanosomatids as *T. brucei,* using classical molecular approaches hugely aided by omics analyzes. Our group is also dealing with this problem, but using in general a full high throughput philosophy: building a complete ORFeome, creating a full protein-protein interaction map using yeast two-hybrid technology, and characterizing differentially expressed genes using a generic framework. Other high throughput initiative is to analyze bioinformatically the whole content of genes with unknown function in all trypanosomatids with available genome, trying to contextualize them and to create guilty-by-association rules. Here, we present our initial bioinformatics analysis of the relationships of genes with unknown function in trypanosomatids, including evolutionary relationships, protein domain architecture, localization prediction, gene expression networks and protein-protein interaction networks. Using these data, we were able to contextualize a significant proportion of *T. cruzi* genes with unknown function (~20%). Taken together, these data represent a powerful approach to orientate the laboratory efforts aimed at functional characterization as well as adding valuable information for gene list analyses of omics data. **Supported by:**CAPES

BM058 - **MOLECULAR FINDINGS OF TRYPANOSOMA CRUZI I IN EXPLANTED HEARTS FROM BRAZILIANS PATIENTS**

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Chagas disease has a variable clinical course and the cardiac involvement are the most serious and frequent manifestation. Heart transplantation is the proper treatment for end-stage heart failure. Molecular evaluation of the Chagas heart disease (CHD) manifestations by molecular profiling of *T. cruzi* populations in explanted tissues have been indicated a predominance of TcII in patients from Minas Gerais, Brazil. This aspect is different in some other Latin America countries as Colombia and Venezuela where TcI is more frequently found associated with CHD manifestations. Herein, we present molecular data, which indicate the presence of TcI also in CHD patients from Minas Gerais, Brazil, unlike what is normally reported. We observed the presence of TcI in heart explants of two of the 50 CHD patients transplanted at the Hospital das Clínicas da UFMG in the last six years. For one of them TcI was found in atrium-ventricular junction, left ventricle; apex of the left ventricle and interventricular septum, and in the second patient TcI was found in the interventricular septum. These results represent the first reported cases of TcI directly detected in heart of CHD patients, in Brazil. Besides these two cases, we found also one case of TcVI, one case partially identified as TcV/VI, one mixed infection of TcII plus TcVI in explanted tissues of patients. Together these findings suggest that the molecular epidemiology of CHD in Minas Gerais is more complex than initially expected. Ongoing studies have been done to enlarge the number of analyzed CHD patients in order to discriminate the intra-specific variability of *T. cruzi* populations by LSSP-PCR and microsatellites **Supported by:**FAPEMIG, Capes, CNPq

BM059 - **INVESTIGATION OF RESERVOSOME TARGETING OF P-TYPE H+-ATPASE IN *TRYPANOSOMA CRUZI***

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The endocytic pathway of *Trypanosoma cruzi* displays a unique compartment, the reservosome, a lysosome-like organelle which accumulates internalized nutrients and proteases. However, molecular markers for reservosomes that allow detailed investigation of the organelle biogenesis and function are still lacking. Previous studies with the *T. cruzi* Y strain identified a pair of tandemly linked genes that encode P-type H^+-ATPases, called TcHA1 and TcHA2, which are homologous to fungal and plant proton-pumping ATPases. Previous studies using immunofluorescence and immunoelectron microscopy suggested that the gene product of TcHA1 is localized mainly in the reservosomes, among other cellular membranes, whereas TcHA2 localized only to reservosomes. Since TcHA2 displays an additional 50 amino acids insertion at the N terminal region, we asked if this region could be responsible for the targeting of TcHA2 to reservosomes. To address this issue, we cloned the TcHA2 gene from Dm28c *T. cruzi* and constructed transfection cassettes of different domains of TcHA2 in fusion with eGFP. Genomic DNA from *T. cruzi* Dm28c was used as template for PCR reactions to amplify the full length genes of TcHA1 e TcHA2. Those genes share over 90% sequence similarity with the respective genes of the Y strain. Four PCR products encompassing: i) the 50 aas N-terminus insertion, ii) 363 aas, iii) 592 aas, iv) 916 aas or full length TcHA2 were generated by PCR and fused to eGFP. The transfection cassettes are currently being cloned in pTEX for parasite transfection. In parallel, a 780 bp fragment corresponding to an internal region conserved between TcHA2 and TcHA1 was used to produce recombinant Histidine-tagged protein by heterologous expression in *E.coli* as inclusion bodies. The recombinant fusion protein was denatured, purified and used to produce anti-sera to *T. cruzi* P-type ATPases. The analyses of P-type ATPase protein expression in *T. cruzi* is ongoing. **upported by:**cnpq

BM060 - **SINERGISTIC ACTION OF METALLIC COUMARIN COMPOUNDS AGAINST DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*.**

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Objective: Study of susceptibility of different strains of the protozoan Trypanosoma cruzi to metallic compounds and their associations in the elimination of amastigotes form, responsible for the chronic phase of disease For this we compared the activity of coumarin compounds complexed to metals and Benzonidazol against the form amastigotes of different strains. Using combinations of two of the metallic compounds obtained by our group, we studied the synergistic action of them. We are conducting in vivo experiments using compounds with higher index security (IS) for the treatment of mice.
Methods and results: In vitro studies were performed with different strains of amastigotes of T. cruzi treated with benznidazole, coumarin, 4-hydroxy-3-nitrocumarin (4H3NC) and 4H3NC associated with metals (copper, iron, zinc or nickel). The synergistic action was assessed by associations of different compounds. Infected Vero cells (106/ml) were incubated in 24-well plates with RPMI medium containing the compounds for 24 hours and the infection rates (IF) calculated. Compounds 4H3NC-Cu, coumarin and 4H3NC-Fe showed significant differences between the strains in IF. The use of metal compounds related to the treatment of amastigotes showed a greater effectiveness in relation to benznidazol, as well as differences between the strains.
Conclusion:The statistical analysis showed significance in the anti-amastigote activity of compounds 4H3NC-Cu, coumarin and 4H3NC-Fe compared to control and benznidazol in different strains. Experiments associations of metallic compounds showed highly significant results of antiparasitic action. Tests are in progress to modulation of the concentration of different metal compounds in looking for similar activity among different strains. The continuation of these studies in vivo using Swiss mice infected with T.cruzi is ongoing. Our results can contribute to the use of these compounds for the chronic form of Chagas disease. **Supported by:**UNIVERSIDADE BANDEIRANTE ANHANGUERA, UNIBAN

BM061 - **INVESTIGATION OF THE PARTICIPATION OF GALECTIN-3 IN APOPTOSIS PATHWAYS OF CELLS INFECTED BY *TRYPANOSOMA CRUZI*.**

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Introduction: The etiologic agent of Chagas disease is the protozoan *Trypanosoma cruzi,* in humans the pathogenesis advances with intracellular development in several tissues. The stages of infection involve adhesion, recognition, signaling and invasion, through interactions between host receptors and the parasite surface molecules. In the host cell, a varied set of molecules become active during the adhesion and recognition, as galectin-3 that mediate host-parasite interactions in dendritic and smooth muscle cells. **Objective**: Our experiments aim to investigate the role of galecin-3 over apoptosis signalling pathways in cells infected by *T. cruzi*. **Methods:** To analyze the consequences of infection by *T. cruzi,* tissue-culture trypomastigotes expressing GFPreporter were used to infect HeLa cells or HeLa cells transduced with lentiviral vectors for RNAi, which express a shRNA Gal-3 or shRNA scramble. After infections, GFP-positive infected cells were analyzed by flow cytometry in order to estimate the percentages of cells at *early stage apoptosis* by measuring the exposition of phosphatidylserine (PS) staining with PerCP-Cy™5.5 Annexin V. The late stage apoptosis was measured by incorporation of 7-AAD, which intercalates into double-stranded DNA of dying or dead cells. **Results and Conclusions:** Cells showed different percentage of Annexin V positive staining: 63,2% for etoposide as a positive control, and 18,9%, 83,9%, 69,1%, at 8, 16 and 24 hours post-infection. To 7AAD, the percentages were 87,3% for etoposide as a positive control, and 24,4% 67,9% 88,3% at 8, 16 and 24 hours post-infection. Additional analyses are in course to confirm activation of the extrinsic or intrinsic pathway of apoptosis during infection in normal and transduced HeLa cells. The use of GFP-positive parasites allowed us to estimate by multi-fluorescence-channel flow cytometry the host apoptosis mediated by galectin-3. Knowledge about the roles of lectins as galectina-3 may contribute to a better understand of the pathophysiology in Chagas' disease. **Supported by:**PROCIÊNCIA-IFRJ, FAPERJ