PV-03 - **Endosymbiosis in trypanosomatids: the presence of the symbiotic bacteria suppresses the overflow metabolism in *Angomonas deanei***

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The coevolution of *Angomonas deanei* with a symbiotic bacterium represents an important model for studying the origin of organelles and parasitism. There is an intense metabolic exchange between both partners and the symbiont completes essential biosynthetic pathways of the host protozoan. In this work, we investigated the influence of the symbiont on the intermediary and energetic metabolism of *A. deanei* through ultrastructural and biochemical analyses. The wild-type (AdWt) and apossymbiotic (AdApo) strains were compared under different nutritional conditions. Results showed that AdWt incorporated and consumed less glucose than AdApo. Nuclear Magnetic Resonance (NMR) revealed that ethanol is twice more excreted by AdApo than by AdWt. Proteomic analyzes indicate that fermentation pathway enzymes were upregulated in AdApo, while AdWt upraised Krebs cycle and oxidative phosphorylation enzymes. A 24% reduction in ATP production was observed in AdWt cultured in SDM80 with glucose or in Warren medium after KCN inhibition, but this result was not repeated when cells were cultured with proline or submitted to fasting. AdApo showed no significant variation in ATP levels after growth under different conditions or inhibition by KCN. O_2 consumption by AdWt cultured with glucose was higher than that obtained with proline or submitted to fasting, while in AdApo no differences were observed in these culture conditions. Ultrastructural analyzes suggest that in the presence of proline or in fasting state, cells showed enlarged mitochondrial cristae, whereas in SDM80 with glucose mitochondrial swelling and approximation between glycosomes and mitochondrion were observed. Data indicate that the symbiont promotes the recovery of intermediate metabolites, reducing the overflow and optimizing the metabolism of *A. deanei*. **Supported by:**CAPES / 88887.604982/2021-00 **Keywords:** endosymbiosis;intermediate metabolism;energy metabolism.

PV-12 - **GENOMIC STUDY OF THE INTERMEDIATE METABOLIC PATHWAYS IN *Angomonas deanei*, A SYMBIONT-HARBORING TRYPANOSOMATID**

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Some protozoa of the Trypanosomatidae family, as Angomonas deanei, maintain a mutualistic relationship with a symbiotic bacterium, representing an excellent model for studying the origin of organelles and cellular evolution. During the cell cycle, the bacterium maintains a close proximity with glycosomes, special types of peroxisomes that optimize ATP production by compartmentalizing most enzymes of the glycolytic pathway. Such organelles also participate in fatty acid oxidation, pyrimidine biosynthesis, carbon dioxide fixation, gluconeogenesis, succinate fermentation and contribute to the synthesis of compounds such as glycerol-3-phosphate. The present work evaluated in silico the influence of the symbiont on the intermediary metabolism of the host trypanosomatid and its possible role of integration with other cellular structures, to better understand the intense metabolic exchange that characterizes this relationship. To achieve this goal, we performed a genomic study of the glycolytic pathways, gluconeogenesis, pentoses, Krebs cycle, purine and pyrimidine metabolism of A. deanei and its symbiont, other trypanosomatids and prokaryotes served as comparative models. By aligning protein sequences, we observed that the symbiont is phylogenetically closer to prokaryotes, especially other trypanosomatid symbionts, than to its eukaryotic host. We also observed that in A. deanei all investigated pathways are practically complete, whereas the symbiont suffered gene loss in most synthesis routes, as those of energy metabolism. The symbiont has a very small, but highly functional genome, completing host pathways for the production of essential metabolites, as amino acids and vitamins. **Supported by:**CNPq, FAPERJ **Keywords:** endosymbiosis; trypanosomatid; intermediate metabolism.

PV-13 - **Cell cycle particularities in *Kentomonas*, a genus that contains symbiont-harboring trypanosomatids**

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Kentomonas is a new genus of monoxenic trypanosomatids whose species host a bacterial symbiont in their cytoplasm. The β-Proteobacterium, also present in *Angomonas* and *Strigomonas*, maintains a mutualistic relationship with the protozoan, that is characterized by intense metabolic exchanges and ultrastructural changes. Furthermore, during the cell cycle, the bacterium division is coordinated with other host cell structures, so that each new cell contains a single symbiont. The main objective of this work is to analyze, from a structural point of view, aspects of the cell cycle in the *Kentomonas* genus, comparing the recently isolated *K. deaneorum* with *K. sorsogonicus* and also with *A. deanei* and *S. culicis*, the best characterized species. Results obtained by transmission electron microscopy and FIB-SEM showed that the *K. deaneorum* symbiont has a great proximity to the mitochondrial branches and that its kinetoplast has a disk shape, whereas in *K. sorsogonicus*, the symbiotic bacterium has an association with glycosomes and the kinetoplast has a trapezoidal format. In *A. deanei* and *S. culicis*, the symbiont shows proximity to glycosomes and also to mitochondrial branches. In all these species, the symbiont is close to the nucleus, indicating that the bacterium uses this organelle as a topological reference during the division process. It is important to mention that, curiously, *K. inusitatus* does not contain an endosymbiont, which may indicate that the symbiosis in *Kentomonas* is facultative and not mutualistic, as in the other genera. We conclude that *Kentomonas* species present intriguing particularities, constituting excellent models to study cell evolution and the diversity in trypanosomatids. **Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Código do Processo: 161032/2021-4
Keywords:endosymbiont-harboring trypanosomatids;Kentomonas genus;ultrastructural characterization of cell cycle.

PV-14 - **Location and expression of KAPS during Trypanosoma cruzi metacyclogenesis**

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Trypanosoma cruzi has a complex life cycle, which assumes different forms of development during metacyclogenesis. During this process of cell differentiation, epimastigotes present in the insect gut transform into metacyclic trypomastigotes, which are capable of infecting vertebrate hosts. Important morphological and ultrastructural changes occur throughout the *T. cruzi* metacyclogenesis. In epimastigotes, the kinetoplast that contains the mitochondrial DNA (kDNA) has a disk shape, whereas in metacyclic trypomastigotes this structure becomes globular. Furthermore, the kDNA topology changes from highly condensed to a looser arrangement. These changes are related to the activity of small basic proteins, called Kinetoplast Associated Protein (KAPs). KAP4 and KAP7 are considered universal since they are present in all trypanosomatids so far analyzed. In this work we obtained protozoa which express KAPs fused to a fluorescent tag, thus allowing the evaluation of protein localization and expressions during metacyclogenesis. In epimastigotes, KAP4-mNG is located both in the kDNA network, in a semi-circle distribution, and in antipodal sites. In intermediate forms of the protozoan, which already present the globular kinetoplast, the same semi-circle location was observed, but the antipodal sites were not labeled. In metacyclic trypomastigotes, no labeling was observed for KAP4-mNG. In epimastigotes KAP7-mNG was observed punctually at the antipodal sites, while in intermediate forms of the protozoan and metacyclic trypomastigotes the labeling was not observed. Relative expression analysis showed that both KAPs were downregulated throughout metacyclogenesis, however this occurred in different ways. After nutritional stress, the expression of KAP4-mNG gradually decreased, while KAP7-mNG abruptly reduced and remained constant during the rest of the process. We conclude that the remodeling of the kDNA network is related to the negative regulation of universal KAPs. **Supported by:**CNPq and FAPERJ
Keywords: Trypanosoma cruzi;metacyclogenesis;kinetoplast.

PV-15 - **Leishmanistatic in vitro activity of triclabendazole**

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Leishmaniasis is a tropical neglected disease that causes approximately 1 million new cases and 30,000 deaths per year. Given the lack of adequate medication for the treatment of leishmaniasis, it is essential to use drug repositioning to save time and money in the search for new drugs, especially in the neglected disease scenario. Available treatments are still far from being fully effective in treating their clinical forms and are parenterally administered, which makes it difficult to carry out the complete treatment, in addition to being extremely toxic and, in some cases, leading to death. Triclabendazole (TCBZ) is a benzimidazole used in the oral treatment of fasciolosis in adults and children and presents lower toxicity in comparison with Amphotericin B (AmpB). When associated with the possibility of oral administration, it becomes a desirable candidate for the treatment of other parasitosis. The mechanism of action for TCBZ is not yet well understood, although it may have microtubules, polyamines, or ergosterol biosynthesis in the parasite as a pharmacological target. TCBZ has already shown antiproliferative activity against *T. cruzi*, *T. brucei,* and *L. infantum*. Within this context, this work aimed to evaluate the *in vitro* anti-*Leishmania* effects of TCBZ on *L. amazonensis* strain. The selectivity index (SI = CC_{50}/IC_{50}) was similar when compared with SI of AmpB. The evaluation of the cell cycle showed an increase of up to 10% of cells concentrated in S and G2, and morphological analysis by scanning microscopy showed high rates of dividing cells. Ultrastructural analyzes demonstrated large intracellular lipid concentrations, indicating alterations in lipid metabolism. Considering that TCBZ has the advantage of being cheaper and orally administrated, these results suggest that TCBZ is a promising candidate for use in the treatment of leishmaniasis. **Supported by:**CAPES E FIOCRUZ **Keywords:** LEISHMANIASIS; DRUG REPURPUSING; TRICLABENDAZOLE.

PV-16 - **Endosymbiosis in trypanosomatids: the coordinated division of the bacterium depends on the host microtubules' dynamism**

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Some trypanosomatids, such as *Angomonas deanei*, co-evolve with a symbiotic bacterium in a mutualistic relationship. The bacterium presents coordinated division with other host cell structures, such as the nucleus and the kinetoplast, so that each protozoan contains a single symbiont. The use of microtubule polymerization inhibitors,as well as the RNAi system for tubulin, revealed that the symbiont division is cytoskeletal-dependent. In this work, Trichostatin A (TSA), an inhibitor of Histone Deacetylases (HDACs), was employed to verify the importance of microtubuledynamic instability for symbiont division. HDACs are enzymes that catalyze the removal of acetyl radicals (deacetylation) from proteins such as histones and tubulin, thus influencing the microtubule dynamism. The CRISPR-Cas9 system was also used to delete the HDAC6 gene, however, it was not possible to obtain null mutants for this protein, whichseems to be essential for *A. deanei* survival. The TSA treatment promoted inhibition of proliferation, but not of viability. Morphological and ultrastructural changes were observed in treated protozoa analyzed by different electron microscopy techniques and by fluorescence optical microscopy, such as symbiont filamentation and alterations in the cytoskeleton arrangement. Cell cycle arrest in the G1 phase was also observed. Similar structural changes were also reported in single allele-deleted mutants. In conclusion, the inhibition of deacetylation affects the division synchronicity of the symbiont with otherhost protozoan structures and that the bacterium division depends on the microtubule dynamism. **Supported by:**FAPERJ e CNPq **Keywords:** Symbiosis in trypanosomatids; coordinated division; microtubule cytoskeleton.

PV-17 - **Could the differentiation occur preferentially at some cell cycle stage of Trypanosoma cruzi?**

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The Trypanosoma cruzi is constantly submitted to the selective pressures that make them adapt to different host environments.The differentiation of epimastigotes into metacyclic trypomastigotes is triggered by cell starvation, pH, and temperature.The parasites change their morphology, metabolism, and gene expression.Many molecular mechanisms that could control differentiation,for example,the cell cycle,remain unknown.The cell cycle consists in a cascade of events that may interfere with how the parasites respond to the environmental stimulus.We aim to verify if the differentiation trigger occurs preferentially in a specific cell cycle stage of T.cruzi.We synchronized exponential epimastigote cultures (CL Brener) in different cell cycle stages using hydroxyurea (HU).Immediately after the drug release,most parasites were in G1/S transition. After four and eight the parasites were in S and G2/M, respectively. We confirmed the cell synchronization by submitting the parasite samples to flow cytometry.We also used exponential and stationary epimastigotes without HU treatment as a control.Following the cell cycle synchronization,we submitted the parasites to in vitro differentiation.Our findings suggest a higher number of metacyclic trypomastigotes in G1/S and G2/M synchronous culture when compared to those submitted to differentiation in the S stage.Interestingly, the culture supernatant and adhered parasites were mostly in G1/S no matter what cell cycle stage the differentiation stimulus was given.The differentiation in the exponential phase was enriched in intermediated parasites on the culture supernatant.In contrast,the stationary phase culture has had only undifferentiated epimastigotes and metacyclic trypomastigotes.These preliminary results have shown that parasites in G1/S and G2/M phases could be more responsive to differentiation stimuli.We are now researching other aspects of these intriguing molecular mechanisms that connect the cell cycle to differentiation. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo / 2019/21354-1 **Keywords:**Trypanosoma cruzi; Differentiation; Cell cycle.

PV-18 - **Study of the role of Multicopper Oxidases genes in first stage nymphs of *Rhodnius prolixus* infected with *Trypanosoma cruzi*.**

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Multicopper oxidase (MCO) are a family of copper-containing oxidases that in insect have the functions associated to essential physiological process including cuticle sclerotization, iron metabolism and immunology. However, the participation of MCO in parasite-insect interactions has notbeen deeply studied. Through *in silico* analysis of gut transcriptome of first instar nymphs of *Rhodnius prolixus*, a vector of *T. cruzi*, we identified the presence of 11 genes in members of the families of MCO. Of these genes, 2 genes RPRC 002327 and RPRC000040 showedsignificantly increased expression after infection with the *T. cruzi*. Through the qPCR analyses, we confirmed that the gene RPRC000040, here denominated rpMCO2b, had a significant expression increase when infected with different parasite loads with trypomastigote stages of the parasite. Interestingly, comparison of specific relative expression of different organs showed the MCO2b is expressed in all organs excepted heart, with the highest levels of expression found in the posterior midgut, anterior and rectum followed by the testicles and fat body. MCO2b knockout did not change the course of hemoglobin digestion or embryogenesis, but resulted in a ten-fold increase in the size of resident microbiota. This result suggests the involvement of MC2b in midgut immune response in *R. prolixus*.
Supported by:CNPQ **Keywords:**Multicopper oxidase; Rhodnius prolixus;Trypanosoma cruzi.

PV-19 - **Metabolic enzymes in the nuclear compartment: a putative link with epigenetic changes in *Trypanosoma cruzi***

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Studies associating epigenetics and gene expression are already well known in many organisms. However, this connection is not so evident in Trypanosomes, whose gene expression regulation is based on post-transcriptional mechanisms. Compared to other eukaryotes, Trypanosomatids harbor peculiarities both in their epigenetic and metabolic programs. Different environments make the availability and use of metabolites very different in each life stage of the *Trypanosoma cruz*i parasite, which transits between invertebrate and mammalian hosts during its cycle. Therefore, we aimed to delve deeper into the relationship between epigenetics and metabolism searching metabolic proteins enriched in chromatin and nuclear proteomics public datasets. Specially enzymes related to the metabolism of acetyl-CoA, α-ketoglutarate, and succinyl-CoA, that are important for the acetylation, methylation, and succinylation processes, respectively. We found that 16 enzymes belonging to glycosome, mitochondria, and cytoplasm involved in glycolysis, TCA cycle, and SAM metabolism were found in the nuclear compartment and associated with chromatin in *T. cruzi*. Using the Tryp Tag platform, we found that 35,3% of these proteins in *Trypanosoma brucei* have nuclear localization using the neon green tag. To confirm the nuclear localization of these enzymes, we are currently performing activity measurements with epimastigote nuclear extracts. Preliminary analysis confirms nuclear hexokinase activity, while the pyruvate kinase (negative control) activity was not detected in the nuclear extracts. We choose six enzymes found in the proteomes to evaluate their enzymatic activity. In addition, we are currently using CRISPR-Cas9 to tag these selected metabolic targets to confirm their nuclear location. We intend to confirm the location and respective activities of these selected metabolic enzymes in the nucleus, showing a new interplay between metabolism and histones post-translational modification in *T. cruzi.* **Supported by:**FAPESP 2021/12469-0 **Keywords:**Trypanosoma cruzi; metabolism;epigenetic.

PV-20 - **Oxidative stress response mechanisms in phosphorylation of eIF2α and AMPK in T. cruzi**

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Trypanosoma cruzi epimastigotes that proliferate in the gut of triatomines undergo metacyclogenesis to become infective to mammalian host. This occurs through external stimuli mediated by different types of stress, including nutritional and oxidative stress. In eukaryotes, specific protein kinases are activated and phosphorylate the eukaryotic translation initiation factor (eIF2) which causes translation repression with the activation of stress response factors. AMPK (AMP-activated protein kinase) also acts as an energy sensor in cells that is activated by AMP/ADP increase through phosphorylation of a conserved threonine residue, resulting in several catabolic processes to regenerate ATP. To verify the role of these two pathways in T. cruzi, we obtained eIF2α-mutated parasites from Y and DM28c strains, in which the phosphorylated residue of eIF2α was replaced by alanine using CRISPR-Cas9. Y-strain parasites became highly susceptible while DM28c was less affected by hydrogen peroxide-induced stress. Oxidative stress induced AMPK phosphorylation in wild type eIF2α of both strains. In contrast, AMPK is more phosphorylated in eIF2α mutant of Y than of the DM28c strain. We also found higher levels of in oxidative species in the mutant of Y strain than in DM28c. These results indicate that eIF2α phosphorylation induces oxidative stress responses that can prevent activation of AMPK. Therefore, both pathways might be relevant for parasite differentiation into infective forms. **Supported by:**Fapesp - 2021/12515-1 **Keywords:**eIF2;AMPK;stress reponse.

PV-21 - **The tDNA chromatin changes positively correlate with transfer RNA expression levels in *Trypanosoma cruzi* life forms.**

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The tRNA genes (tDNAs) contain the genetic information for transfer RNA (tRNAs) expression, which function as an adapter molecule linking a specific amino acid to its corresponding mRNA codon during the protein synthesis. *T. cruzi,* the etiologic agent of Chagas Disease, has a complex life cycle with different life forms living in vertebrate or invertebrate hosts. Recently, we detected by the FAIRE-seq analysis that the open chromatin regions associated with tDNAs are more enriched in epimastigote (Epi) than in metacyclic trypomastigote (MT) forms. Interestingly, during the differentiation of Epi to MT life forms, a decrease in their translatome and transcriptome occurs. We hypothesized that the closed tDNAs chromatin observed in MT might be associated with these events and could play a key role in parasite differentiation. Therefore, this work aimed to analyze whether the tDNA chromatin changes are important to regulate tRNA expression and to determine the moment, during metacyclogenesis, that tDNA's chromatin became closed. Thus, we applied global nascent RNA transcriptome (Gro-seq) obtained in Epi forms in association with mature tRNA expression analysis by the northern blot in different life forms of *T. cruzi*. The tDNA chromatin changes were monitored by FAIRE-qPCR of three tDNA loci during the metacyclogenesis. The results showed a positive correlation between open tDNA chromatin and tRNA nascent expression levels in Epi. We also verified that the Epi has about two times more mature tRNA transcripts than MT. Furthermore, tDNA's chromatin became closed within 48 hours of metacyclogenesis induction. Studies are in progress to determine whether the closed tDNA chromatin is an important step to decrease transcription and translation in MT forms or a consequence of other events not yet fully understood. **Supported by:**FAPESP 21/11419-9 **Keywords:**Trypanosoma cruzi;tRNA expression;cell differentiation; .

PV-22 - **Could Hi-C data analysis indicate putative *Trypanosoma cruzi* centromeric regions?**

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The genome organization of trypanosomatids is peculiar due to the lack of classical RNA Pol II promoters regions; thereby, post-transcriptional mechanisms are the main strategy for regulating gene expression. Coding protein genes are transcribed as polycistronic units, and little is known about their disposal and composition in centromeric regions. Recent literature pointed out the chromatin tridimensional organization as a new layer of epigenetic regulation and highlight chromosomes grouped in nuclear territories, and centromeric DNAs often in close contact in the nucleus. Centromeres of only three chromosomes in *T. cruzi* have been identified so far. We are studying the role of chromatin topology in gene expression, the chromosomal regions more likely to interact spatially, and the putative centromeres in *T.cruzi* Brazil A4 by using genome-wide chromosome conformation capture (HiC) public data. We identified active and inactive compartments, topologically associated domains (TADs), and DNA loops. The first principal component analysis (PCA) plots can reflect the rough structure of chromosomes, dividing them on the chromosome arms and revealing the centromeric positions. Our results suggest eleven chromosomes with PCA1 typical of chromosome arms. Functional genomic analysis (e.g., GO and REVIGO) reveal lesser functional pathways heterogeneity for these centromeric regions. We do not observe significant differences in the number of genes on putative centromeric segments compared to their juxtaposing areas. We performed BLASTn searches using the retroelements VIPER and SIRE, and the repetitive regions TRS of centromeres from other *T.cruzi* and *T.brucei* strains. We also developed bash scripts to process the BLASTn results and filter the best matches. We observed the spatial interaction of centromeres in the nucleus is also true for *T. cruzi.* Our work reinforces the Hi-C relevance for exploring centromeres and expands our knowledge of genome organization on trypanosomes. **Supported by:**FAPESP, Projects 2021/03219-0 and 2018/15553-9 **Keywords:**high throughput chromatin conformation capture;centromere;Trypanosoma cruzi.

PV-23 - **Characterization of TgSRS12B, a surface protein predominant in Brazilian isolates of Toxoplasma gondii.**

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Toxoplasma gondii is the etiological agent of toxoplasmosis that affects ¼ of the world population. Toxoplasmosis is very dangerous in immunosuppressed patients, such as HIV patients, transplanted patients, or pregnant women. In Brazil, the high genetic variability makes these “atypical strains" cause more severe symptoms compared to North Hemisphere strains. Toxoplasma is part of the Apicomplexa phylum, characterized by the Apical complex, releasing several proteins related to invasion, parasitophorous vacuole formation, and maintenance. However, the first step of invasion is the adhesion to the cell host surface. One family protein that participates in that step is the SAGs. More than a hundred SAGs are annotated, and anchored by GPI, but few are characterized. The most characterized is the SAG1, the most abundant in reference strains and a promising candidate for diagnostic and vaccines. We analyzed the surface proteins of 5 isolates and references strains by mass spectrometry to understand the Brazilian strains. We identified more than 20 SAGs, and several transmembrane proteins still not identified. We confirmed that SAG1 is the most expressed SAG also in Brazilian strains. Most importantly, we identified some SAGs that are differentially expressed in Brazilian strains compared with the reference strains. One of these was the SRS12B, present in all 5 Brazilian strains but not in the reference strains RH and ME49. In order to study SRS12B, we are performing overexpression and soon knockout using CRISPR technology to understand the role of this SAG in the infection. It is important to notice that most SAGs that are differentially expressed are more present in bradyzoites (ToxoDB), and we did not induce differentiation. However, we noted that some Brazilian strains have a spontaneous differentiation by immunofluorescence. Understanding these specific Brazilian strains can reveal some proteins that can improve or better diagnose toxoplasmosis in Brazil. **Supported by:**Fiocruz, Capes, CNPq e Fundação Araucária **Keywords:**Toxoplasma gondii;Diagnosis;SAGs.

PV-24 - **Effects of meiosis-related genes deletion in Leishmania hybridization**

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Clinical outcomes of leishmaniasis are known to be related to *Leishmania* species diversity and the emergence of new strains. Previously, a cryptic sexual cycle has been described involving promastigote stages developing in the sand fly vector. The generation of hybrids was demonstrated in laboratory during sand fly infections and, more recently, in culture after DNA damage. We previously identified a subgroup of DNA stressed cells that upregulated a number of meiosis-related genes. Here, we used *L. tropica* parental strains MA37 and L747 that have a high mating efficiency to generate CRISPR-Cas9 competent cell lines, to delete the meiosis-related genes HAP2-1, HAP2-2, SPO11, MND1, DMC1, HOP1 and HOP2, and investigate their respective roles in genetic exchange. We were able to generate null mutants for each of these genes in both strains by substituting the whole CDS for Puromycin N-acetyltransferase (PAC) gene. For *in vitro* crossings, we used the null mutants from one of the strains in combination with a control line containing the Blasticidin S deaminase (BSD) gene integrated into the SSU rRNA locus, and selected for cells resistant to both PAC and BSD. For null mutants of *L. tropica* MA37, two null mutants showed a significant decrease in the minimum frequency of hybridization-competent cells: DHAP2-2 (2.2-fold lower, $p = 0.0021$) and DHOP1 (no hybrids recovered). By contrast, L747 null mutants showed a reduction for DHAP2-2 (1.5-fold lower, $p < 0.0001$), DDMC1 (1.9-fold lower, $p < 0.0001$), DHOP2 (5.5-fold lower, $p=0.0003$) and DHOP1(no hybrids recovered). *Lutzomyia longipalpis* infections indicated that the *L. tropica* L747 DHOP1 mutant was impaired for hybridization *in vivo.* These findings implicate the involvement of protein components of the meiotic machinery in *Leishmania* hybridization. Further experiments, including the generation and testing of re-expressor lines, are being performed to further investigate the role of meiosis-related genes in genetic exchange. **Supported by:**National Institutes of Health **Keywords:**Leishmania;Sexual cycle;Meiosis.

PV-25 - **Translation initiation factors EIF4E3 and EIF4E4 associate to different proteins and mRNAs targets in *Trypanosoma cruzi*.**

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In Trypanosomatids the control of gene expression is mainly performed by post transcriptional mechanisms and the total mRNA levels not always are according to corresponding protein levels, indicating a specific mRNA selection. Particularly, translation initiation seems to be regulated in trypanosomes, performed by several eIF4F-like complexes, whose roles in translation regulation have not being fully elucidated. When exponentially growing *T. cruzi* cells are subjected to nutritional/pH stress, a global repression of translation occurs, but a few transcripts are still associated to ribosomes. Here we investigated the two eIF4E paralogues, EIF4E3 and EIF4E4, in exponentially growing and stressed cultures by immunoprecipitation assays and analyzing the protein partners and the associated mRNAs. The results have shown that EIF4E3 forms a complex with eIF4G4 and both PABP1 and PABP2. On the other hand, eIF4E4 forms a complex with eIF4G3, eIF4A1 and PABP1. Both complexes are resistant to RNAse A treatment, suggesting they are formed by direct interactions. Several other translation initiation factors were found associated with EIF4E3 complex, indicating a role on translation, while eIF4E4 was mostly found with other RNA binding proteins. Both eIF4F complexes proved to be stable in stress condition, indication that complex composition was not the mechanism of the global repression of translation. However, some interactions are absent or reduced in stressed cells. Transcriptome analysis for both complexes have shown that EIF4E3 complex associates with several transcripts' classes. Alternatively, eIF4E4 associates preferably to ribosomal protein mRNAs. Surprisingly, under stress conditions a seemly higher abundance of mRNAs was captured, but composed to similar transcripts types for both factors, suggesting that the eIF4F-mRNA interaction was more stable. The data acquired indicates that both eIF4F complexes seem to have complementary roles in translation in *T. cruzi*. **Keywords:**T; cruzi;Translation Initiation;eIF4E.

PV-26 - **Characterization of *Giardia intestinalis* cytoskeleton: A new extra-axonemal structure evidenced by high resolution microscopy**

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Giardia intestinalis is an intestinal protozoan parasite that causes a diarrheal disease named giardiasis. During its life cycle, this protozoan has two morphologically distinct developmental stages: the cyst, which is the infective form, and the trophozoite responsible for colonization of the host intestine. *Giardia* cytoskeletal is composed primarily of microtubules organized in unique structures that are involved in a wide range of functions as cell motility and adhesion. *Giardia* flagella, displayed the 9:2 + 2 microtubular pattern, distributed in: anterior, posteriolateral, ventral and caudal flagella. The flagellar pairs differ in length, position, and their association with specific structures. The marginal plate and dense rods are associated with the axoneme of the anterior flagella and funis is associated with the axoneme of the caudal flagella. However, the composition and function of these axoneme-associated structures are unknown. In this work we analyze the externalization region of axonemes of the G. intestinalis and characterized an associated structure using high resolution microscopy techniques. Cells are treated with 2% NP-40 detergent for 10 min to remove the plasma membrane and expose the cytoskeleton. Then, the cells are observed by scanning electron microscopy, atomic force microscopy and transmission electron microscopy. Our observations in no-extracted and extracted membrane cells show that the externalizing region of the flagellum presents a differentiated morphological domain. Negative stain reveals an extra-axonemal structure that is observed associated with all pair of trophozoite flagella. A new extra-axonemal structure is around the axonemes and measures about 50 nm in thickness. This structure remains attached to the axoneme even when the connections between the axoneme microtubules are disrupted after detergent treatment. Our data will contribute to a better understanding of the organization and functional role of the *Giardia* flagella. **Supported by:** CAPES and FAPERJ (88887.512242/2020-00; E-26/202.824/2017). **Keywords:**Giardia intestinalis;high resolution microscopy;G; intestinalis cytoskeleton.

PV-27 - **Biochemical characterization of CRL (Cullin RING ligases)- like E3 ubiquitin ligases in Leishmania infantum**

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The ubiquitin proteasome system (UPS) is responsible for the most of intracellular proteolysis in eukaryotes and the ubiquitination process occurs through the action of three enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-carrying enzyme) and E3 (ubiquitin-ligases) that play a key role in this process, recognizing and transferring ubiquitin to its substrate. In parasitic protozoan, intracellular proteolysis is essential for the alternation of hosts in their life cycles and consequently for the success of parasitism. The *Leishmania* proteasome has a high identity to that of humans, being considered a target for treatment of leishmaniasis, however little is known about UPS in *Leishmania* genus. We aim to characterize the *Leishmania infantum* orthologous to the human genes SKP1, RBX1 and CUL1 respectively, which are components of the CRLs in humans. We evaluate through immunoprecipitation in mammalian HEK293T cells the interaction of the human components of CRLs and the *L.infantum* orthologs. Human F-box protein 7 (FBXO7) interacted with SKP1 from *L.infantum* and human Cullin1 interacted with SKP1 and RBX from *L.infantum*, suggesting that the regions responsible for the interaction among these proteins are conserved in the parasite proteins, suggesting the presence of the CRL complex in *L.infantum*. The parasite was genetically modified through the CRISPR-Cas9 strategy to generate *L. infantum* Cas9T7 lineages hygromycin resistant and it showed the same morphology and growth as the wild-type. After that, *L. infantum* Cas9T7 was used to produce *L. infantum* lineages with SKP1, Cullin 1 or RBX in fusion with 3xmyc-mCherry. These lineages will be used to develop immunoprecipitation of the parasites lysates with anti-myc resin aiming the identification of the CRLs components partners in *L.infantum* throught mass spectrometry. Our interaction results indicates a conservation of CRLs components in *L.infantum*, however their partners were not identified yet. **Supported by:**CNPq
Keywords: Leishmania;Ubiquitin proteasome system (UPS);E3 ubiquitin-ligase.

PV-28 - **Localization of the protein myosin C (TcMyoC) along the life cycle of *Trypanosoma cruzi*.**

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Myosins constitute a superfamily of motor proteins capable of moving on actin filaments, involved in several functions including endocytosis. Besides Myosin 1, conserved in several eukaryotes and Myosin XIII class, particular to kinetoplastids, *Trypanosoma cruzi* has 8 myosin genes (TcMyoA to TcMyoH-d), which arose from the expansion of Myosin XIII class. Due to the absence of these expanded genes in *L. major* and *T. brucei*, an association with the endocytic pathway was suggested. Recently, the participation of MyoF in the endocytic pathway of *T. cruzi* was demonstrated. Aiming at identifying the participation of TcMyoC in the endocytic traffic, we generated a mutant strain fusing the mNeonGreen (mNG) tag gene and 3 c-myc sequences to the N- terminal region of the *TcMyoC* gene by CRISPR-Cas9. Tagged epimastigotes observed alive showed TcMyoC close to the preoral ridge region. Endocytosis assays with transferrin-CF555 under different conditions, showed that TcMyoC followed the tracer, from the uptake at the cytostome-cytopharynx complex to the lysosomes., After detergent fractionation and Western blotting with anti-myc, TcMyoC was found mostly, but not exclusively, in the insoluble fraction, suggesting an association with the cytoskeleton. Immunogold labelling, with anti-mNG antibody, in negative stained cytoskeleton preparations, showed TcMyoC at the anterior region of the cytopharynx microtubules, at the preoral ridge. During metacyclogenesis, we found TcMyoC protein at the posterior region of stressed epimastigotes and intermediate forms. Metacyclic trypomastigotes were devoid of mNG::TcMyoC signal. After infection of mammalian cells, TcMyoC was found along the cytostome-cytopharynx complex of intracellular amastigotes. Extracellular amastigotes showed a punctual signal at the anterior region and trypomastigotes from the culture supernatant presented no signal. These results strongly suggest the close association of TcMyoC to the endocytic pathway of *T. cruzi*. **Supported by:**CNPq e 161037/2021-6
Keywords: TcMyoC;actin cytoskeleton;Trypanosoma cruzi.

PV-29 - **Production of *Trypanosoma cruzi* lineages expressing a panel of mutant versions of the TcRlp Ras-like GTPase.**

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Ras family GTPases are molecular switches that regulate cellular proliferation and differentiation by cycling between GTP and GDP-bound states. Ras family proteins also undergo isoprenylation (geranylgeranylation or farnesylation) on a c-terminal CaaX motif that enables membrane association, which is essential for their activity. The parasite *Trypanosoma cruzi* has only one Ras gene, which encodes the protein TcRlp (*T. cruzi* Ras-like protein), whose function is unknown. TcRlp has the CaaX motif CVLL, which is a target to geranylgeranylation. Our aim is to start the characterization of the TcRlp protein. In order to investigate the ability of TcRlp to bind and hydrolyze GTP, we have produced 3D homology-based models of wild-type TcRlp, as well as of mutants predicted to have impaired GTPase activity (TcRlp-G12V and Q61K – positive dominants) or increased affinity for GDP (TcRlp-S17N – negative dominant), and docked them *in silico* with GDP and GTP. The Q61K and S17N mutants presented molecular interactions which are consistent with positive and negative dominants, respectively. Next, we have produced mutants of the TcRlp gene, cloned them in the pTEX-GFP vector and transfected into Dm28c epimastigotes. Thus, the strains GFP-TcRlp-WT (wild-type), GFP-TcRlp-G12V, GFP-TcRlp-Q61K, GFP-TcRlp-S17N, GFP-TcRlp-dCaaX (non-isoprenylated) and GFP-TcRlp-CQLF (a farnesylated mutant), as well as the double mutants GFP-TcRlp-Q61K-dCaaX, GFP-TcRlp-Q61K-CQLF and GFP-TcRlp-G12V-CQLF were successfully produced. The intracellular localization of mutant proteins was investigated by fluorescence microscopy. TcRlp-WT and Q61K displayed a cytoplasmic-vesicular distribution, while TcRlp-G12V oscillated between cytoplasm and nuclei. TcRlp-S17N, -CQLF, -Q61K-CQLF and -G12V-CQLF all presented nuclear localization, and TcRlp-dCaaX and -Q61K-dCaaX were dispersed in cytoplasm. As selected mutations affected TcRlp localization, these strains will be valuable to investigate the cellular role of TcRlp. **Supported by:**PIBIC-UFRJ **Keywords:**Ras family GTPases;Trypanosoma cruzi;Protein prenylation.

PV-30 - **Characterization of a Fatty-Acid Binding Protein-like in *Leishmania amazonensis***

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Leishmania parasites are the causative agents of a group of diseases collectively known as leishmaniases. These neglected tropical diseases are endemic in 98 countries, where more than 1 billion people are at risk of infection. There are no vaccines and therapy remain limited due to drug toxicity and parasite's resistance. During their life cycle, *Leishmania* alternates between invertebrate and vertebrate hosts. Hence, they must adapt to different environments and compete with their hosts for several essential nutrients, such as lipids and fatty acids (FAs). Considering the crucial role of Fatty-Acid Binding Proteins (FABPs) in lipid metabolism, we looked for proteins containing FABP-like domains in the *Leishmania* genome. We identified a putative *L. mexicana* gene (LeiFABP) encoding a conserved hypothetical FABP-like domain. Therefore, our goal is to functionally characterize LeiFABP in *L. amazonensis*. We cloned constructs of LeiFABP fused to GFP in the *Leishmania* expression plasmids pXG-GFP+ and pXG-GFP2+. With these, we have been investigating the subcellular localization of LeiFABP in *L. amazonensis*, and characterizing LeiFABP overexpressing parasites regarding replication and virulence. We observed that LeiFABP expression is differentially modulated during in vitro growth of *L. amazonensis* promastigotes. LeiFABP characterization may help elucidate the distinctive features of *Leishmania*' FAs metabolism and trafficking, which may help to uncover new targets for the development of better therapeutic strategies against leishmaniases. **Supported by:**FAPESP Nº de Processo 2020/07465-2 **Keywords:** Leishmaniasis;lipid metabolism; neglected tropical diseases.

PV-31 - **Comparative genomic analysis of two *Leishmania Infantum* Brazilian strains: MHOM/BR/74/PP75 and HUUFS14.**

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Visceral leishmaniasis (VL) is defined as a non-contagious infectious disease caused by the protozoan of the genus Leishmania. Comparative genomic analyses compare the composition of genomes, e.g. presence or absence of genes, gene organization, gene duplication, genetic variations and relate these findings to the functional characteristics of the species analyzed. Here, our aim was to perform a comparative genomic analysis between two L. infantum Brazilian strains with the JPCM5 reference strain (Spanish). For this study, two Brazilian strains were analyzed: HUUFS14 isolated in 2009 from a VL patient in Sergipe and MHOM/BR/74/PP75, isolated in 1974 from a child in Bahia. Genomic DNA samples of the MHOM/BR/74/PP75 and HUUFS14 were sequenced in Illumina platform (MHOM/BR/74/PP75: 2x150bp, 490x, 6.2Gb; HUUFS14: 2x 150bp, 150x, 4.3Gb). Genomes were assembled using the Companion software. The total number of genes, protein coding genes, pseudogenes and ncRNA were analyzed and compared. The copy number variation (CNV) was performed in Artemis BamView software by mapping the paired-end reads in the JPCM5 reference genome. Subsequently, the genes were functionally annotated as type of gene and metabolic pathways using TriTrypDB. Genomic analyses showed a decrease in genes, protein coding and ncRNA number of MHOM/BR/74/PP75. However, the increase in the number of pseudogenes of both strains is remarkable when compared to JPCM5. In total, 332 genes had a decrease in CNV, in which 302 were protein-coding genes, 28 ncRNA and 2 pseudogenes. Interestingly, the genes with the greatest decreases in CNV observed were: Amastin-like (n=31), Elongation factor (n=15) and GP63 (n=7). We found 288 metabolic pathways, with purine metabolism the pathway with the highest single gene count (n=27), followed by the cysteine biosynthesis pathway (n=16). Genome annotation of Brazilian strains is important for comparative genomics of clinical isolates obtained from VL patients in Brazil. **Supported by:**FAPESP- IC: 2021/14615-3 ; FAPESP- JP: 2016/20258-0
Keywords: Leishmania Infantum; comparative genomic; reference genome.

PV-32 - **Pseudopod projection and modulation in the testate amoeba *Arcella intermedia* (Arcellinida:Amoebozoa)**

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Testate amoebae are unicellular eukaryotes that have a shell (test) covering the organism's cell. These organisms use projections of their cytoplasm called pseudopods for locomotion, feeding, and other complex behaviors. The pseudopod is considered a key morphological character, and it is vastly used to describe, distinguish, and classify amoebae groups. Despite this pivotal role of pseudopods, the intra-specific morphological variability of this structure and the relationship between this variability and other features of the organisms has been poorly explored. Also, the correlation between this variability and the type of substrate on which the organism locomotes is unknown. In this preliminary work, we used the testate amoeba specie *Arcella intermedia* (Arcellinida:Amoebozoa) in three different types of substrate (i.e., agar plates, plastic bottles and glass plates with liquid media) to investigate i) the morphological variability range regarding the organism performance (i.e., locomotion speed), ii) whether the shell attributes and distinct types of substrate correlate to the performance, iii) in case correlations are found, describe how the correlation is given and its relations among the variables.
Thus, we measured the shell diameter and area, pseudopod number per individual, and pseudopod length and width of 30 individuals from each substrate. We computed the cell's measurements and the locomotion speed as variables in generalized linear models, known as GLM, for further analysis, building a model for each type of substrate.
The results showed that the locomotion speed average is very similar among the three substrates and revealed a negative correlation between the locomotion speed and the number of pseudopods. The average number of pseudopods was noticeably higher in individuals of agar plates. The previous results suggest that these individuals can modulate their pseudopods according to the substrate, actively managing to sustain a performance rate. **Supported by:**CAPES-PROEX - 88887.659012/2021-00
Keywords:cellular motility;microeukaryotic plasticity;variability.

PV-33 - **Study of phospholipases A_2 in *Rhodnius prolixus* midgut during *Trypanosoma cruzi* infection**

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Rhodnius prolixus is a hematophagous insect known to be a vector of *Trypanosoma cruzi*, etiologic agent of Chagas's disease. Fighting against the vector is one way to prevent this disease. Finding insect vectors' molecules that are crucial for the infection is necessary for the control of this disease. In this scenario, previous studies from our group demonstrated the involvement of lysophosphatidylcholine (LPC), in different aspects of the infection. Thus, the aim of this work is to study a superfamily of enzymes that can promote the release of this lysophospholipid (LPL), phospholipases A_2 (PLA_2). These enzymes can catalyze the hydrolysis of glycerophospholipids in the *sn-2* position, releasing fatty acids and LPLs. In the present study, we investigated whether the infection by *T. cruzi* modulates the gene expression of secreted PLA_2 ($sPLA_2$) in the anterior (AM) and posterior (PM) midgut of *R. prolixus*. Adult females were fed with blood containing 10^8 parasites/ mL and 3, 10, 15 and 21 days after feeding insects were dissected. AM and PM were collected and submitted to total RNA extraction, cDNA synthesis and real time PCR for the analysis of four $sPLA_2$, $RpPLA_2$ 4037, $RpPLA_2$ 9995, $RpPLA_2$ 8617 and $RpPLA_2$ 8619 gene expression. It was observed that, in the AM, the expression of $RpPLA_2$ 4037 was significantly reduced 21 days after feeding and $RpPLA_2$ 8617 gene expression significantly increased 10 days after feeding. In the PM, a significant reduction in the expression of $RpPLA_2$ 4037, $RpPLA_2$ 8617 and $RpPLA_2$ 8619 on the third day after infection was observed. Furthermore, the expression of $RpPLA_2$ 4037 reduced fifteen days after infection, $RpPLA_2$ 9995 reduced ten and fifteen days after and $RpPLA_2$ 8617 increased 21 days after infection. These results demonstrate that *T. cruzi* can modulate the gene expression of $sPLA_2$ in the midgut of the vector and more studies are necessary to evaluate the importance of some of those genes for parasite's proliferation and differentiation. **Supported by:**CAPES, CNPq, FAPERJ **Keywords:**Rhodnius prolixus;Trypanosoma cruzi;Phospholipase A2.

PV-34 - **The role of SIRT1 in *Rhodnius prolixus* lipid metabolism during *Trypanosoma cruzi* infection**

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Rhodnius prolixus is a mandatory hematophagous triatomine found in tropical regions such as Central and South America. It is one of the main vectors of Chagas disease caused by the protozoan *Trypanosoma cruzi*. After a blood meal on an infected vertebrate host, the insect ingests trypomastigotes, and the whole development of the parasite in the insect is limited to the intestinal environment. *R. prolixus* metabolism during *T. cruzi* infection is poorly understood, and classic energetic molecular sensors have not yet been described in this model. Sirtuins are a family of histone deacylases dependent on the availability of NAD+ regulating different metabolic pathways. In mammals, 7 sirtuins (SIRT1-7) have been described with different cell localization. For the first time, we described 4 sirtuins in the genome of *R. prolixus*, which were classified as RpSIRT1, RpSIRT5, RpSIRT6, and RpSIRT7 through phylogenetic analysis (unpublished). Furthermore, qPCR analysis showed that *T. cruzi* infection increased the expression of RpSIRT1 and RpSIRT7 in the hindgut. In the fat body, an increase in the expression of RpSIRT1 and RpSIRT5 was observed in males infected with *T. cruzi*. According to these results, only RpSIRT1 showed higher expression in the fat body and hindgut in insects infected with the parasite. From now on, we decided to analyze the impact of RpSIRT1 expression on the lipid metabolism of *R. prolixus* infected with *T. cruzi*. For this analysis, a dsRNA will be used to knockdown RpSIRT1 expression in *R. prolixus* females. The insects will be divided into two groups: infected with *T. cruzi* and uninfected. The insects will be dissected into the hindgut and fat body, which will be used for cDNA synthesis. Afterward, a qPCR analysis will be performed using key lipid metabolic enzymes to understand the impact of RpSIRT1 knockdown. Our project seeks to continue exploring modulators of lipid metabolism to contribute to enabling new targets for vector control. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Rhodnius prolixus;Trypanosoma cruzi;Sirtuins.

PV-35 - **The impact of multi-mapping reads on transcriptomic and epigenomic datasets in *Trypanosoma cruzi*.**

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Trypanosomatids have a genomic structure organized in polycistronic regions (PTU). Specific promoters for each gene have not been described so far, thus post-transcriptional mechanisms are mainly responsible for gene expression regulation. Nascent transcript analysis could elucidate the real impact of transcription regulation on the expression levels of different coding DNA sequences (CDS), PTUs, as well as RNA classes in *T. cruzi***.** We obtained nascent transcripts in a Global Run-on (GRO) assay by Br-UTP incorporation in epimastigotes (EPI). GRO-seq were sequenced in the Illumina NextSeq platform, and reads were processed and mapped to the *T. cruzi* Dm28c genome masking the rRNAs and removing reads with low-quality scores (MAPQ10) leading to an underestimation of certain gene sets due to an overrepresentation of multi-mapping reads (35% of total mapped reads). Many strategies have been described to deal with multi-mapped reads, resulting in greater gene/transcriptional quantification accuracy. The impact of the multi-reads on GRO and RNA-seq analysis was compared by considering either keeping or removing all multi-mapped reads or using algorithms, such as the Kallisto package and MMR, that use maximization expectation and read coverage based methods, respectively. We observed an important difference in the expression of transcripts from disruptive and core compartments. Now we are applying the same strategy to evaluate the impact of multi-mapping reads on epigenetic datasets, such as FAIRE-seq, and H2B.V ChIP-seq. In our initial analysis, this approach suggests a degree of correlation, however, we need to find the best mapping representation of these data sets, to obtain a real estimation of genome coverage. Future analysis will be carried out to compare transcript abundance of CDSs and PTUs, and finally, integrate them with RNA-seq, FAIRE and Chip-seq datasets to shed light on the impact of transcription regulation on the gene expression of *T. cruzi*. **Supported by:**FAPESP 18/15553-9
Keywords: Gro-seq;Trypanosoma cruzi;Multi-mapping.

PV-36 - **Genome scale metabolic models: A tool for metabolic comprehension**

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Genome-scale metabolic models (GSMM) allow for computational inspection of how metabolic networks build themselves around their constitutive reactions and metabolites. In addition, they allow for hypothesizing and for a better understanding of non-obvious metabolic connections between reactions that are directly or indirectly related. Additionally, simulations of GSMM can be experimentally validated. Aiming to understand the role of the metabolic pathways of *Trypanosoma cruzi* that are absent in *Trypanosoma brucei*, we used a *T. brucei* 927 GSMM containing 1986 reactions and 1687 metabolites as a descriptive and predictive tool of the metabolism of *T. cruzi*. As an experimental example, we developed *T. brucei* cell lines expressing the four-step histidine-glutamate pathway of *T. cruzi*, which is naturally absent in *T. brucei*. We then compared the experimental results with the predictions of adding these enzymes in the computational model of the metabolism of *T. brucei*, to determine if the insertion of a completely different pathway could disturb the metabolic flux of other reactions in the computational model. The model objective was set to biomass production with a growth rate at the lower bound of 0.077, calculated using proliferation curve data of *T. brucei* measured in our lab. The cell lines of *T. brucei* expressing the partial His degradation pathway cannot use His as a carbon source; additionally, urocanate, the metabolite generated through His degradation, diminishes cell viability during the nutritional stress assay. Using that previous information we can deploy the computational model to inspect which metabolites might be accumulating to cause this diminished cell viability. These metabolites will be later measured *in vitro*. Observing the metabolism of *T. cruzi* through a scaffold model can be one of the keys to comprehending the metabolic and evolutionary advantages this parasite may have compared to others. **Supported by:**FAPESP **Keywords:** trypanosoma;genome-scale metabolic models; metabolism.

PV-37 - **Alterations in lipid metabolism of *Rhodnius prolixus* during *Trypanosoma rangeli infection.***

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Trypanosoma rangeli infects triatomine bugs, including *Rhodnius prolixus* (*Reduviidae, Triatominae*), and a variety of mammals. It is widely distributed in Central America and in the Northern part of South America. The geographical distribution of *T. rangeli* overlaps that of *T. cruzi* and the parasites share reduviid bugs as vectors. Once these parasites are transmitted by the same triatomine vectors, mixed infections may occur in both vertebrate and invertebrate hosts. *T. rangeli* has a complex life cycle, especially in the invertebrate host. After being ingested by a triatomine vector feeding on the vertebrate host, the parasite establishes an infection in the bug intestine. Proliferating epimastigotes and long trypomastigotes may penetrate the gut wall, invade the hemocoele, and produce numerous epimastigotes and trypomastigotes. In the hemolymph, parasites undergo constant division and transformation to metacyclic trypomastigotes. Subsequently, some of them invade the salivary glands from where they can be transmitted to the vertebrate host in the next feeding cycle. The objective of this work is to understand the alterations in lipid metabolism of *R. prolixus* caused by *T. rangeli infection*. *R. prolixus* were infected with *T. rangeli* (10^4 parasites/mL) and three days after infection the fat body and hemolymph were collected. The organs were subjected to lipid extraction and lipids classes were analyzed by thin-layer chromatography. We observe a decrease in the amount of triacylglycerols at the fat body of infected insects. This result was confirmed by fluorescence microscopy of the lipid droplets. In the fat body of infected insects, a smaller number of lipid droplets was observed. Our results demonstrated that *T. rangeli* was able to modulate the amount of triacylglycerol in *R. prolixus* fat body. Possibly, the parasites are modulating the lipid metabolism of their insect vector to supply their lipid demands **Supported by:**CAPES, CNPq, FAPERJ **Keywords:** Rhodnius prolixus; Trypanosoma rangeli;Lipid.

PV-38 - **Characterization of the translation initiation complex eIF3 in Leishmania infantum: identification of conserved and divergent elements within the interactome from six distinct subunits.**

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The initiation of protein synthesis in eukaryotes depends on the action of the translation initiation factors, also known as eIFs, where the largest is the eIF3 complex, formed by 13 subunits in mammals (eIF3a to eIF3m). eIF3 has various and distinct roles in translation, such as binding to the 40S ribosomal subunit and facilitating its association with the mRNA, a role that requires its interaction with the eIF4F complex, bound to the mRNA 5' end. In trypanosomatids, single homologues were identified for 12 of the eIF3 subunits. Here, to better understand the diversity of eIF3 interactions in these parasites, six eIF3 subunits (EIF3A, EIF3D, EIF3E, EIF3G, EIF3I and EIF3J) were selected for expression in transgenic *Leishmania infantum* cell lines, fusioned to an HA epitope. Cytoplasmic extracts from each cell line were used to immunoprecipate each HA-tagged bait with anti-HA beads, with co-precipitated protein partners identified by mass spectrometry. Three of the baits co-precipitated with most of the other eIF3 subunits, but EIF3E, EIF3J and EIF3A showed more restricted profiles. Multiple eIF4F-like complexes are present in trypanosomatids, based on different eIF4E and eIF4G subunits, and these eIF3 subunits seems to associate differentially with them. For exemple, EIF3D and others co-precipitated with EIF4E3/EIF4G4 whereas EIF3E co-precipitates preferentially with EIF4E4/EIF4G3 and EIF3I with none. Some RNA binding proteins (such as RBP12, PUF7 and PUF10) co-precipitated with all baits, while others were found with specific subunits, such as EIF3I (RBP29 and RBP35) and EIF3J (PUF5). Several protein kinases, RNA helicases and even ribosomal proteins were also found differentially associated with the tagged baits. These results not only increase the knowledge about the interaction network for each of these eIF3 subunits, but also provide evidence that distinct eIF3 complexes, with different interacting partners might be active during translation in trypanosomatids. **Supported by:**FACEPE (APQ-1662-2.02/ 15), CNPq (401888/2013-4; 400789/2019-1; 310032/2019-9). **Keywords:**translation initiation;protein synthesis;regulation of gene expression.

PV-39 - **IDENTIFICATION OF A *Trypanosoma cruzi* UNIQUE PROTEIN KINASE GENE THAT IS ESSENTIAL FOR PARASITE DIFFERENTIATION, EDK1**

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Protein kinases (PKs) are enzymes with fundamental roles in biology. In trypanosomatids there are >200 genes encoding PKs of various families, and a recent genome-wide kinome screen in *Leishmania mexicana* pointed to several PKs essential for parasite survival or for successful infection. In *Trypanosoma cruzi*, little is known about the role of PKs. From the *T. cruzi* genome, we identified 19 unique PK genes that are absent from *Leishmania* and *T. brucei*. We investigated the role of a selected putative unique PK, using CRISPR/Cas9 to generate null mutants in *T. cruzi* Dm28. Template DNA for guide RNAs targeting the 5' and 3' regions of the gene and for repair cassettes containing antibiotic-resistant genes were generated by PCR and introduced in epimastigote forms of the *T. cruzi* line constitutively expressing SpCas9 and T7 RNA polymerase. Populations resistant to both antibiotics were cloned and gene deletion in both alleles was verified. Null mutants were found to be incapable of differentiating from epimastigotes to metacyclic trypomastigotes (metacyclogenesis) *in vitro*, both spontaneously and by induction with TAU and TAU3AAG media. Discrimination between the different forms of the parasite was made by analyzing the kinetoplast position in relation to the nucleus, revealing that the population of the parental Dm28SpCas9 line had 24% of metacyclics, 33% of intermediate forms (Ia, Ib and/or Ic) and 43% of epimastigotes. In contrast, no metacyclic or Ic forms were observed for the null mutants. Based on this phenotype we named this PK, Essential for Differentiation Kinase (EDK1). To further validate its role, re-expressor lines were generated by re-introducing one copy of EDK1 in the tubulin locus of the null mutant. The complemented line differentiated to metacyclics and was resistant to lysis by human serum, indicating that it restored differentiation to fully functional trypomastigotes. This is the first description of a PK essential for metacyclogenesis in *T. cruzi*. **Supported by:**UK RESEARCH AND INNOVATION (GCRF); CNPq; FAPERJ **Keywords:**KINASE;DIFFERENTIATION;UNIQUE.

PV-40 - ***Leishmania braziliensis* RuvB-like1: the biological relevance of arginine methylation**

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RuvB-Like 1 is a protein belonging to the AAA+ family of ATPases. This protein is conserved from yeast to humans and is known to play essential roles in several cellular processes, such as DNA repair, transcription regulation, chromatin remodeling and apoptosis. Furthermore, RuvB-Like 1 proteins are characterized by the presence of conserved motifs identified as Walker A, Walker B, sensor I and sensor II, responsible for ATP binding and hydrolysis. In previous work we identified RuvB-Like 1 as a potential target of the *Leishmania braziliensis* Protein Arginine Methyltransferase 5 (*Lbr*PRMT5), which methylates arginine residues. Thereby, our goal is to characterize the RuvB-Like 1 in *L. braziliensis* (*Lbr*RuvB1) and the effect of arginine methylation on this protein. The alignment of amino acid sequences from humans, yeast, *Plasmodium falciparum* and *Leishmania spp*. showed that this protein is conserved in *Leishmania* parasites and 64,25% identical to the human enzyme. Also, *Leishmania* RuvB-Like 1 protein possesses the motifs Walker A, Walker B, sensor I and sensor II, suggesting they are functional. Despite the observed conservation, an overlapping of predicted structures of *Lbr*RuvB1 and human RuvB1 revealed some differences. To investigate the *Lbr*RuvB1, we added an N-terminal myc tag to *Lbr*RuvB1 and confirmed its integration by PCR, using CRISPR/Cas9 system. Western blotting assays revealed a protein of the predicted size (50 kDa) and showed that it is expressed in procyclic, metacyclic and, to a lesser extent, in axenic amastigote forms. In addition, transcripts for *Lbr*RuvB1 were present in all three biological forms and were elevated in amastigote. Preliminary results from immunofluorescence assays indicate that LbrRuvB1 displays a cytosolic localization. *Myc-LbrRuvB1:LbrPRMT5*-knockout mutants are being generated, which will allow us to assess the relevance of arginine methylation for the expression, stability and localization of LbrRuvB1. **Supported by:**Fapesp - 2020/14059-0 **Keywords:**RuvB-Like1;Leishmania;Protein Arginine Methyltransferase.

PV-41 - **Analysis of target transcripts identified through comparative proteomics of Lutzomyia umbratilis, the main vector of Leishmania (Viannia) guyanensis in the Amazonian region, in populations with different vectorial capacities.**

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The epidemiological profile of the American Cutaneous Leishmaniasis in the Amazonian cities of Manacapuru (MNP) and Rio Preto da Eva (RPE), separated by the Negro River, differs, RPE being an endemic area of the disease and MNP showing no significant number of cases. We performed midgut proteomic analysis using sand flies from both cities. A total of 3,292 proteins were identified, 3,252 of which are found in sandflies from both locations, 30 are exclusively present in MNP and 11 only in RPE. Among the proteins shared in both populations, four were selected for transcriptional validation, based on the criteria of having a fold change greater than 2 or less than 0.5 and a potential to impact on the vectorial capacity of insects. Of these four proteins, SL9B2, PATH and PEPT1-Like may be correlated with the process of alkalinization of the insect's intestinal lumen, affecting the parasite development, and Paramiosin is correlated with the protective capacity of the sand fly's digestive tract muscles, which would also impact the vector-parasite interaction and thus explain the epidemiological difference observed in the two cities. With these targets defined, RT-QPCR was performed using insects from the two locations to compare the level of transcripts in carcasses and intestinal tracts. Our results showed that PATH, PEPT1-Like and Paramiosin are upregulated in MNP, similar to the data from the proteomic analysis and these being probably relevant in the mechanism of parasite transmission by the insect. On the other hand, the protein SL9B2 had more transcripts in sand flies from RPE, different from the proteomic result and, although it is known that levels of transcription are not always related to abundance of proteins, the influence of this protein in the interaction of the parasite with the insect still needs to be better analyzed. **Supported by:**FAPERJ **Keywords:**Leishmania;Sand fly;Vectorial capacity.

PV-42 - **Restoring of a pseudogene encoding a nucleoside hydrolase enzyme from the parasite *Trichomonas vaginalis***

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In order to survive and to successfully infect the human host, the parasite *Trichomonas vaginalis* depends on purine and pyrimidine salvage pathways, and nucleoside hydrolases (NHs) are the main enzymes involved in nucleoside metabolism to obtain new nucleobases. The genome of *T. vaginalis* (G3 strain) encodes four NHs (TvNH1-4), as well as a more distant related pseudogene (TvNH5), which has its ORF interrupted by a stop-codon. We have sequenced TvNH5 from two *T. vaginalis* strains (JT and FMV1) and confirmed the same interruption in both genes. Furthermore, we found an apparently intact orthologue of TvNH5 in *Tritrichomonas foetus* genome (Belfast strain), sharing 63% similarity in aa sequence. Thus, the inactivation of TvNH5 gene seems to have occurred at the basis of *T. vaginalis* speciation. This study aims to investigate the activity of an artificially restored TvNH5, in order to understand putative selective pressures that caused its inactivation. First, we have produced a 3D homology-based model of a potentially functional TvNH5 (TvNH5-T544C), inserting a gln residue in the stop-codon position, based in the corresponding sequence from *T. foetus*. TvNH5-T544C was built using the Swiss-Model server, based on an NH structure from *Gardnerella vaginalis* (6BA0). Refinement was performed with the Galaxy WEB server and the quality of the model was availed using Verify 3D, PROCHECK and Prosa-Web servers. The nucleoside substrate specificity of TvNH5-T544C will be evaluated by molecular docking. Next, using site-directed mutagenesis, we have produced the TvNH5-T544C gene, replacing the TAA stop-codon by a CAA gln codon. The TvNH5-T544C gene was fused with the iLOV fluorescent gene and cloned in the *T. vaginalis* expression vector pMasterNeo, in order to study cellular localization and possible phenotypic effects. TvNH5-T544C was further cloned in the vector pET15b, for heterologous expression and production of a recombinant protein to test in vitro for substrate specificity. **Keywords:**Trichomonas vaginalis;nucleoside hydrolase;pseudogene.

PV-43 - **Geographical Origin of *Leishmania* Ross, 1903 (Trypanosomatidae)**

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Several hypotheses have been proposed on the origin of *Leishmania*. The Neotropical hypothesis places its origin in the Neotropical region, with mammals as their first vertebrate host. The Palearctic hypothesis proposes an origin in the Palearctic region during Cretaceous and reptiles would be their first vertebrate hosts, dispersing via terrestrial bridges subsequently. The Supercontinent hypothesis suggests an origin in Gondwana, with mammals as their first vertebrate host and vicariant events playing a major role in the lineage distribution. The aim of this work is to discuss the geographical origin of *Leishmania*, suggesting historical processes that may have influenced current patterns of distribution of the genus based on hypotheses of phylogenetic relationships and reconstructing of ancestral areas. Gene sequences from gGAPDH, HSP70 and V7V8 were retrieved from GenBank and TriTrypDB in a total of 45 terminal taxa (29 from *Leishmania*). Genes were aligned on ClustalX and MAFFT7. Evolutionary models were accessed on jModelTest and Bayesian analyses were performed on MrBayes 3.2.7a. Reconstruction of ancestral areas was performed on RASP 4.2 using a Statistical Dispersal-Vicariance analysis (S-DIVA). Bayesian analysis recovered *Leishmania* and its subgenera as monophyletic with high support values. *Sauroleishmania* placement deep within the tree suggests mammals as the first vertebrate hosts of the genus. S-DIVA analysis does not support a Palearctic origin. Highest probability areas recovered were Afrotropical+Neotropical region (p=22%), or Neotropical region (p=21%) or Neotropical+Oriental region (p=21%). Recovered geographical events follow the Supercontinent hypothesis. *Viannia* lineage remained on Neotropical region while *Leishmania+Sauroleishmania* dispersed from Africa to other regions. Geographical history of *Mundinia* could not be recovered by the S-DIVA analysis. Further molecular clock analyses are needed to better understand *Leishmania* evolution. **Supported by:**CNPq
Keywords: Molecular Phylogeny;Biogeography;S-DIVA.

PV-44 - **Antileishmanial activity of metal complexes and sidnones in Leishmania amazonensis**

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Leishmaniases are classified as neglected diseases, as they are not attractive for the pharmaceutical industries, since there is no prospect of a return on financial investment. Furthermore, there is no licensed vaccine for use in humans, the current treatment is expensive, toxic, painful and many cases of resistance have been reported, making treatment difficult. With the advance in inorganic chemistry, metal complexes have been rescued as interesting alternatives for drugs. In fact, gold, platinum, and silver complexes have shown good activity against tumors and some infections. On the other hand, sidnones constitute the first class of synthesized mesoionic compounds, having important activity biology and are extensively studied. This work proposes structure/activity studies to evaluate the effect of organometallic complexes and mesoionic derivatives in *Leishmania amazonensis*, the species responsible for the cutaneous form. *L. amazonensis* promastigotes were incubated with the molecules at different concentrations for 72 hours and the parasite growth was evaluated with resazurin. To evaluate the anti-amastigote activity, murine peritoneal macrophages were infected for 4 hours, treated with molecules for 72 hours, and the activity was evaluated microscopically. For cytotoxicity assays, murine peritoneal macrophages were incubated with the molecules for 72 hours and cell viability was evaluated by resazurin. In silico pharmacokinetic and toxicological analysis was also performed for the active substances, as a selection parameter for candidates to be evaluated in vivo. Of the 15 sidnones evaluated in this work, eight showed activity, with IC_{50} below 100µM, as well as the silver complex with an IC_{50} of 34µM. These promising compounds will be next evaluated in vivo. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018
Keywords: Mesoionic compounds;organic-metallic complexes;sidnones.

PV-45 - **Sugar nucleotide biosynthesis pathways diverge among trypanosomatids**

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Trypanosomatids have a variety of glycoconjugates that play pivotal roles on these species infectivity, virulence and survival within hosts. Based on the description of the sugar nucleotide biosynthesis (SNB) enzymes on the TriTryps genomes (*Trypanosoma brucei, Trypanosoma cruzi*, and *Leishmania major*), we have comparatively evaluated genes coding for 21 enzymes involved in SNB among 18 trypanosomatid species, and their close relative species, *Bodo saltans*. BLASTp analyzes were carried out using the TriTryps SNB sequences as query against the TritrypDB database (v57), following search for conserved domains using the CDD Websearch tool. All analyzed species contain genes required for production of UDP-GlcNAc, GDP-Man, and UDP-Galp, while *Leishmania, Endotrypanum, Porcisia, Crithidia*, and *Leptomonas* species lack the gene encoding for GDP-L-fucose-synthetase that leads for GDP-Fuc generation. Some SNB-related genes like phosphoglucomutase, involved in the biosynthesis of UDP-Glc, and the components of UDP-Galf synthesis pathway were not observed on the African trypanosomes. The last was also absent in *Angomonas deanei*, *Blechomonas ayalai*, and *Paratrypanosoma confusum*. While genes coding for UDP-Rha and UDP-Xyl biosynthesis were observed in T*. cruzi, T. rangeli, T. grayi, P. confusum*, and *B. saltans*, in *B. ayalai* only the UDP-Rha coding gene is observed. The unique known gene in the GDP-Ara biosynthesis pathway, fucose (arabinose) kinase, is absent in *P. confusum, B. saltans, A. deanei*, and all African trypanosomes. The differences observed on sugar biosynthesis pathways among trypanosomatids reflects their genome plasticity and might be related to distinct nutritional requirements or availability across a variety of hosts and vectors involved on their distinct life cycles. Thus, detailed investigation of sugar nucleotide pathways in trypanosomatids will contribute to enlighten important metabolic routes, metabolites and general glycobiology of parasites. **Supported by:**CNPq, CAPES, FINEp **Keywords:**Monosaccharides;Trypanosomatidae;Evolution.

PV-46 - ***Herpetomonas muscarum* in *Stomoxys calcitrans* (Linnaeus, 1758): isolation, identification and interaction aspects.**

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Stomoxys calcitrans (Diptera) or stable fly is a cosmopolitan insect and the only species of the genus that occurs in America. Adult females and males are hematophagous, being mechanical vectors of a wide range of pathogens of veterinary interest as viruses, bacteria, protozoa and helminths. Their bites are painful and cause a lot of stress to livestock hosts affecting weight gain and causing reduced milk production. The Trypanosomatidae family includes both heteroxenic protozoa of medical and veterinary importance, as well as monoxenic representatives found only in arthropods. Monoxenic protozoa have potential as a strategy for biological control of insect vectors. Recently, we observed the presence of a trypanosomatid, molecularly characterized by our group as *Herpetomonas muscarum* in *S. calcitrans* hemolymph of field and F1 colony flies kept in our laboratory. The isolates were cultured and cryopreserved in liquid nitrogen and deposited in the Protozoa Collection of Fundação Oswaldo Cruz, RJ. In the present work, besides describing the isolation and identification, we analyzed the *in vitro* and *in vivo* interaction of *H. muscarum* with *S. calcitrans* guts. *H. muscarum* remained bound to the fly guts in higher numbers when compared to *Leishmania amazonensis* and was detected in the fly guts until three days after being ingested by the insect. Ultrastructural and histological aspects of *H. muscarum – S. calcitrans* guts are being analyzed by electron microscopy. Moreover, we observed that *S. calcitrans* hemocytes are classified as plasmatocytes, granulocytes, oenocytoids and prohemocytes and we intend perform differential quantification of these cell types in flies infected or not with the trypanosomatid. *S. calcitrans* is considered a pest of livestock and yet there are not available effective ways of controlling its proliferation and we expect to better understand its relationship with *H. muscarum* and how it affects the insect biology. **Supported by:**Capes, Faperj e PROPPG-UFRRJ **Keywords:**mechanical vector;monoxenous trypanosomatids;immune system.

PV-47 - **The evolution of FLA1 and FLA1BP proteins among Trypanosomatids**

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Trypanosomatid's single flagellum is attached to the cell body membrane through a specialized region called the flagellum attachment zone (FAZ). In *Trypanosoma brucei* procyclic forms, the Flagellar Adhesion Glycoprotein 1 (FLA1) and FLA1-Binding Protein (FLA1BP), are localized in the cell body and flagellar membrane, respectively, and interact to attach the flagellum to the cell body through their extracellular regions, which contain an NHL domain. FLA1 and FLA1BP have a transmembrane region and an intracellular tail, which is important for their FAZ localization. A paralogous set of FLA proteins are expressed in *T. brucei* bloodstream forms, with similar functions to FLA1/FLA1BP. However, little is known about the pattern of FLA protein conservation in other trypanosomatids. Comparative sequence analysis of the 7 FLA proteins from *T. brucei* revealed that FLA1 and FLA1BP are single copy genes in all analyzed species, except for *Trypanosoma congolense*, for which, paralogous genes are observed in two distinct chromosomes. This gene expansion may have allowed the stage-specific gene expression regulation between the different life cycle stages of *T. brucei*. The sequence of FLA1BP varies among species; however, the NHL domain within the extracellular region is conserved, likely mediating the FLA1-FLA1BP interaction. While the intracellular region of FLA1BP varies in sequence and size among species, the initial 13 residues after the transmembrane region (IR^{13}) are highly conserved. To assess the importance of this region, we overexpressed in *Trypanosoma cruzi* RFP tagged *Tc*FLA1BP and *Tc*FLABP1ΔIR^{13}. Overexpression of *Tc*FLA1BP resulted in altered parasite growth and morphology while parasites overexpressing *Tc*FLABP1ΔIR^{13} were less affected. Taken together, our results show that FLA1/FLA1BP gene family have expanded in *T. brucei* and *T. congolense*, while other trypanosomatids have a single pair of FLA1/FLA1BP. **Supported by:** CNPq, CAPES, FINEp, Oxford Brookes University **Keywords:**Trypanosomatidae ;Flagellar adhesion; Intracellular junction.