HP – 001 - **Elucidating the role of Golgin-63 (TcG63) in Golgi structure and function in *Trypanosoma cruzi***

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The characterization of molecular mechanisms regulating the Golgi complex in *Trypanosoma cruzi* is limited, despite its importance for cell survival. The Golgi complex has a prominent function in the processing, transport, and sorting of intracellular proteins. The Golgi's stacked cisternae are held together by structural proteins, such as golgins, which are vital for membrane fusion, transporter docking, and providing structural support. While the location and ultrastructure of the Golgi in *T. cruzi* are well described, its biogenesis and protein composition remain poorly understood. In this work, we performed a search of the *T. cruzi* genome for orthologous Golgi complex proteins already characterized in other trypanosomatids. Accordingly, we selected golgin 63 (TcG63), a homolog of *T. brucei* G63. We used gene editing strategies with the CRISPR/Cas9 system to generate parasites tagged with mNeonGreen and Myc (mNG::TcG63) and knockout parasites (TcG63$^{-/-}$) to identify its cellular location throughout the parasite's life cycle and evaluate phenotypes resulting from the absence of the protein. The mutant parasites were generated by co-transfection with an SgRNA template and a donor DNA to induce homology-directed repair in epimastigotes of the T7Cas9 cell line. The expression of TcG63::mNG was confirmed by flow cytometry and western blot. Live parasite fluorescence assays revealed the location and conformation of TcG63::mNG in the main forms of *T. cruzi*, suggesting a location at the sides of the Golgi complex stacks, as observed in other organisms. The deletion of the TcG63 gene was verified by PCR, and the absence of the corresponding protein was confirmed by Western blot analysis. Although no observable changes were found in the growth curve or metacyclogenesis, TcG63$^{-/-}$ parasites showed exacerbated infectivity. These results point to a role for TcG63 in regulating processes such as protein glycosylation and vesicular trafficking.
Supported by: CNPQ 130724/2025-4 **Keywords:** Trypanosoma cruzi;Golgi complex;Molecular Biology.

HP – 002 - **Re-expression of *Trans*-sialidase activity in knockout mutants confirms its essential role as virulence factors during *Trypanosoma cruzi* infection**

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Trans-sialidases (TS) belong to the largest gene family in the *T. cruz*i genome in which only few sequences encode enzymatically active TS (aTS). aTS are responsible for transferring sialic acid (SA) from host glycoconjugates to mucins present on the parasite surface. Using CRISPR/Cas9 technology (premature stop codon insertion), we generated, aTS knockout parasites (aTS-KØ) that display undetectable levels of TS activity. aTS-KØ are unable to establish infection even in the highly susceptible IFN-ɣ KØ mice. Moreover, data suggest that aTS mutants represent a highly attenuated lineage capable of generating protection in aTS-KØ-immunized animals subsequently challenged with a virulent strain. Although aTS-KØ parasites are capable of infecting different cell types, they exhibit inhibited differentiation from amastigotes to trypomastigotes and impaired parasite release from infected cells. To investigate the role of aTS in infection and validate the attenuated phenotype of aTS-KØ parasites, TS re-expressor lines (aTS-KØ AB) were generated by restoration of the previously disrupted reading frame using again CRISPR/Cas9 gene editing. aTS-KØ AB re-expressors localize aTS on the parasites surface and can partially rescue aTS activity and SA transference. aTS-KØ AB partially reverts the *in vitro* differentiation and release phenotypes of TCTs, as well as the *in vivo* infection phenotype in comparison to WT. Comparative RNAseq analyses were performed between WT and aTS-KØ TCTs to identify if the aTS depletion affected unrelated genes. No significant changes were observed in the core region (basal metabolism). Regarding, the disruptive compartment, aTS-KØ showed an increased expression of MASP (25), non-active TS (12) and mucins (7) gene families, indicating an adaptive response to the absence of aTS. These findings confirm the role of aTS as essential virulence factors in *T. cruzi* and highlight the importance of new gene editing methodologies for the study of this parasite, especially regarding multigene families. **Supported by:** Capes, CNPq, FAPEMIG, INCTV
Keywords: Active trans-sialidases (aTSs);multigene family knockout;re-expressors.

HP – 003 - **DEVELOPMENT OF RNA VACCINES FOR CHAGAS DISEASE BASED ON SEQUENCES FROM *TRYPANOSOMA CRUZI* TRANS-SIALIDASES**

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Trans-sialidases (TS) present on the surface of *Trypanosoma cruzi* are responsible for transferring sialic acid residues from host glycoconjugates to mucins on the parasite surface, a mechanism related to parasite evasion from host immune system. TS contains a C-terminal immunogenic domain with amino acids repeats known as SAPA (Shed Acute Phase Antigen). Previously, our group have shown that mice immunization with recombinant TS induces the development of a protective Th1 response and that immunization with the antigen containing only SAPA repeats does not result in protection against a challenge infection. Since RNA vaccines rely on the intracellular synthesis of the antigen and, therefore, induce a potent Th1 response, we compared immunization protocols based on recombinant protein and the corresponding mRNA encapsulated into lipid nanoparticles (LNPs). We also compared the results of RNA immunization with TS sequences with and without SAPA. These sequences were cloned into a mammalian expression vector and used for mRNA synthesis using in vitro transcription protocols. After cell transfection, the capacity of these mRNAs to produce the antigens were evaluated by western blot using antibodies generated against the recombinant proteins. Immunization with mRNA/LNP formulations containing these sequences, induces similar humoral and cellular immune responses compared to immunization with recombinant proteins, as well as similar protection against challenge with a virulent *T. cruzi* strain, as shown by parasitemia and tissue parasitism. However, immunization with TS protein or RNA containing the repeat domain resulted in increased production of IL-10 compared to mice immunized with TS without SAPA, and this increased IL-10 production correlates with a significant reduction in the inflammatory infiltrate in heart tissues of infected animals. These results indicate that the presence of SAPA repeats may contribute to promoting a desirable, more balanced immune response.

Supported by: CNPq/400821/2023-0
Keywords: RNA vaccine;T; cruzi;Chagas disease, immune response.

HP – 004 - **IMPACT OF CONGENITAL TOXOPLASMOSIS ON RETINAL VASCULAR DEVELOPMENT IN MICE**

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Congenital toxoplasmosis (CT) occurs when the parasite *Toxoplasma gondii* is transmitted vertically during pregnancy and is a significant public health concern. CT can lead to severe ocular and neurological malformations, with impacts on neurogenesis and angiogenesis. Herein, we aimed to evaluate the effect of congenital toxoplasmosis on neonatal retinal angiogenesis in mice. Pregnant female C57BL/6 mice were inoculated intragastrically on embryonic day 10 (E10) with two *T. gondii* cysts (ME49 strain). Control animals were inoculated with a brain macerate from an uninfected C57BL/6 mouse. Pups on postnatal days 5 and 14 (P5 and P14) were weighed, and retinas were dissected for western blotting, RT-qPCR, and immunohistochemistry analyses. We observed that mice from infected dams had lower weight at P5, altered brain weight and width at P5 and P14, when compared to age-matched controls. By confocal microscopy, we observed a significant decrease in vessel area, number of vascular junctions, and total vessel length in infected animals at P5. A significant decrease in the number of ascending sprouts from the deep vascular plexus to form the intermediate vascular plexus was also observed in infected retinas at P14, indicating a delay in the intermediate plexus development. In addition, infected mouse retinas (P5) had a significant increase in VEGF receptor 2 and a decrease in GFAP protein levels. Our results indicate that the congenital *T. gondii* infection affects mouse development, including brain malformations and an impact on the retinal vasculature maturation and development. These findings recapitulate pathogenic events observed in human congenital toxoplasmosis.

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Keywords: Congenital toxoplasmosis;angiogenesis;retina.

HP – 005 - **Integration of knock sideways and BioID to elucidate the role and interactors of the transcription factor AP2-O in *Plasmodium falciparum***

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The Api-AP2 transcription factor family is essential for gene expression regulation in *Plasmodium falciparum*, orchestrating the activation and silencing of genes involved in virulence and key parasite processes. However, its mechanisms of action and interaction network remain poorly understood. To investigate the function and identify associated proteins, we monitored the effects of knock sideways and knockdown of *Pf*AP2-O during the intraerythrocytic cycle of *P. falciparum*. The *PfAP2-O* was tagged with 2×FKBP, GFP, and a neomycin/G418 resistance gene in the *P. falciparum* NF54 strain. Following confirmation of correct tag integration, the NF54::AP2-O_2×FKBP_GFP line was supertransfected with the plasmid pLyn_FRB_mCherry, which provides conditional mislocation to the secretory compartment. In addition, we performed a BioID approach transfecting a plasmid expressing the biotin ligase BirA*. In the presence of biotin, proteins close to *Pf*AP2-O were biotinylated and subsequently identified by mass spectrometry, enabling the mapping of its potential interactome. Depletion of *Pf*AP2-O via knock sideways/knockdown (Rapamycin 250nM + Glucosamine 5Mm) led to a significant reduction in parasitemia, the massive presence of pyknotic bodies, extracellular parasites, and delayed intraerythrocytic development. The BioID experiments led to a complex picture of biotinylated proteins, which not only included nuclear proteins but also proteins from the proteasome and many ribosomal factors. Taken together, *Pf*AP2-O is necessary for the normal progression of the parasite life cycle, and its interaction network may reveal novel targets for the development of antimalarial strategies.
Supported by: FAPESP 2021/02298-3
Keywords: Plasmodium falciparum;BioID;ApiAP2.

HP – 006 - **Comparative genomics and functional analysis of mucin-like and TASV-like glycoproteins in Trypanosoma rangeli: Impact on parasite infectivity in the mammalian host**

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Trypanosoma rangeli is a non-pathogenic kinetoplastid parasite that infects a wide range of mammals and triatomine bugs across South and Central America. Despite its inability to cause disease in humans, it shares hosts, vectors, and significant antigenic similarity with *Trypanosoma cruzi*, the causative agent of Chagas disease, making it a valuable model for comparative parasitology. Analysis of the new genome version of *Trypanosoma rangeli* (SC58 V.2) revealed seven mucin-like genes with conserved motifs absent in other kinetoplastids, and 14 TASV-like genes previously considered exclusive to *T. cruzi*, expanding the known surface glycoprotein repertoire of this non-pathogenic hemoflagellate. *Tr*MUC proteins display high predicted O-glycosylation (52.77% ± 13.5) and phosphorylation (43.3% ± 7.22), whereas TASV-like proteins show lower values (8.55% ± 2.28 and 14.27% ± 2.73, respectively) but are enriched in aromatic residues (6.67% ± 1.83). Homologous (*Tr*MUC, *Tr*TASV-like) and heterologous (*T. cruzi Tc*MUC I and II) glycoprotein genes were expressed in *T. rangeli* (Choachí strain). Expression resulted in hyperglycosylated molecules, with heterologous *Tc*MUC proteins being more glycosylated than their homologous counterparts. Infectivity assays in BALB/c mice demonstrate distinct infection outcomes. *Tc*MUC II expression by *T. rangeli* resulted in significantly higher parasitemia from day 1 ($p \leq 0.05$) up to day 7 post-infection ($p < 0.001$), whereas *Tc*MUC I had no effect. In contrast, TrTASV-like1 expression reduced parasitemia between days 2–4 ($p \leq 0.05$), and *Tr*MUC3 notably decreased parasitemia from day 2–8 ($p < 0.0001$) despite almost impaired protein expression. These findings indicate that *Tr*MUC and *Tr*TASV-like proteins modulate *T. rangeli* infection dynamics in mammalian hosts through distinct glycosylation and surface expression patterns, revealing key functional divergences from *T. cruzi* and uncovering novel targets for comparative kinetoplastid research.
Supported by: CNPq (308616/2015-4), CAPES/STINT (23038.003782/2014–11), SRC (202105567)
Keywords: Mucins;TASV-like;Trypanosoma rangeli.

HP – 007 - **High incidence of chronic Chagas' disease in the human population of an Amazon settlement with a silent *T. cruzi* blood circulation**

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Chagas' disease (CD) is a neglected trypanosomiasis endemic to the American continent, caused by *Trypanosoma cruzi*, naturally transmitted through skin by feces and urine of triatomine species during blood feeding in vertebrate animals, including humans. Others route of *T. cruzi* transmission is oral, congenital, and by blood transfusion, and blood derivates. In Brazil, vector transmission of CD decreased substantially after the elimination of the principal triatomine, *T. infestans*. Environmental changes caused by anthropic actions, forced wild triatomines involved in *T. cruzi* wild cycle, proliferate near human population in rural areas, contaminating *in house* production of aliments as Açai and Cupuacu juices, with feces and urine containing *T.* cruzi, causing oral transmission, the principal route of CD infection in the Amazon region. Individuals contaminated by *T. cruzi* can develop acute CD with asymptomatic, mild or severe clinical signs which can evolute to chronic CD, marked by a silent long phase that culminates in a severe physiopathology compromising the cardiac or gastrointestinal systems. Diagnosis in the CD acute phase is important to prevent evolution to chronic CD. Using a molecular and serological approach, a rural Amazonic population localized in Cruzeiro do Sul, Acre, Brazil, was investigated for CD in 2022 and 2024. From 186 individuals in 2022 we found 1 case of serology positive, and 28 of people negative for serology and positive by the parasite. In 2024 the number of serology positive increased substantially to 11 and 32 negative for serology were positive for the parasite nucleic acid. The results revealed a high incidence of CD with silent circulation of the parasite in the blood of human population which need public health support for control of CD in the region. Besides, investigation of the transmission routes of *T. cruzi* in the region with characterization of its genetic pattern is taking place.

Supported by: FAPESP 2022/10392-2; FAPESP
Keywords: Chagas Disease;Trypanosoma cruzi;Molecular diagnosis.

PV – 001 - **Understanding the mechanism underlying oocyst division**

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Plasmodium oocysts develop on the abluminal surface of the mosquito midgut approximately 24 hours after an infectious blood meal. Despite the ingestion of thousands of parasites which will fertilize and traverse the midgut epithelium, only a few dozen oocysts typically form in field conditions [Smith et al, 2014] . Each oocyst then undergoes extensive DNA and organelle replication to produce thousands of sporozoites — the infectious stage that invades the mosquito salivary glands and vertebrate cells. Oocysts are thus key multiplicative stages that replenish the number of parasites during the parasite lifecycle inside the mosquito.
Although oocysts were first identified by R. Ross in 1897, their biology remains poorly understood. Using parasite lines expressing organelle-specific fluorescent reporters combined with ultrastructural, dynamic, and longitudinal *in vivo* imaging, we identified a novel type of asexual division in *Plasmodium berghei* oocysts. The division of oocysts involves the formation of a bud in a process reminiscent of yeast asexual division. Nuclei and organelles migrate into the nascent daughter oocyst generally enveloped by the mother's capsule, a protein structure thought to protect oocysts. Cytokinesis is accompanied by the invagination of both the plasma membrane and capsule, ultimately leading to a second multinucleated cell. In fixed midguts, budding oocysts were detected at frequencies of 0–10% of the total population, reaching 20% in one parasite line, with prevalence inversely correlated with oocyst age and midgut density. In some cases, oocysts were connected by capsule and membrane filaments, potentially representing structural remnants of budding. Longitudinal imaging of live infected mosquitos confirmed the occurrence of this division *in vivo*. We hypothesize that oocyst division constitutes an adaptive strategy that enhances parasite survival under low-density conditions by enabling prolonged sporozoite production, thereby enhancing salivary gland colonization, and ultimately increasing the efficiency and duration of transmission to the vertebrate host.

Supported by: Labex IBEID, ISIDOre
Keywords: Oocyst;Budding;Imaging.

PV – 002 - **TcMK1, TcMK2 and TcMK3: Trypanosoma cruzi Specific Protein Kinases Essential for Metacyclogenesis**

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We have investigated the function of three T. cruzi protein kinase (PK) genes which do not have orthologues in T. brucei and Leishmania. Null mutants for the three genes were generated using CRISPR/SpCas9 and were unable to differentiate from epimastigotes to metacyclic trypomastigotes (metacyclogenesis), thus named TcMKs (Trypanosoma cruzi Metacyclogenesis Kinases). Parental line epimastigotes submitted to TAU3AAG differentiation for 5 days resulted in approximately 45% metacyclics, 20% Ia, 25% Ib, 5% Ic and 5% epimastigotes. In contrast, TcMK1 null mutants submitted to metacyclogenesis resulted in 70% Ia and 24% Ib, while almost undetectable Ic. Metacyclogenesis assays with TcMK2 null mutants resulted in 35% Ia, 59% Ib and less than 1% Ic while TcMK3 null mutants resulted in 30% Ia, 60% Ib and <5% Ic. TcMK1, TcMK2 and TcMK3 null mutants are fully susceptible to lysis by human serum and unable to infect mammalian cells. Meanwhile, the addback lines of TcMK2 and TcMK3, in which one copy of the gene was re-introduced in the null mutant, rescued differentiation phenotype. To evaluate whether TcMK2 activity was required for metacyclogenesis, we generated addback lines where a copy of a mutated TcMK2 in the predicted proton acceptor Asp266Ala was inserted in tcmk2-/- epimastigotes. The D266A mutants are unable to differentiate into metacyclic trypomastigotes. TcMK2 null mutants and the D266A addback line submitted to differentiation using TAU3AAG medium, were fully susceptible to lysis by human serum, do not express metacyclic specific surface proteins, such as gp82 and gp90, do not present typical metacyclic morphology distinguished by positioning of nucleus and kinetoplast. Furthermore, those mutants were unable to infect mammalian cells, consistent with an essential role for fully functional TcMK2 in metacyclogenesis. Taken together our results suggest that we have identified three PKs that are involved in crucial checkpoints during T. cruzi differentiation.
Supported by: capes
Keywords: KINASE;DIFFERENTIATION;MUTANTS.

PV – 003 - ***Functional analysis of IP6K in Trypanosoma cruzi: establishing genotoxic stress baselines to guide future DNA damage studies in IP6K-mutant lineages***

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Trypanosoma cruzi, the etiological agent of Chagas disease, is subjected to multiple genotoxic insults during its life cycle, including oxidative stress, ionizing radiation (IR), and ultraviolet (UV) light. Effective detection and repair of DNA lesions are critical for parasite survival, persistence, and pathogenicity. Inositol pyrophosphates (PP-IPs) have been shown in model eukaryotes to regulate key pathways involved in DNA repair, including homologous recombination and cell cycle checkpoint activation. However, it is not yet known whether PP-IPs also play a role in DNA repair in trypanosomatids. Of note, the most common PP-IPs in trypanosomatids is IP_7, which is synthesized by the inositol hexakisphosphate kinase (IP6K). Here, we used *T. cruzi* (CL Brener) to generate mutant lineages for IP6K kinase: single-KO for IP6K ($IP6K^{-/+}$) and IP6K overexpressor ($IP6K^{+/+/+}$). These lineages were confirmed by PCR and RT-qPCR and showed slight differences compared to the controls relative to the growth curves, DNA content analysis and cell cycle phases duration. Before challenging these lineages, we decided to standardize the concentrations of the genotoxic agents that will be used (50-200 µM H_2O_2, 100-500 Gy ionizing radiation – IR, and 100-1000 mJ UV) performing assays only with the *T. cruzi* WT. Our preliminary findings suggest that *T. cruzi* responds differently to the genotoxic agents: *T. cruzi* can recover from oxidative damage caused by up to 100 µM H_2O_2 and apparently reaches a dormant state in response to all IR doses. Curiously, *T. cruzi* is apparently not sensitized by UV light (up tp 1000 mJ). Currently, we are evaluating the behavior of *T. cruzi* IP6K mutant lineages against these genotoxic agents. We believe that future results of this project could pave the way for future exploration on roles of PP-IPs in *T. cruzi* DNA repair.
Supported by: FAPESP - 2023/00253-8
Keywords: Chagas disease;DNA repair;inositol pyrophosphates.

PV – 004 - **DETECTION OF PHLEBOTOMINAE TRANSMITTED VIRAL COINFECTIONS IN DOGS WITH VISCERAL LEISHMANIASIS IN BRAZIL**

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Leishmaniasis is a parasitic disease caused by protozoans of the Genus Leishmania. The disease is widespread worldwide, and in some countries, it is a significant healthcare concern. The Phebotominae vectors of Leishmania may transmit Phleboviruses and Pacuvirus from the Bunyavirales Order. These viruses also pose a health risk to humans and livestock, causing symptoms such as thrombocytopenia and fever. Previous studies in our lab have shown that coinfection, both in vitro and in vivo, increases both the parasite load and the viral titer of the sample, suggesting an association between more severe cases of leishmaniasis and coinfections with viruses from the Bunyaviricetes order. Dogs are the main reservoir for L. infantum transmission, and we hypothesized the existence of coinfection in transmission foci of Visceral Leishmaniasis with Leishmania and Bunyavirales. To test this hypothesis, we searched for sequences of segment L, the most conserved region of the genomes of both Phleboviruses and Pacuviruses.

We focused on sequences found in the New World transmitted sand file Bunyavirales genomes and designed two sets of PCR primers to detect these viruses. We tested bone marrow and serum samples from 25 VL-positive dogs from the Municipality of Rio de Janeiro. We detected by RT-PCR in 8 animals, thus suggesting that dogs are coinfected with Bunyavirales and Leishmania. Our data show, for the first time in Brazil, that the coinfection in VL foci adds a layer of complexity to the understanding of the factors involved in L. infantum transmission. Our group also isolated one virus from the infected dogs and cultivated it in our laboratory. We successfully sequenced the genome of the viral isopate. We determined that it is a variant of the Pacui virus, initially described in the North of Brazil, from sand flies and rodents. Our data revealed the first description of a Pacui-related virus in Rio de Janeiro, and, importantly, the first case of a dog coinfected with the Pacui virus and *Leishmania infantum. They may uncover a silent cotransmission in the visceral leishmaniasis foci in peri-urban areas. Further* investigation is necessary to assess the impact of this coinfection on the Leishmania life cycle.

More samples from Rio de Janeiro and other States in Brazil are currently in progress.

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Keywords: Leishmania;coinfection;detection.

PV – 005 - **Mitochondrial RNA editing in Arcellinida (Amoebozoa)**

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Mitochondria are essential organelles in eukaryotic cells, originating from an ancestral endosymbiotic event. Mitochondrial RNA (mtRNA) editing is a process that alters mitochondrial transcripts via substitutions or indels. Although observed in several lineages, debates about mechanisms, evolutionary drivers, and functional consequences of mtRNA editing remain unresolved even in model organisms, including kinetoplastids, plants, and *Homo sapiens*. In the eukaryotic supergroup Amoebozoa, mtRNA editing has been documented in the orders Myxogastria and Dictyostelida. Arcellinida is a monophyletic order of shelled amoebae among the most diverse and ecologically relevant amoebozoan lineages. Preliminary evidence suggests the presence of mtRNA editing within the group, but the lack of high-quality reference mitochondrial and nuclear genomes limits the characterization of this phenomenon. Here we present preliminary mitogenomic and transcriptomic data to characterize mtRNA editing in free-living testate amoebae. Arcellinida has a unique mtRNA editing pattern in comparison with other/external Amoebozoa orders, but internally consistent even in distant infraorders, suggesting an ancestral synapomorphy in the group. Our analysis suggests that those lineages edit their mtRNA by inserting, deleting and substituting various nucleotides, giving sense to protein coding sequences and structure to RNA sequences. This study generates foundational resources for the investigation of Arcellinida and provides key insights into the diversity and evolution of mtRNA editing in general, while additionally contributing to broader goals in protistology by optimizing cultivation and sequencing methods for poorly characterized lineages.

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Keywords: mitochondrial genome;RNA editing;Amoebozoa.

PV – 006 - **EXO1 Facilitates Efficient DNA End Resection and Damage Signaling during Double-Strand Break Repair in Trypanosoma brucei**

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Introduction: Double-strand break (DSB) repair is crucial for genome integrity in *Trypanosoma brucei*, impacting chromosome stability and antigenic variation. DNA end resection, a key step in homologous recombination, is mediated by nucleases such as EXO1, but its precise role in *T. brucei* remains unclear.
Objectives: To investigate the role of EXO1 in DNA damage signaling, DNA end resection, and repair of an induced intrachromosomal DSB generated by the I-SceI nuclease.
Methodology: EXO1 knockout lines were generated from cells expressing inducible I-SceI to create a site-specific DSB. Growth curves were analyzed to assess the impact of EXO1 loss on proliferation. Clonogenic assays tested survival after a single DSB or widespread damage. Resection was quantified by qPCR at sites distal to the I-SceI cut. γH2A levels after a single DSB or etoposide damage were determined by Western blot. DSB repair outcomes were assessed by PCR.
Results: EXO1 is not essential in *T. brucei*, being dispensable for proliferation under normal conditions. EXO1 knockouts survived damage similarly to parental cells, indicating repair without EXO1 is possible. EXO1 was required for efficient DNA damage signaling to distinct DSB types: upon site-specific induction, it was needed for full γH2A accumulation; in contrast, its absence after etoposide damage caused a prolonged γH2A signal, suggesting delayed repair or checkpoint recovery. EXO1 loss delayed—but did not limit—DNA end resection and did not alter the predominance of homologous recombination in repairing a site-specific DSB.
Conclusions: EXO1 contributes to efficient long-range resection in *T. brucei*, facilitating timely repair and proper DNA damage signaling, with redundancy in the resection machinery and interplay among nucleases maintaining genome stability.
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Keywords: Double Strand Break;DNA end resection;DNA repair.

TB – 001 -**Tackling Cutaneous Leishmaniasis: Repurposing Human Kinase Inhibitors**

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Collectively, the shortcomings of current treatments for leishmaniasis make the discovery of new alternative treatments an urgent matter. Leishmaniasis is a neglected tropical disease and therefore the resources for drug discovery are limited. In this context, repurposing remains as a viable strategy to mine for new effective treatments. Kinases have been increasingly studied as druggable targets for the development of cancer treatments. This led to the current panel of approximately 100 molecules that were approved over the world for clinical use against different types of cancer. In general, their mechanism of action revolves around the inhibition of the kinase ATP-binding domain, which blocks its cell signalling pathways. More recently, in *Leishmania*, kinases have been explored as potential therapeutic targets due to their essentiality for parasite survival. Therefore, we aimed here to investigate the therapeutic potential of kinase inhibitors that were previously approved for clinical use and could be repurposed for the treatment of leishmaniasis. In total, 121 compounds were triaged *in vitro* against promastigotes of *L. amazonensis*, from which 13 led to less than 50% parasite viability at 1 µM. These were evaluated against bone marrow-derived macrophages for their cytotoxicity, as well as their activity against intracellular amastigotes. Two compounds, A and B, were found to be highly selective against the parasite, with and EC_{50} < 1 µM and a SI > 10. Both compounds are currently being assessed regarding their efficacy in treating infected mice. Although their mechanisms of action have been elucidated for another disease, these molecules have never been investigated for leishmaniasis. For that reason, these drugs could have different targets in the parasite than in humans. Therefore, we have selected mutant parasites that are resistant to either compound A or B via chemical mutagenesis, and searched for mutations on a gene of interest. Missense mutations on this gene were found in all resistant parasites analysed, suggesting that its inhibition may potentially be the main mechanism of action of these drugs. Ongoing efforts are towards the genetic validation of these findings.
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Keywords: Leishmania;Repurposing;Drug discovery.

TB – 002 - **Cyclic peptide Li1 blocks macrophage invasion by metacyclic Leishmania amazonensis and reduces parasite burden in mice**

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Leishmaniases are neglected tropical diseases caused by protozoa of the genus *Leishmania*. The cyclic peptide Li1, selected by phage display for binding to *Leishmania infantum*, has emerged as a promising inhibitor of parasite entry into host cells (Verga et al., 2024). On this basis, we evaluated the effect of Li1 in cutaneous leishmaniasis caused by *L. amazonensis*. Parasites were subjected to enrichment procedures to increase the proportion of metacyclic forms and then pre-exposed to Li1 or its linear analogue Li1nc. Both peptides were synthesized by Fmoc-based solid-phase peptide synthesis, purified by reverse-phase high-performance liquid chromatography, and confirmed by electrospray ionization mass spectrometry. *In vitro*, pre-exposure of enriched metacyclics (0.5 mg/mL) to Li1 significantly reduced infection of murine and THP-1–derived macrophages, without cytotoxicity (CC_{50} > 1 mg/mL) or promastigote growth inhibition (IC_{50} > 1 mg/mL). Localization assays showed a discrete and punctate distribution of Li1 on the parasite surface, contrasting with the diffuse binding pattern of Li1nc. For *in vivo* assays, parasites were pre-incubated with peptides, thoroughly washed to remove unbound molecules, and only then used for infection of BALB/c mice. This prior interaction was sufficient to reduce parasite burden by 55.2% with Li1 and 31.1% with Li1nc, without evidence of hepatic or renal toxicity. Taken together, the findings demonstrate that Li1 consistently reduces parasite internalization in macrophages and attenuates infection *in vivo* across different *Leishmania* species and clinical forms. The more defined and punctate interaction of the cyclic peptide with the parasite surface, in contrast to the diffuse and less specific pattern observed for the linear analogue, underscores that cyclization enhances the strength and specificity of peptide–parasite interaction. Importantly, the observation that brief pre-exposure of infective forms to Li1 was sufficient to decrease subsequent parasite burden highlights a mechanism with direct implications for prophylactic applications. Altogether, these results support the potential of Li1 as a promising candidate for innovative strategies aimed at controlling leishmaniases, including vector-based approaches such as paratransgenesis.
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Keywords: Cyclic peptide;L; amazonensis;Metacyclic forms.