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SPC – 001 - **Biosynthesis of isoprenoids in *Plasmodium falciparum***

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Research into the various biosynthetic pathways for proteins, lipids, and carbohydrates in *Plasmodium falciparum* is one strategy for identifying potential targets for antimalarial drug development. It is crucial to identify biosynthetic pathways unique to the protozoan—pathways not shared with the host. Work in our laboratory has focused on the biosynthesis of N-linked glycoproteins and isoprenoids in the intraerythrocytic stages of P. *falciparum*. While N-linked glycoprotein biosynthesis in *P. falciparum* closely resembles that of other eukaryotic cells—offering few potential antimalarial targets—we made the interesting discovery that the processing of the glycan core parallels the maturation of the Golgi apparatus throughout the intraerythrocytic cycle. The lipid carrier involved in the biosynthesis of glycoprotein N-linked is a polyisoprene (dolichol) containing 60–65 carbons, differing from human cells, where dolichols consist of 110– 120 carbon chains. This finding prompted us to investigate isoprenoid biosynthesis in *P. falciparum*. Using metabolic labeling techniques with a panel of different radioactive precursors we describe the biosynthetic steps of the methylerythritol phosphate pathway (MEP) leading to isopentenyl pyrophosphate, as well as several complex isoprenoids, in P. *falciparum* blood stage cultures. Other polyisoprenoids identified included dolichol, ubiquinone, carotenoids, menaquinone, tocopherol, phylloquinone, and cytokinins, which are related to distinct physiological functions in the parasite, such as N-linked glycoprotein biosynthesis, the electron transport chain, antioxidant activity, and the regulation of parasite growth and development, respectively. As these products are absent from or differ in the human host, the enzymes of this metabolic pathway are probably promising new targets for antimalarial drugs. Whereas in *P. falciparum* the isoprenic side chains of ubiquinone contain 7–9 isoprenic units, 10-unit side chains are found in humans. In a search for the *P. falciparum* enzyme responsible for the biosynthesis of isoprenic side chains attached to the benzoquinone ring of ubiquinones, we cloned and expressed a putative polyprenyl synthase. The recombinant protein, as well as *P. falciparum* extracts, showed an octaprenyl pyrophosphate synthase activity, with the formation of a polyisoprenoid with eight isoprenic units. The recombinant enzyme could be competitively inhibited in the presence of the terpene nerolidol. This is the first report that directly demonstrates an octaprenyl pyrophosphate synthase activity in parasitic protozoa. Given the rather low similarity of the *P. falciparum* enzyme to its human counterpart, decaprenyl pyrophosphate synthase, this octaprenyl pyrophosphate synthase likely represents a promising drug target. Another enzyme identified, farnesyl diphosphate synthase, catalyzes the synthesis of farnesyl diphosphate (FPP) as well as geranylgeranyl diphosphate (GGPP); this activity was inhibited by risedronate. A plasmodial enzyme that presented phytoene synthase activity, responsible for the biosynthesis of carotenoids, was also identified and characterized, and was inhibited by norflurazon. We identified a gene in the *P. falciparum* genome that encodes a transmembrane prenol kinase (PolK), an enzyme that phosphorylates farnesol (FOH) and geranylgeraniol (GGOH). PolK is thought to be used by the parasite when these alcohols diffuse from the host's plasma into the parasite, representing a novel type of enzyme involved in isoprenoid metabolism. In conclusion, over the past two decades our laboratory has shown that the intraerythrocytic stages of *Plasmodium falciparum* synthesize N-linked glycoconjugates and a broad range of isoprenoid end-products – including dolichols, ubiquinones, carotenoids, menaquinone, tocopherol, phylloquinone and cytokinins – through enzymes, intermediates and lipid carriers that differ substantially from those of the human host. Key examples include a markedly shorter dolichol chain, an octaprenyl pyrophosphate synthase with low similarity to the human decaprenyl pyrophosphate synthase, and parasite enzymes such as phytoene synthase and the prenol kinase PolK, which have no clear human counterpart. Collectively, these findings establish the isoprenoid biosynthetic and salvage pathways of *P. falciparum* as a rich, largely unexploited source of parasite specific enzymes – several of which are already validated as druggable through their sensitivity to inhibitors such as nerolidol, risedronate and norflurazon – reinforcing their potential as targets for the rational design of new antimalarial drugs. **Supported by:** FAPESP (2024/09997-2), CNPq and CAPES **Keywords:** Malaria;P; falciparum;Isoprenoids.

CO – 001 - **Mosquito midgut response to blood feeding at a single-cell resolution**

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Mosquitoes are anautogenous insects: they can sustain metabolism on nectar but must consume blood to complete the gonadotrophic cycle and lay eggs. Blood feeding triggers a rapid metabolic shift, particularly in the midgut—the first tissue to encounter blood and any pathogens, such as *Plasmodium*. The midgut is essential for digestion, nutrient absorption, and reproduction, and is also critical for *Plasmodium* establishment and persistence. We performed single-cell RNA sequencing of midgut cells from sugar- and blood-fed mosquitoes, 24 hours post-feeding at peak digestion. Our goal was to identify midgut cell types and their transcriptional profiles. We developed a dissociation protocol yielding high-quality cells and profiled over 13,000 cells. We validated markers for enteroendocrine cells, stem cells, and anterior midgut enterocytes. Defining markers for posterior midgut enterocytes is more complex, as their transcriptional profiles vary with function. Our data show that enterocytes undergo a marked transcriptional shift after blood feeding, alternating between digestive and immune states. Furthermore, transcripts of digestive enzymes such as trypsin localize to the apical region, suggesting a conserved mechanism to enhance translation. Altogether, our data show that blood feeding primarily reshapes enterocyte transcription to enable digestion, nutrient uptake, tissue homeostasis, and microbiota control.

CO – 002 - **Glycerol metabolism as a novel regulator of trypanosome development and transmission**

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Transmission of Trypanosoma brucei to the tsetse fly is classically thought to depend on quorum sensing: at high parasite density, proliferative slender bloodstream forms differentiate into growth-arrested stumpy forms pre-adapted for development in the insect vector. Quorum sensing therefore couples transmission competence to parasite density while limiting parasite expansion within the mammalian host. However, human infections with T. b. gambiense are typically chronic and characterised by extremely low, sometimes intermittently undetectable, parasitaemia that appears insufficient to trigger quorum-sensing-dependent stumpy formation. Despite this apparent absence of the canonical transmission stage, infected individuals can remain a source of parasites for tsetse flies. This long-standing transmission paradox suggests that transmission competence can be generated or maintained independently of parasite density, potentially within extravascular tissue reservoirs. Using pleomorphic *T. b. brucei*, we investigated whether the metabolic environment of extravascular tissues provides an alternative route to transmission competence. At low parasite density, physiological concentrations of glycerol and glucose mimicking adipose and hypodermal interstitial fluids (0.2 and 4 mM, respectively) induced a distinct proliferative population, termed slender-Glyc. These parasites displayed a proteome intermediate between slender and stumpy forms and acquired reversible competence to differentiate into procyclic forms. Importantly, slender-Glyc parasites established tsetse midgut infections at a frequency comparable to quorum-sensing-induced stumpy forms (40%

versus 32%), whereas parasites maintained under blood-like glycerol conditions were almost non-infective (0.7%). This developmental response required glycerol uptake and metabolism, linking transmission competence to active metabolic sensing rather than glycerol exposure alone. Glycerol also acted at high parasite density. Tissue-like glycerol concentrations prolonged the otherwise short lifespan of quorum-sensing-induced stumpy forms, and this protection was lost in glycerol kinase-null parasites. Thus, glycerol can both generate proliferative transmission-competent forms before the quorum-sensing threshold is reached and extend the persistence of stumpy forms after quorum-sensing commitment. These findings identify host-tissue metabolism as a regulator of parasite developmental fate. We propose that adipocyte-derived glycerol in the skin complements quorum sensing through two distinct effects: at low parasite density, it generates proliferative transmission-competent slender-Glyc forms, whereas after quorum-sensing commitment, it prolongs the survival of stumpy forms and thereby extends the transmission window. This model dissociates transmission competence from the requirement for high parasitaemia and offers a mechanistic explanation for how T. b. gambiense may remain transmissible during chronic human infections. More broadly, it reframes the transmission paradox as a consequence of considering parasite density without accounting for the metabolic heterogeneity of host tissues. Identifying glycerol-dependent forms in infected human tissues will now be essential to establish their contribution to natural transmission. **Keywords:** Trypanosoma brucei; transmission paradox; glycerol metabolism.

CO – 003 - **TRAMP-CSS complex is essential for hepatocyte and erythrocyte infection by Plasmodium zoites**

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Plasmodium sporozoites invade mosquito salivary glands, mammalian blood vessels in the skin and the hepatic parenchyma to ultimately enter inside hepatocytes and produce thousands of erythrocyte-invasive merozoites. Despite the importance of these steps in early malaria infection, little is known about their molecular basis. Here, using a rodent model and in vivo conditional mutagenesis, we show the role of the TRAMP and CSS (TC) complex in *P. berghei* (Pb) sporozoite and merozoite infectivity. Conditional gene excision of these molecules did not significantly alter invasion of mosquito salivary gland by sporozoites, nor their motility or cell wounding activities. However, conditionally knocked out (cKO) sporozoites displayed impaired cell entry and development inside hepatoma cells. Similarly, cKO merozoites could not establish blood infection in vivo, indicating that the Pb TC complex is essential for erythrocyte invasion. The multi-stage and essential role in hepatocyte and erythrocyte infection renders this complex a promising vaccine target and opens novel perspectives to unravel the mechanisms underlying Plasmodium entry into different cell types.

CO – 004 - **Genomic merger: How Leishmania hybridization drives parasite evolution and immune escape**

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A critical yet often overlooked factor contributing to the emergence and adaptability of Leishmania is the generation of hybrids through a cryptic sexual cycle within the sand fly vector. Although hybridization in Leishmania is recognized as a key driver of genetic innovation with unpredictable consequences on pathology, epidemiology, and disease outcomes in natural populations, the mechanisms by which the merger of two genomes promotes disease emergence remain largely understudied. Here we apply comparative genomics and transcriptomics approaches on parasite hybrid strains that have evolved in the field, inside experimentally infected sand flies or in culture to assess the role of genetic introgression on parasite adaptation and fitness gain. Short- and long-read sequencing analyses of L. infantum field isolates causing visceral and cutaneous diseases in immunocompetent individuals across the Maghreb and the Italian peninsula revealed a shared hybrid structure of these genomes. Integrating metagenomic, BLAST, and in silico multi-locus sequence typing (MLST) analyses uncovered significant genetic introgressions from *L. donovani* and *L. tropica*, indicating a complex history of hybridization. Tracing the evolutionary origins of these geographically distinct hybrid strains assessing SNP profile, SNP frequency, or genetic admixture grouped these strains into different clusters, suggesting independent genetic origin. Surprisingly, despite their distinct evolutionary histories, resolving the mosaic architecture of these hybrid genomes identified shared introgressed regions enriched in genes encoding fast evolving surface proteins and virulence factors, including members of the amastin gene family. Conceivably, these introgressions may be under natural selection to promote immune escape, thereby driving convergent evolution among independent hybrid strains. Experimental sand fly infections with hybrid strains identified gene conversion and recombination as key mechanisms underlying haplotype shuffling and convergent allelic selection. RNA-seq analyses of hybrids enabled us to distinguish gene dosage–dependent expression changes from genome-independent alterations in transcript abundance. These findings reveal an important and underappreciated role for post-transcriptional regulation in promoting phenotypic adaptation and mitigating the genomic shock associated with hybrid formation. In conclusion, our results highlight the role of hybridization in enhancing Leishmania fitness during clinical infection and reveal a novel form of antigenic escape that may confer selective advantages immediately after transmission, rather than during chronic infection. Our study provides a computational framework capable of exploiting hybridization as a powerful genetic tool to identify loci under convergent selection, capable of revealing clinically relevant pathogenicity factors with potential diagnostic or prognostic applicability. **Keywords:** Leishmania hybridization; fitness gain; comparative genomics.

CO – 005 - **Energy status as a signal for adaptation and differentiation in *Trypanosoma cruzi***

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Trypanosoma cruzi alternates between environments that impose profound changes in nutrient availability and, consequently, in cellular energy status. Rather than representing only a constraint that parasites must overcome, changes in energy availability may constitute regulatory signals that couple metabolic state to adaptive decisions, including proliferation, stress responses, autophagy and differentiation. We propose that the parasite does not simply respond to nutritional stress by restoring energetic homeostasis, but can translate changes in its energetic state into signals that determine alternative cellular programs. In this framework, the AMP-activated protein kinase (AMPK), a central sensor of cellular energy status, represents a plausible component of the signaling network that links nutrient availability to parasite adaptation and developmental transitions. *T. cruzi* AMPK is a heterotrimeric complex composed of α, β and γ subunits and is unusual in containing two catalytic α isoforms. Phylogenetic analyses indicate that duplication of the α-subunit genes occurred independently in kinetoplastids, while the α1 isoform accumulated a greater number of sequence changes, suggesting possible lineage-specific functional diversification. To investigate whether the two isoforms contribute differently to the energetic response, we generated α1- and α2-knockout parasites using CRISPR-Cas9. Following clonal selection, the correct editing of these genes, as well as the absence of off-targets, was evaluated by whole-genome sequencing using nanopore technology. Loss of α2 produced a marked reduction in parasite growth during both exponential and stationary phases. This phenotype was partially alleviated by supplementation with glucose, linking α2-dependent AMPK function to carbon availability. Moreover, α2-deficient parasites displayed increased phosphorylation of the remaining α1-associated species, suggesting functional compensation between the two isoforms. Together, these observations indicate that the two catalytic subunits are not functionally equivalent and support a role for AMPK in translating changes in nutrient availability into cellular responses. A broader relationship between nutritional stress, autophagy and developmental differentiation is supported by our recent studies of the TcVps34–TcVps15 complex. Nutritional stress induced during metacyclogenesis is accompanied by increased autophagic activity, and parasites overexpressing TcVps34 or TcVps15 displayed enhanced autophagy during the early stages of differentiation, together with increased metacyclogenesis. These parasites showed increased numbers of Atg8.1-positive structures and enhanced hydrolytic activity of degradative compartments. Pharmacological modulation further supported the functional connection between autophagy and differentiation: inhibition of PI3K activity reduced the autophagic response during differentiation and negatively affected metacyclogenesis. Interestingly, enhanced autophagy and differentiation were not necessarily associated with increased infectivity, since TcVps34- and TcVps15-overexpressing parasites displayed reduced establishment of infection in Vero cells. Taken together, these findings support a model in which energetic and nutritional states are not merely passive consequences of environmental conditions, but can be integrated into signaling networks that reshape parasite physiology and developmental fate. AMPK, with its unusual expansion of catalytic α subunits, may represent an important entry point for understanding how *T. cruzi* senses and interprets energetic states. We propose that the relationship between energy sensing, autophagy and differentiation provides a framework to investigate how metabolic information is converted into developmental decisions during the complex life cycle of this parasite. **Keywords:** Energy sensing;Nutritional stress;Parasite development.

CO – 006 - **From parasite biology to therapy: druggable targets in malaria**

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Malaria remains a major global health threat, causing more than 240 million infections and over 600,000 deaths each year, largely due to *Plasmodium falciparum*. Rising resistance to current antimalarial drugs highlights the urgent need for new therapies that target novel parasite pathways. Although target-based drug discovery has long guided the development of safer and more effective treatments, phenotypic profiling can link drugs, targets, and resistance mechanisms. Targeting the *P. falciparum* ring stage is especially valuable because it can prevent progression to severe disease and interrupt parasite transmission. We have identified a novel class of antimalarials active against both proliferating and dihydroartemisinin (DHA)-induced quiescent ring stages in DHA-sensitive and DHA-resistant *P. falciparum* strains. This class has shown no in vitro selection for resistant parasites and has a favorable oral pharmacokinetic profile. Our studies, which integrate medicinal chemistry, chemoproteomics, and microscopy, indicate that these compounds act through a fast-killing mechanism. Together, this work demonstrates the value of interdisciplinary approaches for developing innovative, effective therapies. **Keywords:** Malaria, drug discovery; proliferating; artemisinin-induced quiescent ring stages.

CO – 007 - **The sweet side of immune evasion in African trypanosomes**

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African trypanosomes evade host immunity by constantly switching expression of variant surface glycoproteins (VSGs) to generate extreme antigenic sequence diversity. Previous work showed that *O*-glycosylation of Class B VSGs also modulates antibody recognition and influences infection outcome, but the responsible enzyme has remained elusive. VSGs are co-transcribed with twelve expression site-associated genes (ESAGs), of which only ESAG6/7 and ESAG4 have defined functions. Here, we identify ESAG3 as the VSG *O*-glycosyltransferase, the third ESAG to be functionally characterised. We show that ESAG3 depletion *in vivo* abolishes VSG *O*-glycosylation and trypanosome recognition by an *O*-glycosylation-specific monoclonal antibody, while complementation restores both. Recombinant ESAG3 has strict UDP-glucose donor specificity, acting on serine/threonine residues within surface-exposed VSG loops. Cryo-electron microscopy reveals that ESAG3 assembles into an unprecedented octadecameric ring with C3 symmetry at 3.4 Å resolution. Structure-guided mutagenesis shows that this ring architecture regulates catalysis since disrupting the contact interface increases substrate turnover. Furthermore, we show that VSG3*O*-glycan occupancy correlates with susceptibility to complement-mediated lysis, linking this modification directly to a functional outcome of the host antibody response. ESAG3 is encoded within VSG expression sites, so when parasites switch expression sites, VSG sequence changes alongside co-transcribed *O*-glycosylation machinery, integrating genetic and chemical variation into a unified immune evasion strategy. Since ESAG3 belongs to a glycosyltransferase family found exclusively in kinetoplastids, including *Trypanosoma cruzi*, we are now testing whether its restriction to this lineage has functional consequences for immune evasion across the phylum. **Keywords:** O-glycosylation;African trypanosomes;immune evasion.

CO – 008 - **Trypanosomal acetate:succinate CoA transferase: from biochemical and structural characterization to a metabolic engineering tool for bioenergetic studies across organisms**

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Acetate:succinate CoA transferase (ASCT) is a mitochondrial enzyme conserved in trypanosomatids and several anaerobic or microaerophilic eukaryotes, where it functions together with succinyl-CoA synthetase (SCS) to generate ATP through substrate-level phosphorylation independently of oxidative phosphorylation. Because mammals lack ASCT and instead possess the related enzyme succinyl-CoA:3-ketoacid CoA transferase (SCOT), understanding the biochemical and structural basis of ASCT function has important implications for parasite biology, drug discovery, and metabolic engineering. Our work has established the biochemical foundation of the trypanosomal ASCT/SCS cycle by developing an in vitro reconstituted assay and demonstrating that this pathway efficiently couples acetate production with mitochondrial ATP synthesis. Kinetic analyses revealed a remarkably high catalytic turnover, supporting the hypothesis that the ASCT/SCS cycle serves as an alternative ATP-generating system capable of sustaining mitochondrial energy metabolism when oxidative phosphorylation is impaired. In addition to ATP production, the cycle provides acetate required for cytosolic acetyl-CoA synthesis and de novo fatty acid biosynthesis, highlighting its central role in trypanosome metabolism. More recently, we solved crystal structures of Trypanosoma brucei ASCT in complex with its substrates and products, providing the first comprehensive structural framework for substrate recognition and catalysis. Comparative structural and mutational analyses identified residues governing oligomerization, CoA binding, and reaction specificity. Remarkably, substitution of a single amino acid generated a dramatic gain of SCOT activity, revealing the molecular determinant that distinguishes ASCT from its mammalian counterpart despite their highly conserved overall fold. These findings provide a mechanistic explanation for the evolutionary divergence of two homologous enzymes catalyzing distinct metabolic reactions and establish a framework for selective inhibitor design targeting parasite ASCT. Beyond parasite biology, we demonstrated that trypanosomal ASCT can function as a synthetic metabolic module. Heterologous expression of ASCT in Mycobacterium smegmatis partially restored intracellular ATP levels under ATP synthase inhibition and conferred tolerance to low concentrations of bedaquiline, demonstrating that the ASCT/SCS cycle can be functionally integrated into a phylogenetically distant organism to reprogram cellular bioenergetics. This work establishes ASCT as a versatile metabolic engineering tool for probing the contribution of ATP homeostasis to antimicrobial mechanisms of action. Collectively, these studies establish ASCT as more than a parasite-specific metabolic enzyme. By integrating biochemistry, structural biology, and synthetic metabolic engineering, our work positions the trypanosomal ASCT/SCS cycle as a unique platform for investigating mitochondrial energy metabolism across diverse organisms, dissecting ATP-dependent cellular processes independently of oxidative phosphorylation, and enabling the rational design of novel antiparasitic therapeutics. **Keywords:** acetate: succinate CoA transferase; mitochondrial bioenergetics; metabolic engineering.

CO – 009 - **Mitochondrial butterfly effect: how changes in the organelle physiology impacts the fitness of trypanosomatids**

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Trypanosomatids colonize a wide range of host environments and are continuously exposed to diverse stress conditions, requiring extensive metabolic remodeling. The mitochondrion has a decisive contribution to the oxidative stress, due to the electron leakage from the electron transfer system. This organelle exhibits several functional peculiarities in trypanosomatids, making it an attractive target for therapeutic intervention. In the last 20 years, our group has been studying the mitochondrial susceptibility and adaptative processes of trypanosomatids, in special *Trypanosoma cruzi*. Similar to *T. brucei*, the mitochondrion of *T. cruzi* undergoes significant functional changes throughout its life cycle. Epimastigotes depend more heavily on mitochondrial metabolism than bloodstream trypomastigotes, as demonstrated by their greater susceptibility to the electron transport chain inhibitor antimycin A. In contrast, bloodstream trypomastigotes display lower oxygen consumption and mitochondrial membrane potential (ΔΨm), but higher mitochondrial reactive oxygen species (ROS) production and greater resistance to hydrogen peroxide challenge. These findings indicate that mitochondrial remodeling throughout *T. cruzi* life cycle is a key metabolic adaptation that promotes parasite survival in distinct environments. Clearly, oxidative stress also plays an important role in the interaction between trypanosomatids and insects. Strigomonas culicis ROS resistant strain shows enhanced mitochondrial metabolism and reduced oxidative injury, and high antioxidant capacity, leading to an increased colonization of Aedes aegypti, favoring parasite persistence in the invertebrate host. The mitochondrion of trypanosomatids is the most recurrent target organelle of antiparasitic compounds, commonly showing phenotypes as ultrastructural swelling and ΔΨm loss. Our group investigated the trypanocidal mechanisms of naphthoquinones and their derivatives. Naphthoimidazoles derived from beta-lapachone consistently impaired T. cruzi mitochondrial function by inhibiting electron transport chain activity, reducing oxygen consumption, and increasing ROS generation, ultimately disrupting parasite bioenergetics, data corroborated by proteomic analyses. Notably, although mitochondrial dysfunction is the predominant phenotype, naphthoquinones and their derivatives exhibit distinct mechanisms of action that are closely related to their chemical structures. Hydroxyl naphthoquinone (HN) induced early mitochondrial dysfunction in T. cruzi, characterized by ultrastructural alterations and reduced oxygen uptake, but no increase of ROS production was observed. The preincubation with antioxidants protected the parasite from HN trypanocidal action and the inhibition of trypanothione reductase activity, suggest a disruption of thiol redox homeostasis, rather than direct ROS generation. Up to now, our structure-activity analysis of highly active naphthoquinones presented no direct correlation among the presence of specific moieties, mitochondrial injury and trypanocidal effect. Although benznidazole (Bz) has remained the Chagas disease first-line treatment for decades, its mechanism of action remains incompletely understood and the contribution of oxidative stress to parasite killing and drug resistance is still controversial. Bz induced increased ROS levels, mitochondrial swelling, reduced ΔΨm and oxygen consumption, effects absent or attenuated in Bz-resistant T. cruzi. Our functional data together with proteomic analysis indicate oxidative damage induced by this drug is not primarily of mitochondrial origin, despite the organelle is a major target in the parasite, playing a central role in the development of Bz resistance. Novel perspectives on trypanosomatid-targeting chemotherapy could be developed based on better comprehension of mitochondrial oxidative regulation processes. **Keywords:** trypanosomatids; mitochondria; oxidative stress, naphthoquinones.

CO – 010 - **Benzoxaboroles target CPSF3 for degradation through a SUMO-dependent pathway in trypanosomes**

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Current and emerging pathogens threaten public health, and neglected tropical diseases (NTDs) remain a significant burden. Collective efforts have decreased the global burden of several NTDs, including African trypanosomiasis and malaria, with the former at historical lows. Two new compounds recently advanced through the pipeline for the treatment of African trypanosomiasis; fexinidazole and acoziborole. Acoziborole is a benzoxaborole (BoB), a drug class that emerged as potent microbicides against a wide spectrum of infections, spanning viral, bacterial, protozoan and fungal agents, and acoziborole has been recently licensed for use in the clinic. Significantly, BoBs demonstrate activity towards both kinetoplastida and apicomplexan parasites, despite their evolutionary divergence. A common target, cleavage and polyadenylation specificity factor 3 has been identified for BoB activity against multiple protists. CPSF3 is part of a large and widely conserved complex, with endonuclease activity recognising the pre-mRNA 3'-cleavage site prior to polyadenylation. CPSF3 is the sole catalytic component of the CPSF complex and the target of several distinct BoBs in multiple unicellular protozoan parasites, including *Plasmodium falciparum*, *Toxoplasma gondii*, *Trypanosoma brucei* and *T. cruzi*. Several metabolic pathways in trypanosomes process and activate specific BoB derivatives, serving to retain them within the parasite and hence potentiate trypanocidal activity. SUMO (small ubiquitin-like modifier) is widely distributed across eukaryotes and influences protein location, stability and function, and is associated with stress. In most taxa, including trypanosomes, SUMO is located primarily in the nucleus. Humans possess four SUMO paralogs but trypanosomes have a single SUMO gene as well as single E1, E2 and E3 ligases. In T. brucei SUMO is closely associated with transcriptional and post-transcriptional regulation proximal to the expression site body (ESB). Significantly, the ESB contains multiple proteins responsible for securing monoallelic expression of VSG, while sumoylation is associated with proteins located at VSG-encoding chromatin. SUMO is important for translocation of mRNA to the NPC in animal and fungal cells, and while not demonstrated experimentally, there is sufficient conservation within trypanosomes to expect similar mechanisms to operate. Genome-wide screens previously identified two classes of trypanosome genes implicated in BoB sensitivity. Examples of prodrug processing include metabolism by parasite aldehyde dehydrogenase or serine carboxypeptidase, and are highly sensitive to BoB structure. Blocking these pathways leads to much lower levels of BoB sensitivity compared to a BoB-resistant CPSF3 mutant (100-fold versus 6-fold), suggesting additional mechanisms operate in the latter case. Here, using comprehensive genetic screens in Trypanosoma brucei, we establish a functional connection between the boron-heterocyclic scaffold shared by all BoBs and protein sumoylation genes, which represent a pan-BoB association rather than prodrug processing pathways that are structure specific. We observe a rapid, transient increase in global protein sumoylation following BoB exposure. While we do not observe direct CPSF3 sumoylation, we find that acoziborole and other BoBs rapidly and specifically destabilise CPSF3 in a SUMO- and proteasome-dependent manner. **Keywords:** Acoziborole; CPSF complex; benzoxaborole, proteasome.

CC – 001 - **DNA metabolism in trypanosomatids: why so many differences?**

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Trypanosoma cruzi, the etiological agent of Chagas disease (CD), infects approximately 8 million people worldwide and is exposed to a wide range of environmental stresses throughout its life cycle, including nutrient deprivation, pH fluctuations, oxidative stress, and host immune responses. Many of these conditions induce DNA damage, requiring the activation of DNA damage response (DDR) pathways that coordinate DNA repair with critical cellular outcomes, such as cell cycle arrest, dormancy, or programmed cell death. Understanding how *T. cruzi* senses and responds to distinct types of DNA lesions is essential for elucidating parasite biology and may reveal new therapeutic opportunities. Our recent findings indicate that different classes of DNA damage trigger distinct biological responses in *T. cruzi*. Transcription-blocking DNA lesions activate signaling pathways leading to programmed cell death, whereas DNA double-strand breaks promote the establishment of a dormant state. This observation is particularly relevant because dormant intracellular parasites have been proposed to contribute to drug tolerance and treatment failure in Chagas disease. This study investigates the roles of genes involved in homologous recombination and transcription-coupled DNA repair in mediating these stress responses. By characterizing the molecular pathways that determine the parasite's fate following different types of DNA damage, we clarify the relationship between DNA repair, stress signaling, dormancy, and cell death in *T. cruzi*. These findings will advance our understanding of parasite adaptation to hostile environments and may identify molecular targets for the development of novel therapeutic strategies against Chagas disease. **Supported by:** CNPq, FAPEMIG (APQ-05883-24, APQ-02533-24). **Keywords:** Trypanosoma cruzi;DNA damage response;transcription-coupled DNA repair.