SPC-1 - **Molecular Biology approaches to understand *Leishmania***

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The purpose of this lecture is to present the paths of a research line, which aims to understand how the protozoan *Leishmania* maintains itself in nature and how it establishes relationships with its hosts. We chose to show these paths as branches of a “tree”, representing the relationships of the “hypothetical entities”. The “root of the tree” occupies a special place since it is the source entity. Thus, we intend to show how “gene characterization” represents the origin of research lines of our lab and how it evolved in two branches: one for the identification of the pathogen, linked to the diagnosis and phylogeny of these parasites, and another related to the physiology of these organisms and their interaction with the hosts.

We started with the characterization of the ribosomal cistron (rDNA), which contains the sequences that will be exclusively transcribed by RNA Polymerase I (RNA polI) to originate RNAs that constitute the ribosome. In this characterization, we used the evolutionary conserved characteristic of the rDNA to search for regions that could be discriminatory among the species of *Leishmania* [1] [2]. However, the SSU rRNA sequence (RNA of the small subunit of the ribosome) is highly conserved and so the desired discrimination was not achieved for the species level. The same was true for the establishment of phylogenetic relationships, which generated unresolved trees. Nevertheless, using information from the SSU rRNA sequence, we confirmed that *L. tarentolae*, a species that infects lizards, belongs to a sub-genus previously identified as *L. (Sauroleishmania)* [3].

Using the rDNA gene characterization, we went to the other branch of our “tree” to study the regulation of rRNA transcription, determining the promoter region of RNA polI of some *Leishmania* species. The functional analysis of RNA polI promoter regions, through transient transfections of constructs containing a reporter gene under promoter region control, showed that RNA polI recognition is species-specific [4]. Additionally, we observed that heterologous RNA polI promoter was more active than its homolog, leading us to propose the existence of a species-specific repressor controlling rDNA transcription [5].

Since discrimination and identification of species was still our goal for later establishment of a diagnostic method using DNA, we moved back to the root of our “tree” to search for other target sequences. We identified Glucose-6-phosphate dehydrogenase (G6PD) coding region that allowed the discrimination of *L. (Viannia) braziliensis* from other *Leishmania* spp. [6] and provided the tool to identify its mammalian reservoir [7]. But we still have not come up with a robust target for species identification. Other sequences were tested, and finally, using the *hsp70* sequence as target in a PCR-HRM (Polymerase Chain Reaction coupled to High Resolution Melting) analysis, we describe a protocol that allowed the identification of all species that circulate in a given territory (Americas or Eurasia) [8]. This protocol was transposed from research benchtop to patient’s samples allowing epidemiological studies and an individual physician conduct in face to patient's prognosis [9].

In our search for species-specific targets, we characterized the arginase coding-gene. Previously, Prof. Erney Camargo’s group demonstrated how urea cycle enzymes have different expression profiles among trypanosomatids genera with *Leishmania* genus expressing arginase, an important enzyme of the urea cycle. Our study indicated that arginase gene sequence was not a good discriminatory target, however, moving to the other branch of the "tree", the arginase gene characterization of *L. (L.) amazonensis* allowed the heterologous expression of the active enzyme and its biochemical and biophysical characterization [10] [11]. With the purified recombinant enzyme, we obtained an anti-arginase polyclonal serum used to determine its localization at the glycosome, trypanosomatids’ exclusive organelle, confirming what had already been predicted by the presence of the SKL-addressing signal at its C-terminal [12].

The next step comprised the understanding of physiological role of arginase and showed its importance in the infection and maintenance of the parasite in the host cell, obtaining a *L. (L.) amazonensis* arginase knockout parasite [12]. We were able to obtain this knockout mutant only under culture supplementation with putrescine, what indicates the essential role of arginase in the synthesis of polyamines for parasite replication and supports arginase potential as a therapeutic target [13]. A more detailed study of the physiological role of arginase was obtained using the null arginase knockout in “omics” approaches, to study the interaction of the parasite with its host. We obtained the transcriptomic [14] and metabolomic [15] profiles of wild-type and knockout parasites and the data allowed an improved understanding of metabolic relationships and regulation of the expression of genes related to the polyamine pathway [16], as well as with virulence factors such as parasite recognition, growth and differentiation [17]. We also identified a possible salvation pathway, which should be considered for the proposed use of inhibitors of parasite’s arginase as therapeutics.

Considering the pathways involved in arginine metabolism and its potential role in host inflammatory processes and immune response, the transcriptomic [18] [19] and metabolomic [20] [21] profiles of BALB/c or C57BL/6 macrophages, infected with wild-type or arginase *L. amazonensis* knockout, indicated the importance of the host genetic background for the infection progression.

In another approach to study the host-parasite interaction, we used a panel of 84 miRNAs to determine the miRNA's profile of infected macrophage [22]. This profile is involved in the macrophage response to infection and, in some way, to parasite's arginase activity. The miRNAs we found in the study were also found in the plasma of *Leishmania*-infected patient [23] [24]. Furthermore, we demonstrated the role of mice miRNAs in regulating NOS2, an enzyme that also uses arginine as a substrate for the synthesis of NO, a potent microbicide. It is interesting to note that the human NOS2 presents a mutation in its mRNA 3'UTR, indicating that the miRNA we identified cannot act in this mRNA [25].

L-arginine, the arginase's substrate, is also essential for *Leishmania* survival. The uptake of arginine from the environment, either from the insect or the mammalian macrophage, is crucial for the parasite. We characterized the gene that encodes Amino Acid Permease 3 (AAP3), the main arginine transporter. We identified two copies of this gene on chromosome 30 and showed that one copy is regulated by extra or intracellular arginine levels [15], changes in temperature and/or pH [26], parameters that naturally change when the parasite passes from one host to another. For this gene, we started characterizing its physiological role and later we showed its species discriminatory potential with the same PCR-HRM approach [27].

Concerning the identification/diagnosis "branch", we are currently exploring the possibility of associating different targets, such as *hsp70* and *aap3*, to obtain an improved identification test for the development of an identification kit, in a multilocus approach. As well as, new genes identified from the "omics" data, are being characterized for their physiological role in studies that can indicate potential chemotherapeutic targets.

Taken together, this "phylogenetic tree" represents the evolution along the years of how gene characterization became a solid root with well-established branches.

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CO-01 - **Organization and dynamics of the *Trypanosoma cruzi* genome**

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Trypanosoma cruzi is able to invade almost any kind of cell, freely circulate in the blood or extracellular matrix, pass through the digestive tract of its insect vector and survive after being eliminated in faeces. This stressful lifestyle strongly requires fine regulation of gene expression, which in turn is reflected in its genome organization. Elucidation of the *tritryps* genomes was already representative that the genome of *T. cruzi* had taken particular evolutionary paths: while the genomes of *T. brucei* and *L. major* allowed in-depth comparative studies at the level of synteny and chromosomes, the genome of *T. cruzi* remained highly fragmented due to an expansive effect that results in the presence of multicopy genes. In particular, the surface proteins, which play crucial roles in the invasion, infection and modulation of the immune system, constitute the most expanded families, being encoded by hundreds - and more than a thousand in some cases- of genes. For a long time, it was thought that these genes were located in subtelomeric regions, as occurs in African trypanosomes, which would give them greater reorganisation/recombination capacity. However, a detailed analysis of the nuclear genome of several strains by using long-read sequencing methods led us to the observation that in *T. cruzi* the genome is organized into two clearly differentiated compartments: core and disruptive. The core compartment is composed of conserved genes either from known function or not (e.g.: hypothetical proteins), and they exhibit a high degree of synteny with the rest of the trypanosomatids. In opposition, the disruptive compartment is composed mainly of *transialidase*, *mucin* and *masp* genes and, as is known, synteny is lost or, in other words, synteny disruption occurs.
Long read sequencing allowed chromosome level assembly, and there are chromosomes composed completely for only one compartment, being most of them "mixed" chromosomes, but with a predominance of one of the compartments. When the comparison is made between lineages of *T. cruzi*, also disruption of synteny takes place, meaning that chromosomes predominantly disruptive cannot be numbered indistinctly, so we propose a new nomenclature for them. In addition, this organization is not just a feature of the genome architecture, but significant differences are found between compartments in chromatin organization, intrachromosomal interactions, nucleosome positioning, and methylation patterns, and these differences directly affect stage-specific gene expression levels. **Keywords:** Trypanosoma cruzi;Genomics;Epigenetics.

CO-02 - **Treating Latent Infection: Can We Kill Parasites in Their Sleep?**

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The ability of pathogenic protozoa to undergo differentiation into latent forms is critical to transmission and pathogenesis. Dormant stages of parasitic infection can lead to chronic disease that is intractable to current drug therapies. Here, we present evidence that a stress signaling pathway that mediates control of protein synthesis drives the formation of latency stages. We show that the development of latent stages in *Toxoplasma gondii* and *Plasmodium falciparum* is accompanied by the phosphorylation of eukaryotic initiation factor-2 (eIF2), which prompts changes in translation that produce factors to reprogram the genome. In *T. gondii*, replicating tachyzoites differentiate into latent bradyzoites that persist in the host and give rise to life-threating episodes of reactivated infection. The conversion to bradyzoites is triggered through TgIF2 phosphorylation by a series of eIF2 kinases that recognize different types of cellular stress. We show that pharmacological inhibition of one of these eIF2 kinases, an essential PERK orthologue, inhibits parasite replication and bradyzoite development in vitro and can subvert artemisinin-induced latency in *P. falciparum*. Furthermore, we find that inhibitors of eIF2 dephosphorylation, such as guanabenz, have anti-parasitic activity that includes destruction of bradyzoite cysts in vitro and in mouse models of latent toxoplasmosis. The discovery of a drug that dramatically decreases brain cyst counts allowed us to examine whether the number of brain cysts is associated with well-documented behavioral changes in rodents harboring latent toxoplasmosis. Unexpectedly, we found that brain cyst counts do not correlate with changes in host behavior; rather, behavior changes are associated with neuroinflammation. Altogether, these findings underscore the relevance of translational control through eIF2 as a key switch between proliferation and latency, and holds great promise to be exploited chemotherapeutically. **Supported by:**NIH **Keywords:** Toxoplasma;translation ;latency.

CO-03 - *Trypanosoma cruzi* infection causes cellular stress and a senescence-like phenotype in fibroblasts

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Trypanosoma cruzi infects and replicates within a wide variety of immune and non-immune cells. Here, we investigated early cellular responses induced in NIH-3T3 fibroblasts upon infection with trypomastigote forms of *T. cruzi*. We show that fibroblasts were susceptible to *T. cruzi* infection and started to release trypomastigotes to the culture medium after 4 days of infection. Also, we found that *T. cruzi* infection reduced the number of fibroblasts in 3 day-cell cultures, by altering fibroblast proliferation. Infected fibroblasts displayed distinctive phenotypic alterations, including enlarged and flattened morphology with a nuclei accumulation of senescence-associated heterochromatin foci (SAHF). In addition, infection induced an overexpression of the enzyme Senescence-Associated β-galactosidase (SA-β-gal), an activation marker of the cellular senescence program, as well as the production of cytokines and chemokines involved with the senescence-associated secretory phenotype (SASP) such as IL-6, TNF-α, IL-1β, and MCP-1. Infected fibroblasts released increased amounts of stress-associated factors nitric oxide (NO) and reactive oxygen species (ROS), and the treatment with antioxidants deferoxamine (DFO) and N-acetylcysteine (NAC) reduced ROS generation, secretion of SASP-related cytokine IL-6, and SA-β-gal activity by infected fibroblasts. Taken together, our data suggest that *T. cruzi* infection triggers a rapid cellular stress response followed by induction of a senescent-like phenotype in NIH-3T3 fibroblasts, enabling them to act as reservoirs of parasites during the early stages of the Chagas disease.

CO-04 - The role of the kynurenine pathway at the host-microbiota interface in blood-feeding insects

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Hematophagous insects ingest blood meals that are several-fold their weights before feeding. Excluding water, protein content accounts for 85 % of the composition of vertebrate blood, and therefore, digestion releases exceptionally large amounts of free amino acids. We previously showed that the tyrosine degradation pathway is essential to adapt insects to homeostatically handle dietary tyrosine. Here we studied the role of the tryptophan degradation pathway, the kynurenine pathway (KP). In mammals, intermediates of the KP have been ascribed multiple roles, including modulation of host/microbiome symbiosis. Enzymes of the KP are highly induced in *Aedes aegypti* midgut after a blood meal. We analyzed the phenotypes of a natural kynurenine hydroxylase (*Kh*) mutant in *Aedes aegypti* and CRISPR/Cas9 engineered mutants in *Anopheles stephensi* and *Culex quinquefasciatus*. All species showed a marked loss of fitness after feeding a blood meal. Aedes and Anopheline mosquitoes present different levels of severity, from reduced lifespan and fertility to a compromised midgut permeability barrier function in *An. stephensi*. Kh^- displayed an increase in microbiota when compared to kh^+ insects, without increased expression of canonical immune genes. Metagenomic analysis of *A. aegypti* from Kh mutants and wild type midgut, however, showed relative conservation of relative prevalence of most abundant species, suggesting an effect on microbial community size. Additionally, life span/mortality effects were rescued by antibiotic treatment, revealing a causative role for intestinal dysbiosis. Feeding with xanthurenic acid (XA), one of the KP end products, rescued lethality in both species of mosquitos and limited microbiota expansion in *Ae. aegypti*. These data reveal a unique role of the KP and XA in the regulation of the host/microbiota interface. A role for the aryl hydrocarbon receptor pathway as a downstream target of the KP was investigated.
Supported by:CNPq, FAPERJ **Keywords:** Aedes aegypti;Kynurenine pathway;microbiota.

CO-05 - **APPLYING CRISPR/CAS9 TO STUDY GENE FUNCTION IN *Trypanosoma cruzi*: INSIGHTS INTO THE FUNCTION OF PEPTIDASES, INHIBITORS, AND PROTEIN KINASES**

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The study of gene function in pathogenic protozoa is a fundamental step to understand parasite biology, its interplay with the host, mechanisms of pathogenesis, and to identify new drug targets. While sophisticated genetic tools are widely used in *Leishmania* and *Trypanosoma brucei*, the genetic manipulation of *T. cruzi* has proved to be more time-consuming and less efficient. As a result, from 1990-2018, gene deletion attempts had only been made for 36 *T. cruzi* genes [1]. Lately, the use of CRISPR/Cas9 has led to a remarkable enhancement in the timing and efficacy of genetic manipulation of *T. cruzi* [2-5]. We have adopted this approach to investigate gene function in *T. cruzi* Dm28 and here, we will describe our findings on the roles of the ecotin-like inhibitor of serine peptidases, ISP2, cathepsin B (CATB), and some protein kinases. We generated ISP2-null mutants (Δ*isp*2) in *T. cruzi* using CRISPR/Cas9, and Δ*isp*2 epimastigotes were more susceptible to lysis by human serum than the parental line or transgenic lines re-expressing ISP2. The Δ*isp*2 tissue culture trypomastigotes (TCTs), were more infective to human muscle cells *in vitro*, which was reverted by addition of camostate mesylate, suggesting that epitheliasin (TMPRSS2) is the target of ISP2. We found that increased host cell invasion is mediated through the crosstalk between the Protease-Activated Receptor 2 (PAR2) and Toll-like Receptor 4 (TLR4), confirmed by siRNA of PAR2, TLR4, or TMPRSS2. Furthermore, Δ*isp*2 induced greater tissue edema in the footpad of BALB/c mice and higher levels of KC, MCP1, TNFα, and IFNγ in the spleen. We propose that ISP2 contributes to protect *T. cruzi* from the antimicrobial effects of human serum and to prevent the engagement of inflammatory receptors in host cells, resulting in the modulation of host cell invasion and decreasing inflammation during acute infection. With regards to the cysteine peptidase cathepsin B, RNAi in *T. brucei* had previously shown that a 32% decrease of TbCATB was sufficient to prevent the death of infected mice, suggesting a possible role in infection. We successfully generated TcrCATB-null mutants (Δ*Tcrcatb*) showing that TcrCATB is not essential in *T. cruzi* epimastigotes. Δ*Tcrcatb* epimastigotes grew normally and did not show alterations in the shape and electron density of reservosomes. Δ*Tcrcatb* TCTs were less infective than the parental line, suggesting that this peptidase may play a role in the interaction with the host. We are currently applying CRISPR/Cas9 to investigate protein kinases (PKs), enzymes that play a predominant regulatory role in cell biology. We used transcriptomic data of Dm28c to select PK genes that were upregulated in the infective stages, and also focused on *T. cruzi* unique PKs (absent from *Leishmania* and *T. brucei* genomes). From this, attempts have been made to generate knock-outs for 13 PK genes. Four null mutants have been achieved, while numerous attempts for other genes did not yield null mutants, potentially indicating gene essentiality. We have discovered a *T. cruzi* unique PK that is vital for the differentiation to the metacyclic form, and has thus been named **E**ssential for **D**ifferentiation **K**inase 1 (EDK1). Likewise, *T. cruzi* lacking a unique STE kinase appeared to have impaired metacyclogenesis, and this PK was named EDK2, while another mutant displayed slow growth as epimastigotes. Among the candidates for essentiality, some will be selected for further validation through facilitated null mutant or unforced plasmid shuffle. Taken together, these findings highlight the current *era* of more efficient and timely analysis of gene function in *T. cruzi* to generate new knowledge on parasite biology.

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CO-06 - **Extracellular vesicles released from *Leishmania* infected cells (LiEVs); Biogenesis and putative functions in visceral leishmaniasis**

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Visceral leishmaniasis is a neglected tropical disease that causes significant morbidity and mortality. Iconic images of the distended abdomen of infected individuals due to an enlarged liver and spleen underscores the tissue remodeling in these infections. It is not known how *Leishmania*-derived molecules contribute to this disease presentation. We hypothesized that extracellular vesicles (EVs) released from *Leishmania*-infected cells (LiEVs) that contain parasite derived molecules, induce cellular responses that promote tissue remodeling. Two major mechanisms for exosome cargo loading have been described: the endosomal sorting complex required for transport (ESCRT) pathway and the ESCRT independent pathway ferry molecules that are eventually released from cells in exosomes. Recent studies have begun to elucidate the molecular details of these pathway. There is interest to determine which of these pathways is most important for the loading of molecules from *Leishmania*, which are intravacuolar pathogens. To address this gap in knowledge, we have monitored the intracellular trafficking of *Leishmania donovani* vasohibin (LdVash) in cells expressing fluorophore-tagged components of the ESCRT dependent and ESCRT independent machinery. Our studies have also commenced the investigation of the functional effects of LiEVs. These studies have included evaluation of their capacity to polarize macrophages. I*n vitro* studies thus far have suggested that LiEVs released from *Leishmania*-infected cells have the capacity to induce macrophage polarization to the M2 type. Complimentary *in vivo* studies have also shown more widespread IL4R expression of macrophages in infected tissue, which has provided strong evidence of M2 polarization in *L. donovani* infected tissues, which would ensure parasite persistence.

CO-07 - **Single-molecule analysis of DNA replication in the binucleated *Giardia lamblia* trophozoite**

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DNA replication is a highly precise process that must be completed in a timely and accurate manner once each cell cycle. In eukaryotes, DNA replication starts at multiple sites (replication origins) distributed throughout the chromosomes. DNA are RNA synthesis use the same DNA template and can therefore interfere with each other altering gene transcription program and causing replication stress. *Giardia lamblia* trophozoites are binucleated cells that alternate between a tetraploid (2x2N) and an octoploid genome (2x4N) during the S phase of the cell division cycle. Although a previous study suggested that DNA replication in *Giardia* occurs synchronously between the two nuclei, the dynamics of DNA duplication in this organism is poorly understood. Using single-molecule techniques (DNA combing and nanopore-based sequencing followed by D-Nascent analysis) we deeply investigated the spatio and temporal organization of replication units and the progression of replication forks in *G. lamblia* trophozoites. Our data revealed few active origins (between 3-6) on most DNA stretched molecules, showing that chromosomes are fully replicated from a minimum number of origins. Mean Inter-origin distances (IODs) was 335.7 kb and mean replication rate was faster (3.64 ± 1.32 $kb.min^{-1}$) than in any other protozoan parasite analyzed so far. Further, our precise analysis of DNA replication in single trophozoite cells confirmed that both nuclei replicate synchronously (78.49% of cells) with a variation of the stochasticity of replication timing in the other 21.51% of the population. Detection of BrdU incorporated on nascent DNA (D-NAscent) showed that from a total of 11,400 sequenced genes, 5,739 (50.3%) were identified in genomic regions without replication-transcription (RT) collisions collision while 5,661 (49.7%) can be a source of replicative stress by potential head-on RT collisions, meaning that genome organization of *G. lamblia* does not show a co-orientation bias of replication-transcription. Finally, Gene ontology (GO) analysis of genes at sites of HoRT collisions revealed a significant enrichment for GO terms: microtubule-based processes, cell cycle regulation and DNA replication/repair. Most of these genes are key to important cellular processes as cell division, transport of cellular cargo, organelle positioning, and cell migration. Altogether, our combined analyzes provided a whole-genome map of replication dynamics in *Giardia lamblia.*
Supported by:FAPESP and CNPq **Keywords:** Giardia;binucleated cells;DNA replication dynamics.

CO-09 - **Cell cycle control by ApiAP2 transcription factors in the apicomplexan parasite *Toxoplasma gondii*.**

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All apicomplexan parasites have complex life cycles exhibiting division characterized by a tightly regulated cell cycle control and resulting in the emergence of a various number of daughter parasites. They have evolved efficient and distinctive strategies for intracellular replication where the timing of emergence of the daughter cells, a process termed “budding”, is a decisive element. However, the molecular mechanisms that provide the proper timing of parasite budding remain unknown. Using *Toxoplasma gondii* as a model Apicomplexan, we identified a master regulator that controls the budding process. We show that an ApiAP2 transcription factor, TgAP2IX-5, regulates cell cycle events downstream of centrosome duplication including organelle division and segregation. TgAP2IX-5 binds to the promoter of hundreds of genes and controls the activation of the budding specific cell cycle expression program. TgAP2IX-5 also controls the expression of other ApiAP2 transcription factors, including one that participates in the repression of the developmental switch toward the latent form of the parasite. We are currently investigating the role of these other ApiAP2 transcription factors in controlling the following steps of the cell cycle. **Keywords:** Toxoplasma;cell cycle;apicomplexa.

CO-10 - **10-Year Follow-up study of *Plasmodium vivax* DBPII immune response in the Brazilian Amazon**

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The development of vaccines against *Plasmodium vivax* may be critical in the success of a research agenda to underpin malaria elimination. *Plasmodium vivax* blood stage vaccine strategies have concentrated on region II of Duffy Binding Protein (DBPII) because for reticulocyte invasion most *P.vivax* isolates depends of the interaction between DBPII and Duffy receptor for chemokine (Duffy/DARC).Although DBPII is a major vaccine candidate, we provided evidence that the DBP immune response is strain specific and short lived. While DBPII is weakly immunogenic and induces strain specific immunity, few individuals develop a strain-transcending immune response (“elite responders), indicating that DBPII includes non-polymorphic and less immunogenic epitopes that can be targets for a more broadly inhibitory antibody response. To by-pass DBPII polymorphisms, we have engineered a novel synthetic DBPII-based vaccine (DEKnull-2), which was highly recognized by native Amazonian populations, including the elite responders. Here, we are presenting results of a long-term follow-up to study to characterize the profile of antibody and cellular responses to DEKnull-2 focusing on antigen-specific memory B cells. We are confident that results obtained here may contribute to further improvement of DBPII vaccines that can induce long-term protective immune response. **Supported by:**CNPq, FAPEMIG, NIH-RO1
Keywords: MALARIA;PLASMODIUM VIVAX;VACCINE .

CO-08 - **Iron Metabolism at the Leishmania-Host Interface**

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The parasitic protozoa of the Leishmania genus are the agents of the neglected tropical diseases denominated leishmaniases that affect millions of humans around the world, with clinical manifestations ranging from disfiguring cutaneous lesions to visceral disease and death. Therapy remains limited due to toxicity of drugs, poor implementation, and drug resistance. After transmission to a mammalian host by a sand fly vector, intracellular replication within macrophages is critical for leishmaniases pathogenesis. This process requires the acquisition of essential nutrients from the host, such as iron and heme, as Leishmania does not have cytosolic iron storage proteins and lacks the capacity for heme synthesis. Furthermore, both free iron and heme are cytotoxic due to generation of reactive radicals. Leishmania must therefore acquire host heme and iron for survival inside a host environment that restricts availability of these nutrients to the pathogen – a process termed nutritional immunity. Thus, successful colonization requires the pathogen to circumvent
host iron and heme-related defense mechanisms. The study of genes related to iron trafficking in these parasites revealed that the availability of iron plays a central role in the generation of infective parasites. Iron deprivation modulates the expression of several non-characterized genes, providing an excellent opportunity for the identification of additional components of the molecular machinery responsible for iron acquisition, storage, and metabolism in these parasites. So, investigating how these parasites regulate their cytosolic iron concentration to prevent toxicity, we identified and characterized the Leishmania Iron Regulator 1 (LIR1), an iron responsive MFS-type plasma membrane protein with similarity to plant nodulin-like proteins. Consistent with a role in iron efflux, LIR1 deficiency increases parasite sensitivity to iron toxicity and abolishes parasite infectivity. Besides, preliminary data indicate the role of LIR1 in manganese transport and arginase activity. Further analysis of the previously published transcriptome profile of L. amazonensis genes modulated
by iron deprivation is being conducted for identification and characterization of new genes involved in the transport and metabolism of iron, and potentially other transition metals. From this analysis, we identified two genes with predicted glycosomal targeting signals that are currently being studied for elucidation of the mechanisms involved in the metabolism and transport of iron to Leishmania glycosomes. Glycosomes are unique organelles that constitute one of the main differences between parasite and host. These organelles compartmentalize enzymes that use iron as a cofactor, however, the transport of iron into the glycosomes has not yet been characterized. These studies are central for understanding the physiology of these parasites and should reveal novel targets for leishmaniasis therapeutic intervention. **Keywords:** Heme;ion transport;transition metals.

CC-**Endosymbiosis in Trypanosomatids and Cell Evolution: Mutual Benefits of Living Together**

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During the last decades, knowledge about the cell biology of trypanosomatids has mostly derived from research on parasitic diseases. Here I will discuss the body of information that emerged from studies of the cell biology of non-pathogenic protozoa, with a particular focus on the metabolic and structural adaptations that symbiotic bacteria and monoxenic trypanosomatids underwent during their coevolution. The study of the cooperative interactions between primitive organisms is essential to understand the mechanisms underlying the increased complexity and diversification of eukaryotic cells. Starting with basic facts, there are currently 20 well-characterized genera in protozoa, of which only 5 include species that are pathogenic to mammals and plants. This implies that monoxenic protozoa, which commonly inhabit in a single host throughout their life cycle, usually insects, comprise the vast majority of currently known trypanosomatid species. Importantly, there are 7 species, belonging to different genera, containing a single symbiotic bacterium in their cytoplasm. One of the central themes of evolutionary biology is to understand how symbiosis contributed to the origin of the organelles of eukaryotic cells. Endosymbiosis in trypanosomatids is a classic example of a mutualistic relationship, since neither the bacterium and the protozoan can live separately. As shown by our group, this coevolutionary process was shaped by morphological and biochemical changes that ensured a harmonious relationship between the bacterium and the trypanosomatid host. Remarkably, the intense metabolic exchange that takes place between both partners has turned the trypanosomatid host into an undemanding cell from a nutritional point of view. In a recent study, we obtained evidences that the intracellular bacterium optimizes the energy metabolism of the trypanosomatid host while inducing changes in the structural arrangement of the mitochondrial DNA contained in the kinetoplast (kDNA), as well as in the cytoskeleton of microtubules. Another example of the mutual benefits of “living together” is the precise synchronization between the division of the symbiont with that of trypanosomatid structures, so that each new protozoan generated always contains a single bacterium. Using the RNAi and CRISPR-Cas9 systems to manipulate gene expression of key trypanosomatid proteins, we recently found that the coordination of cellular and symbiont division is achieved at expense of increased dynamism of microtubules. Now enriched by the full-length sequencing of the genome of both partners, the field of endosymbiosis in trypanosomatids stands itself as an essential branch of research in evolutionary cell biology.
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