SPC-001 - **Genome studies of Trypanosoma cruzi: what have we learned about the parasite biology and host-parasite interactions**

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Trypanosoma cruzi, the causative agent of Chagas disease, is a protozoan pathogen that multiplies in the lumen of the insect midgut and within the cytoplasm of different types of mammalian cells. As a vector-borne disease, the parasite is disseminated among hundreds of mammalian species, besides humans, and within different species of insects belonging to the Triatominae family. To adapt to such wide variety of cellular environments including an obligate intracellular stage in the mammalian host, T. cruzi has developed sophisticated regulatory mechanisms to rapid change its repertoire of about 12,000 genes. Being an early divergent eukaryote, its genome is organized in polycistronic transcription units, which implies that gene expression regulation relies on post-transcriptional mechanisms mainly exerted by RNA binding proteins (RBPs). Together with a ratter limited number of research groups, our laboratory has been studying the T. cruzi genome and the molecular factors involved with gene expression and regulation. I will discuss the progress towards unraveling the T. cruzi biology as well as the mechanisms involved in host-parasite interactions, which have occurred thanks to parasite genome sequencing and studies involving gene manipulation approaches. I will also discuss more recent studies that begin to unveil molecular mechanisms used by the parasite to change its molecular composition, to subvert host defense mechanisms and manipulate host cellular programs so that it can benefit of living in several distinct environments. Besides characterizing genes encoding virulence factors, our studies have been focused on regulatory RBPs involved with changes in gene expression that are associated with parasite differentiation and the expression of virulence genes. These studies provide the framework necessary for the identification of new targets and the development of new strategies thar may result in more efficient methods to prevent and control a disease that still poses a significant threat to public health worldwide.
Keywords: Trypanosoma cruzi.genome.gene expression

CO-001 - **Defining the composition of Transitional Fibres in *Trypanosoma brucei***

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Transitional fibres are appendage structures surrounding the distal end of the basal body at the proximal end of the flagellum in *Trypanosoma brucei*. They are involved in connecting the flagellum to the flagellar pocket in Trypanosomes and there are analogous structures in mammalian centrioles – called distal appendages. There is very little understanding of the importance of these structures in Trypanosomes. The genome-wide protein localisation study, TrypTag, has been used to identify a cohort of proteins that localise to the basal body region of *Trypanosoma brucei*. Each protein has been endogenously tagged with an mNeonGreen fluorescent protein and co-localised in a cell line expressing SAS-6::mScarlet – a known basal body marker. A total of 35 putative transitional fibre proteins have so far been identified. Extracted cytoskeletons have been used in conjunction with automated image analysis to rapidly measure the dimensions of transitional fibre signals in thousands of cells and expansion microscopy was used to clearly identify the location on the transitional fibres. RNAi analysis of this cohort reveals the importance of proteins at the transitional fibres for flagellum morphogenesis.

CO-003 - **Inflammasomes, Leishmania and Autophagy: manipulation of host signalling pathways by Leishmania RNA Virus 1**

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Parasites of Leishmania genus have developed various strategies to overcome host immune response favoring its infection and development toward leishmaniasis. With an array of virulence factors, those parasites modify host macrophage signaling and functions. Depending on the species involved, visceral or cutaneous leishmaniasis will develop. Species such as Leishmania guyanensis and Leishmania braziliensis can be naturally infected with the endosymbiotic virus Leishmania RNA Virus 1. The presence of this virus was found to cause a particularly aggressive form of South-American mucocutaneous leishmaniasis. Data to be presented will report how the virus-containing parasites modulate innate immune sensors and signalling pathways including TLRs, TRIF, Type I IFN, autophagy and NLRP3 inflammasome networks that explain in part the exacerbated skin pathology caused by this particular parasite.
Supported by:FAPESP and CNPq **Keywords:** Leishmania.Inflammasome.LRV; Leishmania RNA Virus

CO-004 - **Combining transgenesis with paratransgenesis to fight malaria**

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Malaria is among the deadliest infectious diseases and Plasmodium, the causative agent, needs to complete a complex development cycle in its vector mosquito for transmission to occur. Two promising strategies to curb transmission are **transgenesis**, consisting of genetically engineering mosquitoes to express anti-malarial effector molecules and **paratransgenesis**, consisting of introducing into the mosquito, commensal bacteria engineered to express anti-malarial effector molecules. Whereas both approaches restrict parasite development in the mosquito, it is not known how their effectiveness compares. Here we provide an in-depth assessment of transgenesis and paratransgenesis and evaluate the combination of the two approaches. Using the Q-system to drive gene expression, we engineered mosquitoes to produce and secrete two effectors – scorpine and the MP2 peptide – into the mosquito gut and salivary glands. We also engineered Serratia, a bacterium capable to spread through mosquito populations, to secrete the same two effectors into the mosquito gut. Human parasite *Plasmodium falciparum* oocyst and sporozoite intensity and mosquito prevalence were strongly reduced by expression of the anti-malaria effectors. Mosquito fitness, as measured by longevity, fertility, fecundity, and blood uptake, was not affected by the genetic modifications. Critically, substantially stronger reduction of P. falciparum development was achieved when transgenesis and paratransgenesis were combined. Most importantly, transmission of *P. berghei* from infected to naïve mice was strongly reduced. The combination of transgenesis and paratransgenesis promises to become a powerful tool to combat malaria. **Supported by:**AI127405 from the National Institute of Allergy and Infectious Diseases (NIAID) **Keywords:** Transgenesis.paratransgenesis.malaria transmission

CO-005 - **The arginine sensing pathway in *Leishmania***

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Parasitic protozoa of the genus *Leishmania* are obligatory intracellular parasites that cause human leishmaniasis. During their life, *Leishmania* cycle between the acidic phagolysosome of mammalian macrophages, where they reside as round amastigotes, and the mildly alkaline mid-gut of female sand flies, living as elongated promastigotes. A few years ago, my laboratory discovered that depletion of arginine from growth media induces *Leishmania* promastigotes and amastigotes to rapidly up-regulate expression and activity of the arginine transporter, AAP3. This arginine deprivation response (ADR) is also activated in intracellular amastigotes following macrophage infection and involves a mitogen activated protein kinase 2 (MPK2)-mediated signaling cascade. The *Leishmania* genome contains two identical copies of *AAP3* genes (AAP3.1 and 3.2), but only *AAP3.2* is responsive to arginine deprivation. Deletion of the *AAP3.2* locus, yielded mutants, which retain a basal level of arginine transport (from AAP3.1) that is not responsive to arginine deprivation. These mutants were avirulent to both macrophages and mice. Interestingly, ADR activity during infection influence the expression of SLC38A9, the host lysosome arginine sensor. Silencing the *SLC38A9* gene attenuated ADR in intracellular parasites and increased macrophage susceptibility to infection. My talk will provide an update on the ADR pathway and the crosstalk between host and parasite arginine sensing machinery.

CO-006 - **Disruption of Kharon and Trypanin genes and upgrade of the conditional knockout based on CRE-lox system in *Trypanosoma cruzi*.**

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Essential genes for parasite survival in mammalian host are key targets for pathogen attenuation or as target drug development. Kharon has been identified in other trypanosomatids as a cytoskeletal protein that acts on trafficking of some flagellar proteins and also crucial for parasite viability in mammalian stages (bloodstream in *T. brucei* and amastigotes in Leishmania). Trypanin, which was initially described as T Lymphocyte Triggering Factor (TLTF), functions as part of the dynein regulatory complex and it is lethal in the bloodstream form trypanosomes as shown by Trypanin silencing through RNAi. It is difficult to study gene essentiality in mammalian stages due to the lack of conditional knockout tools in *T. cruzi*. In this talk, it is going to describe our advances on developing an efficient CRE-lox system, a site-specific recombinase technology, widely used to generate conditional gene activation, deletions, inversions, insertions, translocation, and other genetic modifications. The strategy we have developed enabled the control of gene expression in tissue culture trypomastigotes, which are more difficult to transfect than epimastigotes. Towards the functional characterization of Kharon and Trypanin from *T. cruzi*, we have disrupted these genes using the CRISPR-Cas9 system and analyzed the phenotypic changes. Our results show that Kharon and Trypanin mutants behave differently from their counterparts in *T. brucei* or *Leishmania*. **Supported by:**Fundação Araucaria, UFPR, CNPq, and CAPES

CO-007 - **Advances on the impact of multiple blood meals on Leishmania inside the sand fly**

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Disease vectors transmit pathogens as they blood feed, and most vectors take multiple blood meals during their lifetime. A subsequent uninfected blood intake results in a remarkable effect to the Leishmania parasite inside the sand flies. Within 24h after blood feeding by a sand fly carrying a mature parasite infection, the metacyclic promastigotes, previously considered a terminally differentiated infectious stage, dedifferentiate to a leptomonad-like stage: the retroleptomonad promastigote. RNAseq analysis of retroleptomonads showed a distinct transcript expression profile to the other paraite promastigote stages inside of sand flies, validating it as a bona fide developmental form. Also, in the absence of a second blood meal, the small numnber of Leishmania parasites naturally acquired by feeding on an infected host are mostly lost before they develop into the infectious stage. This shows how crucial a second blood meal is and how important is the rise of retroleptomonads to increase the vectorial capacity of sand flies. These data reveal a novel and fundamental role for multiple blood meals in establishing the pathogen, and most importantly, in enhancing infectivity of the insect vector. These findings also place blood sources from other animals where infected sand flies would feed as a critical element in in the transmission of vector-borne pathogens. **Keywords:** Leishmania.Multiple Blood Meals.Vector competence

CC-001 - **From São Paulo to Bahia: a journey into the world of leishmaniasis, a neglected tropical disease**

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Leishmaniasis is a neglected tropical disease, linked to economically disadvantaged populations in tropical regions. It is a diverse human disease caused by many species of *Leishmania* parasites, which are transmitted by the bite of an infected sand fly. The clinical manifestation of leishmaniasis depends upon the species of the parasite and ranges from physical disfigurement to death if left untreated. The high prevalence of this disease is directly linked to the triad host–vector-parasite: *Leishmania* parasites manipulate the vertebrate immune system and exploits sand fly vector component, all favoring establishment of disease. Our laboratory has been involved in studying this interface, looking into the vertebrate immune response to sand fly salivary components and into the pathogenesis of Cutaneous Leishmaniasis, caused by the particularly vicious *Leishmania braziliensis*. I will present our findings in these areas, ranging from works conducted in experimental models to works in the field and how, in our opinion, we can move forward towards understanding the complexity of this relationship **Keywords:** Host-pathogen.sand fly saliva.ulcer