SPC - **TRYPANOSOMA CRUZI: A MODEL SYSTEM FOR CELL DIFFERENTIATION AND GENE EXPRESSION REGULATION STUDIES**

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Since the pioneer work of Carlos Chagas in 1909, it was described that *Trypanosoma cruzi* presented distinct morphological and functional types, indicating that cell differentiation occurs during the life-cycle of the parasite. The stages alternation between infective and replicative forms in the vertebrate and in the invertebrate host makes *T.cruzi* a very interesting model system for studying cell differentiation and differential gene expression. However the initial challenge was how to obtain *in vitro* the amounts of parasites required for biochemical studies and how to reproduce the differentiation processes observed *in vivo*. The development of axenic culture conditions for the production of epimastigotes was an important breakthrough and the subsequent development of differentiation protocols allowed important progresses in *T.cruzi* and Chagas disease research. Due to the possibility of obtaining large amounts of epimastigotes, several studies were focused on the transformation of epimastigotes into metacyclic trypomastigotes. This led to the development of a very efficient chemically defined medium based on the ionic composition of triatomine urine (Contreras et al., 1985, MBP., 16:315) and supplemented with a few amino acids (TAU3AAG medium) (Bonaldo et. al. 1988, JCB 106:1349), that allows obtaining bona fide metacyclic trypomastigotes. The availability of reasonable amounts of distinct developmental stages of the parasite allowed a better biochemical characterization of *T.cruzi* and the isolation of stage specific genes. More recently, with the remarkable progresses in genomic science leading to the sequencing of *T.cruzi* genome and the development of functional genomic tools, new perspectives were opened for our understating of the mechanisms involved in gene expression regulation and consequently stage-specific gene expression. It is well-established that post-transcriptional mechanisms play a major role in the regulation of gene expression in trypanosomes. Recent findings indicate that several mechanisms modulating *T.cruzi* gene expression occur at the cytoplasmic level and involve the interaction of specific RNA binding proteins (RBP) with mRNAs. These messenger ribonucleoprotein (mRNP) complexes can be found either associated to polysomes and actively translated or maintained in the cytoplasm as RNA granules for either storage or degradation, depending on the associated proteins. Interestingly, our data indicate that mRNAs encoding proteins related in function are associated to specific sets of RBPs providing support to the existence of post-transcriptional operons (regulons) in T.cruzi.

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CO.01 - **IMMUNOLOGICAL CONTROL OF PHOSPHATIDYLSERINE EXPOSURE BY AMASTIGOTES OF LEISHMANIA (L) AMAZONENSIS**

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Leishmania amazonensis amastigotes make use of surface-exposed phosphatidylserine (PS) molecules to promote infection and alternative activation of macrophages (MΦs), leading to uncontrolled intracellular proliferation of the parasites. This mechanism was quoted as apoptotic mimicry. Now we provide evidence that the increased PS exposure observed on amastigotes from BALB/c mice lesion is due to the generation of a non-polarized MΦ stimulation by cytokines produced by CD4+ T cells. The presence of very low concentrations of NO as a result of iNOS activation is sensed by intracellular amastigotes, triggering PS exposure, while simultaneous arginase I expression and polyamine synthesis are necessary for maintenance of parasite viability and persistence in the host. In order to assess if apoptotic mimicry also operates in a clinical setting we analyzed PS exposure and its consequences, in amastigotes derived from isolates obtained from patients with localized cutaneous leishmaniasis (LCL) and diffuse cutaneous leishmaniasis (DCL) due to infection with Leishmania (L) amazonensis.
Diffuse cutaneous leishmaniasis (DCL) is a rare clinical manifestation of leishmaniasis, characterized by an inefficient cell-mediated immunity to parasite antigens, leading to uncontrolled parasite growth. Amastigotes collected from F1(Balb/c x C57Bl/6) MΦs infected with promastigotes from DCL patients presented a consistently higher PS exposure at 24 h after infection than those derived from patients with LCL. Furthermore, results showed that DCL isolates, which contained a higher PS exposure on their surface, were derived from patients with the higher number of lesions and duration of the disease. These data suggest that, in human infections, parasites that are capable of exposing higher amounts of PS tended to induce a more severe and persistent disease.
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CO.02 - **HOST REMODELING BY MALARIA PARASITES**

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Malaria parasites extensively remodel their host erythrocytes. Our interests focus on mechanisms of remodeling and their implications for disease. Current work focuses on dynamics of parasite invasion ligands that target host components and mediate signaling during invasion. We are also investigating invasion ligands that contribute to disease pathologies of anemia and cerebral malaria in model animal hosts and host inflammatory mechanisms engaged in disease processes. Finally we have a long standing interest in how a secretome of hundreds of effector proteins of the human malaria parasite *Plasmodium falciparum* constitute a 'secretome', carrying a host-targeting (HT) signal that can be exported from the intracellular pathogen into the surrounding erythrocyte. Cleavage of the HT signal by a parasite endoplasmic reticulum (ER) protease, plasmepsin V, has recently been proposed to be the export mechanism. Data will be presented that the primary host-targeting mechanism is rather a novel interaction with lipids in the ER that precedes cleavage and plasmepsin V can be dispensed with, in export. This provides the first description of novel lipid domains within the ER, with functions in secretion and pathogenesis.

CO.03 - **SELENOCYSTEINE SYNTHESIS IN PROTOZOA: A PECULIAR METABOLISM AND ITS IMPLICATIONS**

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Selenium (Se) is an essential element mainly found in selenoproteins as the 21st amino acid (Selenocysteine – Sec – U). The selenoproteins generally participate in the cellular redox state balance, playing an important role on cell growth and proliferation. These proteins, as well as the components of the Sec synthesis pathway, are present in members of the Bacteria, Archaea and Eukaryote domains. Although not a ubiquitous pathway in all organisms, it was also identified in several protozoa, including the Kinetoplastida and *Naegleria*. By RNA interference of the *T. brucei* selenophosphate synthetase *SPS2*, we found a requirement under sub-optimal growth conditions involved in oxidative stress protection of the parasite and its absence severely hampers the parasite survival in the presence of an oxidizing environment. This finding is sugestive of the Sec synthesis pathway enzymes as an interesting target for metabolic investigation. A candidate compound that inhibits the action of selenoproteins revealed effective against *Trypanosoma cruzi* cells in vitro and in vivo and is been tested in *Leishmania* cells as well. The publication of *Naegleria gruberi* (ATCC 30224) genome allowed us to investigate the presence of the Sec-incorporation pathway in this “primitive” eukaryote. Using a thorough bioinformatics approach; we identified various genes involved in Sec-incorporation machinery. Among these genes, it was identified a homolog of Selenophosphase synthase (SelD or SPS). The structure of the selenocysteine pathway and the comparative investigations of this methabolic pathway in different protozoa will be discussed.

CO.04 - **REGULATION OF GENE EXPRESSION IN AFRICAN TRYPANOSOMES**

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The African trypanosome Trypanosoma brucei multiplies extracellularly in the bloodstream of the infected host, where it is protected by a dense Variant Surface Glycoprotein (VSG) coat. An individual trypanosome has more than 1500 VSG genes, of which one is expressed at a time from one of about 15 telomeric VSG expression site (ES) transcription units. For antigenic variation to work, it is key that all VSGs except one are kept transcriptionally silent. There is very little transcriptional control in African trypanosomes, and the bulk of the T. brucei genome is arranged as extensive polycistronic transcription units constitutively transcribed by RNA polymerase II. We are investigating the molecular machinery behind VSG silencing, whereby a stringent control operates on the active VSG ES. Chromatin structure was not originally thought to play a significant role in ES regulation in bloodstream form T. brucei. However our lab and the Cross lab (Rockefeller University) have recently shown that this is not correct, and that the active ES is highly depleted of nucleosomes. In addition, we and others have recently identified a growing number of chromatin proteins or chromatin remodeling complexes which play critical roles in ES control. These include the chromatin remodeler TbISWI, the FACT complex, the nucleoplasmin-like protein NLP and histone H1. I will discuss our recent experimental data investigating how VSG expression site control could be operating.