SPC1 - **EXPLORING THE CONTENT OF NONCODING RNAS, PUTATIVE REGULATORY ELEMENTS, IN THE PROTOZOAN PARASITE *LEISHMANIA***

CRUZ, A.K.[*1]
1.USP-FMRP, RIBEIRAO PRETO, SP, BRASIL.
e-mail:akcruz@fmrp.usp.br

Understanding how the protozoan parasite *Leishmania* survives in the extremely diverse and hostile environments of the insect gut and vertebrate macrophages is a challenge for molecular parasitologists. *Leishmania* genetic organization is crucial for the parasite success, and this organization has several peculiarities. Leishmania genes are arranged as long polycistronic units, and mature mRNAs are produced from the primary transcripts by trans-splicing and polyadenylation. There are no canonical promoters for mRNA transcription, and modulation of gene expression happens at the post-transcriptional level through a combination of distinct mechanisms. Our laboratory is focused on understanding some of the layers at which regulation of gene expression occurs in Leishmania. Serendipitously, studying a group of short unannotated and polyadenylated transcripts from Leishmania major, we identified and partially characterized one of them arising from the 3'UTR of one of the two copies of a ribosomal protein gene (RPS16). This transcript, named ODD3, occurs as an independent ~150 nucleotide-long transcript and preliminary results suggest it interacts with proteins involved in elongation of transcription. In addition, we are currently studying possible regulatory differences for each copy of the duplicated RPS16 ribosomal protein coding gene driven by the 3'UTR differences. Non-protein-coding RNAs (ncRNAs) have been shown to act in a diversity of molecular processes, as demonstrated in different eukaryotes, but are poorly explored in trypanosomatids as putative regulatory elements. Therefore, we extended the search for similar UTR-derived or uaRNAs to *Leishmania donovani* exploring the non-polysomal ribonucleoprotein fraction. Subsequently, we conducted an in-depth study on the modulation of gene expression across the life cycle stages of *Leishmania braziliensis* covering coding and noncoding RNAs (ncRNAs). Analyses of differentially expressed (DE) genes revealed that most prominent differences were observed between the transcriptomes of insect and mammalian proliferative forms (6,576 genes). A computational pipeline and different ncRNA predictors allowed the identification of 11,372 putative ncRNAs. Most of the DE ncRNAs were found between the transcriptomes of insect and mammalian proliferative stages (38%). Of the DE ncRNAs, 295 were DE in all three stages and displayed a wide range of lengths, chromosomal distributions, and locations; many of them had a distinct expression profile compared to that of their protein-coding neighbors. The presence of 22 of the predicted transcripts of similar length was confirmed by northern blotting analysis. Knockout (KO) and tagging of 5 transcripts were obtained using CRISPR/Cas9 editing machinery, and the parasites' phenotypes are under analysis. Modification of the macrophage in vitro infection profile was witnessed for one of the evaluated ncRNA KO, and pulldown assays were conducted to identify ncRNA binding proteins. Some of the novel putative ncRNAs revealed in L. braziliensis might be regulatory elements to be further investigated.
Supported by:FAPESP (2013/50219-9), CNPq and CAPES

CO1 - **REVERSED IMMUNOGLYCOMICS FOR IDENTIFICATION AND SYNTHESIS OF α-GAL BIOMARKERS AND VACCINES FOR CHAGAS DISEASE**

Susana Portillo [1,*], Igor E. Silva [1,*], Uriel Rodriguez-Ortega [1,*], Alba Montoya [2,*], Brenda G. Zepeda [1], Eva A. Iniguez [1], Roger A. Ashmus [2], Jerry A. Duran [1], Nathaniel S. Schocker [2], Janet J. Olivas [1], Nasim H. Karimi [1], Alexandre F. Marques [1,3], Julio Alonso-Padilla [4], Luis Izquierdo [4], Maria-Jesús Pinazo [4], Maria Tays Mendes [1], Cameron C. Ellis [1], Claudia Manriquez Roman [1], Jane F. VandeBerg [5], Otacilio C. Moreira [6], Laura-Isobel McCall [7], Belkisyolé Alarcón de Noya [8], Oscar Noya [8], John L. VandeBerg [5], Rosa A. Maldonado [1], Faustino Torrico [9], Joaquim Gascón [5], Katja Michael [2], and Igor C. Almeida [1,¶]

[1] Department of Biological Sciences, University of Texas at El Paso, El Paso, TX, U.S.A.;
[2] Department of Chemistry and Biochemistry, University of Texas at El Paso, El Paso, TX, U.S.A.;
[3] Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil;
[4] Barcelona Centre for International Health Research (ISGlobal), Barcelona, Spain;
[5] University of Texas Rio Grande Valley, Edinburg, TX, U.S.A.;
[6] Laboratório de Biologia Molecular e Doenças Endêmicas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil;
[7] University of Oklahoma, Norman, OK, U.S.A.;
[8] Instituto de Medicina Tropical, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela;
[9] Fundación CEADES and Universidad Mayor de San Simón, Faculty of Medicine, Cochabamba, Bolivia;
e-mail:icalmeida@utep.edu

Chagas disease (CD), caused by Trypanosoma cruzi, affects 6-7 million people in Latin America. The great majority of those infected with T. cruzi are asymptomatic; however, 20-30% will develop cardiac and/or gastrointestinal complications, leading to death or permanent disability. Infected individuals can now be found in many other parts of the world, including the U.S.A., Europe, and Japan, due to intense globalized migration in the last several years. Currently, there are two drugs available to treat CD, benznidazole and nifurtimox, which are primarily used in endemic countries in Latin America. While both drugs are highly effective in the acute stage of the infection, they are less effective for the treatment of chronic stage. Moreover, chemotherapy with these drugs is also linked with serious adverse events, which may lead to the discontinuation of the treatment in 10-20% of the patients. Furthermore, negative seroconversion using conventional serology following chemotherapy takes 10-20 years to occur, which is a poor outcome to support an extensive treatment program of chronic CD patients. Due to these and other reasons, it is estimated that less than 1% of chronic CD patients currently undergo chemotherapy. Therefore, dearth of dependable biomarkers (BMKs) for assessment of therapeutic efficacy following chemotherapy is a major challenge in translational CD. Other major challenges include the lack of effective epidemiological and insect vector control, accurate diagnosis (particularly in nonendemic areas like the U.S., and endemic areas like Mexico), and a prophylactic or therapeutic vaccine.

T. cruzi infective trypomastigote forms have a surface heavily coated with highly immunogenic glycosylphosphatidylinositol (GPI)-anchored glycoproteins such as tGPI-mucins (or TcMUCII mucins), mucin-associated proteins (MASP), and trans-sialidases (TS). tGPI-mucins contain immunodominant α-Gal glycotopes, such as Galα(1,3)Galα(1,4)GlcNAcα (Galα3LN) and several branched ones, which induce high levels of protective, lytic anti-α-Gal antibodies that control parasitemia in both acute and chronic phases of CD. Recently, we have demonstrated that a synthetic neoglycoprotein (NGP) containing Gaα3LN induces 100% survival following parasite challenge in α1,3-galactosyltransferase-knockout (α1,3-GalT-KO) mouse model, which mimics the human response against α-Gal glycotopes that are absent in humans and Old-World nonhuman primates. These α-Gal glycotopes are also highly conserved throughout *T. cruzi* genotypes and field strains, since patients with chronic CD from very distinct endemic and nonendemic regions universally produce high levels of *T. cruzi*-specific anti-α-Gal antibodies. Here, using the reversed immunoglycomic approach, we have identified, synthesized, and evaluated the efficacy of several α-Gal-based neoglycoproteins (α-Gal-NGPs) as potential biomarkers for early assessment of chemotherapy outcomes in chronic CD in various preclinical and clinical studies. We also assessed these synthetic α-Gal-NGPs as vaccine candidates in both α1,3-GalT-KO mouse and nonhuman primate models of CD. Taken together, our data strongly indicate that α-Gal-NGPs are very promising BMKs not only for early evaluation of chemotherapeutic outcomes but also as potential prophylactic vaccine candidates. **Supported by:**National Institutes of Health, and Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation **Keywords:** Chagas disease; glycobiology; biomarkers

CO2 - **THE BENEFIT OF BEING DIFFERENT: UNDERSTANDING CELL-TO-CELL HETEROGENEITY IN PATHOGENS**

SIEGEL, N.[*1]
1.UNIVERSITÄT MÜNCHEN, MUNICH, ALEMANHA.
e-mail:n.siegel@lmu.de

Driven by enormous technological advances that permit the study of individual cells, an overwhelming revelation of modern-day biomedical research is the overarching heterogeneity that includes all realms of biology. Single-cell transcriptomics have led to the characterization of specific cell types within complex tissues, as well as to the discovery of new cell states. Yet, cellular heterogeneity is not restricted to multicellular organisms.
Cellular heterogeneity is a major driver in the adaptation of pathogens to ensure successful infections. Viruses, bacteria, and protozoan parasites all face similar challenges when infecting a susceptible host, including the evasion of the host immune response. This common challenge has led to the evolution of remarkably similar survival strategies even among evolutionarily distant pathogens. One of these strategies is antigenic variation. Antigenic variation refers to the capacity of an infecting organism to systematically alter the identity of proteins displayed to the host immune system making it difficult or impossible for the host to eliminate the pathogen. Successful antigenic variation relies on tight expression of antigens and frequent recombination among antigen genes. Both homologous recombination and gene expression are affected by 3D genome architecture and local DNA accessibility. However, processes that link local chromatin conformation, 3D genome architecture and antigenic variation are not well understood.
We are interested in mechanisms that control cell-to-cell heterogeneity in pathogens and how pathogens benefit from such heterogeneity. To this end we study how differences in genome sequence and chromatin organization influence the expression of genes in different unicellular eukaryotes. One key question of our research is how pathogens regulate antigenic variation at the genomic and transcriptional level. As a model we use the protozoan parasite *Trypanosoma brucei*, the causative agent of African sleeping sickness. Employing different genome-wide approaches such as Hi-C to study 3D genome organization, ATAC-seq to study local chromatin compaction, and single-cell RNA-seq to study antigen expression, we have recently identified histone variants as a molecular link between global genome architecture, local chromatin conformation and antigenic variation.
Supported by:ERC and DFG **Keywords:** Trypanosoma brucei; chromatin; antigenic variation

CO3 - **DRUG DISCOVERY FOR NEGLECTED TROPICAL DISEASES**

ANDRICOPULO, A.D.[*1]
1.USP-IFSC, SAO CARLOS, SP, BRASIL.
e-mail:aandrico@ifsc.usp.br

Scientific and technological breakthroughs have compelled the current players in drug discovery to increasingly incorporate knowledge-based methods. Structure-based drug design (SBDD) and ligand-based drug design (LBDD) approaches have been successfully used to identify hits and optimize ligands into drug-like compounds, providing groundbreaking drugs for many therapeutic areas, including highly complex conditions and diseases that were previously considered a death sentence. Neglected tropical diseases are a diverse group of infectious conditions prevalent in tropical and subtropical areas, and affect more than one billion people worldwide, disproportionately among the poorest populations and those living in remote and rural areas, almost exclusively in the developing world. Although these diseases are responsible for a considerable global burden, the development of therapeutic products is disproportionately low.
To that end, we have developed forefront research on highly prevalent and life-threatening neglected tropical diseases. This conference will provide a perspective on the central aspects of medicinal chemistry and drug discovery for Chagas disease and leishmaniasis, exploring the fundamentals and applications of SBBD and LBDD.
Keywords: Drug discovery; chagas disease; leishmaniasis

CO4 - **A LIGHT IN THE DARK: USING REAL-TIME IMAGING TO DEFINE THE *IN VIVO* INFECTION DYNAMICS OF *TRYPANOSOMA CRUZI*.**

LEWIS, M.D.*[1]
1.LSHTM, LONDON, REINO UNIDO.
e-mail:michael.lewis@lshtm.ac.uk

Efforts to understand the pathogenesis of Chagas disease and to develop new treatments have been hindered by insufficiently tractable animal models of *T. cruzi* infection. We developed transgenic parasites expressing a codon-optimised, red-shifted luciferase transgene, which enabled us to track their *in vivo* infection dynamics with unprecedented sensitivity and accuracy, in combination with minimal sampling bias. Acute infections present subtle variations depending on transmission route and parasite form, but typically lead to the same characteristic dynamic equilibrium between host and parasite in the chronic phase. *Ex vivo* bioluminescence imaging can be used to rapidly quantify tissue-specific parasite loads. This has led to the discovery that the gastrointestinal tract and the skin are the primary sites of long-term parasite persistence, whereas other organs, such as the heart, appear subject to episodic cycles of parasite (re-)invasion and clearance. By coupling infection imaging with histopathological analyses and comparing combinations of different mouse and *T. cruzi* strains, we have found that host and parasite genetics shape the severity of pathology in both cardiac and digestive forms of Chagas disease. Studies using dual bioluminescent-fluorescent reporter parasites are now enabling us to visualise host-parasite interactions at the cellular scale, particularly in studies of GI tract infections. Overall, our real-time imaging studies lead to the conclusion that the complex quantitative, spatial and temporal dynamics of *T. cruzi* infections are central to understanding the pathogenesis of Chagas disease and to optimisation of treatment strategies.

Keywords: Chagas disease; imaging; host-parasite interaction

CO5 - **TOWARDS THE UNDERSTANDING OF BENZNIDAZOLE RESISTANCE IN *TRYPANOSOMA CRUZI***

TRIANA, O.*[1]; MEJÍA JARAMILLO, A.M.[1]
1.UDEA, MEDELLIN, COLÔMBIA.
e-mail:omar.triana@udea.edu.co

The improvement of Chagas disease treatment is focused not only on the development of new drugs but also in understanding mechanisms of action and resistance to drugs conventionally used. Although several studies aimed at understanding drug resistance in T. cruzi have been carried out in recent years, so far there are only a few genes known to confer drug resistance. The most important of these include the mitochondrial NADH -dependent type-I nitroreductase (NTR I), which play critical role in the activation pathways of both drugs, leading to the formation of glyoxal (GO), a highly reactive metabolite, which generates guanosine-glyoxal adducts. Despite these discoveries, the mechanism of action of this drug continues to be poorly understood. Global analysis of the differential expression of genes between sensitive and resistant parasites, coupled with functional genomics experiments, is a key strategy for identifying the genes that participate in the mechanisms of action and resistance to Bz in T. cruzi.

In order to identify differentially expressed transcripts between sensitive and resistant parasites, a massive pyrosequencing of the T. cruzi transcriptome was carried out. We used a natural T. cruzi population resistant to benznidazole, which has clones with different susceptibilities to this drug without alterations in the NTR I gene. Additionally, susceptible parasites submitted to selective pressure with different benznidazole concentrations until obtain resistant parasites were used. Moreover, the 2D gel electrophoresis profile of sensitive and resistant parasites was analyzed and the data were supported with functional genomics. The results showed 133 differentially expressed genes in resistant parasites. The transcriptome and proteome analyses revealed the regulation of different genes with several functions and metabolic pathways, which could suggest that resistance in T. cruzi is a multigenic process. Interestingly, we found that two T. cruzi proteins previously described to be related to Bz resistance, the aldo-keto reductase (TcAKR) and alcohol dehydrogenase (TcADH), were overexpressed in the resistant clones. The results showed that the

overexpression of these proteins enhances resistance to benznidazole and glyoxal in T. cruzi. Moreover, a decrease in mitochondrial and cell membrane damage was observed, accompanied by a drop in the intracellular concentration of reactive oxygen species after treatment. Our results suggest that these proteins are involved in the mechanism of action of benznidazole. Moreover, APRT, SOD, PUF3, PUF6, TcOYE, and MPX were also regulated between both groups of parasites. The role of these genes is broadly discussed. Intriguingly, alterations in the NTR I gene were not found in Bz natural resistant parasites isolated from humans, suggesting the participation of other genes in this process.
This study, together with other genomic research, offers a good approach to identify new genes participating in resistance to Bz and the mode of action of this drug. Finally, the whole transcriptome analysis, coupled with proteome and functional genomic analyses, could help identify and demonstrate the roles of certain genes involved in T. cruzi resistance to Bz.
Keywords: Trypanosoma cruzi; benznidazole; drug resistance

CO6 - **WHY IS UNDERSTANDING THE DIVERSITY AND PHYLOGENY OF PARASITES THAT CAUSE LEISHMANIASIS IMPORTANT AND NOT JUST AN ACADEMIC EXERCISE?**

SHAW, J.[*1]
1.USP, SAO PAULO, SP, BRASIL.
e-mail:jayusp@hotmail.com

Progressive understanding of diversity depends on the interpretation of results generated by new technologies. Different clinical forms of leishmaniasis were recognized for hundreds of years but it was the microscope that showed they were caused by small round intracellular parasites. As people looked more closely at their morphology it became evident that they were not all the same and that some behaved differently in mammals and insects. But there were limits to the usefulness of these methods for differentiating parasites. This became evident as more parasites were isolated. Studies using biochemical and molecular techniques in the 1970s heralded an exciting new era in our understating of the amazing diversity of parasites commonly known as leishmania. It also opened up the possibility of using this data to study their phylogeny. Advances in phylogenetic analysis raised an important question – Is it justiciable to use of the name Leishmania for parasites isolated from mammals or sand flies that grow as promastigotes in culture? An array of molecular genetic markers confirm that some parasites originally named as Leishmania are better accommodated within a 3-genus. These parasites belong to the monophyletic subfamily Leishmaniinae. Phylogenetic studies using multigene datasets show that the isolation of ancestral groups at different times led to the present-day distribution in which some are universal, while others are restricted to a continent. Subsequently there has been secondary expansions in which some old-world parasites have migrated and evolved to the Americas. An appreciation of Leishmaniinae diversity and phylogeny is fundamental to a better understanding of leishmaniasis in man. It is clearly no longer acceptable to extrapolate results obtained with one species to another for the development of new treatments, diagnostic methods and control. On the other hand, phylogenetic information allows us to extrapolate potentially useful similarities. **Supported by:**CNPq **Keywords:** Diversity; phylogeny; leshmaniinae

CO7 - **TRYPANOSOMES CAN INITIATE MRNA EXPORT CO-TRANSCRIPTIONALLY**

GOOS, C.[*1]; DEJUNG, M.[2]; WEHMAN, A.M.[1]; M-NATUS, E.[1]; SCHMIDT, J.[1]; SUNTER, J.[3]; ENGSTLER, M.[1]; BUTTER, F.[2]; KRAMER, S.[1]

1.UNIVERSITY OF WÜRZBURG, WÜRZBURG, ALEMANHA; 2.IMB, MAINZ, ALEMANHA; 3.OXFORD BROOKES UNIVERSITY, OXFORD, GRA-BRETANHA.

e-mail:susanne.kramer@uni-wuerzburg.de

Trypanosomes are among the most ancient eukaryotes and had a billion years to evolve their own, very special biology. This includes their unusual genome architecture with epigenetically defined promoters that thrive polycistronic transcription. These polycistrons are subsequently processed into mature mRNAs by trans-splicing. As transcription is largely uncontrolled for the majority of genes, gene regulation is almost entirely posttranscriptional. In the Kramer lab, we are interested in understanding the regulation of gene expression in trypanosomes, focusing on spatial aspects of mRNA metabolism.

We have recently visualized nuclear export of mRNAs in trypanosomes by intra- and intermolecular multi-colour single molecule FISH. We found that the initiation of nuclear export requires neither the completion of transcription nor splicing and can occur co-transcriptionally. Nevertheless, we show that unspliced mRNAs are mostly prevented from reaching the nucleus-distant cytoplasm and instead accumulate at the nuclear periphery in cytoplasmic nuclear periphery granules (NPGs). Further characterization of NPGs by electron microscopy and proteomics revealed that the granules are located at the cytoplasmic site of the nuclear pores and contain most cytoplasmic RNA-binding proteins but none of the major translation initiation factors. Our data are consistent with NPGs being the 5' ends of polycistronic mRNAs stuck in the nuclear pore by slowed or inhibited nuclear export, perhaps by a yet unknown quality control system that recognises export-incompetent mRNA regions at the basket site of the pore.

Our data indicate that trypanosomes regulate the completion of nuclear export, rather than the initiation. This is in striking contrast to the regulation of mRNA export in opisthokonts that mainly occurs at the initiation stage. **Supported by:**DFG

Keywords: Trypanosoma brucei; mrna export; mrna fish

CO8 - **IMMUNE MECHANISMS DRIVING ANEMIA IN *PLASMODIUM VIVAX* INFECTIONS: UNDERSTANDING THE DESTRUCTION OF NON-INFECTED ERYTHROCYTES**

BRAGA, E.M.[*1]

1.UFMG, BELO HORIZONTE, MG, BRASIL.

e-mail:embraga@icb.ufmg.br

Plasmodium vivax is the most widely distributed species that cause malaria in humans, accounting for approximately 75% of the cases registered in the Americas in 2017. One of the major complications of *P. vivax* infection is anemia, which is thought to arise from the destruction of both infected and non-infected RBCs (nRBCs). The study of potential immune mechanisms involved in clearance of nRBCs during *P. vivax* infections has been an ongoing research topic of our group for the last years. One element of the adaptive immunity that may take part in the n RBC destruction is the presence of autoantibodies, molecules induced to autologous components of RBCs. In this direction, we have shown anti-RBC IgG and IgM are both increased in anemic patients with acute vivax malaria and both antibodies are able to decrease the deformability of nRBCs, but only IgG can induce in vitro erythrophagocytosis. Such effects are enhanced in type O erythrocytes, suggesting that individuals from this blood group infected with *P. vivax* malaria may be more susceptible to develop anemia. We have also demonstrated that band 3 and spectrin are the main targets of autoantibodies elicited during vivax infection. Moreover, by in silico analysis we have identified *P. vivax* proteins that share homology with human RBC proteins such as spectrin, suggesting that infection drives autoimmune responses. These findings offer potential avenues to a better understanding of the immunopathological mechanisms involved in the destruction of nRBCs, leading to anemia in *P. vivax* infections. **Supported by:**CNPq and Fapemig

Keywords: Autoantibodies; erythrophagocytosis; band 3

CO9 - **SCREENING FOR ANTIPARASITIC LEADS FROM A LIBRARY OF NATURAL PRODUCTS FROM TEMPERATE ZONE PLANTS**

HORROCKS, P.[*1]
1.KEELE UNIVERSITY, STAFFORDSHIRE, REINO UNIDO.
e-mail:p.d.horrocks@keele.ac.uk

There is an urgent need to identify and evaluate novel chemical scaffolds to seed the drug discovery pipeline for parasitic diseases. Complementing international efforts to explore the potential of huge commercial chemical libraries, the search for new leads also encompasses the evaluation of natural products. PhytoQuest, a UK-based Industrial Biotechnology small to medium-sized enterprise, has produced a library of approximately 1000 molecules, isolated predominantly from temperate zone plants. As such, this library represents a unique resource for lead discovery of high value chemicals from temperate zone plants against parasitic diseases, with previous studies focusing largely on plants from tropical and subtropical zones. The library comprises a wide range of chemical classes, two thirds of which are novel, and the remaining third not commercially available. Critically, the compounds are pure, overcoming common issues with screening fractions of complex mixes, and have been selected to reflect potential development, with a high degree of functionality and physiochemical properties that match Lipinski's Rule of Five. A subset of approximately 650 compounds have been screened against the intraerythrocytic stages of Plasmodium falciparum and axenic amastigotes of Leishmania mexicana, with a further screen against Trypanosoma brucei now underway. Here I report a characterization of our hits against P. falciparum and L. mexicana.
Supported by:BBSRC (HVCFP) **Keywords:** Drug discovery; natural products; screening

CO10 - **BROMODOMAINS: NEW TARGET FOR SERCHING TRYPANOCITIC DRUGS**

SERRA, E.C.[*1]; PEZZA, A.[1]; RAMALLO, A.[2]; GARCIA, P.[2]; TAVERNELLI, L.[1]; DOCAMPO, R.[3]; ESCALANTE, A.[2]; FURLAN, R.[2]; ALONSO, V.[1]
1.INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE ROSARIO, UNR-CONICET, ROSARIO, ARGENTINA; 2.FACULTAD DE CIENCIAS BIOQUÍMICAS Y FARMACÉUTICAS, UNIVERSIDAD NACIONAL DE ROSARIO., ROSARIO, ARGENTINA; 3.DEPARTMENT OF CELLULAR BIOLOGY, UNIVERSITY OF GEORGIA, ATHENS., GEORGIA, USA.
e-mail:eserra@fbioyf.unr.edu.ar

The discovery of new therapeutic options against Trypanosoma cruzi, the causative agent of Chagas disease stands as a fundamental need. Drugs currently available, benznidazole and nifurtimox, have significant toxic side effects and their efficacy against the life-threatening symptomatic chronic stage of the disease is under discussion.
Bromodomains are protein modules that bind to acetylated lysine residues. Proteins containing bromodomains are often found as components of larger protein complexes with roles in fundamental cellular processes including transcription and cell cycle regulation, among others.
Trypanosoma cruzi has seven genes that code for bromodomain-containing factors (TcBDF1-7). Six of them are considered essential after CRISPR/Cas9 knock out assays. Among them, we have centered our work on TcBDF2 and TcBDF3. TcBDF2 is nuclear, binds to acetylated histones and has preference for H4 acetylated in lysine 10 and 14. In contrast, TcBDF3 is cytoplasmic and binds acetylated alpha tubulin. The overexpression of dominant negative mutants of both proteins inhibits parasite growth, confirming their essentiality. To validate bromodomains as targets, we assayed different already known bromodomain inhibitors against epimastigotes. Two compounds, GSK-iBET151 and JQ1(+), showed trypanocidal activity. The binding of both compounds to TcBDF2 and TcBDF3 was assayed in vitro by quenching of intrinsic fluorescence and thermal shift assays. Using a fluorescence quenching assay adapted to a 96-well fluorimeter, we screened a collection of chemically engineered plant extracts and a dynamic combinatorial library, against TcBDF3. By these approaches we identified two new compounds that bind to TcBDF3 and show trypanocidal activity. These compounds are being used as leaders to search for better inhibitory activities.
Keywords: Trypanosoma; bromdomains; trypanocide

CC1 - ***LEISHMANIA*** **AND SAND FLY: UNRAVELLING A COMPLEX RELATIONSHIP**

TRAUB-CSEKO, Y.M.[*1]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:ytraub@ioc.fiocruz.br

Leishmaniasis is a serious worldwide problem and is considered an emerging disease. There has been a significant increase in the number of human cases of visceral leishmaniasis in recent years, which in Brazil is almost always caused by Leishmania infantum chagasi, transmitted by Lutzomyia longipalpis. Disease control mechanisms are faulty in both vector control and treatment, and there is no effective vaccine. There is therefore a demand for the development of new approaches for the control of this disease. The control of insect-borne diseases depends largely on our ability to control the vector, or to interfere with parasite-vector interactions. There are limitations on the use of traditional vector control methods, such as insecticide resistance, costs, etc., which could be overcome by new approaches. For that, it is necessary to know the mechanisms involved in the parasite-vector interplay. Our laboratory has been involved in the study of many molecular aspects of this interaction, largely unknown until the recent past. Here I will present some of our findings, from basic aspects of the vector's immune responses to the parasite or the identification of molecules expressed by the parasite inside the insect, to our more recent interest in the development of a transmission blocking vaccine. **Keywords:** Leishmaniasis; lutzomyia longipalpis; vector-parasite interaction