MC1 - ARGININE DEPRIVATION INDUCES AN MAPK2-MEDIATED SIGNALING PATHWAY IN *LEISHMANIA*

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Arginine is an essential amino acid for Leishmania, but not for its mammalian hosts. Parasites import arginine via a mono-specific amino acid transporter (LdAAP3) and direct it primarily into the polyamine pathway to provide precursors for trypanothione biosynthesis. For both promastigotes and amastigotes, arginine depletion of the growth medium induces a rapid up-regulation of LdAAP3 expression and activity; along with concomitant increase of mRNA and protein levels for a small group of other genes (encoding mostly metabolic enzymes and RNA binding proteins). Proteomic analyses show that ~40% of the phosphopeptides that increased in abundance 5-15 minutes after arginine depletion contain a serine-proline (SP) motif, suggesting activation of a protein kinase (PK) pathway involving MAPK2 and MAPK10, which have this substrate specificity. Our preliminary studies also indicate that an external sensor activates the pathway via an on/off mechanism. Significantly, this arginine availability pathway is activated during macrophage invasion, suggesting that it plays an important role in adaptation of Leishmania amastigotes to intracellular growth. We hypothesize that sensing arginine availability plays a critical role in Leishmania virulence by activating a rapid metabolic reaction in response to the lower arginine concentration of the macrophage phagolysosome. This allows the invading amastigote to further deplete the macrophage arginine pool, thereby suppressing host production of cytotoxic nitric oxide, and to increase production of a key parasite anti-oxidant (trypanothione).

MC2 - GENOMICS AS A TOOL TO STUDY CELL-SURFACE VARIABILITY IN TRYPANOSOMA CRUZI

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The ability of Trypanosoma cruzi to survive in the mammalian host is in part due to the presence of a diverse surface membrane coat. In fact, a remarkable feature of the T. cruzi genome is the massive expansion of genes that encode polymorphic surface proteins, which include the trans-sialidase and sialidase-like superfamily (TcS), mucin-associated surface protein (MASP), TcMUC mucins, among others. These T. cruzi gene families are often clustered into large haploid and heterogeneous arrays that are enriched by retroelements. These regions, which contain elements not found in the Trypanosoma brucei and Leishmania genomes, encode key players in the T. cruzi-host interaction. Several members of these families are known to be involved in cell invasion and intracellular survival and/or are targets of both innate and adaptive immune response. These genomic regions are also highly polymorphic in the two haplotypes of the CL Brener hybrid strain, suggesting that they are subject to intense rearrangement. To gain insights into the level of polymorphism of these gene families, we performed sequence clustering analysis, and detected distinct patterns of diversity, suggesting that different evolutionary mechanisms have shaped the evolution of these families. Our data suggest that MASP is the most polymorphic T. cruzi multigene family, followed by TcS and TcMUC. TcS members form robust subgroups with polymorphic 3' flanking regions, whereas MASP and TcMUC coding regions display a continuous gradient of diversity and their 3' flanking regions are highly conserved. The possible involvement of recombination events in the generation of this pattern of diversity will be discussed. Functional studies on the MASP family will also be presented. We speculate that the large repertoire of MASP sequences exposed at the trypomastigote surface may contribute to the ability of T. cruzi to infect several host cell types and/or participate in host immune evasion mechanisms. We have investigated the expression profile of MASP members during in vivo and in vitro T. cruzi infections. Monoclonal antibodies raised against B5 and H5 peptides, each one present in a single MASP member and known to be antigenic, were generated and used in immunofluorescence assays. Approximately

30% of the parasites were labeled, indicating that the expression of a MASP member containing one of these peptides is limited to a subset of the population. These antibodies were also used to carry out the enrichment of the parasite population expressing the B5 or H5 peptide. The enrichment effect on the infectivity profile in vivo was evaluated by infection of C57BL/6 mice with WT trypomastigotes and the population enriched for each one of the MASP variants. Preliminary results suggest alterations in the parasitemia in mice infected with enriched trypomastigote population compared with the WT group. We have also performed B-cell epitope prediction on MASP proteins and found that several members are differentially recognized by sera from acutely infected mice. We speculate that variations in the large repertoire of potentially antigenic peptides derived from MASP family may favor the parasite escape the immune response during the acute phase of infection. **Supported by:**CNPq, FAPEMIG, INCTV, CAPES

MC3 - 15 YEARS OF MEDICAL ACTION IN CHAGAS: PREVENTION, DIAGNOSIS, TREATMENT AND CURE

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More than a decade ago, MSF decided to break the silence surrounding this disease and attend to the neglected populations. Providing treatment is possible, even in the most isolated areas. The majority of the estimated 8 to 10 million people afflicted with Chagas disease are poor and live in rural Latin America. The triatomine bug that transmits the parasite Trypanosoma cruzi that causes the disease thrives in the adobei housing in which the majority of patients live. Chagas disease is often known as an “invisible disease”, as most patients do not present symptoms. It often becomes known only when people die suddenly of the consequences of the disease, the conditions to which Chagas has left them vulnerable. In recent years, as migration and travel to other parts of the world have increased, people in Europe, North America and Asia have also been diagnosed. But they are a very small minority of all cases, and the primary profile of a Chagas patient remains the same. Patient access to health care for diagnosis and treatment is extremely limited in rural areas of disease-endemic countries. There are several factors that exacerbate this situation: many doctors and nurses are not aware of what Chagas disease is, or that it can be treated; health posts in rural settings lack the necessary diagnostic tools and treatments; and the disease is often asymptomatic for many years. More than a decade ago, MSF decided to break the silence surrounding this disease and attend to the neglected populations. Since 1999, MSF has screened more than 90,000 people and treated more than 8,000 patients. By adapting models of intervention to the context, raising awareness of the disease and building capacity within MoH health systems, MSF has shown that providing treatment is possible, even in the most isolated areas. According to the World Health Organization (WHO), diagnosing Chagas disease during the chronic phase involves performing two serological conventional tests that detect circulating IgG antibodies (immunoglobulin). These include Enzyme Linked Immunosorbent Assay (ELISA), Indirect Immunofluorescence Assay (IFA) or indirect hemagglutination (IHA) methods. Such laboratory tests require qualified staff, as well as specific equipment and infrastructure. These are either unaffordable or unavailable in many settings impacted by Chagas disease, meaning that there is not enough diagnostic capacity at present to enable timely treatment. Currently, there are several rapid diagnosis tests (RDTs) available for detecting T. cruzi antibodies in whole blood, serum or plasma. The tests are qualitative or semi-quantitative and rely on different principles—immunochromatography, particle agglutination, immunofiltration or immunodot—and all deliver results in 15 to 30 minutes without the need for electrical equipment. In its 15 years of experience in various programs of prevention, diagnosis and treatment of Chagas disease in resource limited settings (including within existing primary health care systems), MSF has collected a sizable store of information that highlights the safety for the antiparasitic treatment of Chagas disease using benznidazole. It is important to consider that of the more than 8,000 patients treated with benznidazole as first-line therapy in our projects, there were no deaths, and only 1% of patients had serious adverse effects. This is clear evidence that doctors and nurses should not hesitate to treat patients for fear of the side effects caused by these medications if they do a proper follow up of the course of treatment. These findings were supported by several scientific publications that showed that the antiparasitic treatment of adults presenting with no clinical signs of disease is indeed viable. The second drug available is nifurtimox which remains a second-line treatment option since safety, specifically in adult patients remains a big concern.

MC4 - ENDOSYMBIOSIS IN TRYPANOSOMATIDS: AN ETERNAL LOVE AFFAIR

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One subject that presents a great interest in Sciences is the understanding of how primitive microorganisms, the non-nucleated prokaryotes, evolved to give rise to more complex and compartmentalized cells, the eukaryotes. The study of two or more different species that live together in a close symbiotic relationship, gives support to better answer this question, especially if both partners maintain a long-term association. Trypanosomatids are protozoa that have been extensively studied because they can cause diseases to men, animal and plants. In contrast, monoxenic species, which predominate in this family, present a single invertebrate host during all its life cycle. Six species of insect trypanosomatids bear an obligate intracellular bacterium in their cytoplasm that co-evolves with them in a mutualist relationship, thus representing an excellent model to study cell evolution and the origin of organelles. Such species are grouped in two genera Angomonas and Strigomonas and the symbiont from different protozoan species share identity and are derived from an ancestral of a β-Proteobacterium that belongs to the Alcaligenaceae family. The symbiont is free in the host cytoplasm and its envelope is composed by two membranes and a reduced peptidoglycan layer, which facilitates intense metabolic exchanges between the host cell and the symbiotic bacterium. Biochemical and genomic studies revealed that the endosymbiont contains enzymes that complete essential metabolic pathways of the host protozoan, such as the urea cycle, for aminoacid production and routes for vitamin biosynthesis. The presence of the prokaryote influence lipid and energetic metabolism of the trypanosomatid and also modifies its surface charge and carbohydrate composition. Live together assumes concessions, thus ultrastructural alterations are observed in the host protozoan, which presents a reduced paraflagellar structure and a typical array of the kinetoplast DNA network. The synchrony in cellular division is another striking feature of this symbiotic relationship. The bacterium divides in coordination with other host cell structures, thus each daughter cell carries only one prokaryote. The symbiont depends on protein factors produced by the trypanosomatid to divide and the coordination of the bacterium division varies according to the host species, being related to the cell cycle phases.
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MC5 - THE INTERSECTION OF PARASITE AND HOST METABOLISM IN THE *TRYPANOSOMA CRUZI* INTRACELLULAR GROWTH CYCLE.

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The intracellular amastigote stage of Trypanosoma cruzi is a critical target for the development of vaccines and therapeutic interventions aimed at combatting human Chagas' disease. Despite their relevance in infection and disease, knowledge of the biology of intracellular *T. cruzi* amastigotes is limited. With a view to defining the metabolic dependencies of intracellular *T. cruzi* amastigotes, we have applied genome-scale approaches to: (a) follow the dynamic remodeling of the *T. cruzi* transcriptome during the establishment and maintenance of intracellular infection and (b) to identify host susceptibility factors that facilitate the intracellular *T. cruzi* infection process. Transcriptomic analysis of an intracellular infection time course in human fibroblasts reveals that the transition from invasive extracellular *T. cruzi* trypomastigotes to replication-competent amastigotes is accompanied by a major shift in parasite gene expression. Metabolic functions including nucleic acid and lipid biosynthesis are increased in replicating amastigotes as are amino acid and fatty acid catabolism, TCA cycle function and mitochondrial ATP synthase genes indicative of active respiration in intracellular amastigotes. Metabolic predictions for trypomastigotes and amastigotes were validated by conducting extracellular flux measurements on live parasites. RNA interference screening in mammalian cells reveals a critical role for host metabolic networks centered around energy production, nucleotide metabolism and lipid metabolism as central processes supporting intracellular *T. cruzi* growth in mammalian host cells. Current studies are exploring the dynamics between host fatty acid metabolism and intracellular amastigote growth and survival using induced pluripotent stem cell derived human cardiomyocytes as a tool.

MC6 - TRYPANOSOMATID KINOMES; NEW APPROACHES FOR TARGET VALIDATION

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There are many genetic tools available for *Leishmania*, which have aided in the understanding of unique aspects of the molecular and cellular biology of these organisms. The “toolkit” encompasses the ability to carry out transient and stable transfection with high efficiency, the availability of episomal and integrating expression vectors, the use of up to 8 positive selectable markers and crucially the ability to carry out gene knockouts via homologous recombination. The analysis of essential genes, however, has proved challenging as the tetracycline on/off inducible expression system, whilst established, suffers from leaky expression such that it has not been widely adopted. RNA interference (RNAi) has proved to be a useful tool for investigating essential genes in *Trypanosoma brucei*; however the RNAi pathway has been lost in many *Leishmania* sp. We have addressed these limitations by developing an inducible gene knock-out system based on dimerised Cre recombinase (diCre) in *L. mexicana*. This method allows the excision of a gene flanked by LoxP sites (“floxed”) following induction of Cre recombinase activity through rapamycin mediated dimerisation. The method has been validated by targeting a “floxed” gene encoding CRK3, a cdc-2 related kinase necessary for cell cycle progression. Induction of diCre and subsequent excision of the loxP flanked *CRK3* gene results in a dramatic cell cycle arrest phenotype and severe morphological defects. These data show that diCre mediated knockout of *L. mexicana* genes is feasible, thereby representing an important advance in the analysis of essential genes, with impact on future drug target validation studies. The application of this technology to the characterisation of *Leishmania* protein kinases will be discussed, in comparison with a kinome-wide RNAi screen in *T. brucei*.
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MC7 - DEEP THROAT: THE CYTOSTOME COMPLEX OF *TRYPANOSOMA CRUZI*

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In *Trypanosoma cruzi*, the cytostome-cytopharinx complex is the main site of endocytosis. It is present in the proliferative forms, epimastigotes and amastigotes, but disappear when they differentiate to trypomastigotes. We used advanced electron microscopy techniques to reconstruct the entire cytostome-cytopharinx complex, including the surrounding cytoskeleton and vesicles. Focusing on epimastigotes that had taken up gold-labeled tracers, we produced 3D snapshots of the process of endocytosis. The cytoskeleton was composed of two microtubule sets: a triplet that started underneath the cytostome membrane and a quartet that originated underneath the flagellar pocket membrane and followed the preoral ridge before reaching the cytopharinx. The two sets accompanied the cytopharinx forming a `gutter` and leaving a microtubule-free side, where vesicles were found associated. Cargo was unevenly distributed along the lumen of the cytopharinx, forming clusters in regions of enlarged diameter. The cytostome-cytopharinx complex undergone remodeling along epimastigotes proliferative cycle and along the differentiation steps, metacyclogenesis and amastigotes to trypomastigotes. Curiously, in all cases the membrane of the complex disassembled before the microtubules, suggesting that it is a highly dynamic membrane domain.
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MC8 - GENOME-WIDE CO-ORDINATION OF DNA REPLICATION INITIATION IN KINETOPLASTID PARASITES

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DNA replication is central to the propagation of life and initiates by the designation of genome sequences as origins, sites where DNA synthesis begins. Eukaryotic linear chromosomes are replicated from potentially thousands of origins, which are designated by binding of the Origin Recognition Complex (ORC) to defined genome sites. In Trypanosoma brucei, we have mapped replication origins in the nuclear genome through next generation sequencing, comparing read depth in DNA from S and G2 phase cells. This approach revealed ~40 origins, a remarkably small number for a eukaryote, all of which flank the multigene transcription units in the core of the genome. Moreover, we find that the T. brucei origins represent only a fraction of the mapped binding sites for one ORC factor (ORC1/CDC6), and that some origins initiate replication more efficiently than others. These data suggest that origin redundancy and a temporal order of firing are found in T. brucei, phenomena in common with replication initiation in other eukaryotes but poorly understood. In common with most eukaryotes, we can find no consensus sequence for ORC1/CDC6 binding or origins. To try and understand replication further, we have mapped origins in the genomes of Leishmania major and L. mexicana, both of which display considerable gene syntenty with T. brucei, but also notable structural differences: chromosome numbers and size are different between the parasites, as is ploidy stability. We find a very similar number of total origins in both Leishmania species compared with T. brucei, and many localise to syntenic regions, suggesting conservation of origin location. However, origin usage is strikingly different: we find only a single origin in each of the Leishmania chromosomes, and each origin appears to fire with equal efficiency. Moreover, in Leishmania we are able to identify features that are specific to origin-active loci, distinct from T. brucei. We believe that analysis of replication in kinetoplastids reveals adaptations that reflect the different genome architectures and provides insight into the evolution of multiple origins for genome replication of eukaryotes, including the machinery that is used to co-ordinate the firing of multiple origins.

MC9 - IMPROVED T. CRUZI COMPARATIVE GENOME SEQUENCING REVEALS MECHANISMS OF GENETIC EXCHANGE AND THE FULL SURFACE ANTIGEN REPERTOIRE

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The protozoan parasite Trypanosoma cruzi is the causative agent of Chagas disease, a devastating neglected disease that affects more than 10 million people, mainly in Latin America but it is spreading to other parts of the world. As a part of a large project to investigate T. cruzi strain diversity and epidemiology, we have performed comparative whole genome sequencing of several T. cruzi strains. These include the first sequence of a strain, Sylvio X10/1, from the TcI clade, which is the dominating clade north of the Amazon, and a clone of the bat-specific subspecies T. cruzi marinkellei. I will here present the genome sequences of several TcIV strains from Venezuela and Brazil that have revealed the hybrid nature of this clade. While TcIV originates from a hybrid formation event, most of the allelic polymorphism has been eliminated over time. The origin of each gene, as well as the polymorphism pattern provides insights into the processes that control genetic exchange in this parasite. I will, in addition, present expanded comparisons of TcI strain genomes from multiple geographic locations, in order to explore the extensive genome-wide variation within TcI. As a part of this project, an improved TcI reference genome has been produced using long read single molecule sequencing. The resulting assembly has shown that we are able to resolve complex repetitive regions for the first time and clarify the complete chromosome structure of the parasite. I will discuss how to utilize these data for the study of surface molecule genes and their importance for immune evasion and pathogenesis.

MC10 - PLASMODIUM INVASION OF HOST CELLS: AN UPDATE

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Plasmodium, like many other parasites of the Apicomplexan phylum, is an obligatory intracellular parasite that needs to invade host cells to multiply. The parasite invades host cells by inducing the close apposition of the plasma membranes of the parasite and host cell in a ring-like fashion, called tight junction (TJ). The parasite then slides through the circular TJ using its own actin-myosin motor into an invagination of the host cell surface that becomes the parasitophorous vacuole, where it can multiply. The TJ is thought to constitute a bridge between the cortical cytoskeletons in the two cells and to act as a traction point for the motor. Although the TJ was first described more than 35 years ago, its molecular composition remains elusive. In the last ten years, different experimental approaches have led to two contradictory models of the composition of the TJ. In this talk, I will present and confront the two models and discuss the approaches that might be undertaken to help resolve the issue.