MINI-CONFERENCES

[October, 2008-10-28 – 11h30 - RUBI ROOM]

MC01 - A role for DHH1 in regulating gene expression in *Trypanosoma brucei*

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Gene expression in trypanosomes is largely regulated post-transcriptionally. Differential expression of individual genes in various life cycle stages is achieved by regulation of mRNA half-life and translation. For example, *GPI-PLC* is expressed in bloodstream but not procyclic forms and is regulated by a ≥10 fold difference in mRNA half life. The RNA helicase *DHH1* was identified in an RNAi screen for gene necessary for complete suppression of *GPI-PLC* mRNA in procyclics and also in a screen for cytoplasmic mRNA processing body (P-body) components. The function of *DHH1* was tested by the expression of loss of activity and RNA-binding mutants. Here, the phenotype of a helicase mutant, dhh1 E182Q, containing a mutation in the DEAD box (DEAD->DQAD) is described.

Expression of dhh1 E182Q at levels ~equal to endogenous DHH1 produced a dominant negative phenotype with a rapid arrest of cell proliferation, reduced polysomes and an increase in the size of P-bodies. The mutant protein itself had impaired localization to P-bodies. There was a specific increase in steady state levels of a subset of mRNAs, a microarray experiment identified 78 mRNAs that increased more than two fold. Developmentally regulated mRNAs were more than five fold over-represented in this group, suggesting that control of life cycle stage specific mRNAs is particularly dependent on DHH1.

One developmentally regulated mRNA, *ISG75*, that increased on the expression of dhh1 E182Q was characterized in more detail. The 3.3-fold increase in *ISG75* mRNA was caused by an increase in mRNA half-life and there was a ~5-fold increase in ISG75 protein. The majority of *ISG75* mRNA was present in polysomes both before and after induction of dhh1 E182Q expression. Thus, the increase in *ISG75* mRNA half life on dhh1 E182Q expression cannot be explained by re-location to polysomes. In the simplest model, dhh1 E182Q expression reduces the rate at which *ISG75* mRNA is routed to P-bodies resulting in an increased half life.

[October, 2008-10-28 – 11h30 - RUBI ROOM]

MC02 - Exploring the Tritryp kinome: biology and drug targets

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Protein kinases are critical molecules in regulating intracellular processes, such as the cell cycle, and responses to the environment, such as stress and cell signaling in response to environmental stimuli. They have been the focus of numerous campaigns to develop drugs to combat cancer and other chronic diseases. A number of drugs that target protein kinases have been approved for human use. As a result, large libraries of compounds targeting kinases exist throughout the pharmaceutical research community. Previous studies have identified approximately 150 protein kinases in *Trypanosoma brucei* and somewhat more in *Leishmania* and *Trypanosoma cruzi*, most of which fall into the same groups as those found in the human host. However, dedicated tyrosine kinases appear to be absent and only a handful of protein kinases appear to possess transmembrane domains. The functions of only a few trypanosomatid protein kinases are known, primarily those related to cyclin-dependent kinases and MAP kinases. Based on work in *Leishmania*, which showed specific MAP kinases to be essential, we targeted two of the four MAP kinase kinases for deletion in *T. brucei*. Neither was essential in the bloodstream stage. In contrast, four protein kinases that possess transmembrane domains were examined, and all appeared to play important roles in the pathogenic bloodstream form. Two are localized to the endoplasmic reticulum, one to the flagellar pocket and undulating membrane and one to the lipid monolayer of lipid bodies. Our data indicates that this last protein kinase is required for the formation of these enigmatic organelles and thus it appears to be critical to intracellular lipid homeostasis.

[October, 2008-10-28 – 11h30 - SAFIRA ROOM]

MC03 - *Toxoplasma gondii* infection in humans, a smart parasite versus the immune system.

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Toxoplasma gondii is an obligate intracellular parasite that infects a wide range of warm-blooded vertebrates and causes disease in agricultural animals and humans. Some individuals infected with *Toxoplasma gondii* develop ocular lesions. To study the relationship between the immune response and this parasite, we selected infected individuals with and without ocular lesions and matched non-infected controls, subjects were divided into groups on the basis of presence of serum antibodies to *T. gondii*, presence of ocular lesions, and clinical history. We have shown that the production of interleukin-12 and interferon-□ is associated with protection against the development of ocular lesions. On the hand, patients with congenital disease present a decreased cellular response to toxoplasma antigens, measure by lymphocyte proliferation, delayed-type hypersensitivity and cytokine synthesis. Nevertheless the humoral immune response did not show any differences related to the time of infection. We have also shown that the development of an autoimmune response against retinal antigens, although it is associated with the presence of ocular lesions, it is also indicative of milder lesions. However, differences in the immune response alone cannot account for the percentage of patients that present ocular lesions secondary to *T. gondii* infection in Brazil. Therefore we decided to evaluate the parasite genetics and we have shown that strains causing ocular toxoplasmosis in Southern Brazil are significantly different from those found in patients in the Northern Hemisphere. Furthermore, these genetic differences are reflected in the parasites antigenic makeup, as measured by *T.gondii*-specific antibody repertoire and specific modifications are associated with severe disease. These findings now allow us diagnostic tools that will allow for better follow up of infected individuals.

[October, 2008-10-28 – 12h00 - SAFIRA ROOM]

MC04 - Induction and Maintenance of T cell Memory in Chagas Disease

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CD8+ T cells specific for *Trypanosoma cruzi* are activated slowly after the initial infection but develop into very robust, highly focused contributors to protective immunity. In long-term mouse infections, *T. cruzi* -specific CD8+ T cells are retained primarily as effector memory T cells (Tem) in the blood and lymphoid tissues and become activated to effector function in tissues where parasite persist, presumably as a result of encounter with *T. cruzi*-infected host cells. Despite parasite persistence, a fraction of parasite-specific CD8+ T cells are maintained as central memory T cells (Tcm), based upon their expression of CD127 and CD62L. Curative drug treatment of chronically infected mice results in a shift in phenotype of *T. cruzi*-specific CD8 T cells to a predominantly Tcm phenotype, thus providing a marker for cure in this chronic infection. Unfortunately, monitoring of *T. cruzi* –specific T cell responses in chronic human infections is considerably more difficult than in the mouse but nevertheless can be used as one method to assess treatment efficacy.

[October, 2008-10-28 – 11h30 - ESMERALDA ROOM]

MC05 - RHYTHMS, LOVESONGS AND INSECT VECTORS

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The daily activity rhythms of insect vectors are controlled by the endogenous biological clock. A number of genes involved in the control of circadian rhythms have been isolated in the model species *Drosophila melanogaster*. However, despite the medical importance of hematophagous insects, very little is known about the molecular genetics of their circadian clocks. Previously, we analyzed the activity rhythms and daily expression of clock genes in the sand fly *Lutzomyia longipalpis*

(Diptera: Psychodidae), the main vector of visceral leishmaniasis in Latin America. Currently, we have been studying the molecular control of circadian rhythms in mosquitoes (Diptera: Culicidae). We are comparing the activity rhythms and circadian expression of the main clock genes in *Aedes aegypti*, a diurnal mosquito that is vector of dengue and yellow fever, and in *Culex quinquefasciatus*, a nocturnal mosquito vector of filariasis. Our group is also studying the speciation in insect vectors. There is extensive evidence that *L. longipalpis* is a species complex although the number and distribution of the different siblings in Brazil is still somewhat unclear. Our previous analysis of the copulation songs produced by *L. longipalpis* males and population genetic studies of lovesong genes have provided strong evidence for the existence of at least four sibling species in Brazil. We are currently extending this analysis to a number of other Brazilian populations of this species complex.

[October, 2008-10-28 – 12h00 - ESMERALDA ROOM]

MC06 - Bromeliad tanks: an unexplored habitat proves protist endemism and significantly increases the number of ciliate species

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Bromeliads (Bromeliaceae) are epiphytic and terrestrial rosette plants, possibly related to the Liliidae (lilies). With few exceptions, they occur only in Central and South America. The rosette collects rain water and particulate material, mainly plant litter, in the tanks (cisterns) formed by the coalescing leaf axils. These tanks form an extensive, highly compartmentalized water and humus body above the ground and are inhabited by many ordinary and endemic organisms, ranging from protists to amphibians. However, bromeliad protists are very poorly explored, and my group was the first reporting on endemic ciliates from tank bromeliads (Foissner et al. 2003). Meanwhile, we discovered about 40 new ciliate species in the cisterns of about 50 bromeliad species from the Dominican Republic, Jamaica, Ecuador, Brazil, Venezuela, and Peru. The new species belong to a huge variety of ciliate groups, for instance, haptorids, tetrahymenids, peritrichs, astomates, colpodids, and hypotrichs. New species have been found not only in tank water but also inside and outside of various metazoan inhabitants, such as insect larvae and oligochaetes. Since there are 2,000 – 3,000 bromeliad species with very different lifestyles, they are likely to contain hundreds of novel ciliate species, though a first detailed analysis in Jamaica show that some “typical” bromeliad ciliates occur in bromeliads from various genera. The distribution of species, described and undescribed ones, seem to be is very patchy. Several of the new species found are impressive “flagships”, that is, they have such large size and specific morphology that they would have been found in Europe, if they were there. Consequently, these species must have a restricted geographical distribution, disproving the old hypothesis that microscopic organisms are cosmopolitan. Many of the new species have close relatives in freshwater and semiterrestrial habitats, and some evolved to distinct evolutionary units, i.e., genera and families, arguing for a long-lasting, independent evolution driven by ecological constraints and spatial isolation. Interestingly, the high morphological and ecological diversity of tank bromeliad ciliates is only partially recovered by small-subunit (18S) ribosomal RNA (rRNA) nucleotide sequences, indicating decoupling of morphological and genetic evolution. Almost half of the new species can switch from bacteriophagous, microstome morphs to a predatory, macrostome lifestyle, likely due to the strong competition in these peculiar habitats, especially when the tanks desiccate and space and food become less and less. Thus, there occur macrostomes also in groups traditionally lacking this ability, for instance, in *Platyophrya bromelicola*, a new colpodid ciliate discovered in Jamaican bromeliads. Detailed investigations are needed to answer some of the many questions posed by the bromeliad habitat. Fortunately, we were successful in obtaining a good grant from the Austrian Science Foundation (FWF) to continue our studies on taxonomy and ecology of ciliates from cisterns of bromeliads. (Supported by the Austrian Science Foundation, Project P20360-B17.)

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[October, 2008-10-29 – 11h30 - RUBI ROOM]

MC07 - Chagas disease in the global world: From Central and South America to the develop countries of North America, Australia, and Europe

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INTRODUCTION. Human *T. cruzi* infection is in retreat in most endemic countries of South and Central America thanks to advances in the interruption of vectoral and transfusion transmission through the coordinated action of governments, WHO, and multilateral and bilateral agencies. Economic hardship, political problems, or both, have spurred migration from Chagas endemic countries to developed countries. Therefore, it is important to explore the impact of this migration on the public health of receiving countries.
OBJECTIVE. To estimate the impact of migration on the potential of transmission of human *T. cruzi* infection in non endemic countries
RESULTS. Estimates indicate that in Australia 1,089 of the 65,705 Latin American immigrants (17 per 1000) may have been infected with *T. cruzi,* in 2005-2006. In Canada, 1,218 of the 131,135 immigrants (9 per 1000) whose country of origin was identified may have been also infected in 2001. In Spain, a magnet for Latin American immigrants since the year 2000, 6,141 of 241,866 legal immigrants (25 per 1000), could have been infected in 2003. In the USA, 38,777 to 339,954 of the 7,20 million legal immigrants (8 to 50 per 1000), depending on the scenario, from the period 1981- 2005 may have been infected with *T. cruzi.* On the other hand, 33,193 to 336,097 of the estimated 5,6 million undocumented immigrants (6 to 59 per 1000) could have been infected in 2000.
CONCLUSIONS. Non endemic countries receiving immigrants from the endemic ones should develop policies to protect organ recipients from *T. cruzi* infection, prevent tainting the blood supply with *T. cruzi*, and implement secondary prevention of congenital Chagas disease. Legislation might have to be modified, so that immigrants are not discriminated against at their places of employment due to their infection .

[October, 2008-10-29 – 12h00 - RUBI ROOM]

MC08 - Why and how *Anopheles darlingi* is an efficient malaria vector in the Amazon Region.

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Anopheles darlingi is described in epidemiologic and entomologic studies as the important malaria vector in the Brazilian Amazon Region. In the meantime, studies on the presence of this *Anopheline* species in natural ecosystems and in artificial environments created by human activity show that *An. darlingi* is an extremely rare species in the Amazon forest (Lourenço de Oliveira et al., 1996), but it is always the dominant anopheline species in human communities of malaria endemic areas in the Region. (Deane et al.,1986; Tadei et al., 2000; Gil et al., 2003, 2007). Laboratory studies of *Anopheline* mosquitoes from Rondônia showed a good vector capacity of *An. darlingi* when compared to other anopheline species found as minor populations in malaria endemic areas (Klein et al., 1991). In the majority of field studies comparing outdoor and indoor's anopheline densities it is shown that outdoors' densities are always five to ten times higher than the indoor ones (Tadei et al, 2000; Gil et al, 2003, 2007). These and other observations originated the conviction on the important participation of outdoor malaria's transmission in the Amazon region, that explains the high levels of malaria incidences observed in diversified human habitats and ecosystems structures including rural riverine areas, periphery of cities, degraded invaded forest, agro industrial settlements etc..However, in the same field studies where malaria transmission is evaluated by esporozoite infection rate (EIR), it is always observed a surprising low rate, usually less than 1, in areas with API (annual parasite index) values of 500 to 1000. These figures contrast with those found for *An. gambiae* in African urban and suburban endemic areas of equivalent APIs values showing, for instance, ERI values o 15 to 77 in suburbs of cities (Robert et al., 2003). Theoretical speculation, in this respect raises the question of the absence of co-evolution of human malaria parasites and anopheline vectors in the Amazon Region. In Africa, malaria vectors like *An. gambiae*

and *An. f**unestus* species have participate in a close co-evolutionary complex composed of man, malaria parasites and mosquito vector, all of them locally originated and evolved for millions of years of convergent process. In the American continent, where man arrived relatively recently, *An. darlingi* probably survived establishing co-evolutionary complexes with wild mammals and/or birds. In the present communication are presented data of longitudinal field studies in highly endemic area of vivax and falciparum malaria at the riverine Madeira river area. Malaria cases were registered in one urban and two suburban human communities. Longitudinal survey identified the residence and date of each malaria case. Monthly captures in each locality defined the seasonal *Anopheline* densities' variation and infection rate by malaria parasite correlated to malaria incidence. The results indicate a fundamental role of indoors' transmission and suggest a low vector capacity of *Anopheles darlingi* in this area which needs to be compensated by its extremely high densities to maintain the observed high malaria incidence. Efficiency of *An. darlingi* in malaria transmission in the reverine areas of the Amazon Region seems to be dependent on its capacity to reach high densities promoted by human degradation of natural sites rather than from a putative vector capacity of the local malaria vector in the area. Possible implications of these contradictory observations will be discussed.

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[October, 2008-10-29 – 11h30 - SAFIRA ROOM]

MC09 - The Redox Biochemistry of Peroxynitrite and Peroxiredoxins during macrophage-*T. cruzi* interactions

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Macrophages play a central role in the control of the infection mediated by *Trypanosoma cruzi*. Among other effector mechanisms, free radicals and oxidant species including superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and nitric oxide ($^{\bullet}NO$) constitute relevant cytotoxic mediators, the formation of which relies on enzymatic systems that respond to the interactions of the macrophage with the parasite and/or the presence of cytokines produced by inflammatory cells. In this context, we have described that peroxynitrite anion ($ONOO^-$), the product of the diffusion-controlled reaction between $O_2^{\bullet-}$ y $^{\bullet}NO$, is a key intermediate formed inside the phagosome to generate nitroxidative damage in the parasite and promote its death within the first hours of infection. The biological chemistry of peroxynitrite is rather complex and it participates in biomolecular damage in the target cell by both direct and secondary free radical-dependent processes which mainly result in oxidation and nitration reactions. On the other hand, we have characterized potent enzymatic peroxynitrite detoxifying system in the parasite such as the peroxiredoxins (cytosolic and mitochondrial) which contain critical thiols in the active site that serve to readily react and reduce peroxynitrite to nitrite; sometimes, the catalytic elimination of a significant fraction of macrophage-derived peroxynitrite determines that the parasite resists the oxidative challenge created by the mammalian cell, being able to later escape to the cytosol to proliferate and sustain the infection process. In fact, parasites overexpressing peroxiredoxins cope well with the macrophage cytotoxic response; recently, we have also found that natural and highly virulent strains of *T. cruzi* contain large levels of the various components of the trypanothione-dependent antioxidant enzyme network, including the peroxiredoxins. Altogether, our results indicate that the delicate redox balance established between the oxidative response of the infected macrophage and the endogenous antioxidant systems of the parasite is a critical factor that can decisively influence the control or propagation of the infection.

[October, 2008-10-29 – 12h00 - SAFIRA ROOM]

MC10 - PROTEOMIC ANALYSIS OF ACIDOCALCISOMES of *Trypanosoma cruzi AND T. brucei* AND THE CONTRACTILE VACUOLE of *T. cruzi* REVEALS THE EXPRESION OF NOVEL ENZYMES AND TRANSPORTERS

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Acidocalcisomes have been conserved during evolution from bacteria to man and play essential physiological roles (1) but little is known about their protein composition. Acidocalcisomes resemble lysosome-related organelles (LRO) from mammalian cells in many of their properties. For example, we found that platelet dense granules, which are LROs, are very similar to acidocalcisomes (2). They share a similar size, acidic properties, and both contain pyrophosphate, polyphosphate and calcium. Recent work that indicates that they also share the system for targeting of their membrane proteins through adaptin-3 reinforces this concept. In addition, acidocalcisomes possess several proteins that are potential novel targets for chemotherapy (3). Acidocalcisomes interact with other organelles in protozoan parasites, like the contractile vacuole in *T. cruzi.* The contractile vacuole is an organelle whose presence in *T. cruzi* has been neglected until recently (4). Proteomic analysis of acidocalcisomes of *T. cruzi* and *T. brucei* and the contractile vacuole of *T. cruzi*, identified a number of enzymes and transporters including acidocalcisome markers such as V-H^+-PPase, vacuolar calcium ATPase and aquaporin. A selected set of genes of identified proteins was cloned and the proteins expressed with epitope tags to confirm their localization. We detected amino acid transporters, cation transporters, and homologues of yeast vacuolar transporter chaperones in acidocalcisomes, and a V-H^+-ATPase, SNAREs, and other proteins in the contractile vacuole. Studies with the vacuolar transporter chaperones in *T. brucei* confirmed their acidocalcisome localization, and RNAi experiments revealed a role of these proteins in acidocalcisome biogenesis and cell growth (5). Interestingly, our analysis of *T. cruzi* subcellular proteome revealed the expression of 70 dispersed gene family proteins, a large number of proteins that heretofore has been severely underrepresented in previous proteomic analyses. Of 697 *T. cruzi* proteins sequenced by tandem mass spectrometry, 220 were annotated as hypothetical proteins in the *T. cruzi* genome. BLAST searches of the hypothetical proteins identified $TcIP_3R$, a putative receptor with significant similarity to inositol 1,4,5-trisphosphate and ryanodine receptors. A similar protein ($TbIP_3R$) was detected in the subcellular proteome of *T. brucei.* Two potential inositol 1,4,5-trisphosphate binding sites are present in $TcIP_3R$ as well as a membrane-spanning region characteristic of ion transport proteins. Expression of fragments of $TcIP_3R$ allowed the characterization of its ligand binding characteristics and confirmed its subcellular localization.

Keywords: acidocalcisome, contractile vacuole, *Trypanosoma cruzi, Trypanosoma brucei*

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[October, 2008-10-29 – 11h30 - ESMERALDA ROOM]

MC11 - IRON-SULFUR CLUSTER ASSEMBLY IN PARASITIC PROTISTS

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The formation of iron-sulfur clusters is a novel fundamental function of mitochondria, which is required for maturation of FeS proteins. Studies of organismal diversity showed that a number of "textbook" mitochondrial pathways could be highly modified or absent under specific environmental conditions or at certain developmental stages. In contrast, FeS cluster assembly was suggested to be the only essential mitochondrial function, which is invariably present in all eukaryotes. To test this hypothesis we investigate FeS cluster assembly machinery in parasitic protists from distant eukaryotic supergroups, which contain either mitochondria or mitochondria-like organelles such as hydrogenosomes or mitosomes. Phylogenomic analyses revealed that FeS cluster assembly machinery of mitochondrial type (ISC) is present in all eukaryotes with mitochondria or hydrogenosomes and cell localization studies confirmed its presence within the organelles. Functional studies showed that the mechanisms of FeS cluster assembly is highly conserved from humans (Opisthoconta) to trypanosomes (Excavata). In addition to ISC machinery, alternative SUF machinery was found in organisms with apicoplast. However, in organisms with mitosomes several unique modifications have been found. In microsporidian *Trachipleistophora hominis*, the key ISC component IscU and frataxin were displaced from the organelle to the cytosol. In entamoebids as well as related free-living *Mastigamoeba balamuthi*, the bacterial NIF system was found in the cytosol, while mitochondrial ISC machinery was absent. This survey confirmed that FeS cluster assembly is the essential eukaryotic function, however, the mitochondrial machinery could be exceptionally replaced by the cytosolic systems in anaerobic organisms with highly reduced mitochondria.

[October, 2008-10-29 – 12h00 - ESMERALDA ROOM]

MC12 - Human Resistance to Infection by African Trypanosomes

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African trypanosomes (prototype: *Trypanosoma brucei*) are protozoan flagellates that infect a wide range of different mammals. In humans these parasites have to counteract innate immunity because human serum possesses efficient trypanolytic activity. Resistance to this activity has arisen in two *T. brucei* subspecies, termed *T. b. rhodesiense* and *T. b. gambiense*, allowing them to infect humans where they cause sleeping sickness in East and West Africa respectively. The study of the mechanism by which *T. b. rhodesiense* escapes lysis by human serum led to the identification of the trypanolytic factor, which turned out to be an ionic pore-forming apolipoprotein L1 (ApoL-I) associated with some HDL particles. Humans lacking ApoL-I, due to frameshift mutations in both apoL-I alleles, are susceptible to infection by non-pathogenic trypanosomes, and their serum is devoid of any trypanolytic activity. Recently, we have identified the trypanosomal receptor responsible for the uptake of the ApoL-I-containing HDL particles. The specific ligand to this receptor is the haptoglobin related protein (Hpr), also contained in these HDL lytic particles, bound to haemoglobin. The parasite's glycoprotein receptor, bound to the haptoglobin-hemoglobin complex, mediates the uptake and incorporation of heme into intracellular hemoproteins. In mice, this receptor was required for optimal parasite growth and the resistance of parasites to the oxidative burst by host macrophages.