ROUND-TABLES

[November, 09 - 10:30 - Room A]

RT1A - Tales of three trypanosomatids: global comparative genomics of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*

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TriTryp Sequencing Consortium

The TriTryp sequencing consortium (TIGR, Sanger Institute, Seattle Biomedical Research Institute and Karolinska Institute) has completed the first draft of the three reference trypanosomatid genomes: *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*. This landmark achievement opens a unique opportunity for genome-wide comparisons yielding insights into the evolutionary forces that have shaped the genome architecture of these parasites. In this context, the role of directional clusters, multi-gene families, mobile elements and structural RNAs will be discussed. While these genomes generally display a striking level of gene synteny (conservation of gene order), micro synteny breakpoints are evident in many regions. In addition to expansion of species-specific multi-gene families, gain and loss of specific genes may be linked to the distinct life strategies adopted by each one of these parasites. In the second part of the presentation, an overview of the *T.cruzi* genome project activities will be presented. The hybrid and repetitive nature of CL-Brener genome, the reference strain for the *T.cruzi* genome project, posed new challenges. New assembly strategies and algorithms were developed in order to assemble the two haplotypes separately. Sequencing of *T. cruzi* Esmeraldo (a parental strain of CL-Brener) at low sequencing coverage was also performed as a strategy to validate the assembly and identify regions derived from Esmeraldo versus those corresponding to the other haplotype. From a total of 61 Mbases (Mb) of annotated sequence, approximately 31 Mb correspond to heterozygous regions, 2 Mb correspond to homogyzous regions and 22.5 Mb represent repetitive regions. In addition, 5.4 Mb of the contigs represent regions where the two haplotypes were merged. The average identity difference between heterozygous regions is 5.4 % indicating this is the most polymorphic genome sequenced to date.

[November, 09 - 10:30 - Room A]

RT1B - Comparative genomic analysis of *Leishmania* spp

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Complete genome sequencing of the reference strain for *Leishmania major* (www.genedb.org) has been followed very quickly by the complete genome shotgun sequencing projects for both the visceralising species *Leishmania infantum* and the causative agent of muco-cutaneous leishmanisis in the Americas, *Leishmania braziliensis*. This will allow comparative genome analysis of members of the three main pathogenic groups that are responsible for the spectrum of diseases caused by species within this genus. The genome of *L. major* is not only essentially complete but also annotated to a high level using both manually reviewed similarity and domain searches and comparison to the related kinetoplastid organisms, *Trypanosoma brucei* and *Trypanosoma cruzi*. In the latter instance, conservation in synteny between these related organisms has allowed improved definition of gene prediction and product assignment. Using the usual methodologies involved in pre-finishing shotgun data and the *L. major* genome as a template, comprehensively annotated genomes of the other two *Leishmania* species should be possible. Comparison of the three *Leishmania* genomes will help determine those genes that are species specific and identify conserved non coding regions that will be putative regions for regulation of gene expression. This type of analysis will also help to identify more subtle sequence differences in orthologs that may also influence the difference in host pathologies.

[November, 09 - 10:30 - Room A]

RT1C - What telomeric organization of *Trypanosoma cruzi* tells us about the parasite genome evolution

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Many organisms and in particular parasitic protozoa such as *Plasmodium* and *Trypanosoma brucei* have developed sophisticated mechanisms to adapt to the hostile environment posed by the host, such as exposing variable surface antigens to evade the immune system. In these two cases, genes coding for surface antigens are located at subtelomeric regions, and it has been speculated that this preferred location facilitates gene switching and expression, and the generation of new variants (Cano 2001; Barry et al. 2003). In previous works we have described the basic elements of *T.cruzi* telomeres, and found that they were enriched in (pseudo)genes from the ts (trans-sialidase)-like family and sequences related to VIPER (vestigial interposed retroelement) (Vazquez et al. 2000, Chiurillo et al. 1999 and 2002). Members of ts-like gene family display great sequence diversity and encode many surface proteins related with cell invasion, virulence, and evasion from the host immune system (Weston et al. 1999, Frasch 2000). We speculated that the preferred telomeric location of ts-like family genes could be connected to the generation

of variants via non-homologous recombination (Chiurillo et al. 1999 and 2002). The recent release of the whole sequence of *Trypanosoma cruzi* Cl Brener has allowed us to study the detailed organization of the telomeric and subtelomeric regions of this parasite, where we confirmed the presence of two types of telomeric ends describe by Chiurillo et al. 1999, in addition, subtelomeric regions appeared to be enriched in (pseudo)genes of RHS (retrotransposon hot spot) (Bringaud et al., 2002), TS (trans-sialidase)-like proteins, and the putative surface protein DGF-1 (dispersed gene family-1, Winker et al. 1992). Sequence analysis of the ts-like genes located at the telomeres suggested that *T. cruzi* chromosomal ends could have been the site for generation of new gp85 variants, an important adhesin molecule involved in the invasion of mammalian cells by *T. cruzi*. Finally, a model for the generation of *T. cruzi* telomere by chromosome breakage and telomere healing is discussed.

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[November, 09 - 10:30 - Room A]

RT1D - A survey of *Leishmania braziliensis* genome

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The current status of trypanosomatids genome projects strongly supports comparative analysis of content and organization of genome from different organisms. We conducted a pilot project of shotgun sequencing of *L. braziliensis* genome. Approximately 15% of the genome has been analyzed and compared to *L. major*-Friedlin. The comparative analysis revealed differences in G+C content and BlastN searches showed that 60.2% of the clusterized GSSs displayed similarity to *L. major* genomic sequences. BlastX showed that 45.3% of the predicted proteins showed similarity to annotated proteins of *L. major*. Coding regions are much more conserved than non-coding ones. Furthermore, a strategy for systematic gene trapping has been tested using the Tn5 in vitro transposon system. Four cosmids have been subjected to in vitro transposition with high efficiency. Sequencing of insertion events was used for investigating synteny with *L. major* genome and for systematic gene trapping. Primer island sequencing revealed the high synteny of the evaluated genomic regions. On the other hand, preliminary analyses carried out with ORFs annotated on the extreme regions of *L. major* chromosomes suggest that order and profile of gene duplications observed on the extremes of chromosomes do not happen in a similar way in *L. braziliensis*. Recovered *L. braziliensis* transfectants carrying trapping events are currently under analyses. In addition a similar gene trapping strategy using GFP is under use to understand the role of a putative ribosomal protein, which is preferentially expressed in amastigotes of *L. major* but apparently constitutive in *L. braziliensis*.

[November, 09 - 10:30 - Room B]

RT2A - Immune responses to *Plasmodium vivax*duffy binding protein in the amazon area

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Plasmodium vivax Duffy binding protein (PvDBP) is functionally important in the erythrocyte invasion process and provides a logical target for vaccine-mediated immunity. Studies on the immune response to the PvDBP have been carried-out essentially among lifelong residents of highly endemic areas of malaria in Asia. In the present study, we demonstrated that PvDBP is naturally immunogenic in different populations of the Brazilian Amazon, and the proportions of PvDBP IgG positive subjects increased with exposure to malaria transmission, reaching a peak in those subjects with long-term exposure (¿15 years) in the Amazon area. This profile of antibody response was significantly different from the one observed for the *P. vivax* merozoite surface protein 1 (PvMSP1_19, 19kDa fragment), which was relatively uniform in areas with markedly different levels of malaria transmission. In a small sample of adults with symptomless *P. vivax* infection, we could not detect any significant correlation between antibodies against these *P. vivax* proteins and asymptomatic infection. In a follow-up of a population exposed to a P. vivax malaria outbreak, outside of the Amazon area, we have found that individuals serologically negative to the rPvDBP became positive after a *P. vivax* relapse. These results point toward that boosting of the anti-DBP antibody response is achieved at the time of a new *P. vivax* episode. Finally, our study provided an additional insight by demonstrating cumulative exposure as a determinant that acts independently of host age in generation of anti-PvDBP IgG response among migrants into the Amazon area.

Support by: WHO/TDR, FAPEMIG, CNPq

[November, 09 - 10:30 - Room B]

RT2B - The murine infection by Sylvio X10/4 clone of *Trypanosoma cruzi*: a chronic myocarditis model

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Chagas' disease is a chronic infection caused by *Trypanosoma cruzi* that represents an important public health burden in Latin America. Frequently the disease evolves undetectable for decades, while in a significant fraction of the affected individuals it culminates in death by heart failure. Here, we describe a novel murine model of chronic infection by *T. cruzi* using a stable clone isolated from a human case (Sylvio X10/4). The murine infection by Sylvio X10/4 courses without patent parasitaemias, but the parasite can be indirectly detected in the blood, striated muscle or heart, or directly in tissue sections, at different moments after the infection. The reasons that determine the absence of circulating parasites in this infection model are not known, but whatever the mechanism might be, it seems to be exhausted when the parasite load becomes very high, such as in IFN-γ-KO mice. Besides IFN-γ, IL-12 is also involved in the control of Sylvio X10/4 parasites. While the requirement for IL-12 could be related to its IFN-?-inducing properties, it is not clear which are the IFN-γ effector functions crucial for Sylvio X10/4 control. Apparently, the protective role of IFN-γ is not majorly related to nitric oxide production, since iNOS-deficient mice display an infection course identical to that of WT mice. Nevertheless, Sylvio X10/4 parasites seem to be sensitive to death inside activated macrophages, as observed from in vitro experiments with adherent peritoneal cells. Of interest was to find that infected CD4-KO mice did not display increased parasitaemias (even though some of the animals die because of a wasting disease). On the other hand, CD8-KO mice were shown to be highly susceptible to infection by Sylvio X10/4 parasites, all the animals dying relatively soon after infection (around 18 days). The protective effect of CD8+ cells, however, seems to be unrelated to the mechanism that promotes the control of circulating parasites, inasmuch as CD8KO mice display no enhanced parasitaemias. Of interest, infection in the C3H/HePAS mouse results in intense cardiac inflammatory lesions that recapitulate the chronic cardiac pathology observed in the human disease. Heart lesions are absent in the first months of infection but they progressively increase at later stages. Striated mus-

cle lesions are also present in C3H/HePAS mice, but these show different kinetics than heart pathology. Viable parasites are detected and recovered from the heart lesions of C3H/HePAS mice, supporting the notion that development of chronic heart pathology in Chagas' disease is related to reactivity towards the parasites that persist in the affected tissue. Interestingly, and in contrast to C3H/HePAS mice, in infected A/J mice, chronic inflammatory lesions are targeted to the liver and the skeletal muscle, while pathology and parasites are undetectable in the heart. The phenotypic analysis of F1 (A/J X C3H/HePAS) and F2 (A/J X C3H/HePAS) mice suggests that the genetic predisposition to develop the inflammatory lesions caused by *T. cruzi* (Sylvio X10/4 clone) is heterogeneous because the heart and liver pathology segregate in the F2 generation. These findings raise the hypothesis that the pathology heterogeneity observed in humans with Chagas' disease (absence and presence of cardiac or digestive chronic lesions) may be attributable to host genetic factors.

[November, 09 - 10:30 - Room B]

RT2C - Tryps across the human blood-brain barrier

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African trypanosomes are major pathogens of humans and animals. *Trypanosoma congolense* and *Trypanosoma vivax* are pathogens of cattle but do not infect humans. Two subspecies of *Trypanosoma brucei*, *T. b. rhodesiense* and *T. b. gambiense*, are human pathogens while the closely related subspecies *T. b. brucei* is non-infective to humans. The latter is frequently used as a model for trypanosomiasis in laboratory animals. Each of the above parasites plays an important role, sometimes devastating in the health and welfare of people and cattle throughout large areas of sub-Saharan Africa. Although there were indications that human African trypanosomiasis (HAT), commonly called African sleeping sickness, was under control during the middle of the 20^{th} century, the World Health Organization reports that several countries are seeing a resurgence of trypanosomiasis of epidemic proportions. Because death is inevitable if a patient is untreated, human trypanosomiasis has been claimed to be more deadly than other vector-borne diseases, such as malaria. Despite its importance, the mechanism by which the African trypanosomes enter the CNS remains an unresolved issue. *In vitro* models of the BBB have clearly become important tools for identifying the cellular and molecular elements that may be possible targets for interventions for the transmigration of many pathogens into the CNS. We have previously shown that human infective *Trypanosoma brucei gambiense* strain IL1852 crosses *in vitro* models of the human blood-brain barrier (BBB) consisting of human brain microvascular endothelial cells (BMEC) grown in Transwell^{TM} inserts more aggressively than the animal infective *T. b. brucei* strains 427 and TREU 927 (Grab DJ et al., J Parasitol, Oct 2004). On addition to human BMEC the parasites cause rapid changes in monolayer integrity as well as rapid transient or oscillatory changes in human BMEC intracellular Ca^{2+} levels; $[Ca^{2+}]_i$.) The $[Ca^{2+}]_i$ rise is blocked by pretreatment of the human BMEC with the phospholipase C (PLC) inhibitor U73122. Furthermore, pretreatment of human BMEC for 2 hrs with U73122 prior to washing and addition of the parasites blocked the transmigration of the trypanosomes across human BMEC monolayer. While trypanosome crossing of human BMEC was observed at 90 min, no parasites were detected in the U73122 pretreated human BMEC, even at the 3 hr time point. We also monitored human BMEC integrity over the course of the experiment by measuring Transwell^{TM} transendothelial electrical resistance. We found that pretreatment of BMEC with U73122 tightened the barrier to the trypanosomes. In line with the known function of PLC, the inhibition of trypanosome migration across human BMEC pretreated with BAPTA (an intracellular Ca^{2+} chelator), or calphostin C (a protein kinase C inhibitor), lends further support for PLC's role in BBB transmigration. The data upholds the concept that activation of the host's G-protein coupled receptor(s) (GPCR-PLC system) may play a role in the parasite transmigration process. The identification of specific receptors will allow us to design new therapies that will stop the parasites from entering the brain and prevent many of the agonizing neurological symptoms which eventually lead to death.

[November, 09 - 10:30 - Room B]

RT2D - Immunoregulation of human leishmaniasis: the role of T cell subpopulations and their products

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Regulation of the immune response directed against the pathogen *Leishmania* is critical for the establishment of effective control of the pathogen while limiting the development of pathology. It is likely that some types of immune responses directed against *Leishmania* can lead to severe clinical forms of leishmaniasis causing pathology (mucosal) and/or a poor control (disseminated and visceral) of the pathogen. The studies presented here have been designed to examine the role that T cell subpopulations, and their products, play in this important process of immunoregulation following infection by *L. braziliensis* in cutaneous (CL) or mucosal (CL) leishmaniasis. We have examined T cell responses and immunoregulation in human leishmaniasis with the aim of defining: 1) cellular mechanisms involved in regulation of the disease; 2) immune profiles associated with clinical indicators of disease; and 3) immunoreactivity related to different clinical forms of disease.
PBMC from individuals with *L. braziliensis* infection, were analyzed for the frequency of activated and memory T cells, as well as antigen specific, cytokine producing T cells and

monocytes. Following the determination of these immune reactivity indicators, the above mentioned studies were carried out.

Briefly, studies of immunoregulation in human CL showed: 1) a positive correlation between *ex vivo* CD45RO frequencies and antigen specific cytokine (IFN-gamma or IL-10) producing cells; 2) a positive correlation amongst SLA specific, IFN-gamma or TNF-alpha and IL-10 producing lymphocytes with one another; and 3) a higher frequency of IL-10 producing, parasite specific (anti-SLA or anti-LACK), lymphocytes are correlated with a lower frequency of TNF-alpha producing monocytes, demonstrating an antigen specific delivery of IL-10 inducing negative regulation of monocyte activity. Next, to understand the clinical relevance of these indicators of immune reactivity, correlation studies were performed comparing immune indicators with clinical measurements (Montenegro skin test (MST) and lesion area). The analysis demonstrated a positive correlation between MST area and the frequency of ex vivo activated $CD69^+CD4^+$ T cells. In contrast, higher frequencies of activated $CD69^+CD8^+$ T cells correlated with a smaller MST area. Finally, larger lesions were correlated with higher frequencies of; 1) SLA specific, inflammatory cytokine (IFN-gamma or TNF-alpha) producing lymphocytes; and 2) higher frequencies of activated T cells as determined by CD69 and $CD40L^+$ expression. Thus, the clinical relevance of several immune indicators was established.

Lastly, studies designed to compare immune profiles between CL and ML patients were performed. Patients with ML presented a higher frequency of activated T cells as measured by ex vivo frequencies of $CD4^+CD69^+$, $CD4^+CD28^-$, $CD4^+$CD62L-, and $CD8^+CD69^+$ than those with CL. Moreover, after stimulation with SLA, patients with ML presented a higher frequency of TNF-alpha producing $CD4^+$ and $CD14^+$ cells than CL individuals. While CL patients displayed a coordinate regulation of the frequency of IL-10 and TNF-alpha producing monocytes, the ML patients did not. This lack of a positive correlation between IL-10 producing and TNF-alpha producing monocytes in ML patients could lead to a poorly controlled inflammatory response *in vivo*. These results corroborate with a model of an exacerbated, unregulated, immune response in ML patients and point to key immunomodulatory leukocyte populations, and cytokine networks that may be involved in the development of immunopathology in ML patients. Financed by: WHO/TDR, CNPq, and TMRC. Corresponding E-mail: kjgollob@mono.icb.ufmg.br

[November, 09 - 10:30 - Room C]

RT3A - The paraflagellar rod of endosymbiont-bearing trypanosomatids: lost but not forgotten

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Cilia and flagella are central to many biological processes in a diverse range of organisms. The main component of cilia/flagella is the 9+2 axoneme - an organelle which was formed very early in eukaryotic evolution and is highly conserved. The Kinetoplastida are very appealing models for the study of flagellar function, particularly in the light of the near completion of 3 typanosomatid genomes. In many organisms, the axoneme is augmented by extra-axonemal structures - for example the fibrous sheath in mammalian spermatozoa, R-fibre in dinoflagellates, and the paraflagellar rod in euglenoids and kinetoplastids. The best characterized of these is the paraflagellar rod (PFR) which is composed of two major proteins, PFR1 and PFR2, and several minor ones. The PFR is necessary for full motility in the kinetoplastids and provides support for metabolic regulators that may influence flagellar beating. However, there is an intriguing puzzle: one clade of endosymbiont containing trypanosomatids apparently lack a PFR yet are able to attach to the invertebrate host epithelia and are as motile as other kinetoplastid species that possess a PFR. We investigated how these organisms are able to locomote despite the apparent lack of PFR and whether the absence reflects a loss of the PFR genes within the genomes of these organisms.

We have identified a single-copy *PFR1* gene in the endosymbiont-bearing trypanosome *Crithidia deanei*. This gene is expressed in *C. deanei* and is able to partially rescue a *pfr1* null mutation in *Leishmania mexicana* cells, demonstrating that the encoded protein is functional. Moreover, antibodies against PFR1 recognise a small extra-axonemal structure in *C. deanei* and careful re-examination of the flagellar ultrastructure reveals the existence of a PFR that is greatly reduced in comparison to non-endosymbiont bearing kinetoplastids. This demonstrates the existence of a cell motility structure that had been missed by previous analyses. We will discuss some thoughts and evidence pointing to interesting conclusions about PFR structure and function in kinetoplastids in general.

Acknowledgements: CAPES, The Wellcome Trust, The Royal Society and BBSRC founding programmes.

[November, 09 - 10:30 - Room C]

RT3B - Flagellar rafts, lipid rafts and flagellar lipid rafts

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The trypanosome flagellum is a multifunctional organelle with well recognized roles in motility and adherence and which is increasingly appreciated as the primary surface by which the parasite perceives its environment. It is long established that the trypanosome flagellar membrane is contiguous with, but has a chemical composition different from, that of other domains of the plasmalemma. How this is achieved and its importance to parasite biology has been widely reflected upon. Our work demonstrates that the specialized composition of the flagellar membrane reflects, and is dependent upon, increased concentrations of so-called lipid rafts. These discrete regions of lateral heterogeneity in plasma membranes, sometimes referred to as microdomains, are characterized by detergent resistance and high liquid order. They are widely perceived as platforms or scaffolds for concerted membrane functions such as signalling. Our initial cell fractionation and fluorescence microscopy studies of the flagellated protozoan parasite *Trypanosoma brucei* indicated that raft-associated molecules, such as sterols, glycosphingolipids and the dually acylated calcium binding "sensor"protein Calflagin Tb1.7 protein are enriched in the trypanosome flagellar membrane. Calflagin Tb1.7 was found to be raft-associated by virtue of its resistance to 1The flagellar localization of Calflagin Tb1.7 could be altered by treatment with chemicals which stabilize or destabilize the membrane. Detergent extraction of cells yielded discrete detergent-resistant membrane patches, which were regularly spaced and localized to the surface of the flagellar axoneme. The mean size of these patches was greater than that estimated for lipid rafts in other cell types but similar to the expected size and localization of intraflagellar transport particles suggesting a role on membrane organizing for these particles. Together, these results provide biophysical and biochemical evidence indicating that lipid rafts are highly enriched in the trypanosome flagellar membrane, providing a unique mechanism for the localization of some dually acylated signalling proteins and illustrating a novel mechanism by which a cell can partition specialized cellular functions to discrete membrane macrodomains. The work serves to emphasize the dual function of the trypanosome flagellum as an environmental sensor and motility organelle and suggests direct proteomic routes to purification of signalling cascade components and virulence factors.

[November, 09 - 10:30 - Room C]

RT3C - Parasites in Motion: Cell Motility in African trypanosomes.

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Trypanin (TPN) is a flagellar protein that is required for directional cell motility in *Trypanosoma brucei*. TPN (-) mutants generated by RNA interference (RNAi) are not paralyzed, but can only spin and tumble in place. The molecular function of TPN is not known. Recently, it was demonstrated that the TPN homolog in *Chlamydomonas reinhardtii*, PF2, is a component of the dynein regulatory complex (DRC). In *C. reinhardtii*, the DRC is thought to regulate the inner dynein arms and is therefore critical for the regulation of flagellar beat. The DRC is a complex of at least seven proteins localized to the base of the second radial spoke in the 9 + 2 flagellar axoneme. The identity of other components of this complex are currently unknown. We are currently using genetic screens to test the hypothesis that TPN is a component of a similar complex in *T. brucei*. We are also working to identify additional components of the DRC in *T. brucei*.

[November, 09 - 10:30 - Room C]

RT3D - Ablation of a cytoskeletal chaperone reveals the cytostome in *Trypanosoma cruzi*

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Trypanosomes are flagellated protozoans and excellent model organisms for the study of organellar biogenesis. Molecular chaperones are involved in organellar protein targeting and translocation across membranes, and in protein folding and assembly in all cellular compartments. Trypanosomes possess four Hsp70 family members and several dozen unique Hsp40 proteins, some of which are organelle specific. Tcj1 is a member of the *T. cruzi* hsp40 family that is specifically associated with the flagellar cytoskeleton via its unique C-terminal substrate-binding domain. The tcj1 mRNA and protein are expressed at very low levels in normal cells and visualization of protein production and localization requires expression of a transgene. Tcj1 is encoded by a single copy gene and deletion of the two allelic copies by homologous recombination yields viable cells having at least two interesting phenotypes. The majority of tcj1-/- parasites released from infected mammalian cells are increased in diameter and the kinetoplast-flagellar complexes are farther from the posterior end of the cell as compared to wildtype. Most strikingly, scanning electron microscopy revealed tcj1-/- epimastigotes to have an invagination in the surface membrane located approximately 500 nm to the "right"of the flagellar pocket relative to the cell's intrinsic left-handed helical pitch. This phenotype is reversed upon complementation by a tcj1-expressing episome. The invagination fits the description of the cytostome, a specialized region in *T. cruzi* epimastigotes involved in nutrient uptake. Indeed, uptake of several small molecules is reduced by approximately 50 % in the tcj1-/- mutant compared to wildtype or complemented lines, further linking the invagination with the process of nutrient uptake and implicating the unique structure as a misplaced

cytostome.

[November, 09 - 10:30 - Room D]

RT4A - Detection of an iron superoxide dismutase excreted by trypanosomatids belonging to the genera *Trypanosoma*, *Leishmania* and *Phytomonas*: A tool for identification and diagnosis

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The invasion by a pathogen triggers a series of mechanisms in a host to eliminate the invader. These include highly conserved processes in the evolutionary chain, such as the production of highly unstable and reactive free-radicals capable of altering the integrity of membranes and DNA in the pathogen. On the other hand, parasitic protozoa have developed highly efficient detoxifying mechanisms to adapt to the host, these involving a number of enzymes (catalase, glutation peroxidase, superoxide dismutase) that act as powerful detoxifiers. Trypanosomatids, like other pathogens, have enzymatic mechanisms to detoxify oxygen radicals, such as superoxide dismutase (SOD).
Two superoxide dismutases (SODI and SODII) have been detected and purified in flagellates isolated from different plants (*Euphorbia pinea, E. characias, Coco nucifera Trifolium* sp., *Mangifera indica* and *Lycopersicon esculentum*) and insects (*Fabrictilis gonagra* and *Veneza zonata*). All these protozoa are considered to belong to the genus *Phytomonas*. The two SODs purified are iron superoxide dismutases, with different molecular weight and isoelectric point (SODI has a Pm of 66 kDa and a pI of 6.8, while SODII has a Pm of 22 kDa and a pI of 3.6). In addition, SODI is located exclusively in the cytosol fraction, while SODII is located not only in the cytosol but also in the glycosomal fraction, and 20% of its activity occurs in the periplasm. By Western blot, polyclonal antibodies of the SODII from Phytomonas isolated in Euphorbia characias, was specific at, high dilution (1/40,000), only for trypanosomes considered members of the genus Phytomonas, while the reaction with the other species of the family Trypanosomatidae (*Herpetomonas samuelpessoai*, *Herpetomonas davidi*, *Trypanosoma cruzi* strain maracay, *Leishmania donovani*, *Crithidia luciliae* and *Leptomonas collosoma*) and other protozoa (*Giardia lamblia* and *Trichomonas vaginalis*) tested negative. Recently, we found that *Phytomonas* isolated from *E. characias* excretes into the culture medium a SOD coinciding with SODII. in pI and Pm. Phytomonas isolated from *Lycopersicon esculentum* also excreted a SOD with the same physico-chemical characteristics as the SOD excreted by *Phytomonas* isolated from *E. characias*. The excretion of a highly immunogenic SOD activity by the species belonging to *Phytomonas* appears to be a generality, and this excretion is undoubtedly related to the mechanisms that these parasites developed to adapt to their host. In tomatoes experimentally infected with Phytomonas isolated from Lycopersicon esculentum in Spain, at 7 days of infection, we were able to detect, using polyclonal antibodies against the SOD excreted by the pathogens, the presence of the parasites in a tomato-fruit extract, as confirmed by light microscopy.
Other trypanosomatids of great importance to human health, such as *Trypanosoma cruzi*, the causal agent of Chagas' disease, as well as different species belonging to the genus *Leishmania* (*Leishmania infantum*) also excrete into the culture medium a SOD with different pI and Pm from each other and different with respect to the SOD excreted by *Phytomonas*. This SOD excreted by *T. cruzi* and *L. infantum* is highly immunogenic. A total of 1029 sera from individuals from the state of Querétaro (Mexico), susceptible to Chagas' disease, plus 12 sera from patients suffering Chagas' disease from the province of La Libertad Peru, were analysed with three different serological tests (ELISA, IFI and HAI) to detect anti-*T. cruzi* antibodies. The seroprevalence (for at least two tests) were: 8.2% in the Mexican sera, and 100% in the Peruvian sera. Western blots gave a positive reaction of the sera reactive against the antigenic fraction of the excreted SOD. The ELISA-Western-blot correlation was 100%. Sera from patients infected with excreted *Leishmania peruviana*, *Toxoplasma gondii* and *Mycobacterium leprae* were negative against this fraction. These results show that the excreted SOD, apart from being a marker of genera for the members of the family Trypanosomatidae, could also be useful in diagnosing parasitism by *T. cruzi* and *Phytomonas* spp. and perhaps for other trypanosomiasis.

[November, 09 - 10:30 - Room D]

RT4B - Demonstration and modelisation of mechanical Transmission of Trypanosomes by Tabanids

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Although the mechanical transmission of *Trypanosoma vivax* is generally admitted, at least in America, it required further demonstration and more comprehensive mechanisme analyse. To demonstrate mechanical transmission of African stocks of trypanosomes by common African tabanids, a series of 3 experiments was carried out in Lahirasso, Burkina Faso, an area of very low tsetse pressure. To avoid any interference of tsetse flies, the experiments were conducted in a corral covered by a mosquito net. Ten heifers (crossbred zebu X Baoulé), free of trypanosome infection, were kept together; two of them were experimentally infected with local stocks of *T. vivax* or *T. congolense*. Tabanids were trapped with 2 Nzi traps in the surrounding area. In the two first experiments, 2 heifers were experimentally infected with a local stock of *T. vivax*. Tabanids freshly-captured with 2 Nzi traps were released into the fly proof corral, during 20 days: on average 32 *Atylotus agrestis* / heifer / day in the first experiment, and

54 *Atylotus fuscipes* in the second one. Mechanical transmission of *T. vivax* was demonstrated in both cases with high incidence rates: respectively 63% and 75%. In a third experiment 2 heifers were experimentally infected with a local stock of T. congolense. On average 29 freshly-captured *Atylotus agrestis* per heifer / day were released in the same conditions. The incidence rate of *T. congolense* infection was 25 %. A mathematic predictive model of the transmission was succesfully established, showing that the ability of both tabanid species as well as that of both *Trypanosoma* sp. were comparable. Mechanical transmission proved to be a fully probabilistic mechanism of which most influent parameters are the level of parasitaemia in donors and the number of potential vectors. email: marc.desquesnes@cirad.fr

[November, 09 - 10:30 - Room D]

RT4C - Where are the limits of the trypanosomatid world in plant and insects? Analogy with the mollicutes.

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Hartrot and Lethal Yellowing are two diseases of coconut in Latin America and the Caribbean. Both show the same syndrome. But one, hartrot, is caused by a trypanosomatid and the other is caused by a "phytoplasma", the trivial name given to plant, phloem-restricted mollicutes (no genus name). The trypanosomatids found in plants were given an arbitrary genus name "*Phytomonas*"as soon they were discovered. The class Mollicutes comprises 4 orders. The order Entomoplasmatales contains two families, Entomoplasmataceae, and Spiroplasmataceae, which can be found in plants, insects and even in animals. For instance one species of *Spiroplasma* is the aetiological agent of a citrus disease. Another *Spiroplasma* species was isolated from flowers without any apparent damage to the flower. And another species is associated with suckling mouse cataract. *Spiroplasma* spp develop in the nectar of flowers or on their surface. Some of these spiroplasmas are acquired by various visiting insects such as butterflies, honey bees, bumble-bees etc. *Spiroplasma melliflorum*, living in the nectar of flowers causes the honey bee "May disease."Some *Acholeplasma* spp, found on plant surfaces or even in soils or sewage are saprophytic, others occur in insects and animals in which they can be associated with pathologies. Phylogenetic studies interestingly show that saprophytic mollicutes of the genus *Acholeplasma* are closer to phloem- restricted and non- culturable phytoplasma associated with diseases than other phloem- restricted mollicutes of the genus *Spiroplasma*, also associated with diseases. It is amazing to see that some trypanosomatids develop in the same habitats and provide the same picture. For instance *Herpetomonas*, formerly known as "monoxenous insect trypanosomatids", also occur on flowers, and multiply in various fruits (1, 2). *Leptomonas* and *Crithidia* other "insect trypanosomatids"have also proved capable of multiplying in fruits and/or seeds and contaminated fruits or flowers can be a source of trypanosomatids for visiting insects (2, 3, 4). From these results, it seems clear that *Herpetomonas*, *Crithidia* and *Leptomonas* are not "monoxenous"insects trypanosomatids anymore. Likewise not all trypanosomatids found in plants are *Phytomonas* and although a genus *Phytomonas* is justified, it must be kept in mind that they are not only "plant trypanosomatids"as they multiply in insects, not only in "insect vectors"but also in some predatory insects (5). Furthermore, *Herpetomonas*, and/or *Leptomonas* have been isolated from rats, dogs and immunosuppressed humans (6,7). As it was shown for viruses (8) we can imagine the passage of trypanosomatids from plant/insect to animals and humans. Taxonomic positions of ex "lower trypanosomatids"will be discussed with regard to the different degrees of mollicute taxonomy.

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[November, 09 - 10:30 - Room D]

RT4D - Unraveling the secrets of *Trypanosoma vivax*: from epizootiology to the genome

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Trypanosoma vivax is a hemoparasite affecting livestock industry in South America and Africa. In the Pantanal, Brazil, it causes economic losses mainly in cattle, and in a lesser extent to buffaloes. Little is know of their maintenance and spread in nature, particularly in terms of reservoirs and means of mechanical transmission. Despite the high economic relevance of the disease caused by *T. vivax*, few researches on its molecular characterization has been done to date compared with human trypanosomes as *T. brucei* spp and *T. cruzi*. The main reason is the difficulty to grow the parasite into laboratory rodents and "in vitro". These characteristics have limited the research on *T. vivax* during the last decades, consequently very few markers have been described for its molecular characterization. During the last years we have been working with descriptive epizootiology and markers discovery. For the latter purpose, a small-insert genomic library was constructed into the BamHI site of pUC18 using the cloned stock ILDat2160. The library was semi-normalized by hybridization with known repetitive regions, then from the negatives colonies 456 GSS of high quality (265 singlets and 67 clusters) were obtained and analyzed. Most common blast hits were: acetyl-CoA carboxylase, actin, adenylyl cyclase, kinase, dynein, phosphoglycerate mutase, histones, flagellar proteins, ubiquitin and ABC transporters. kDNA minicircles were also identified, presenting only 1 conserved region (CR). That CR contains 3 Conserved Sequence Blocks (CSB) named CSB1, CSB2 and CSB3. CSB-3 is highly conserved to their homologs described in other trypanosomatids, and is thought to be origin of replication of

minicircles. CSB-2 is less conserved among T. vivax minicircles and of the eight nucleotides that compose this sequence, the last five are identical. The size of the minicircle is 480 bp, which would be in accordance with the 465 bp reported by Borst et al (1985). From this GSS approach, a number of new markers have been obtained, nevertheless, we are now in the process of sequencing EST of a Brazilian strain, in order to study the transcriptome of this species. Finally, we anticipate the availability of a high number of markers via the ongoing comparative genome sequencing project being held at The Sanger Centre-UK. Financial support: IAEA, CIRAD, IFS.

[November, 09 - 14:00 - Room A]

RT5A - How bioinformatics can help in designing new weapons against tropical diseases

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Tropical diseases affect over three quarters of the worlds population and include diseases caused by bacteria, viruses and several protozoa. These parasites cause enormous mortality and morbidity world-wide and are a major threat to public health, economic growth and development Yet, in contrast to the size and dynamics of the problem, tools for the control of these diseases, such as drugs and vaccines are scarce and rapidly losing their effectiveness: due to the emergence and spread of resistance. most anti-parasite drugs are relatively ineffective, toxic and difficult to administer as most compounds that kill protozoa are also toxic to our own cells. Hopefully, several parasite genomes were recently revealed, so that there is a strong hope that we would be soon able to battle against the parasites with appropriate and efficient weapons. This could be achieved by the combination of several bioinformatics techniques, working on existing genomics and proteomics data to identify new valuable and specific targets against the parasites. In conjugation with molecular modelling techniques, bioinformatics can be utilised to identify the three dimensional structure of these new targets which in turn will be used to identify active sites and their interaction with putative ligands. Associated with in silico combinatorial chemistry and screening techniques, bioinformatics is likely to accelerates the optimisation process for the discovery of new efficient and selective drugs against the parasites. Combinatorial chemistry has experienced an explosive growth in recent years. It provides a powerful methodology for exploring the molecular geometrical and interactional spaces through molecular diversity generation in particular for the discovery of new biologically active substances and medical drugs. Therefore, in the general scheme of rational drug design approach against the parasites, the second step after setting the targets database is to create libraries of existing and/or virtual chemical compounds which will be in silico tested against the target active sites in order to discover possible lead compounds to be next proposed for synthesis to the chemists. As concerns virtual screening, this technique of testing compounds in silico not only reduces costs, as only a small number of compounds need to be next synthesised and tested, but also accelerates the optimisation process for new drugs. This talk will highlight such possibilities by presenting several successful stories in this drug discovery pipeline against tropical deseases.

[November, 09 - 14:00 - Room A]

RT5B - Post genomic analysis of adenylate and arginine kinases: phosphotransfer processes in Trypanosomes.

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Maintenance of cellular energy homeostasis is a crucial cellular process. Cellular energy transfer by diffusional exchange of ATP is kinetically and thermodynamically inefficient since it requires a significant concentration gradient. In this work we propose the existence in *Trypanosoma cruzi*, of an enzymatic 'phosphotransfer network' that communicates the spatially separated intracellular ATP consumption and production processes. Two enzymes with core roles in maintaining energy balance are adenylate kinase (phosphotransferase with a phosphate group as acceptor) and arginine kinase (phosphotransferase with a nitrogenous group as acceptor). Arginine kinase activity provides a spatial and temporal energy buffer that can be readily mobilized during cellular stress. Adenylate kinase is involved in the homeostasis of adenine nucleotides by interconversion of constituents of the adenine nucleotide pool, including ATP synthesis from ADP. Here, we discuss the biological function of arginine kinase in *Trypanosoma cruzi* by a comparative genomic analysis of the arginine kinase gene loci in different protozoan parasites. In order to analyse the role of these enzymes we determined the effect of silencing the expression of all three isoform genes in procyclic *Trypanosoma brucei* by RNA interference. Finally, we identified seven distinct adenylate kinases in *Trypanosoma cruzi*, more than reported for any other organism, and we characterized two of these isoforms.
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[November, 09 - 14:00 - Room A]

RT5C - *Schistosoma mansoni* transcriptome: perspectives for functional genomics

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Schistosomiasis remains a major public health problem in Africa, Asia and parts of South America in spite of the efforts to control its impact on human populations. There is one effective drug, Praziquantel, however it does not prevent re-infection in areas with sustained endemicity. Complementary measures are necessary, and vaccination is considered the most effective means; nonetheless, an effective vaccine has yet to be developed. The paucity of effective

tools to fight this parasitosis has stimulated a consortium of laboratories in São Paulo State, Brazil, to acquire extensive transcriptome data. In the October, 2003 issue of Nature Genetics a full article was published describing the transcriptome of *Schistosoma mansoni* (Verjovski-Almeida et al., Nat. Gen. 35: 148-157, 2003). A total of 163,586 reads was obtained, from six different stages of the life cycle of the parasite (adult worms, eggs, miracidiae, germ balls, cercariae, schistosomula), one order of magnitude more sequences than the adult worm and egg sequences previously available in GenBank (13,000). After filtering out retrotransposon, mitochondria and bacterial contaminants, approximately 125,000 reads were analyzed. These assembled into 30,988 *S. mansoni* assembled ESTs (SmAEs) that were estimated to represent 92% of the transcriptome. The total number of genes was predicted by two methods to be approximately 14,000, comparable to that of the fully sequenced invertebrates *Caenorhabditis*, *Drosophila* and *Ciona*. By similarity searching for orthologs in the public databases we were able to assign functions for 30 % of *S. mansoni* sequences. A very high frequency of sequences (70%) does not present a significant match with known proteins from other organisms and have no assigned functions, probably being novel *S. mansoni* genes or non-coding RNAs. In *S. mansoni*, 8001 distinct SmAEs were classified into gene ontology (GO) categories. Protein kinases were the single most abundant protein family, while among eukarya-conserved sequences (1443 genes) those relating to metabolism were the most numerous. In contrast, the metazoa-specific sequences (645 genes) were predominantly for genes involved in signal transduction, cell-cell interactions, developmental processes and response to external stimuli. A number of bioinformatic tools were developed that allow exploring all the data that was generated (http://bioinfo.iq.usp.br/schisto). A task force has been established in São Paulo to investigate the potential drug targets and vaccine candidates that were postulated in the Brazilian transcriptome project. Thus, Instituto Butantan will investigate DNA vaccines. A group at Universidade de São Paulo has established a microarray facility and is generating a chip containing 4,000 selected *S. mansoni* genes. The microarray will be used to determine stage-specific genes, to monitor gene expression during development of the parasite in the animal host and its change as a result of the host's induced immune response. A group at Universidade de São Paulo, Ribeirão Preto will undertake the immunolocalization of proteins coded by the genes under study. It is anticipated that a wealth of information will be generated by the initiatives described here as well as by many other groups around the world, as we enter the functional genomics and proteomics era.

[November, 09 - 14:00 - Room A]

RT5D - Post genomic analysis of *Trypanosoma cruzi* differentiation

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The transformation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) is a suitable model for studying the molecular basis of *Trypanosoma cruzi* differentiation because the process can be mimicked in vitro resulting in the obtention of bona fide metacyclic trypomastigotes. Most, if not all protein-coding genes in this species lack introns and their transcription is polycistronic. The precursor RNA is processed by a trans-splicing reaction resulting in the addition of a common spliced leader sequence to the 5' portion of all mRNAs and of a poly-A tail to the 3'-end. Gene expression appears to be regulated essentially at the posttranscriptional level. In order to investigate whether the regulation of gene expression during *T. cruzi* differentiation was determined by differential regulation of mRNA turnover or by mRNA mobilization to the polysomes, we have compared several developmental stages of the parasite using microarray analysis. A *T. cruzi* microarray was constructed comprising more than 5,200 distinct sequences in triplicate on each slide. The microarrays were then hybridized to total and polysomal RNAs extracted from several developmental stages of the parasite. The results show that few differences in the complexity of the RNA populations are observed when total RNA populations are compared. However, many mRNA transcripts appear to be differentially expressed when polysomal RNA populations are compared. These results suggest that gene expression is controlled by the selective recruitment of mRNA molecules, very likely stored in the cytoplasm, to the translation machinery.

Financial support: CNPq, PRONEX, Fundação Araucária, NIH, Fiocruz

[November, 09 - 14:00 - Room B]

RT6A - Predicting Geographic Variation In The Risk Of Cutaneous Leishmaniasis Throughout Colombia.

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Approximately 6000 cases of cutaneous leishmaniasis are notified annually in Colombia, representing a several-fold increase since the 1980s. Notifications certainly underestimate true incidence, and their geographic distribution is probably biased by local health service effectiveness. We investigate how well freely available environmental data explain the distribution of cases amongst 1079 municipalities. For each municipality, a unique predictive logistic regression model was derived from the association amongst remaining municipalities between elevation and/or land cover (pre-classified maps derived from satellite images) and the odds of at least one case reported. Land cover had greater predictive power than elevation, but using both datasets improved accuracy. Fitting separate models to different ecological zones, reflecting transmission cycle diversity, enhanced predictions. We derive measures that can be directly related to control decisions, and show how these vary with the threshold selected for predicting a positive municipality. The results identify areas most likely to be under-reporting disease.

[November, 09 - 14:00 - Room B]

RT6B - Current transmission and control of Chagas' Disease and leishmaniasis in Argentina.

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Chagas' disease and leishmaniasis are the two leading endemic diseases transmitted by vectors in Argentina. A program with a nationwide impact for the control of Chagas disease began in Argentina in 1962, and in 1999 an interinstitutional coordinating program at the national level was formed with the aim of coordinating strategies for the control of leishmaniasis transmission. Remarkably has been the progress made over the past 15 years. The control of both vectorial transmission through *T. infestants* and *T. cruzi* transmission through blood transfusion has been consolidated. Furhter, the control of congenital transmission as well as etiologic diagnosis and treatment for the young population were implemented nationwide. In 2003, the infestation of household for triatomines was 3.1 % [0.0% - 39.4%]. Although the interruption of the vectorial transmission in 4 provinces was achieved in 2002, current rates still indicate the existance of high risk areas for infestation, as evinced by the 18 reported cases of vectorial transmission in 2003. Intervention to mitigate vectorial transmistion in the affected areas was intensified. Further, the control of blood for transfusion indicates a prevalence of 4,1% of infection among blood donors. Considering the high risk of accidents through this transmission route, it is necessary to sustain this strategie. Screening of pregnant women increases year after year. A number of 185,362 women were controlled in 2003, and the prevalence of infection was 5.5 % [0,7% - 14,0%]. The current estimate of new cases of Chagas' disease congenitally transmitted range from 800 to 1,700. In the future the congenital transmission will be the only infection route that will cause new cases, when the complete control of vectorial transmission is achieved. The set of actions implemented has shown its efficacy in the reduction of the prevalence in the infant population. In 2003, 14,541 serologic tests were performed in children aged 15 years or younger. Prevalence of infection was 3.4 % in areas with recently implemented surveillance and 0.8 % in areas where surveillance already existed. Clinical research conducted in the earlier 1990s allowed us to incorporate in a programatic fashion etiological diagnosis and treatment in the infected infant and young population. Tegumentary leishmaniasis (TL) with expression Cutaneous (CL) and Muco-cutaneous (MCL) in Argentina is an endemic disease with sporadic outbreaks of epidemic stemming from different reasons. Its clinical manifestation is similar in all endemic provinces. The main species of parasite isolated has been the *Leishmania (Viannia) braziliensis*. The low ocurrance of mucosal forms of the disease would be attributable to the earlier diagnosis and timely treatment offered by the health system. The effective human-vector contact ocurrs in the wild environment as well as in peridomestic and periurban sectors. The former takes places during logging or foresting activities, fishing, or hunting, which is frequent in the northwest region of the country. The latter, is more connected with peoples' proximity to residual vegetation, causing periurban outbreak, as can be seen in the northeast region of Argentina. Potential vectors, as the *Lutzomyia neivai*, are abundant in peridomestic area near closed secundary vegetation environement (modified) and the population dynamics is associated with climate changes. Other species, such as the *Lu. migonei*, *Lu. shannoni* and *Lu. cortelezzii*, could be responsible for the transmission cycle observed in the northwest region. In the northeast, this role could be attributed to *Lu. whitmani*, *Lu. shannoni*, *Lu. cortelezzii*, *Lu. quinquefer* and *Lu. migonei*. The determinants of transmission analyzed varied according to the different study regions. Parallel to the system of cases' detection for early treatment, we propose the design of an entomologic surveillance in those critical areas during the period of endemic transmission. This porpuosal is based on a barrier espatial interventions when vectors' abundance show high risk of epidemic outbreak. 748 cases of tegumentary leishmaniasis were notified in Argentina in 2002 and 348 cases in 2003. In the light of the recent ocurrance of visceral leishmaniasis transmission in Paraguay, a field study was carried out in Clorinda (a village in the Argentinean Province of Formosa, which is bounded by Asunción del Paraguay) to evaluate risk factors. Preliminar data confirmed the presence of the potential vector (*L. longipalpis*),

but no dispersion data, was still obtained. Human or canine migration implying risk was observed. On the other hand, this piece of research has not yet demostrated evidence of autochthonous transmission. Although LV canine (imported) was only detected, no human cases were observed. Further research has been planned to monitor this scenario. Community involvement for the control of these endemic diseases will be crutial to attain the sustainability of the control strategies implemented. E-mail: ssosa@msal.gov.ar

[November, 09 - 14:00 - Room B]

RT6C - Epidemiological Study of Exposed Individuals to Phlebotomines in an endemic area for visceral leishmaniasis

Aldina Barral
FIOCRUZ-Bahia

[November, 09 - 14:00 - Room B]

RT6D - Molecular eco-epidemiology of *Leishmania (Viannia) braziliensis*: genetic diversity and transmission cycle

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Most of the environmental factors affecting the epidemiology of the various leishmaniasis are still poorly understood. Wild mammals serve as reservoirs for most of the New World *Leishmania*, but there is increasing evidence that some of the human pathogenic *Leishmania* can be maintained in both sylvan and urban cycles. In the case of *L. (V.) braziliensis*, there are evidences that some domestic and sylvatic animals may serve as reservoir hosts of this parasite. The existence of an urban cycle involving peridomestic sand fly species for *L. (V.) braziliensis* reflects the ability of these parasites and their vectors to adapt to changes in their original forested habitats with important public health implications. Studies using molecular techniques to characterize *L. (V.) braziliensis* populations from different regions have shown a relationship between level of similarity among the parasite's populations and their geographic range. We recently employed numerical zymotaxonomy and the variability of the internal transcribed spacers (ITS) between the small and large subunits of the rRNA genes to examine strain variation and relationships in natural populations of *Leishmania (V.) braziliensis*. A number of strains of this species from distinct hosts and Brazilian geographic regions were analyzed and these were assigned to 15 zymodemes clustered in two major genetic groups. The great number of isolates placed in zymodeme IOC/Z-27 was collected on the Atlantic coast. The results of the restriction fragment length polymorphism analysis of the ITS depicted considerable genetic variability among these parasites from different regions where they are endemic. The genetic differences in *L. (V.) braziliensis* reflected distinct eco-epidemiological features of the infection since these strains are endemic in the distinct areas studied. In areas where sylvatic animals are not apparently involved with the transmission cycle of *L. (V.) braziliensis* it was observed low level of heterogeneity in the parasite population. However, in areas where it was detected moderate level of heterogeneity in the parasite population it was described the role of small rodents as the primary reservoirs of this *Leishmania* species. *L. (V.) braziliensis* strains from the Amazon Basin showed a high level of diversity and this is probably related to the great number of sandfly vector(s) or animal reservoirs in that region. The link that possibly exists between both the sylvan and urban cycles related with *Lutzomyia intermedia* (probably the main vector of the parasite in Southeast Brazil) is now under investigation through genetic population studies. Genetic analysis of *Lu. Intermedia* was conducted comparing the population structure of this sand fly species from two endemic areas that exhibit distinct eco-epidemiological patterns. The results clearly demonstrate the existence of population genetic substructuring within *Lu. Intermedia* from different regions or habitats, and raise interesting questions about parasite-sand fly vector specificity, epidemiology (e.g., specific transmission cycles of *L. (V.) braziliensis* genotypes in nature), and ecology of the different genotypes of *Lu. Intermedia*. Our studies reinforce the clonal theory for *Leishmania* parasites showing the genetic diversity of this pathogen and an association of *L. (V.) braziliensis* genotypes with specific transmission cycles, probably reflecting an adaptation of different clones to the vector species involved.

Supported by FIOCRUZ/IOC, CNPq-PRONEX, FAPERJ (Brasil)

[November, 09 - 14:00 - Room C]

RT7A - Interaction triatomine-vertebrate host

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Triatominae bugs are obligatory haematophagous insects, taking blood directly from venules and arterioles. The events that characterize the feeding process include detection of the host, active exploratory movements of the mouthparts in the skin surface of the host, biting or penetration (insertion of the mouthparts into the skin), localisation of blood within a blood vessel, ingestion of blood and removing of mouthparts to finish the feeding process (Lavoipierre et al., 1959; Friend and Smith, 1977). Parameters of feeding behaviour are influenced by intrinsic characteristics of the feeding appa-

ratus, such as size of the cibarial pump (which varies between species) and by host factors related to haemostasis, such as probing time, frequency of cibarial pump contractions and interruptions during the engorgement phase. These parameters seem to act together, having a significant impact in the triatominae feeding performance. Among the triatominae species so far studied, ingestion rate of fifth instar nymphs ranged from 3.1±1.6 mg/min for *Rhodnius neglectus* fed on mice to 25.2 ± 6.0 mg/min for Triatoma. infestans fed on pigeons (Guarneri et al., 2000; Sant' Anna et al., 2001). This work evaluated some aspects related to triatomine feeding process on mammalian hosts, such as salivation profile, choice of vessel (arteriole or venule), microvascular changes, leukocytes response and platelet aggregation. In order to access the ear microvasculation of hairless mouse, intravital microscopy technique was used. After fluorescent labelling of saliva (from *R. Prolixus*) with Acridine Orange, we were able to visualise the bolus of saliva during engorgement phase, in an average frequency of 0,51 ± 0,18 Hz. During probing phase, saliva was secreted continuously. It suggests that frequency of the salivary pump is higher in this phase. During the insect feeding process (*R. prolixus* and *T. infestans* nymphs), haemorrhagic regions in the skin were frequently observed (¿75%), occurring mostly during the probing phase. Using vital labels such as Evans' Blue dye or FITC-Dextran, we observed an increase of vascular permeability after insects probing in all experiments. We also observed that both species introduced their maxillae preferably in venules than arterioles. However, when comparing engorgement time either in arteriole or venule, arterioles seem to offer a more satisfactory environment for the feeding process. It was possible to evaluate vascular changes induced by both species. Venules used for *R. Prolixus* feeding presented vasodilation (75%), whereas venules used for *T. Infestans* feeding presented vasoconstriction (80%). Using intravital microscopy, we observed a significant increase of leucocytes rolling and adhesion after feeding of *R. prolixus* on mouse, characterizing an inflammatory process. In these experiments, we also visualised recruitment and aggregation of platelets in the endothelial line of the vessel wall. The insect feeding process consists in a complex mechanism, in which both mechanical characteristics (mouthparts movement or vessels deformation due to the cibarial bomb functioning) and saliva deposition act all together or separately in the host microcirculation.

Supported by: CNPq, ECLAT (CDIA) and CAPES.

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[November, 09 - 14:00 - Room C]

RT7B - Evolution of the Group Leucosphyrus of *Anopheles (Cellia)*, vector of *Plasmodium* in Southeast Asia

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The *Anopheles (Cellia)* includes 224 species among them several important vectors of human malaria parasites. The Leucosphyrus Group belongs to the Neomyzomyia Series of the subgenus *Cellia* and includes 14 named species, 2 geographical forms and six recently described species. Members of the Leucosphyrus Group are primarily jungle breeders and had been incriminated as vectors of human *Plasmodium* in several localities throughout their geographical distribution range in Malaysia, Thailand, Vietnam, Cambodia, Laos, Myanmar, Indonesia, Philippines, China and India. They are mostly primate feeders but also possible bite small canopy mammals. Probably because of their feeding habits, at least seven species are involved in the transmission of non-human malarias in tropical broad leaf evergreen forests and mangrove forests in Southeast Asia as well as in areas of southern India and Sri Lanka. Geographic distribution of non-human primate malarias, especially macaque malaria, is determined by the distribution of the Leucosphyrus Group, which is disjunct and coincident with that of tropical broad leaf evergreen rain forest. *Anopheles dirus* E is vector of macaque malaria in southwestern India and Sri Lanka, whereas *An. Dirus* B, *An hackeri*, *An. Leucosphyrus* A and *An. Introlatus* are vectors in west Malaysia, *An. Balabacensis* in Palawan, and *An. Takasagoensis* is a possible vector in Taiwan. *An. Balabacensis* is also involved in the transmission of human *Plasmodium*, primate non-human *Plasmodium* and filariasis. Fragments of two mitochondrial genes have been used to: 1) to estimate phylogenetic relationships among species of the Leucosphyrus Group; 2) to examine distribution of the species of the Leucosphyrus Group.

[November, 09 - 14:00 - Room C]

RT7C - *Nanos* (*nos*) gene expression in the vector mosquitoes, *Anopheles gambiae*, *Anopheles stephensi* and *Aedes aegypti*

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One approach to genetics-based strategies for the control of vector-borne diseases requires the development of safe and effective means for driving antipathogen effector genes into wild populations of insects. Synthetic transposons whose mobilization is controlled by the DNA elements of developmentally-regulated genes offer a potential solution for introducing effector genes into mosquitoes. Such elements could exhibit sex-, stage- and species-specific trans-

position, thus mitigating some of the concerns associated with autonomous transposition. Transcription products of the nanos homologous genes of *Anopheles gambiae* (Anga nos), *An. Stephensi* (Anst nos) and *Aedes aegypti* (Aene nos) are restricted to developing oocytes in adult females and localized to pole cell region in early embryos. Hybridizations in situ show accumulation of nos mRNAs in the nurse cells and in developing oocytes and early embryos. These features make nos genes promising candidates for donating control sequences to synthetic transposons.

[November, 09 - 14:00 - Room C]

RT7D - Fitness of transgenic anophelines expressing a peptide that inhibits *Plasmodium* development

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One potential strategy for the control of malaria and other vector-borne diseases is the development of transgenic mosquitoes that are refractory to the transmission. Creating insects that have minimal fitness load will be critical to the success of these transgenic-based genetic control strategies. Transgenic mosquitoes expressing the SM1 peptide and the bee venom PLA2 are inefficient vectors in an experimental malaria model. Both transgenes are driven by a carboxypeptidase promoter, which is midgut-specific and induced by a blood meal. To evaluate if these transgenes impose a fitness load in the transgenic mosquitoes several fitness parameters (longevity, fertility, fecundity, and running population cage experiments) were evaluated, and it was shown that while the SM1 transgene did not impose a detectable fitness load relative to the non-transgenic controls, the PLA2 transgene did. Malaria parasites can reduce reproductive fitness of mosquitoes. Thus, mosquitoes that interfere with parasite development should have a competitive advantage when fed on infected blood. To investigate this hypothesis, cage experiments were performed in which SM1 transgenic mosquitoes and non-transgenic ones were fed at every generation with Plasmodium berghei-infected blood. Preliminary results have shown that, under these conditions, the percentage of transgenic mosquitoes increased significantly at each generation. In other words, the transgenic mosquitoes had a significant selective advantage over the non-transgenic ones. Non-transgenic mosquitoes had significant higher mortality than transgenic ones when fed on infected mice. Higher mortality is presumed to be caused by ookinetes at the time of midgut invasion. Moreover, transgenic mosquitoes lay significantly more eggs than non-transgenic ones after feeding on infected blood. These results have important implications for malaria control via genetic modification of mosquitoes. The higher fecundity of these transgenic mosquitoes comparing to non-transgenic ones, when they are fed on infected blood, may act as selective advantage for them after releasing in endemic areas.

[November, 10 - 14:00 - Room A]

RT8A - IMMUNIZATION WITH RECOMBINANT DBL- γ3 INDUCES TRANSCENDING AND ADHESION BLOCKING ANTIBODIES AGAINST FUNCTIONAL DISTINCT CSA-BINDING *Plasmodium falciparum* PARASITES

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Maternal malaria (MM) is associated with sequestration of *Plasmodium falciparum*-infected erythrocytes in the placenta to chondroitin sulfate-A (CSA) via the DBL-γ3 domain of the PfEMP1^{CSA} molecule. Antibodies against CSA-binding infected erythrocytes (IE^{CSA}) correlates with resistance to MM in multiparous women. Based on the FCR3- or 3D7- *P. falciparum* subtypes, we produced recombinant DBL-$\gamma3^{CSA}$ (rDBL-$\gamma3^{CSA}$) in insect cells, which yielded a conformation of the cysteine-rich DBL-$\gamma3^{CSA}$ domain capable of blocking IE^{CSA} binding to CSA. Immunization of mice with rDBL-$\gamma3^{CSA}$-FCR3 and -3D7 domains generated antibodies that recognized homologous and heterologous rDBL-$\gamma3^{CSA}$, indicating conserved epitope(s) that induces a transcending pan-reactive immune response. Mouse monoclonal antibodies (mAb) against both recombinants were pan-reactive with distinct IE^{CSA}, and recognized functionally distinct cloned IE^{CSA} populations regarding their susceptibilities to shear stress. One mAb inhibited efficiently and reversed in vitro IE^{CSA} cytoadhesion to endothelial cells. Hence, the DBL-$\gamma3^{CSA}$ is the target of inhibitory and pan-reactive antibodies. Non-human primates immunized with FCR3-rDBL-$\gamma3^{CSA}$ developed transcending pan-reactive and inhibitory antibodies. Based on these data, and towards the development of an experimental vaccine, we established an in vitro model using placenta cryosections to evaluate potential therapies against MM.

[November, 10 - 14:00 - Room A]

RT8B - Immunoprophilaxy and immunotherapy against canine visceral leishmaniasis with the FML-vaccine. Development of a DNA vaccine.

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The FML-saponin formulation against visceral leishmaniasis had already shown to be safe, immunogenic and protective in Phase I-IIa trials in Balb/c, Swiss albino mice and CB hamsters. In recent Phase III trials of efficacy using the FML-vaccine in dogs induced 92% [18] and 95% [19] protection in naturally exposed vacinees (76% and 80% of vaccine efficacy, respectively). Protection induced by the FML-QuilA vaccine lasted up to 3.5 years after vaccination. The potential effect of the FML-vaccine on immunotherapy of canine visceral leishmaniasis was assayed on 21 *L. chagasi* naturally infected dogs (FML-saponin R vaccine) when seropositive to FML but completely asymptomatic. Twenty-two months after complete vaccination, no obits due to visceral leishmaniasis were recorded and 90% of these dogs were still asymptomatic, healthy and parasite free. On the other hand, 37% kala-azar obits were recorded in a control group that received no treatment during the same period. Normal proportions of CD4 and CD21 lymphocytes were detected in PBMC by FACS analysis, in dogs submitted to immunotherapy, suggesting their non-infectious condition. All animals showed as well significantly increased percents of CD8 lymphocytes as expected for a Quillaja saponin (QuilA) vaccine treatments.[1] The presence of aldehyde groups at C-23 and C-24 of the triterpen aglycon moiety was disclosed in ^{1}H NMR spectra of both the Riedel de Haen saponin (R) (δ 9.336) and Quillaja saponaria QuilA saponin (δ 9.348). The sign of the C-28 acylated linked moiety (δ 176) was present in both saponins, while the δ 171 at C-28 (carboxy group) corresponding to the deacylated saponin, was only detected in the QuilA preparation, indicating 50% of hydrolysis of the ester moiety. The normoterpen moiety was present in both saponins (signals at δ 14-18).The chemical removal of saponin glicidic moieties gave rise to their sapogenin fractions. Their ^{1}H NMR spectra showed the presence of two signals (δ 9.226 and 9.236) for sapogenin R and two signals (δ 9.338 and 9.352) for the QuilA sapogenin. The intensity of the signals suggested two conformational isomers of sapogenin R in the ratio 53% of equatorial aldehyde group to 47The main component of FML is a 36 kDa nucleoside hydrolase (NH36). We tested the immune response and protection induced by the purified FML antigen, the recombinant NH36 (rNH36), both in combination with saponin, and by the NH36 DNA vaccine (VR1012-NH36), against the agents of visceral (*L. (L.) chagasi*) and tegumentar leishmaniasis (*L. (L.) mexicana*) in Balb/c mice. Reduction of parasitic load coompared to controls was achieved after FML and NH36 vaccination (79%; p¡0.01) in *L.(L.) chagasi* infected mice, and by FML vaccination (27%, p¡0.05) in *L.(L.) mexicana* experiment. The highest protection was developed by mice immunised with the VR1012-NH36 DNA vaccine (88%, p¡0.01, against *L.(L.) chagasi* and 47%, p¡0.05, against *L.(L.) mexicana*). Its FACS analysis disclosed normal total CD4+ and CD8+ T cell populations in, with a 2-3 fold increase in IFNγ-producing CD4+ T cells, characteristic of the induction of a Th1 type immune response. Our results showed a strong and specific immunoprotection of the NH36-DNA vaccine against visceral leishmaniasis and a milder but also protective effect against tegumentar leishmaniasis pointing out its potential use in a bivalent vaccine tool for the control of both endemies.[3]

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[November, 10 - 14:00 - Room A]

RT8C - Caspases and apoptosis in experimental Chagas' Disease

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T cells and cytokines play a critical role in the immune response to *Trypanosoma cruzi* in experimental models of Chagas disease (1). Apoptosis occurs in vivo and in vitro in T cells from *T. cruzi*-infected mice, negatively affecting T cell function in vitro (2,3). Moreover, phagocytosis of apoptotic cells exacerbates parasite infection within macrophages and injection of apoptotic lymphocytes increases parasitemia in vivo (4) T cell apoptosis is mediated by death receptor Fas in vitro (5). Fas/FasL interaction initiates apoptosis through activation of caspase-8, which activate death effector caspase-3 (6). Viral-FLIP proteins block caspase-8 and apoptosis mediated by Fas and other death receptors (7). In order to study the effects of apoptosis *in vivo*, we studied transgenic mice, expressing v-FLIP in T cells (8). Experiments also included treatments *in vivo* or *in vitro* with peptides zVAD (general caspase blocker), zIETD (caspase-8 inhibitor) and

zFA (control peptide). We studied caspase activation in T cells. CD4 and CD8 T cells from *T. cruzi*- infected mice expressed activated caspase-3 in vitro. zVAD reduced caspase-3 activation and increased recovery of viable T cells *in vitro*. Injection of zVAD in vivo reduced lymphocyte apoptosis in *T. cruzi*-infected mice. Furthermore, treatment with zVAD reduced parasitemia in infected mice and inflammatory infiltrates in their hearts. These results suggested that inhibition of caspases and apoptosis increased resistance to experimental Chagas disease. Caspase-8 inhibitor z-IETD also blocked activation-induced cell death (AICD) in T cell cultures from infected mice. However, higher levels of parasitemia were observed in zIETD-treated mice, compared to mice treated with control peptide. Alterations in the immune responses were investigated. zIETD reduced IL-2 production by activated T cells in vitro and affected cell signaling that regulate cytokine expression, reducing NF-κB activation in T cell cultures. Furthermore, inhibition of caspase-8 increased type 2 (IL-4/IL-10) cytokine responses to *T. cruzi* infection. These results correlated well with *T. cruzi* infection in transgenic mice expressing a caspase-8 inhibitor in T cells. Increased susceptibility to *T. cruzi* infection and Th2 responses, but reduced T cell expansion were observed in v-FLIP mice. Therefore, caspase-8 plays a critical role in the regulation of T cell expansion and cytokine responses, affecting resistance to *T. cruzi* infection. Support: WHO, CNPq, FAPERJ, PRONEX and Howard Hughes Medical Institute.

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[November, 10 - 14:00 - Room A]

RT8D - The 30 kDa antigen from *Leishmania (L.) chagasi*: a very suitable tool for kala-azar diagnosis and protection studies.

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Previous studies from our laboratory demonstrated the implication of a 30 kDa cysteine proteinase from *Leishmania (L.) chagasi* (p30) in partially protective cellular immune responses in BALB/c mice[1]. The gene encoding the p30 antigen, *Ldccys1*, was amplified by PCR, a product of 1.3 kb was obtained and sequence analysis of the clone showed a high identity to sequences of other *Leishmania* cysteine proteinase genes. Subcloning and expression of clone 1.3 in the pHIS vector resulted in a 47 kDa recombinant protein (rLdccys1) which, in Western blots, was recognized by the monoclonal antibody 2E5D3 reactive with the native p30 from *L. (L.) chagasi*. Serum obtained from BALB/c mice immunized with rLdccys1 reacted with bands of 27 and 30 kDa from promastigote and amastigote *L. (L.) chagasi* extracts, showing that the *Ldccys1* gene corresponds to the *L. (L.) chagasi* p30 gene. Cellular localization of the Ldccys1 by use of mouse anti-rLdccys1 serum in immunolabeling experiments at electron and confocal microscopy level showed that the protein is confined to megasomes of *L. (L.) chagasi*, thus supporting the presence of this organelle in *L. (L.) chagasi* previously described by our group[2]. Immunization of BALB/c mice with *Ldccys1* gene induced a strong humoral response with production of IgG1, IgG2a and IgG2b isotypes. Spleen lymphocytes from these animals released IFN-γ, IL-4 and IL-10, indicating a mixed Th1/Th2 response. Challenge with *L. (L.) chagasi* amastigotes resulted in a significant decrease in the parasite burden of animals immunized with the *Ldccys1* gene when compared to those which received PBS. Nevertheless, there was no difference in parasite burdens of animals immunized with empty pcDNA3 or *Ldccys1*/pcDNA3 plasmid. ELISA tests with sera from patients with VL showed that the sensitivity for detection of antibodies to *L. (L.) chagasi* using rLdccys1, *L. (L.) chagasi* promastigote lysates and amastigote lysates was 80%, 98% and 99%, respectively. The rLdccys1 antigen showed high specificity (96%), no cross-reactivity with sera from Chagas' disease patients, and very low rates of positive reactions with sera from cutaneous leishmaniasis and tuberculosis patients[3]. The lymphoproliferative responses elicited by rLdccys1 were also evaluated in peripheral blood mononuclear lymphocytes from naturally infected people and dogs presenting several clinical signs of visceral leishmaniasis (VL). The rLdccys1 elicited higher T lymphocyte responses in individuals presenting asymptomatic and oligosymptomatic VL compared to those observed in symptomatic patients. Similar results were observed in lymphocyte cultures from *L. (L.) chagasi*-infected dogs. Lymphokine analysis showed a predominance of IFN-γ in the lymphocyte supernatants from asymptomatic individuals, whereas lymphocytes from symptomatic patients released significant levels of IL-4 and IL-10. Intermediary values of IFN-γ, IL-4, and IL-10 were observed in lymphocyte supernatants from oligosymptomatic patients. Lymphocyte supernatants from *L. (L.) chagasi*-infected dogs exhibited similar lymphokine values. These results show that the rLdccys1 from *L. (L.) chagasi* is able to induce and discriminate cellular responses in individuals and dogs naturally infected with *L. (L.) chagasi*, opening perspectives to test the rLdccy1 antigen in protection studies in endemic regions of VL.

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[November, 10 - 14:00 - Room B]

RT9A - Signaling Pathways in malaria parasites.

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Cell signaling pathways interact to integrate and regulate information by evoking complex sinergistic or antagonist responses. Parasites have adapted during evolution to subvert the host for its own benefit. One of the most striking features of malarial infections is the periodicity of the fevers they engender. These occur at multiples of 24 hours (Hawking et al, 1968. Trans Royal Soc Trop Med Hyg 62, 731-760). We have proposed a novel mechanism that allows the intracellular protozoan *Plasmodium* to use the host endocrine regulation to coordinate the regulation of its cell cycle and proliferation. The hypothesis is based on the finding that melatonin synchronizes intraerythrocytic malaria parasites in a Ca2+-dependent manner (Hotta et al, 2000 Nature Cell Biol. Jul (7): 466-8). Unravelling the signal(s) at the basis of this synchronicity represents not only a challenging biological question but might offer new targets against malaria. Of interest, we have identified in *P. falciparum* an ortholog of the receptor for activated C kinase (RACK) that anchor diverse signaling proteins and are involved in modulating cell cycle in mammalian cells (Madeira et al, 2003 Biochem and Biophys Res Commun 306, 995-1001). Changes in intracellular signaling messengers such as Ca^{2+} encode a myriad of informations inside the cell. The ubiquitous role of Ca^{2+} as an intracellular messenger requires precise control of its concentration in the cytosol. The ionic environment where *Plasmodium* divides and differentiates is unlike that encountered by any other eukaryotic cells. The question then arises as to how can *Plasmodium* escapes such a fate and manages to duplicate happily in a low Ca^{2+} environment, the RBC cytossol. We hypothesized that the solution to the above question may indeed reside in the nature and sideness of the PVM (parasitophorous vacuolar membrane). If the erythrocyte plasma membrane Ca^{2+} ATPase is still present in the PVM, after parasite invasion of host cells, it should pump Ca^{2+} into the space between the two membranes, thus generating there a high Ca^{2+} environment. To test this hypothesis we entrap Ca2+ fluorescent dyes (MagFura-2 and Fluo 3) selectively in this space during RBC invasion by *Plasmodium*. Using a confocal microscope we identified a high Ca^{2+} microenvironment by measuring the intensity of the fluorescence signal between the parasite membrane and its envelope, the parasitophorous vacuole (PV) (Gazarini et al, 2003 J. Cell Biol. Apr 14;161(1):103-10). Given that malaria parasites require proteolytic activity for key processes as invasion, hemoglobin degradation and merozoite escape from red blood cells (RBC) we next investigated if the hormone melatonin and the rise in cytoplasmic Ca^{2+} could trigger a thiol-protease activity. By using confocal microscopy in real time we detected the presence of thiol protease activity in *Plasmodium* parasites in either free or within RBC using internally quenched fluorogenic (IQF) peptides (Farias et al, unpublished). We known that melatonin is also able to elicit an increase in the levels of the second messenger cAMP of the parasite cells. This increase is not affected by treating parasites with the PLC inhibitor, U73122 which suggests that PLC might not be involved. A cross-talk between Ca^{2+} and cAMP pathways is now proposed as activation of the Ca^{2+}/calmodulin complex is known to inhibit cAMP phosphodiesterase thus increasing the cAMP levels. Furthermore, 8Br-cAMP treatment, similarly to melatonin, induces parasite cell multiplication and an increase on its intracelular Ca^{2+} levels (Beraldo et al, unpublished). Finally, we want to understand how *Plasmodium* signalling pathways interact with one another to put in action a response during its complex life cycle.

[November, 10 - 14:00 - Room B]

RT9B - Using genomics to assess gene transfer in the Apicomplexa : The functional consequences

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Reports of plant-like and bacterial-like genes have speckled the literature over the last few years for a number of parasitic organisms most notably those within the apicomplexa and kinetoplastida. Among the apicomplexan organisms, following discovery of the apicomplexan plastid (apicoplast), the discovery of plant-like genes was less surprising although the extent of transfer and the relationship of transferred genes to the apicoplast remained unclear. We have used emerging genome sequence data to begin a systematic examination of the extent and origin of transferred genes in the Apicomplexa. We have used a phylogenomic approach to detect potential gene transfers in 4 apicomplexan genomes. We have detected genes of algal nuclear, chloroplast (cyanobacterial) and proteobacterial origin. Plant-like genes were detected in species not currently harboring a plastid (e.g. *Cryptosporidium parvum*) and transferred genes were detected that appear to be unrelated to the function of the apicoplast. Analyses of the genes that were transferred and the pathways in which they participate reveals that transferred genes can, and have, substantially altered existing biochemical pathways or provided material for new and unexpected pathways. In the case of *Cryptosporidium parvum*, a prominent apicomplexan AIDS pathogen, genomic analysis revealed that unlike other apicomplexa, the parasite was completely dependent upon salvage from the host for both purines and pyrimidines. *Cryptosporidium* can survive because multiple horizontal gene transfers of salvage pathway genes have occurred. We have experimentally validated these genomic hypotheses. Our results explain why widely used drugs fail in the treatment of *Cryptosporidium* and suggest novel more promising targets. In summary, the successful integration and expression of the transferred genes in apicomplexan or-

ganisms has changed their genetic and metabolic repertoire.

[November, 10 - 14:00 - Room B]

RT9C - *Trypanosoma rangeli*: Addressing the parasite biology by cellular and molecular approaches

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Trypanosoma rangeli Tejera, 1920, is a hemoflagellate parasite transmitted by triatomine bugs, mainly from the genus *Rhodnius*, in a wide geographical area in Central and South America. The parasite is considered harmless for the vertebrate hosts but presenting pathogenic effects for the invertebrate vectors. Infecting a large number of vertebrate species, including humans, *T. rangeli* shares triatomine vectors and mammalian hosts with *T. cruzi*, determining the occurrence of single and/or mixed infections that are of major importance for Chagas disease diagnosis and epidemiology. Furthermore, *T. rangeli* and *T. cruzi* share several soluble antigens, determining a strong serological cross reactivity in diagnostic assays such as indirect immunofluorescence and Elisa (Grisard *et al.*, 1999). During the last years, a variety of techniques have been used by several groups for both intra and inter-specific characterization of *T. rangeli* strains isolated from distinct geographical regions, hosts or vectors species. The use of biological, immunological, biochemical and molecular markers revealed a considerable intra-specific variability, initially allowing the clustering of the studied samples in 2 groups (Grisard *et al.*, 1999; Guhl *et al.*, 2002; 2003; Vallejo *et al.*, 2002; 2003), and recently in 3 distinct groups (Maia da Silva *et al.*, 2004). Moreover, these studies pointed out several molecular markers for specific detection of these parasites, proving their effectiveness even in mixed infections (*T.rangeli* / *T. cruzi*). Nowadays, our group is dedicated to explore the *T.rangeli* genomics and proteomics, having initially working on an *in vitro* metacyclogenesis system in order to obtain *T.rangeli* trypomastigote forms (Koerich *et al.*, 2002), which are scarce in natural infections. Improvement of this protocol reached an in vitro differentiation rate of over 85-90% , revealing the importance of L-glutamine and the involvement of the ornithine decarboxilase (ODC) on the parasite metacyclogenesis (Koerich *et al.*2003 and on this meeting). Inoculation of irradiated mice (600 rads) by intraperitoneal route with *in vitro*-derived trypomastigotes produced high levels of bloodstream trypomastigotes (2-3x10$\hat{6}$/ml) on the 7$\hat{t}$h day of infection. This protocol allowed the purification of large amouns of *T. rangeli* blood trypomastigotes, which are the basis of the current efforts to study the parasite genome. Once having both *in vitro* and *in vivo*-derived trypomastigotes, we have launched the "*Trypanosoma rangeli* EST project"(www.bioinformatica.ufsc.br/trangeli) which is dedicated to address the parasite transcriptome by sequencing expressed sequence tags (ESTs) and ORF ESTs (Orestes) from epimastigote and trypomastigote forms of the parasite and, in collaboration with Instituto Oswaldo Cruz (Fiocruz), the generation of genome survey sequences (GSS). The aim is to characterize the parasite transcriptome and to implement a *T. rangeli* database to support studies of the unknown life cycle of the parasite as well as for comparative analyses of the parasite sequences with other kinetoplastid species (Grisard *et al.*, 2004). For that, we have launched the "*Trypanosoma rangeli* resource database - TrangeliDB"(www.trangelidb.ufsc.br) that aims to be an easy and user-friendly interface dedicated to congregate researchers and research projects in order to ease research on *T. rangeli* biology and molecular biology. In the near future, the parasite transcriptome database will be freely available through this interface. Up to now, the *T. rangeli* EST project has generated more than 3,400 clones from epimastigote forms and around 1,500 clones were sequenced and, after clusterization, 78 clusters and 222 singlets were obtained (656 sequences in total). From this total, 386 (58.84%) showed matches with trypanosomatids, among which, 168 with *T. cruzi* and 20 with *T. rangeli*sequences. Even forming some clusters, a total of 245 sequences presented no significant matches with any available database, possibly representing new *T. rangeli*sequences (Snoeijer *et al.*, 2004). Along with the continuing sequencing of epimastigotes ESTs, cDNA libraries from *in vivo* and *in vitro*-derived trypomastigotes are being constructed for validation and high throughput EST and Orestes sequencing. This work was supported by MCT/CNPq, UFSC, IBMP, IOC/FIOCRUZ and Funcitec.

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and mini-exon gene sequences supports either clonal evolution or speciation of *Trypanosoma rangeli* strains isolated from *Rhodnius colombiensis*, R. *pallescens* and R. *prolixus* in Colombia. Infection, Genetics and Evolution 3: 39-45.

[November, 10 - 14:00 - Room B]

RT9D - The activity of the prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80) is required for parasite entry into mammalian cells

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Distribution of *T. cruzi* throughout vertebrate host tissues is dependent on the ability of the parasite to cross basement membranes and extracellular matrix to reach and invade host cells. An important step in this process is the specific interaction of the trypomastigote form of the parasite with collagens, laminin, fibronectin and heparin, that ensure parasite entry into the host cells. In accordance with these features of the *T. cruzi*-vertebrate host interaction, we have postulated that the parasite synthesizes an enzyme displaying collagenase activity that could facilitate parasite entry into host cells. Such a proteinase has been identified and characterized as a secreted 80 kDa neutral prolyl oligopeptidase (POP Tc80). POP Tc80 shows strong activity on human collagens and less extensively on fibronectin. Moreover, we have demonstrated that the POP Tc80 also mediates native collagen hydrolysis with features comparable to those displayed by the Clostridium histolyticum collagenase. *T. cruzi* shows a significant increase in POP Tc80 expression during its trypomastigote life cycle stage compared to its non-infective epimastigote stage, corroborating previous data showing that trypomastigotes display three times higher enzymatic activity than epimastigotes. These data and the location of the enzyme inside vesicular compartments close to the flagellar pocket have suggested that this secreted protein could be involved in the infection process by facilitating parasite migration through extracellular matrix and interaction with host cell membrane components. Using specific inhibitors against POP Tc80, we have performed preliminary studies of the protein's physiological role in the *T. cruzi*-mammalian host cell interaction. POP Tc80 activity is specifically inhibited by these molecules, precluding host cell infection by trypomastigotes in a dose-dependent manner. Host cell invasion is a complex process that can be divided into two major steps: 1) parasite attachment to the host cell through protein binding to extracellular matrix components or cell surface carbohydrates; 2) parasite internalization involving early signal transduction events. To distinguish the involvement of POP Tc80 in these two steps, we tested the effects of specific POP Tc80 inhibitors on host cell invasion by trypomastigotes, using a parasite in-out immunostaining technique. Infective parasites treated with specific POP Tc80 inhibitors attach to the surface of mammalian host cells, but are unable to infect them. These data suggest that POP Tc80 inhibitors act through the inhibition of the invasion per se of host cells rather than through the inhibition of trypomastigote attachment to the host cell surface This demonstrated that the proteinase is indeed involved in a nonphagocytic mammalian cell invasion process by *T. cruzi*, and could be a potential target for chemotherapy of vertebrate *T. cruzi*-infection. The structure of POPs shows a cylindrical shape consisting of a peptidase and a seven-bladed ?-propeller domains. It has been proposed that the most distinguishing peculiarity of the POP family members is their specificity for oligopeptides no longer than 30 amino acid residues. However, we found that POP Tc80 hydrolyzes triple helical collagen as well as small peptides. To give a thought to how the enzyme interacts with large substrates, we performed molecular modeling studies based on porcine POP. Our previous results suggest that the peptidase and the propeller domains move to create an interface area allowing the collagen interaction and hydrolysis. We are performing site-specific mutagenesis experiments to test the model. Supported by CNPq, CAPES and CNRS jsantana@unb.br

[November, 10 - 14:30 - Room C]

RT10A - Chloroquine, Mefloquine and Quinine resistance in *P. falciparum*: Do we have molecular Markers?

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[November, 10 - 14:30 - Room C]

RT10B - Inhibitors of Protein Farnesyltransferase as anti-Malarial and anti-trypanosomatid agents.

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The enzyme protein farnesyltransferase catalyzes the attachment of farnesyl groups to proteins in eukaryotic cells including malaria and trypanosomatids. For the past several years we have been developing inhibitors of this enzyme as anti-malaria and anti-trypanosomatid agents. We will present extensive medicinal chemistry and pre-clinical data (in vitro parasite growth, studies with parasite-infected rodents and pharmacokinetic studies) showing that inhibitors of protein farnesyltransferase are promising anti-parasite agents. During our studies of these compounds, we have discovered a novel series of anti-T. cruzi agents that turn out to be inhibitors of lanosterol 14-demethylase, an enzyme required

for sterol biosynthesis. Our studies will also show that it is possible to develop anti-parasite drugs mainly in a university environment and with help from the pharmaceutical industry and from the Medicines for Malaria Venture as a source of financial support and guidance. A brief summary of the Medicines for Malaria Venture drug development pipeline will also be given.

[November, 10 - 14:30 - Room C]

RT10C - Phospholipases A2 and peptides from different venomous origins (bee, snakes, spider and scorpion) exhibit anti-*Plasmodium* properties.

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Animal venoms are valuable sources of novel tools for the characterization of biological functions. Among them, secreted phospholipases A2 (sPLA2) and bioactive peptides are able to inhibit in different ways the *in vitro* erythrocytic development of *Plasmodium falciparum*, the most dangerous agent of malaria.
Four venomous sPLA2 from the bee *Apis mellifera* (bee), the snakes *Naja mossambica mossambica* and *Agkistrodon halys*, and the scorpion *Pandinus imperator* (Imperatoxin I) were tested, along with the sPLA2 from hog pancreas, on the *in vitro* cultures of the different chloroquino-resistant strains of *P. falciparum* : FcB1 (Colombia), W2 (Vietnam) and K1 (South Asia). All enzymes displayed inhibitory effects although large discrepancies were observed between respective IC_50 values. The *Apis mellifera* and *Naja mossambica mossambica* sPLA2s were the most active with IC_50 in the 1-10 picomolar range. In all cases, the IC_50 values were beneath the minimum enzymatic concentration required for 1 % hemolysis of healthy erythrocytes. By inhibiting the bee and hog sPLA2s with p-BPB, we could demonstrate that catalytic activity was involved in *Plasmodium* killing (1). Since phospholipids are substrate of sPLA2 and are present in the human serum contained in the parasite culture medium, we investigated the role of these exogenous phospholipids in the enzyme toxicity. Enzymatic pre-treatment of the serum with seven sPLA2s (the sPLA2s cited above plus the snake *Notechis scutatus scutatus* sPLA2 (notexin) and the *Vipera ammodytes* sPLA2 (ammodytoxin A)) induced *P. falciparum* inhibition (1, 2). In good correlation with these results, no toxicity was observed when experiments were conducted in the presence of ALBUMAX II instead of serum, ALBUMAX II being devoid of lipoproteins and containing 10 times less phospholipids than the culture medium with serum (2). Remarkably, the bee venom enzyme toxicity was restricted to the trophozoite stage of *Plasmodium* development, in contrast to the other sPLA2s that appeared toxic for all stages (1, 2).
We also described two novel peptides, purified from the Trinidad chevron tarantula *Psalmopoeus cambridgei*, that inhibit the intraerythrocytic growth of *P. falciparum in vitro* at low concentrations (IC_{50} in the μM range). These peptides, named Psalmopeotoxin I (PcFK1) and Psalmopeotoxin II (PCFK2), contain 33 and 28 amino acids respectively, with three disulfide bridges, and belong to the Inhibitor Cystine Knot superfamily. They are not haemolytic to normal red blood cells nor do they affect growth or viability of mammalian cells. PcFK2 interacts selectively with the infected erythrocytes, in contrast to PcFK1 which adsorbs strongly to normal erythrocytes. The two peptides do not inhibit neuromuscular function, despite their structural similarity to known neurotoxins (3).
Our results show that - 1) sPLA2s inhibit the intraerythrocytic development of *P. falciparum* through lipid by-products generated by enzymatic hydrolysis of lipoprotein phospholipids. Deciphering the molecular mechanisms at play in the phenotypic singularity of the bee venom enzyme toxicity might offer new prospects in anti-malarial fight - 2) Peptides from spider venoms might be useful tools for anti-malaria research and the basis for rational design of new drugs.
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[November, 10 - 14:30 - Room C]

RT10D - Structural studies targeting *Leishmania* salvage pathway enzymes for the discovery of novel inhibitors

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Leishmaniasis is a serious disease caused by parasites of the order Kinetoplastida. According to the World Heath Organization (WHO, 1998), 88 countries are affected, with 12 million people infected. The need for new drugs for the treatment of leishmaniasis comes from a lack of safe drugs. Knowing that the *Leishmania* parasites are purine auxotrophs, our purpose is to explore the salvage pathway as a target for the development of new inhibitors that may be further developed into alternative drugs the treatment of *Leishmania* infections. We have cloned and characterized the phosphoribosyl transferases (PRTases) of *Leishmania* (APRT, HGPRT and XPRT) as a model system. The genes encoding each PRTase have been cloned, expressed and crystallized. The structure of those proteins have been solved and used for the investigation of the binding of different inhibitors isolated from plant extracts. The three PRTases have been used to evaluate the inhibitory capacity of organic extracts from marine invertebrates, plants and microbes collected in Brazil. The screening of extracts was performed using an

easy and fast spectrophotometric enzymatic assay, which allowed the identification of promising PRTase inhibitors. The inhibition of *Leishmania* growth in vivo was tested using the isolated compounds. Those results indicate that the isolated compounds have different inhibitory potential both in vivo and in vitro assays. Molecular docking experiments associated with the planing of new inhibitors are underway. thiemann@if.sc.usp.br Supported by: FAPESP (CEPID), PRONEX.

[November, 10 - 14:30 - Room D]

RT11A - Genomic changes during the dispersion of the principal vector of Chagas disease in South America: *Triatoma infestans*

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The epidemiological importance of Chagas disease vectors (Hemiptera, Reduviidae) largely depends on its spreading ability and adaptation to domestic habitats. Studies on the changes taken place in such domestication and geographical expansion may help to understand the basic process by which some species of Triatominae are able to invade new habitats and colonize human dwellings. *Triatoma infestans* represents the best example of spreading and adaptation to domiciles observed in a triatomine species. It is found almost exclusively in domestic and peridomestic environments. Historical reconstruction and genetic analyses suggest that Bolivian Andean is the site of origin and dispersal of *T. infestans* throughout South America. Recently, we have analysed the genomic changes that have occurred during this dispersal using cytogenetic techniques to localize repetitive DNA (heterochromatin) and flow cytometry for DNA quantification (Panzera et al. 2004: Emerging Infectious Diseases 10: 438-446.). Our data disclosed two chromosomal groups named Andean (Bolivian and Peruvian Andean samples) and non-Andean (samples from Argentina, Paraguay, Brazil, Uruguay, and Bolivian Chaco). These discrete groups can be recognised by differences in their heterochromatin and DNA content. The Andean specimens exhibited consistently more heterochromatin than non-Andean ones and all of them contained more DNA per cell (approximately 30% more) than did non-Andean specimens. Our study suggests that *T. infestans* was originally a sylvatic species with large quantities of DNA and heterochromatin, inhabiting the Andean region of Bolivia. This cytogenetic attribute was not deeply affected during the first phase of its geographic expansion throughout the domiciles of the Andean region. However, the spread of domestic *T. infestans* throughout the non-Andean regions only involved insects with an important reduction of heterochromatin and DNA amounts. We proposed that the genome size decrease observed in *T. infestans* was a successful change as it underwent adaptation to domiciles located in non-Andean lowland regions. The generation of this genomic variant could have also implied some loss of variability in particular loci. Greater domestic dependence, the inability to return to sylvatic ecotopes, and a certain degree of reduced variability could contribute to making these insects more susceptible to control campaigns. In order to understand the basis of this striking genomic change is necessary to perform the molecular characterization of these heterochromatin regions. Using specific fluorescent DNA-binding dyes we could establish their AT or GC base pairs enrichment. Our results indicate that these regions are heterogeneous and can be divided in A-T and C-G rich sub-regions. The distribution of these types of heterochromatin varies between the Andean and non-Andean groups, showing the non-Andean group a differential loss of AT rich sub-regions. Another approach is the identification of the DNA sequences of these heterochromatin regions. Heterochromatin is formed by two broad classes of repetitive DNAs: tandem arrays and interspersed repeats. Tandemly repeated sequences include satellite DNA while interspersed repeats are usually active or defective transposable elements. We have identified in the heterochromatin region of *T. infestans* a sequence belonging to a putative transposable element (LTR retrotransposon). Using in situ hybridisation we establish that this retrotransposon is a major structural component of the heterochromatic regions. The important role of transposable element in restructuring of host genomes observed in other organisms point out the necessity of a deeply analysis to ascertain its significance in triatomine evolution.

This study benefited from international collaboration through the ECLAT network and CDIA projects from Commission of the European Communities (Brussels, EU). E-mail: panzera@fcien.edu.uy

[November, 10 - 14:30 - Room D]

RT11B - The application of molecular markers to triatomine systematics

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Taxonomists can often be classified in two groups: 'splitters' and 'lumpers'. The first category includes those who exercise what is known as 'alfa' systematics, *i.e.* the separation of cryptic species, or subdivision of species into species complexes. Thus, splitters increase the number of existing species. Lumpers, on the other hand, are those taxonomists who have a tendency of being more conservative (or cautious), and tend to interpret variation usually as intra- (as opposed to inter-) specific variation. Thus, lumpers reduce the number of species (but increase their polymorphism). Molecular markers are the splitter's weapons of choice. By applying such markers one has a great advantage in terms of deciding whether a nominal species is composed of a single or multiple taxa. This is due to the genetic nature of the information generated by such markers. Moreover, if such genetic information is used in conjunction with species concepts such as the Biological Species Concept (BSC), then it becomes particularly powerful. The BSC relies on the ability of organisms to reproduce and consequently exchange genes. Therefore, if a barrier to reproduction arises between two populations, gene flow will be interrupted, and their gene frequencies will begin to differ through time (mainly due to genetic drift and selection). But because this genetic divergence is sometimes not paralleled by morphology (at least for our human eyes), it could well pass undetected by the examination of morphological attributes alone. It can, nonetheless,

be easily detected with molecular markers.
Studies applying molecular tools to triatomine systematics have uncovered cryptic taxa, and helped resolve taxonomic conflicts. I will give two recent examples that illustrate the use of such markers.
1. *Rhodnius prolixus* and *R. robustus*
Rhodnius prolixus and *R. robustus* are the pair of Chagas disease vector species that has received the most attention, in terms of studies on taxonomy, in the last 15 years. This is understandable due to: (i) *R. prolixus* is presently the most important Chagas disease vector in Latin America; and (ii) it is virtually indistinguishable, based on morphology, from *R. robustus*, a vector of secondary importance. The phylogeographical structure of these vectors is presented based on a 663 base pair (bp) fragment of the mitochondrial cytochrome b gene. Twenty haplotypes were recovered from 84 samples examined, representing 26 populations from seven Latin American countries. The resulting phylogenetic tree is composed of five major clades, one representing R. prolixus and four representing *R. robustus*. While *R. prolixus* is a very homogeneous assemblage, *R. robustus* has deeper clades and is paraphyletic, with the clade comprising *R. robustus* from Venezuela (Orinoco region) more closely related to the *R. prolixus* clade than to the other *R. robustus* populations from the Amazon region. The *R. robustus* paraphyly was supported further by the analysis of a nuclear gene (D2 region of the 28S RNA) for a subset of specimens. The data support the view that *R. robustus* represents a species complex.
2. *Triatoma brasiliensis* populations
Triatoma brasiliensis is the most important Chagas disease vector in the semiarid areas of Northeast Brazil. Recent genetic (allozyme-based) and ecologic studies have suggested that *T. brasiliensis* is composed of four distinct chromatic populations (*brasiliensis, macromelasoma, juazeiro*, and *melanica*), which could actually represent different species. These studies, however, used specimens collected from the type-localities for the forms and therefore could not exclude the occurrence of natural intermediate forms, nor allow for the assessment of whether they represent the extremes of a morphological and chromatic gradient. In order to investigate possible incipient speciation among the four chromatic forms of *T. brasiliensis*, we collected samples from across the forms' geographic ranges. Therefore, by sampling points in between their type-localities, we aimed at determining whether they represent the extremes of a morphological gradient, or distinct evolutionary entities. A total of 136 specimens representing 16 populations from across the species' distribution were sequenced for a 510 bp fragment of the cytochrome b gene and phylogenetically analyzed. Results indicated that *T. brasiliensis* is composed of four genetically distinct chromatic forms that present inter-population divergence values (0.027 - 0.119, corrected K2-p) and a pattern of haplotype geographic distribution compatible with the existence of a species complex. As a consequence, such forms can be treated as isolated targets in vector control programs.

[November, 10 - 14:30 - Room D]

RT11C - The Rhodnius prolixus genome project

Wim Degrave
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[November, 10 - 14:30 - Room D]

RT11D - BioNotes - Annotation system of biosequences: Its application in the *Rhodnius prolixus* project

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One of the most important tasks of genome projects is the interpretation of experimental data in order to derive biological knowledge from the data. To achieve this goal, researchers typically search public data sources, execute analysis programs and add manual annotations to register their interpretation of the data. The annotation is a description of high level features of a DNA or protein sequence. Useful information includes whether a stretch of DNA contains an amino acid coding sequence and its putative function. The public data sources are heterogeneous, have an enormous volume of data and are in constant growth. The programs do not have good documentation, standardization in the format of the input and output data, and there are often problems during their execution. In this difficult context, researchers can use annotation systems to deal with a large number of molecular biology programs and data sources. BioNotes is an annotation system which is under development at the Catholic University of Rio de Janeiro and is being used in genome projects of organisms like Rhodnius prolixus and Gluconacetobacter diazotrophicus. BioNotes helps a researcher access annotations stored in public data sources; execute analysis programs, manually generate new annotations; and analyze current annotations, with the help of an appropriate and user-friendly interface. The researchers can navigate through hyperlinks to annotations stored in external public data sources. For example, the researcher can see a result from BLAST and click on the name of one of the similar sequences to look at the annotations of that similar sequence which are stored in the NCBI database. The researchers can also locate annotations by "drilling down"the genome data. BioNotes has schemes that show contigs/ESTs and its reads. The researcher can click on the EST image and see its features, such as its length and its sequence. Besides this, BioNotes tabulates annotations assigned by different sources to the same sequence. For example, the user may run BLAST to compare a gene against different data sources. The system can show a table with the annotations of the similar sequences from these different data sources. Therefore, the user can compare all BLAST results and annotate the original gene. The characteristics of the annotations also vary according to the goal of the genome project. Indeed, anno-

tations generated in the context of a project that targets the complete DNA of an organism have different requirements from those created in the context of a project whose goal is to obtain ESTs. The BioNotes architecture and data model is flexible enough to allow the system to be used to annotate any genome organism and any project: EST or complete genome projects. While a genome project is in progress, sequencing of the genome will generate new reads. As a consequence, BioNotes re-executes the analysis programs to generate new versions of the data and offers tools to transfer manual annotations from older to newer versions of the data, as otherwise it would be very discouraging to annotate data from on-going sequencing projects. Besides that, the public data sources are also being constantly updated, so, to remain up-to-date, BioNotes refreshes its data warehouse from time to time by re-accessing the data sources to retrieve new data. Since BioNotes has a Web interface, data distribution is possible. This fact is important because it makes the data access, control, sharing and interpretation more efficient. The goal of this paper is to briefly describe BioNotes and its application in the *Rhodnius prolixus* project. It will be presented the main analysis programs and data sources which is being used in this project and how BioNotes helps researchers annotate the genome of *Rhodnius prolixus*.

[November, 10 - 14:30 - Room D]

RT11E - Transcriptome of *Rhodnius prolixus* (initial characterization)

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Rhodnius prolixus is a blood-sucking insect, one of the main vectors of Chagas' disease, an incurable illness that afflicts millions of people in Middle and South America. Morbidity caused by this disease imposes a heavy social and economic burden on countries with weakened or deteriorating economies. According to WHO, since the early 1980s, it is recognized that the only feasible way of controlling Chagas' disease is through control of populations of the insect vector. In order to increase the knowledge about *Rhodnius prolixus* biology and its interaction with the parasite *Trypanosoma cruzi*, an insect transcriptome project was initiated. This project aims the characterization of insect gene expression from different tissues, especially the ones involved with blood digestion, insect - parasite interactions and oogenesis. Here we present initial results from randomly sequencing of 800 ESTs from female adult midguts and 960 ESTs from follicular epithelium-cDNA libraries. Individual EST sequences were clustered by the CAP3 program and annotated using Blastx similarity analysis and InterPro Scan. Three different databases, Genebank nr, COG (clusters of othologous groups) and GO (gene ontology) were used for annotation. This data set provides new insights regarding insect physiology such as blood digestion, antioxidant defense, insect immunity and insect-parasite interaction, being the first study on gene expression by transcriptome of a hemimetabolous and of an exclusive blood-sucking insect. Annotated clones will be used in *Rhodnius prolixus* chromosomes physical map elaboration by FISH, as part of Triatomine Genome Initiative, a South American project launched in November 2003, that aims genome sequencing of different species of Triatominae, important for Chagas' disease transmission in Latin-America, focusing on *Rhodnius prolixus*. Supported by CNPq, FAPERJ, PRONEX, PADCT, HHMI.