CONFERENCES

[October, 2008-10-28 – 16h30 - REAL ROOM]

CO1 - T*RYPANOSOMA CRUZI*: ANCESTRAL GENOMES, POPULATION STRUCTURE AND THE PATHOGENESIS OF CHAGAS DISEASE

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There is extensive and well-characterized genetic diversity in *Trypanosoma cruzi*. We propose that the acquisition of detailed knowledge about this variability and about the structure and evolution of *T. cruzi* populations is essential for understanding both the epidemiology and pathogenesis of Chagas disease.
Although capable of recombination in vitro, *T. cruzi* reproduces predominantly by binary fission and consequently its diploid nuclear genotype is transmitted *en bloc* to the progeny. Thus, the parasite presents extreme degrees of linkage disequilibrium and in the past genotyping of nuclear markers in *T. cruzi* has been limited to characterization of multilocus genotypes. Using Bayesian approaches we could dissect the multilocus genotypes of *T. cruzi* into their constituent haploid genome blocks. Our results, obtained from detailed molecular study of 75 strains of the parasite allow us to propose the following model for current *T. cruzi* populations. In a distant past there were a minimum of three ancestral lineages that we may call *T. cruzi* I, *T. cruzi* II and *T. cruzi* III. These persist to this day as zymodemes Z1, Z2 and Z3. At least two hybridization events involving *T. cruzi* II and *T. cruzi* III produced evolutionarily viable hybrid progeny. In both of these, the mitochondrial recipient (as identified by the mitochondrial clade of the hybrid strains) was *T. cruzi* II and the mitochondrial donor was *T. cruzi* III.
To try to dissect host and parasite factors that determine the pleomorphic clinical presentation of Chagas disease, we have developed genetic typing of *T. cruzi* directly in infected tissues using molecular techniques such as LSSP-PCR, rDNA or microsatellite analysis. Studies in both humans and experimental animals have shown striking tissue tropism of *T. cruzi* clones. To correlate the clonal variability of *T. cruzi* with the clinical characteristics of Chagas disease we have proposed a "clonal-histotropic model", based on the concept that strains of the parasite in fact represent swarms of clones with different tropism for diverse tissues (heart, esophagus, rectum, etc.). A determining factor determining the clinical course of disease might be the specific 'constellation' of infecting clones in the swarm and their specific tropisms. We have gathered considerable experimental evidence for this model, including demonstration of different clones infecting the heart and esophagus of the same patient.

[October, 2008-10-28 – 17h10 - REAL ROOM]

CO2 - Retention and loss of RNA interference pathways in Trypanosomatid protozoans

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RNA interference pathways are widespread in metaozoans but the genes required show variable occurrence or activity in eukaryotic microbes, including many pathogens. While most Leishmania lack RNAi activity and key genes encoding Argonaute or Dicer, we show that Leishmania braziliensis and other species within the Leishmania subgenus Viannia encode active RNAi machinery. This provides some optimism for the application of this system as a tool for the systematic experimental manipulation of gene expression in these predominantly diploid asexual organisms, and potentially as a tool for selective chemotherapy. The Leishmania offer an attractive system for testing hypothesis about forces leading to the evolutionary loss of RNAi, with practical implications to the emergence of drug resistance and parasite virulence.

[October, 2008-10-29 – 16h30 - REAL ROOM]

CO3 - PROTEIN GLYCOSYLATION IN TRYPANOSOMATIDS AND YEASTS: SOME CURIOUS RESULTS

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The enzyme involved in the transfer of glycans to Asn residues (*N*-glycosylation) in the lumen of the endoplasmic reticulum (oligosaccharyltransferase,

OST) is a membrane bound complex, closely associated to the translocon, formed by eight subunits in *S. cerevisiae* and in mammals, five of which are essential for the viability of the former organism. The OST from most eukaryotes transfer the complete glycan ($Glc_3Man_9GlcNAc_2$) preferentially over biosynthetic intermediates containing lower number of glucose units. One of the essential proteins (Stt3p) is the catalytic subunit, actually responsible for the transfer of the glycan because: a) crosslinking between the OST components Ost1p, Ost3 and Stt3p and an acceptor polypeptide chain was obtained when a photoreactive reagent was engineered close to a *N*-glycosylation site; b) a Stt3p homologue (PglB protein) was found to be encoded by the *Campylobacter jejuni* genome. The transfer of a variety of glycans from polyprenol-diphosphate derivatives to asparagine residues in the canonical consensus sequence N-X-S/T is operative in this bacterium. Moreover, transfer of PglB to *E. coli* conferred to this last bacterium the capacity of protein *N*-glycosylation, and c) Stt3p is the only protein of the entire OST complex encoded by the genomes of trypanosomatid protozoa. These last microorganisms synthesize and transfer to protein non glucosylated glycans, ($Man_{6,7\ or\ 9}GlcNAc_2$, depending on the species). Cell-free assays showed that trypanosomatid OSTs transfer $Man_{7-9}GlcNAc_2$ and $Glc_{1-3}Man_9GlcNAc_2$ at the same rate. Is the capacity to transfer all glycans at the same rate a feature determined specifically by trypanosomatid Stt3ps or alternatively, is it the complex that determines the preferential transfer of $Glc_3Man_9GlcNAc_2$ in mammalian, plant, fungal and other eukaryotic cells?. To answer this question we replaced *S. cerevisiae* Stt3p by the *Trypanosoma cruzi* homologue in the fungal OST complex. Results obtained show that the complex thus formed preferentially transfers the complete glycan both *in vivo* and *in vitro*. Preference for $Glc_3Man_9GlcNAc_2$ is thus a feature determined by the complex and not by the catalytic subunit. In the parasitic protozoon *Leishmania major* there are four Stt3p paralogues, but, the same as in other trypanosomatids, no homologues to the other OST components seem to be encoded in the genome. We expressed each of the four *L. major* Stt3p proteins individually in *S. cerevisiae* and found that three of them, LmStt3pA, LmStt3pB, and LmStt3D were able to complement a deletion of the yeast *STT3* locus. Furthermore, LmStt3pD expression suppressed the lethal phenotype of single and double deletions in genes encoding other essential OST subunits. Contrary to what happened with *T. cruzi* Stt3p, LmStt3 proteins did not incorporate into the yeast OST complex but formed a homodimeric enzyme, capable of replacing the endogenous, multimeric complex of the yeast cell. Therefore, these protozoan OSTs resemble the prokaryotic enzymes with respect to their architecture but they utilized substrates typical for eukaryotic cells: N-X-S/T sequons in proteins and dolichol-diphosphate-linked high mannose oligosaccharides as acceptor and donor substrates, respectively. As expected for a catalytic subunit not forming part of an OST complex, LmStt3pD expressed in yeasts transferred glucosylated and non glucosylated glycans at the same rate in cell free assays. Why do most eukaryotes display a multimeric OST complex in which, at least in yeasts, several of the component subunits are essential for viability when the whole complex can be replaced by a single protein is a mystery for the moment.

[October, 2008-10-29 – 17h10 - REAL ROOM]

CO4 - Shape, form and function in relation to pathogenicity of kinetoplastid parasites

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The kinetoplastid parasites adopt different cell types according to the particular niche they occupy in the host or vector. In the host *Trypanosoma brucei* appears to be completely extracellular although an important differentiation occurs leading to the non-proliferative stumpy form. *Leishmania* and *T. cruzi* adopt both extracellular (for primary or subsequent cellular infections) and intracellular (for proliferation) cell types. Overall, these trypomastigote, promastigote and amastigote forms have rather different cellular architectures and yet have an underlying unity. The cellular forms represent variable arrangements of a set of 6 units: the sub-pellicular microtubules, flagellum, flagellar attachment zone, flagellar pocket, kinetoplast, and nucleus. In the case of the nucleus and kinetoplast it is the position that varies. The kinetoplast's position is determined by the attachment via the tripartite attachment complex to the basal body complex. In turn, this basal body position determines the direction of flagellum polarity, whilst the extent of flagellum growth and attachment to the cell body provides the variability that differentiates promastigote,

amastigote, epimastigote and trypomastigote forms. In addition, there are specific differentiation steps associated with the transition of one cell type to another.

We have used extensive scanning and transmission electron microscopy and electron microscope tomography, proteomics and monoclonal antibodies and analysis of the phenotype of RNAi mutants to:

- Define the organisation of cellular components providing a three dimensional view of the position of cytoskeleton components, basal body, flagellum, FAZ and the nucleus and kinetoplast.
- Show how the cytoskeleton influences the membrane architecture to define the flagellum pocket
- Show how the cytoskeleton is remodelled, replicated and segregated during cell division resulting in the formation of a new flagellar pocket and ultimately the new cell.
- Define important components of the flagellum, flagellum attachment zone, basal body and sub-pellicular array of microtubules.
- Construct a set of RNAi mutants for each of these proteins and use these to examine the effect of depletion of the protein on cellular architecture.
- Reveal a set of dependency relationships between of these different cytoskeletal components during their construction.
- Reveal the importance of particular structures in defining particular cell forms.
- Define the importance of particular cell architectures in pathogenicity.