MEETING OPENING

Meeting opening - Plenary conference

MO-01 - From vessel to tissue: the travels of *Trypanosoma cruzi*

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The protozoan *T. cruzi* is the etiological agent of Chagas' disease of major medical significance throughout South to Central America. The disease was discovered by Carlos Chagas 100 years ago. In 1909, the Brazilian physician described the parasite in the gut of an insect belonging to the Reduviidae family. This discovery is one of the few examples in the literature where the disease, the parasite, the insect vector and the cell cycle were described in a single manuscript. Modern description of the complex dixenic life cycle of the parasite is basically the same, as originally published.

Different isolates from *T. cruzi* have demonstrated that the parasite populations are extremely polymorphic. This polymorphism has been correlated with the pathology observed in different clinical forms and distinct geographic areas. Indeed, isoenzyme and riboprinting analyses, rRNA promoter activity, mini-exon gene sequencing and microsatellite markers have provided evidence that *T. cruzi* is very heterogeneous. This polymorphism has been correlated with the pathology observed in different clinical forms and distinct geographic areas: in Brazil, the asymptomatic form predominates (60-70%), followed by the cardiac (20-30%) and digestive forms (8-10%); in Chile, the digestive form predominates, whereas in Argentina digestive symptoms correspond to only 3.5% of the total chagasic patients. These differences are due to genetic variations of the parasite and to physiological and genetic conditions of the host, but the contribution of each is a matter of debate.

Whichever are the differences among clones and hosts in the development of the disease in humans it should be stressed that *T. cruzi* must express at the surface ligands that recognize specific host receptors allowing adhesion to the extracellular matrix macromolecules and, finally, to cells.

In the beginning of the 1980s our laboratory described the existence of a group of closely related glycoproteins, originally called Tc-85, which were specifically expressed at the surface of the infective trypomastigote stage. Monoclonal antibodies against these glycoproteins were able to inhibit partially the interiorization of parasites into host cells. Further studies by several groups detected a gene superfamily of which trans-sialidase is a member. This group was called the gp85/trans-sialidase gene superfamily. The existence of this family was entirely demonstrated by the TriTryp effort showing the existence of approximately 700 genes and similar number of pseudogenes with high degree of homology, performing 1-2% of the parasite genome. These genes express the corresponding glycoproteins at the parasite surface with a half-life of 3.5 hours after which they are shed in membrane vesicles. One of the cloned members of the gp85/trans-sialidase superfamily has an affinity to laminin, but not to gelatin or fibronectin and it was found that the binding site is mainly located at the N-terminal sequence. Another clone produced a protein with higher affinity for fibronectin in comparison to laminin. An independent approach using the SELEX method provided RNA aptamers with strong affinity to trypomastigotes that were displaced by fibronectin, laminin, heparan sulfate and thrombospondin, respectively, showing expression of ligands with specific receptor affinities on the surface of the parasite. These aptamers, notably the one displaced by laminin, were able to inhibit partially the entrance of trypomastigotes into epithelial cells.

The whole superfamily possesses a constant peptide sequence that is invariant among members, located at the carboxyterminus, VTV*X*NVFLYNR, which is called FLY domain, for short. This peptide binds to epithelial cells in a saturable manner. An attempt to identify the receptor of that sequence on the cell surface led to cytokeratin-18 (CK-18), although its existence on the membrane is debated. The binding of the peptide promotes dephosphorylation of CK-18 that leads to cytoskeleton reorganization and activation of the ERK1/2 signaling cascade. As a result, there is an increase of parasite entry into epithelial cells.

From the site of insect bite where parasites reside almost undetected by the immune system (stealth parasites) until reaching lymphonodes or the arterial circulation, the trypanosomes will eventually have to cross the arterial walls and fall into the extracellular matrix. *T. cruzi* invades almost any cell, including experimentally enucleated cells, but does not invade red blood cells. This promiscuity needs, at least, ligands with different specificities and equally specific receptors. If one imagines a sequential series of bindings and detachments it may require also, as one of the possible mechanisms, hydrolysis by protein and carbohydrate hydrolases. Thus, the parasite could be freed of the

successive haptotactic interactions, allowing its migration through the basal lamina and the extracellular milieu.

The literature is rich in data involving many molecules at the trypomastigote surface that bind to fibronectin, laminin, heparan sulfate, heparin, thrombospondin-1, collagen, sialic acid, TGFβ and bradikynin receptors, among others. Certainly, *T. cruzi* developed many different paths to progress inside a vertebrate to reach the host cells of the target tissues.

Financial support: FAPESP and CNPq

PLENARY CONFERENCE

Plenary conference

P – 01 - HYDROGENOSOMES, MITOSOMES AND IRON-SULFUR CLUSTER ASSEMBLY

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It is generally accepted that mitochondria are eukaryotic organelles, which evolved from alpha-proteobacterial endosymbiont. However, a mutual benefit for the host and its endosymbiont which led to evolution of mitochondrion is widely debated. Various benefits have been proposed including an oxygen-detoxification, a hydrogen-production or generation of ATP. Here it is considered FeS cluster assembly pathway (ISC) as an alternative benefit which might raise the original selection pressure for retaining an endosymbiont by the host. Studies of organismal diversity showed that majority of typical mitochondrial pathways could be highly modified or absent under specific environmental conditions or at certain developmental stages. In contrast, FeS cluster assembly seems to be the only essential mitochondrial function, which is invariably present. This pathway is conserved across all eukaryotic supergroups from unicellular eukaryotes to humans. Proteins of ISC machinery are conserved even in highly modified or reduced mitochondria such as hydrogenosomes and mitosomes, which were found in parasitic as well as free living protists. In mitosomes of *Giardia intestinalis*, *Spironucleus vortens*, microsporidia and *Cryptosporidium parvum*, the FeS cluster assembly is the only known function of these organelles. Phylogenetic analyses suggested that all key components of ISC machinery are of proteobacterial origin. Another pathway mediating FeS cluster assembly is bacterial NIF system primary involved in maturation of nitrogenases. As an exception, in *Entamoeba histolytica* as well as related free-living *Mastigamoeba balamuthi*, the mitochondrial type of ISC machinery was replaced, by the bacterial NIF system, which operate in their cytosol. Finally, SUF machinery has been found in eubacteria, archaebacteria and plastids. Interestingly, SUF is the only system involved in FeS cluster assembly in a specific group of archaebacteria called the *Crenarchaeota* (eocytes) which was suggested as the prokaryotic antecedents of eukaryotic cells. Thus, the acquisition of ISC system from alpha-proteobacterial endosymbiont might be profitable for such a host.

P – 02 - The central role of protists in terrestrial nutrient cycling

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Decomposition of organic matter, derived from previously living tissues, recycles nutrients through roots and as respired carbon dioxide back to the atmosphere for photosynthesis. At the base of the decomposition food web, bacteria and fungi secrete enzymes that begin the process. The food web is complex and interconnected. Most soils have 10^6 to 10^8 cells.g^{-1} active protists with peak periods of activity that can reach 10^{10} cells.g^{-1}. Most species of soil protists are bacterivores, with fewer species contributing to cytotrophy, fungivory or invertebrate predation. Species with pseudopodia and filopodia explore and feed in crevices inaccessible to the cell itself. Thus the grazing impact on the bacteria biomass is not negligible. The main competing grazers are bacterivorous nematodes and a few other invertebrates. Data on field abundance dynamics, trophic interactions, stable isotope and radioactive isotope analysis are useful tools to decipher the interactions and quantify biomass turnover and nutrient cycling. We can then estimate the protist contribution to biogeochemistry through grazing. In considering the functional ecology of soil protists several questions challenge us. Why so many bacterivorous species? How much niche overlap is there among protists? What opportunities does environmental genomics offer protist ecology?

P – 03 - Deep phylogeny of protozoa and the evolution of body plans

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I shall discuss the deep phylogeny of protozoa and other protists with special reference to the results of recent multi-gene sequence trees, the evolution of protozoan body plans, and the still uncertain position of the root of the eukaryote evolutionary tree. Eukaryotes have been divided into two major groups, the unikonts and bikonts. The unikonts primarily comprise the Amoebozoa, Choanozoa, animals and Fungi, whereas the bikonts comprise the Excavata, Plantae, chromalveolates and Rhizaria. Recent multigene trees suggest that Rhizaria and centrohelid heliozoa may be derived ultimately from chromalveolates by independent losses of the red algal chloroplast that was acquired symbiogenetically by the ancestral chromalveolate. I shall give special attention to evidence for the monophyly and internal phylogeny of Amoebozoa and Rhizaria (especially Cercozoa and its remarkable diversity), to the paraphyly of Choanozoa, to Heliozoa, and to the possible polyphyly, relationships, and special evolutionary significance of Apusozoa (Apusomonadida, Planomonadida, Micronuclearia).

P – 04 - Making home into a cell: the apicomplexan way

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Host cell invasion by Apicomplexa follows a unique pathway that is not fully understood despite significant progress in the recent years. A major clue resides in its being essentially parasite driven, and leading to highjacking host cell functions and/or remodelling the host cell structure soon after entry. The major contributors to the process are two secretory organelles named micronemes and rhoptries, together with a sub-pellicular actin-myosin motor named glideosome driving a gliding type of motility. Invasion proceeds by building a vacuole derived from both host cell membrane and rhoptry components, which the invader enters using a device named moving junction. This junction between the parasite and host cell membranes is built from a complex of micronemes and rhoptry neck proteins (MICs and RONs) exocytosed sequentially at the onset of the process, and inserted respectively in both membranes. The moving junction is believed to be connected to both the glideosome and to the host cell sub membranous cytoskeleton but those links have not been discovered yet. The trigger for microneme exocytosis is related to calcium stores whereas the one for rhoptry contents release is unknown. The rhoptry bulb proteins (ROPs) are injected in the host cell cytosol following the RONs, but the precise way this occurs is still unknown, as some of the ROPs are eventually found in the parasitophorous vacuole lumen whereas others are found on the other side of the PVM, in the host cell cytoplasm or even in the host cell nucleus. ROPs proteins have recently been shown to play a major part in host cell control and parasite development, especially in *Toxoplasma gondii,* where they are more amenable to experimental investigations. In particular, the contribution of enzymes, especially kinases and pseudo-kinases has been shown to be involved in host cell genome expression changes and in parasite virulence. Despite these advances, the apicomplexan invasion process requires much more investigations to fully unravel its basic features and the diversity of its adaptations to the various niches occupied by these parasites.

CONFERENCE

Conference

CO - Chagas Disease: from discovery to the future.

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In 1908 Carlos Chagas described the flagellate *Schizotrypanum cruzi* in the mid gut of *Panstrongylus megistus*, during a malarian campaign in Lassance town, Brazil. In few months he experimentally established the whole cycle of the agent in the vector and in mammalian hosts. In the beginning of 1909, taking into account the anthropophylic habits of the vector, Chagas went back to Lassance to find his first human case of a new disease. In few years more, Chagas described the chronic forms and the congenital transmission of the disease, studying also the diagnosis, the pathogenic processes, the epidemiologic and the prophylactic basis of the new trypanosomiasis.

The celebration of this unique and magnificent scientific work is absolutely meritorious in its centenary. Following Chagas, his disciples and numerous scientists such as Emmanuel Dias clarified the clinical aspects of the disease, improved its diagnosis and launched the first governmental campaigns against the domestic vectors, started in Brazil at the beginning of the 50's. In that decade also were described the chronic digestive Chagas Disease, suspected by himself thirty years before. The first effective drugs for specific treatment appeared in the 1960 decade, as well as the modern techniques for serological diagnosis. Blood banks began to be controlled in the 80's, the same decade in which the modern drugs for chronic heart disease management appeared. Also in the 80's national programs against the vector were expanded in Latin America endemic countries, coinciding with the emergence of modern insecticides of the pyrethroid group. At the beginning of the 90's the very favorable control results obtained in Brazil and some regions of Chile, Uruguay and Argentine, stimulated South American scientists and health authorities to launch an intergovernmental collaborative initiative against vector and transfusion transmission, firstly in Southern Cone, with PAHO direct assistance and coordination. Some years more, other similar initiatives appeared in Andean, Central America and Amazonian regions, followed by Mexico and non endemic countries.

In consequence, from the highest epidemiologic data (1970-80) until today, the incidence, the prevalence and the morbidity of the disease have been decreased along all the worked areas, also contributing for this the rural-urban migration and the progressive improvement of rural dwellings, in many endemic areas. For example, PAHO (2007) estimate that the annual incidence in 19 American countries decreased from 700,000 cases in 1980 to 41,200 in 2006, as well as the prevalence from 30 million to 15 million, and the annual mortality from >45,000 to 12,500 cases. In Brazil, at present time, the main domestic vector (*T. infestans*) is considered eliminated and the detection of indoors triatomines is very low (<3%) in endemic areas. More than 98.5% of blood banks are effectively controlled and less than 0.5% of infected blood donors are found in pre transfusion screening. The congenital transmission also seems to be very low (less than 1% risk) and the infected pregnant women are becoming progressively rare in all the country.

The problems to face in the near future are sylvatic and peridomestic vectors, sustainability of epidemiological surveillance, the detection and treatment of sporadic oral transmitted outbreaks and the adequate medical care for about 2.5 million of already infected chronic patients. Research is needed for new and better specific drugs, new insecticides, more sensible and specific diagnosis, better management of severe cardiopathy and available tools to access parasitological cure and clinical prognosis. Indeed, 20 or 30 years more are required to maintain the disease prevention, and research.

Few years before his premature death, Carlos Chagas used to comment with his disciples: *"Much more than the academic glory of this discovery, the basic task and my most sincere dream is to face effectively this terrible disease ant to finish with it".* This is another example of the great character and citizenship of the talented scientist, whose work and dream were privileged inherited by ourselves.

MINI-CONFERENCE

Mini-Conference

MC-01A - STUDIES ON *LEISHMANIA* PEPTIDASES IN THE POSTGENOMICS ERA

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The publication of the *Leishmania major* genome in 2005 was a seminal landmark in the study of *Leishmania* parasites. This allowed a detailed analysis of the complement of peptidases in the parasite (the degradome) and has led to an investigation into the role of peptidases in growth, differentiation and survival. One Clan and Family of cysteine peptidases (Clan CA, family C1) play important roles in facilitating the survival and growth in mammals of *Leishmania*. The most abundant enzyme is CPB, a lysosomal stage-regulated enzyme belonging to the papain superfamily. The *Leishmania* lysosome has morphological features and intrinsic properties that change during differentiation of the parasite from the proliferative procyclic promastigote to the non-dividing infective metacyclic form. Lysosomal cysteine peptidases are important for a fully functional autophagy pathway. *Leishmania* contains many genes associated with autophagy and we have used ATG8 as a molecular marker for monitoring the pathway. Autophagosomes are most abundant during differentiation of the cell from multiplicative promastigotes to metacyclic promastigotes and from metacyclic promastigotes to amastigotes, suggesting an important role for autophagy in these remodelling processes. *Leishmania* apparently contains four types of ATG8, which are selectively cleaved by the two ATG4 cysteine peptidases of the parasite. Parasites lacking one ATG4 have a growth defect and fail to undergo metacyclogenesis. The data suggest that autophagy is required for efficient metacyclogenesis and transformation to amastigotes. Autophagy is induced when *Leishmania* is placed in starvation conditions, presumably as a survival mechanism. However, if this is unsuccessful *Leishmania* undergoes cell death. The importance of peptidases, lysosome function and autophagy in life-cycle progression in *Leishmania* will be discussed.
Supported by the Medical Research Council (UK)

MC-01B - Enzymes involved in galactosylation: potential drug targets in *Leishmania*

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The glycocalix of *Leishmania* parasites is intimately involved in parasite survival in their insect vector and in the establishment of infection in mammalian hosts. This glycocalix is extremely rich in galactose containing glycoconjugates such as lipophosphoglycan (LPG), proteophosphoglycans (PPGs) and glycoinositolphospholipids (GIPLs) that require UDP-galactose for their biosynthesis. In *Leishmania major*, the UDP-galactose to UDP-glucose ratio (1:2.2) reflects the equilibrium constant of the UDP-glucose 4´-epimerase suggesting that conversion of UDP-glucose is a major route to UDP-galactose formation. Previously, we described the identification of a leishmanial UDP-glucose pyrophosphorylase (UGP; EC 2.7.7.9) responsible for activation of glucose and have now generated a mutant deficient in this enzyme. Surprisingly, the mutant retained substantial phosphoglycosylation and intact GIPLs highlighting the existence of a UDP-glucose independent UDP-galactose salvage pathway. By database searches, we identified a UDP-sugar pyrophosphorylase (USP) and demonstrated, amongst other, its ability to generate UDP-galactose from galactose-1-phosphate and UTP. The manifest homology of this enzyme with plant USPs suggests a horizontal gene transfer from plant to *Leishmania*. In contrast, USP is absent from human. Because of their central role in galactosylation, specific inhibition of both UGP and USP is anticipated to severely affect glycocalix formation and might constitute an approach for the development of drugs.

MC-01C - UNIQUE STRUCTURE OF THE TRYPANOSOME FLAGELLAR MEMBRANE AND MOLECULAR MECHANISMS OF CILIARY PROTEIN TARGETING

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This research began with a simple question. How is a flagellar protein targeted to its specific intracellular location? The 24-kDa flagellar calcium binding protein (FCaBP) has been studied in several laboratories due to its high immunogenicity and release in shed vesicles. In the 1990s, the advent of transfection technology in trypanosomes permitted us to investigate the molecular determinants of flagellar targeting of this protein. Through a series of transfection experiments employing a variety of FCaBP truncation and point mutants, it was determined that N-terminal myristoylation and palmitoylation and calcium binding are essential for targeting to the flagellar membrane. While dual acylation explained membrane targeting, it did not explain the flagellum specificity. Earlier work by Tetley and DeSouza suggested that the flagellar membrane may be enriched in beta hydroxysterols. To investigate this, we employed the African trypanosome, *T. brucei*, for which gene inhibition by RNA interference is available. An extensive biochemical analysis of the *T. brucei* flagellar membrane revealed high concentrations of sterols and saturated fatty acids, correlating with direct observation of high liquid order by laurdan fluorescence microscopy. Thus, the trypanosome flagellar membrane possesses high concentrations of lipid rafts, discrete regions of lateral heterogeneity in plasma membranes that serve to sequester and organize specialized protein complexes. Consistent with this, the FCaBP orthologues in *T. brucei*, the calflagins, lose flagellar localization if the lipid rafts are disrupted by cholesterol chelation. Further, buoyancy of calflagin on a sucrose gradient is disrupted both by chelation of cholesterol and by inhibition of sphingolipid synthesis, the latter of which also leads to cell cycle abnormalities and disruption of cytokinesis. Examination of detergent-extracted flagella by scanning electron microscopy revealed discrete membrane patches associated specifically with the axoneme, suggestive of intraflagellar transport (IFT) particles. Moreover, inhibition of IFT protein synthesis leads to degradation of two of the three calflagins, indicating a relationship of calflagin to IFT, either as a component of the IFT machinery or as cargo. Additional work on FCaBP sought to explore the requirement for calcium binding in flagellar membrane targeting. Since reduction of calcium leads to a washing out of FCaBP from permeabilized cells, we reasoned that FCaBP is similar to neuronal calcium sensors, proteins that bind to the membrane only in the calcium-bound state, where the fatty acid is extruded and available for membrane association. We therefore predicted that an FCaBP calcium binding mutant would fail to localize to the membrane, due to sequestration of fatty acid as was observed in some NCS proteins. Surprisingly, the FCaBP calcium binding mutant localized to the flagellar membrane. The likely explanation for this is that the mutant can dimerize with endogenous wildtype protein, a notion supported by the finding that only wildtype FCaBP, and not the calcium binding mutant, localizes to the flagellum in *L. major*, which does not have an FCaBP orthologue for dimerization. The final aspect of our work involves the study of the palmitate modification, which is essential for flagellar membrane targeting of both FCaBP and the calflagins. *T. brucei* possesses twelve palmitoyl acyltransferases (PATs). Using PAT-specific RNA interference and calflagin localization, we determined that PAT7 is the enzyme that palmitoylates calflagin. In summary, there are specific properties of both protein and membrane that determine the flagellar membrane targeting of FCaBP and the calflagins: (i) protein dual acylation by myristate and palmitate, (ii) protein calcium binding and/or dimerization and (iii) enrichment of the flagellar membrane in lipid rafts. This work was supported in part by grants from the US Public Health Service and the American Heart Association.

MC – 01D - *Abnormal T cell traffic in Chagas disease*

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Along with the evolution of Chagas disease, lymphocyte populations undergo a series of changes in their dynamics, including expansion, differentiation, death, and migration. In mice infected with *Trypanosoma cruzi*, we showed a massive cell death in the thymus. The *ex-vivo* migration patterns of remaining thymocytes were also altered, with higher migratory responses to extracellular matrix (ECM), in association or not with chemokines. Moreover, thymocytes from infected mice exhibited higher membrane levels of ECM and chemokine receptors, and we found an increase in the intrathymic contents of ECM and chemokine ligands, including fibronectin and CXCL12. Migratory responses to respective ligands were particularly enhanced in $CD4^+CD8^+$ thymocytes, in correlation with the release of thymus-derived $CD4^+CD8^+$ cells into peripheral lymphoid organs. Peripheral $CD4^+CD8^+$ lymphocytes also showed abnormally high densities of fibronectin and CXCL12 receptors, and seem to have by-passed normal intrathymic negative selection, since they express forbidden TCR clones. Moreover, they exhibit an activated phenotype, with high levels of perforin and interferon-γ gene expression, suggesting that they may be at the origin of the autoimmune events reported in both murine and human Chagas disease. Finally, we found that circulating lymphocytes from chagasic patients also exhibit activated phenotypes and enhanced migratory response towards ECM proteins.
Financial support: Fiocruz, Faperj, CNPq/Pro-Sul (Brazil), CONICET (Argentina)

MC-02A - BEYOND "EVERYTHING IS EVERYWHERE"

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There is now overwhelming evidence from "simple" taxonomic and faunistic studies for considerable protist endemism. Thus, the "Everything is Everywhere" model has been replaced by the "Moderate Endemicity" model. Now we have to explore the reasons why some species are cosmopolitan while others have a restricted distribution. It is widely assumed that the minuteness (often < 100 µm) of the dispersal stage, viz., the resting cyst fosters cosmopolitan distribution. Although this might contribute, it cannot be the decisive factor because many "lower plants" (mosses, etc.) have restricted distribution in spite of the minuteness of their dispersal units, viz., the spores (most < 100 µm). Thus, I put forward the hypothesis that it is the "strength" of the resting cysts which decides about the distribution of the individual species. This is shown by the survival rates of ciliate cysts in deserts and rain forests: while the former remain viable for years, the latter die within a year in air-dried samples; the few survivors are typical cosmopolitans with a wide ecological range. This hypothesis also explains the great diversity of protist resting cysts. The lecture will show examples from ciliates, for instance, species which produce cyst wall precursors during the trophic stage and others which "enslave" food vacuoles to finish the cyst wall after actual encystment. Supported by the Austrian Science Foundation, FWF project P-19699-B17 and P-20360-B17.

MC-02B - TAXONOMY AND EVOLUTION OF TINTINNID CILIATES (CILIOPHORA, SPIROTRICHEA, TINTINNINA)

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The taxonomy and classification of the about 1,300 tintinnid species are mostly based on features of the lorica, which, however, is subject to a considerable phenotypic plasticity. Therefore, characters of the cell and the resting cysts, the ultrastructure and chemical composition of the lorica, and gene sequence analyses are also necessary to (i) define the species, (ii) reconstruct the evolution, and (iii) establish a natural tintinnid classification. Among the cell features, the somatic ciliary pattern and the ultrastructure of the capsules (presumably tintinnid extrusomes) are especially important. Although characteristic gene sequences could as yet not be included into the diagnoses of the tintinnid taxa, gene markers might in the future distinctly contribute to elucidate the species limits and to discover cryptic species. The phylogeny of the small subunit ribosomal RNA gene roughly matches the cladistic analysis, indicating that the complexity of the somatic ciliary pattern increased and that the hyaline and agglutinated loricae do not characterize distinct lineages, i.e., the hyaline loricae evolved several times independently. Accordingly, the hyaline as well as the agglutinated loricae can be associated with the most highly developed ciliary pattern comprising a ventral, dorsal, and posterior kinety as well as a right, left, and lateral ciliary field.
Supported by the Austrian Science Foundation (FWF; Project P20461-B17).

MC-02C - ON SOME UNIQUE MORPHOGENETIC PATTERNS OF CILIATES DURING BINARY FISSION

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Over the past two decades, numerous investigations on the morphogenesis of ciliates have been carried out by the Protozoology Group of the OUC and their collaborators. More than 90 taxa have been studies representing groups including scuticociliates, protohypotrichs, hypotrichichs, hymenostomatids and euplotids. Among these some unusual pattern of morphogenesis have been recognized: (1) a non-oxytrichid pattern in *Trachelostyla pediculiformis*: the parental oral apparatus is completely renewed by the oral primordium (OP), and the DK-anlagen develope in an unusual fashion and location, i.e. they are fragmented and develop intrakinetally within the right- and leftmost parental DKs; (2) an intermediate mode is found in *Prodiscocephalus borrori*: New OP is formed epi-apokinetally while the formation of the UM uniquely is formed by transverse division; also the FVT-anlagen develop in a primary mode and the caudal cirri form in a euplotid mode; (3) in *Kiitricha marina*, the UM-anlage in the opisthe derives uniquely from the division of the de-differentiated parental UM, no marginal row or 1st frontal cirrus are formed and the DK are developed in two ways: (i) from the posterior portion of the cirral anlagen which later generate the new DK segments, and; (ii) by the irregular proliferation of dikinetids in the long DKs, represents the ancestral mode for all known stichotrichs-euplotids; (4) in *Diophryopsis hystrix*, the single marginal cirrus is formed uniquely from the posterior end of DK-anlage, i.e. similar to way in which caudal cirri are formed; (5) in *Bergeriella ovata*, three special sets of ventral cirri are formed from the FVT-anlagen: (i) the obliquely positioned heteronomous ventral rows (VR) from the left, (ii) longitudinal heteronomous VRs from the middle and (iii) a non-migratory row from the right; (6) in *Parabirojimia similis* some of the transverse cirri develops from both the FVT- and the marginal cirral anlagen.
Supported by the Darwin Initiative (project no. 14-015).

MC-02D - OPERATIONAL APPROACHES TO SPECIES IDENTIFICATION IN MICROBIAL EUKARYOTES – AND NOW DNA BARCODES

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Darwin's legacy was to provide us with a mechanism for the origin of species. While Darwin himself may have doubted the species category, he seems to have argued that species could be imagined to differ morphologically. Like Darwin, biologists typically use operational approaches to recognize differences significant enough to infer the existence of species. The morphological species concept, the biological species concept, and the genetic species concept have at least been operationally applied to microbial eukaryotes, but there can be significant technical problems. A recent genetic approach – the DNA barcode – promises an efficient and potentially universal approach to identifying species. I will review the successes of DNA barcoding of animal species by the Canada Centre for DNA Barcoding, and then present some recent examples of the application of DNA barcodes, both cytochrome c oxidase 1 and spliced leader RNA, to identifying protists. I will conclude with some discussion of the advantages and disadvantages to the barcode initiative.
Funded by NSERC Canada and Genome Canada

MC-02E - PHYLOGENETIC RELATIONSHIPS AMONG PERITRICH CILIATES: NEW PERSPECTIVES BASED ON SMALL SUBUNIT rRNA GENE SEQUENCE DATA

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The ciliate subclass Peritrichia has long been recognized as a monophyletic assemblage comprising two orders, namely Sessilida (>750 spp.) and Mobilida (>250 spp.). In order to re-evaluate the phylogenetic relationships among the peritrichs, SS rRNA gene sequences were determined for 16 species representing seven genera, i.e. five sessilids (*Epicarchesium, Pseudovorticella, Vorticella, Zoothamnium* and *Zoothamnopsis*) and two mobilids (*Trichodina* and *Urceolaria*). Phylogenetic analyses were carried out on these, and previously published, data and the main findings were as follows. (1) Taxa with stalks that contract independently and in a spiral fashion, and their stalkless relatives, form a well-supported clade representing a refined family Vorticellidae, and separate from those whose stalks have a continuous myoneme and contract in a zig-zag fashion (Zoothmniidae). (2) *Epicarchesium* and *Pseudovorticella*, both of which have reticulate silverline systems, are more closely related to each other than to other vorticellids, suggesting that the silverline system may be phylogenetically more informative than coloniality within the Vorticellidae. (3) The mobilids never clustered with the sessilids, but instead formed a clade that is sister to the peniculines at a basal position within the class Oligohymenophorea. By contrast, the sessilids formed a clade that is sister to the hymenostomes at a terminal position within the Oligohymenophorea. Preliminary data based on α-tubulin gene sequences also support the separation of the sessilids and mobilids. If these findings are verified then it is possible that the similarity of the oral apparatus, and the reduced somatic ciliation, in the mobilid and sessilid peritrichs are a result of convergent evolution driven by their similar life-styles and feeding strategies.
Supported by: the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KZCX2-YW-417); National Natural Science Foundation of China (Nos. 30670280 and 40576072); the '100 Talents Project' of the Chinese Academy of Sciences; The Darwin Initiative.

MC-03A - GTPases and the autonomous mechanism of host cell resistance to *Toxoplasma gondii*.

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Many mammals protect themselves against T. gondii infection by means of a family of interferon-inducible GTPases, the IRG proteins. In this talk I will review the present state of knowledge about the properties and functions of IRG proteins in the context of T. gondii infection. The biochemistry of IRG protein function at the parasitophorous vacuole will be discussed, as well as the mechanism by which IRG function attenuates parasite virulence and enables the host to survive infection. I shall also discuss counter mechanisms from the parasite which in turn attenuate host resistance.

Reference: Zhao et al, 2009. Toxoplasma gondii and the immunity-related GTPase (IRG) resistance system in mice - A Review. Mem. Inst. Oswaldo Cruz. 104: 234-240.

MC-03B - ``Biological surprises" from the Giardia lamblia basal body proteome

Encystation and excystation, which are crucial to the pathogenesis of *Giardia lamblia,* are marked by dramatic cytoskeletal remodeling. However, the underlying signaling pathways are not known. Although basal bodies have been studied for >100 years, their composition and functions remain incompletely understood. As an ancient protist that is motile, differentiates, and causes disease, Giardia makes a valuable model. Since calmodulin, which is needed for excystation, localizes exclusively to the basal bodies in giardial cytoskeletons, we used it as bait to affinity purify and identify interacting proteins that may help regulate differentiation. Among the proteins that bound to calmodulin-Sepharose in a Ca-dependent manner, we found known basal body proteins, validating the approach. We also found several unexpected proteins. Exploring their functions has produced complex and interesting findings. Ribosomal proteins found in basal body proteomes are assumed to be contaminants. However, when we epitope tagged one highly conserved ribosomal protein (RP) that affinity purified with calmodulin, we found that it localized to the nucleoli. Confocal microscopy using co-staining with centrin, showed that the nucleolar RP staining overlapped with a single basal body that had been shown by Benchimol to associate with a nucleus. This suggests distinct function of a single basal body and a novel association between a basal body and the nucleolus, another incompletely understood organelle.

We also identified a unique unknown Giardia protein that affinity-purified and co-localized with calmodulin in vegetative cells. Surprisingly it is targeted to variable numbers of encystation secretory vesicles in encysting cells. This may be the first hint that ESVs may be heterogeneous and is the first direct connection between calmodulin, centrosomes and cyst wall biosynthesis. “Biological surprises” such as these may offer unexpected insights into giardial differentiation and basic cell biology.

SYMPOSIUM

Symposium

S - 01A - DISCOVERY AND HISTORY OF PNEUMOCYSTIS

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When Carlos Chagas investigated an illness of railroad workers in Lassance, Minas Gerais, in Central Brazil in 1909, he found a trypanosome and 8-nucleated cysts in the lungs of guinea pigs inoculated with blood from a patient. He postulated that the 8-nucleated cysts represented a schizogonic stage of the new trypanosome, which he called *Schizotrypanum cruzi*. Antonio Carini at the Pasteur Institute in Sao Paulo, saw similar cysts in the lungs of rats infected with *Trypanosoma lewisi.* He sent slides to the Pasteur Institute in Paris, where the husband and wife team of Pierre and Mme. Delanoě identified the 8-nucleated cysts in the lungs of rats with and without trypanosomes, and named them *Pneumocystis carinii* in 1912. Carlos Chagas redescribed the cycle of *Trypanosoma cruzi* in 1914, as the causative agent of Chagas disease. The first illness due to Pneumocystis was “plasma cell pneumonia” in the 1920s when infants in Europe, usually prematures, remained hospitalized for weeks or months, became malnourished, being bottle-fed, because parenteral nutrition was not well developed. These infants frequently died between 2.5 and 6 months, with their pulmonary alveoli filled with Pneumocystis and plasma cells in the interstitium. Werner Dutz described an outbreak in slightly older children in an orphanage in Shiraz, Iran, which he controlled by prophylaxis with sulfadoxine and pyrimethmine and a normocaloric diet. A second clinical presentation was in immunodeficient children, with congenital agammaglobulinemia, severe combined immunodeficiency, glucocorticoid treatment or chemotherapy for acute lymphocytic leukemia or other malignancies. A third clinical presentation is seen in immunosuppressed adults, either corticosteroid-treated post-transplant, or during chemotherapy with alkylating agents or antimetabolites for lymphomas, leukemias, solid tumors, or with infliximab or other tumor-necrosis-factor-α inhibitors for rheumatoid arthritis. A fourth most common clinical presentation is in HIV patients, where it is an AIDS-defining diagnosis. Because Pneumocystis appears to be host-specific, several species have been described: *P. jirovecii* in man, *P.carinii* and *P. wakefieldiae* in rats, *P. murina* in mice, *P. oryctolagii* in rabbits. Prolonged subclinical infection appears to be common, with hypercorticism, protein-caloric malnutrition or other immunodeficiencies, leading to usually fatal disease. Timely prophylaxis or treatment with the synergistic trimethoprim-sulfamethoxazole is usually effective, with precautions about drug toxicity and hypersensitivity. Atovaquone, dapsone-trimethoprim, clindamycin-primaquine, and trimetrexate-leucovorin have also been found effective with appropriate precautions. - Pneumocystis had been considered a protozoan because of the drugs effective against it, and the lack of effectiveness of amphotericin-B. *P. jirovecii* was originally decribed in 1976 according to the rules of the zoologic nomenclature. However, since 1988 several independent molecular genetic studies, placed Pneumocystis between the Ascomycetes and Basidiomycetes. Pneumocystis is an atypical fungus, because of the absence of ergosterol in its two plasma membranes in all of its developmental stages, the presence of filopodial with which they attach to host cells and each other, and the presence of glycoprotein gene rearrangements producing antigenic diversity. The genus *Pneumocystis* and the species *jirovecii,* have been redescribed in conformance with the International Code of the Botanical Nomenclature [J.Eukariotic Microbiol. 46, 89S-92S, 1999]. Transmission is likely by kissing, speaking, coughing, sneezing, with the airborne route supported by identification of Pneumocystis DNA in air samples. Most children have antibody at 2-3 years.
Organisms can be identified in sections and imprints with monoclonal antibody, by staining with hematoxylin, Giemsa, periodic acid-Schiff (PAS) and the wall of the cysts with PAS or Grocott silver. Polymerase chain reaction is useful for tissue analysis. Prolonged cell culture has not succeeded so far.

S – 01A - Natural history of *Pneumocystis* infection in men and other mammals.

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Pneumocystis was discovered in 1909 by Dr. Carlos Chagas and emerged from being considered an insignificant infection of rodents to a major, life-threatening infection of humans in few decades as a result of immunological changes in the human host. The organism was first recognized in 1942 as the cause interstitial plasma cell pneumonitis a disease killing undernourished infants in post-war orphanages. Recognition of this organism remained restricted to immunocompromised hosts like cancer or transplant patients for the next 40 years until AIDS emerged in 1981. A similar situation occurred in other mammals with mainly rat animal models implemented by administration of steroids and other immunosuppressants to mimic *Pneumocystis* pneumonia (PCP) of humans that have been, and continue to be, instrumental in advancing our knowledge of the life cycle, epidemiology, transmission, immune response, pathogenesis and response to therapy of *Pneumocystis*. Lack of a reproducible microbiological culture system for *Pneumocystis* importantly hampered knowledge beyond PCP for many years. The infection was believed to arise from reactivation of latent *Pneumocystis* organisms remaining in the lungs after the primary infection. Developments in molecular biology opened a window for a deeper understanding of this infectious agent. Now we know that Pneumocystis is a non-zoonotic fungus highly host-specific with likely as many Pneumocystis species as the species of mammalian hosts. Human *Pneumocystis* (*P. jirovecii*) and rat (*P. carinii*) have phenotypic and molecular characteristics that are among the better characterized and furthermore, the strict host specificity of *Pneumocystis* has led to the hypothesis of co-evolution. Beyond the classically known PCP, the asymptomatic infection of the immunocompetent host is increasingly attracting the focus of research. The primary infection in humans and other mammals are being studied and silent forms of *Pneumocystis* infection recognized as colonization or carrier states are increasingly detected in the general population and may have an impact in pathogenesis of chronic lung diseases like COPD. Years to come may advance diagnosis, prevention and treatment of the disease in the immunocompromised host, and bring novel discoveries in pathogenesis of this microorganism as a co-factor in lung diseases of immunocompetent children and adults.
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S - 01C - THE *PNEUMOCYSTIS CARINII* GENOME: INTERNAL VIEW

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The international effort to create a *Pneumocystis* Genome Project was launched during the 5th International Workshop on Opportunistic Protists in Lille, France, 1997 and funded by the NIH in 1999 (J.Eukaryot.Microbiol. 44:7s). There it was decided to use the most common rat population, *Pneumocystis carinii* karyotype form 1, as the primary focus of the project. Cosmids representing the telomeric ends were sequenced at the Sanger Center and published in 2005 (Keely et al. Genetics 170:1589) . The 1st version of the genomic assembly was electronically accessible in 2006 (http://pgp.cchmc.org) The mitochondrial (mt) sequence v1 was reported in 2006 (J. Eukaryot. Microbiol. 53:s154). The transcriptional profile of *P. carinii* during fulminate infection was published in 2007(PLoS 2:e423. The fully assembled and annotated *P. carinii* genome is anticipated in 2009, with reads from a deep sequencing approach (Cincinnati Children's Hospital Medical Center) filling the physical gaps. Annotation of the current sequence information has provided a wealth of information for these host-adapted fungi that are unable to be cultivated outside the mammalian lung. Most standard metabolic pathways and cellular processes were represented, including the tricarboxylic acid cycle, glycolysis, amino acid biosynthesis, cell cycle and mitochondrial function. Several gene homologs associated with mating, meiosis, and sterol biosynthesis in fungi were identified. Genes encoding the major surface glycoprotein family (MSG), heat shock (HSP70), and proteases (PROT/KEX) were the most abundantly expressed of known *P. carinii* genes during fulminate infection. Approximately 10% of the genome is devoted to a family of genes that encode surface antigens. These genes are found on all chromosomes at the telomeric ends. Sequences of 6 telomeric segments began with 1 or more unique genes, followed by members of different repetitive gene families, arranged in a head-to-tail array. PRT1 (protease)-MSR (MSG-related)-MSG (Major Surface glycoprotein) was common, suggesting that duplications of these repeats have contributed to expansion of all 3 families. Lack of synteny among the arrays suggested a rapid divergence after duplication. The intergenic spacers contained sequence motifs also present in subtelomeres, implicated in gene expression and recombination in other species. Long mononucleotide tracts were present in some MSR genes, which can induce frequent frameshift mutations, providing another mechanism to generate antigen variation. *P. carinii* genes were replete with introns which had canonical splice sites and were an average of 48 bp in size. Of note, MSG genes contained no introns. The 23kb mt genome was found to be linear, contained terminal repeats, 4 open reading frames and 20 tRNAs in addition to 17 other genes that included the standard 14 fungal mt genes. A 24-bp unit within the mt genome that repeated from one to five times was identified interior to the ends. The apparent presence of many metabolic pathways in *P. carinii,* sexual reproduction within the host, and lack of an invasive infection process in the immunologically intact host suggest members of the genus Pneumocystis may be adapted parasites and have a compatible relationship with their mammalian hosts. Supported by the National Institutes of Health and the Department of Veterans Affairs

S - 01D - *PNEUMOCYSTIS* BIOCHEMISTRY

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Studies on the biochemical nature of *Pneumocystis* is hampered by the lack of cultivation methods that enables high organism yields. Axenic cultures have been developed, which is valuable in answering some questions concerning basic cell physiology, but no available culture method provides indefinite subcultivation with significant cell proliferation. This limits direct biochemical analyses and genetic manipulations. Currently, there are two major approaches used to study *Pneumocystis* biochemistry. One is by analyzing organisms such as *P. carinii* isolated from immunodeficient laboratory rodents. Limited numbers of human-derived *P. jirovecii* have been obtained by bronchoalveolar lavage or isolated from autopsied lung tissue. The other approach is to utilize information from the organism's genome. PCR is being utilized to identify genotypes of organisms from different geographic regions, from different host species, and to detect drug-resistant strains. In the past few years recombinant proteins have been utilized to circumvent the in vitro culture problem. I will summarize recent advances in three areas of *Pneumocystis* biochemical research: the major surface glycoprotein (MSG) antigens, lipids, and *S*-adenosylmethionine (SAM).

The MSG protein is coded by a family of ~80-100 genes in the nucleus but there is only a single expression site giving rise to a single MSG expressed at the cell surface at any given time. Earlier human serological studies employed MSG of organisms isolated from rats or mice as antigens since antibodies directed against MSG from a given *Pneumocystis* species cross-reacts with MSG of other *Pneumocystis* species. More recently, refinement of human serological analysis is being developed in which recombinant MSG is used as antigen to quantify antibodies specific for *P. jirovecii* MSG.

The cellular localization of ubiquinone (CoQ) biosynthesis de novo has been examined in only a few tissues and organisms. This has been a controversial subject and many investigators currently believe it occurs only in the mitochondrion. However, work on *P. carinii* clearly demonstrated that these compounds are synthesized de novo in both the mitochondrial and the microsomal subcellular fractions.

Unlike most fungi, *Pneumocystis* lacks ergosterol but synthesizes distinct Δ^7 24-alkylsterols. Recombinant enzyme proteins in sterol biosynthesis have been expressed in heterologous systems and the effects of drugs have been tested on transformed yeast cells. Other sterol biosynthesis enzymes that are being studied as potential drug targets include squalene synthase (coded by the *erg7* gene), sterol 14α-demethylase (14DM, coded by the *erg11* gene), and SAM:sterol C-24 methyltransferase (SAM:SMT, coded by the *erg6* gene). The activity of the *P. carinii* SAM:SMT enzyme expressed in bacteria has been characterized and shown to catalyze the transfer of the first and second methyl groups at the C-24 position of the sterol side chain. Recombinant SAM:SMT expressed in *E. coli* and the ciliate *Tetrahymena* is being purified and characterized (humans do not have this enzyme).

SAM plays numerous transmethylation reactions in cells. Although *P. carinii* has the MAT gene required for SAM synthesis from ATP and methionine the pathogen apparently requires more than it actually synthesizes. Hence, the organism scavenges SAM from the culture medium or the host's lung reducing lung and plasma SAM levels. Reduced serum SAM provides a potential novel diagnostic approach to detect putative *Pneumocystis* pneumonia (PcP). Nicotine lowers the level of SAM in the lung alveolus resulting in inhibition of *Pneumocystis* proliferation. Supported by a grant from the NIAID RO1 AI064084.

S - 02A - MATING INTERACTION IN BLEPHARISMA JAPONICUM

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Among cell-cell interaction in ciliated protozoa, mating interaction between cells has attracted many investigators, because 1) vegetative cells usually stop dividing before they initiate mating interaction under a food-deprived condition, 2) conspicuous cell-cell interactions occur between complementary mating-type cells through specific chemical substances, 3) mating interaction often bring the cells to conjugation (sexual reproduction), which is the onset of the new life cycle of the ciliates. However, the molecular mechanism which induces conjugation has not been well investigated. In some ciliate species, conjugation-inducing substances (mating substances, mating pheromones or gamones) play an important role. Conjugation of *Blepharisma japonicum* is induced by specific cell-cell interaction between complementary mating-type cells, I and II, through conjugation-inducing substances called gamones. Gamone 1 is a glycoprotein of about 30 kD, synthesized and secreted by mating-type I cells, and triggers various reactions in type II cells, including cell rounding and pair formation. Gamone 1 is the first glycoprotein discovered as a conjugation-inducing substance in ciliates. We investigated whether the oligosaccharide attached to gamone 1 is indispensable for conjugation-inducing activity. We produced gamone 1 which lacks the oligosaccharide by the following two methods, 1) treatment of native gamone 1 with glycopeptidase F which removes N-linked oligosaccharides from the glycoprotein, 2) construction of a bacterial expression system. The results obtained from both systems are consistent such that the gamone 1 lacking the oligosaccharide showed a much reduced activity. It suggests that the oligosaccharide attached to gamone 1 is indispensable for inducing conjugation. Gamone 1 gene expression is particularly regulated by environmental factors such as nutrient depletion and the complementary gamone (gamone 2) which is secreted by mating-type II cells. We recently identified several genes other than gamone 1 gene, which show a conjugation-specific pattern of expression. We are now investigating the mechanism of regulation of gene expression in gamone-stimulated cells. Taken together, we hope to tell a whole story of mating interaction in *Blepharisma japonicum* in molecular terms. Supported by Grant-In-Aids for Scientific Research from JSPS to T.H.(No. 18570129)

S - 02C - Glucan-mediated self-nonself discrimination system in prey uptake by the heliozoon *Actinophrys sol*

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The heliozoon *Actinophrys sol* is a protozoan that feeds on other protozoa and small algae. The cells have pseudopodia, called axopodia, radiating from the cell body, and a number of secretory granules, or extrusomes, beneath the cell membrane. *A. sol* captures prey with its axopodia, and exocytosis of extrusomes is induced by contact with prey cells. Prey cells adhere to the axopodia, and are finally engulfed in phagosomes. Small ciliates and flagellates are usually recognized as prey, whereas other individuals of *A. sol* and inorganic materials are not ingested, indicating the presence of a system of self-nonself recognition on the heliozoon cell surface. Recently, a 40-kDa glycoprotein (gp40) isolated from *A. sol* was identified as a concanavalin A (Con-A)-binding adhesive substance used for recognizing, immobilizing and ingesting prey organisms. Isolation and characterization of gp40 showed that: 1) gp40 is a major Con-A-binding protein in *A. sol* with a molecular weight of 40 kDa, and is stored in the secretory granules known as extrusomes; 2) gp40 was purified by Con A-affinity chromatography, and the cDNA encoding gp40 was cloned and sequenced; 3) the amino acid sequence of gp40 has a glycosyl hydrolase family 16 domain and is significantly homologous to invertebrate β-1,3-glucan recognition proteins, which are pathogen recognition proteins in the innate immune system; 4) gp40 has an N-linked sugar chain and is capable of specifically binding to β-1,3-glucan, but not to β-1,4-glucan (cellulose) or lipopolysaccharides; 5) prey flagellates adhered to gp40 immobilized on agarose beads; 6) phagocytosis was induced in *A. sol* by gp40 immobilized on agarose beads; and 7) gp40 induced exocytosis of extrusomes and cell fusion in heliozoons. These results demonstrate that gp40 is a multi-functional secretory protein in *A. sol*, and is required for correct targeting to food organisms as well as in self-nonself recognition, indicative of a possible evolutionary relationship between prey discrimination by protozoa and recognition of pathogens in metazoan immunity.

S - 02D - Climacostol: an antitumor molecule from ciliated protozoa

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Until recently, plants and fungi have represented the primary source of resorcinolic lipids (or alkylresorcinols), a large group of natural compounds that have attracted the attention of some researchers for their potential use in the therapy and/or prevention of specific classes of diseases. In fact, antimicrobial, antiparasitic, genotoxic, and antitumor activities have been recognized for this class of compounds.
Climacostol is the first example of a resorcinolic lipid isolated from a protozoan, the freshwater ciliate *Climacostomum virens*, which uses it as a defense toxin against predators (Miyake et al. 2003, Eur. J. Protistol. 39: 25–36). Some years ago the compound was structurally characterized as [5-(Z)-non-2-enyl-benzene-1,3-diol], and chemically synthesized (Masaki et al. 1999, Tetrahedron Lett. 40: 8227–8229; Masaki et al. 2004, Tetrahedron 60: 7041–7048).
The availability of synthetic climacostol allowed us to investigate the *in vitro* cytotoxic activity of the molecule on a panel of five human tumor and one non-tumor cell lines.
The results obtained clearly showed that climacostol effectively inhibited the growth of tumor cells in a time- and dose-dependent manner by inducing apoptosis, with non-tumor cells proving to be significantly more resistant to it.
With regard to the mechanism of action of climacostol, it appears that, depending on the cell lines, the molecule can trigger either a canonical caspases-mediated apoptotis, or an apoptosis-like programmed cell death (PCD). In addition, mitochondria appear to be primarily involved in the pro-apoptotic action of climacostol, that induces a rapid loss of their transmembrane potential and the release of cytochrome c from the organelles.
Taken as a whole, the experimental data suggest that climacostol exerts its action on tumor cells by triggering a mitochondrion-dependent PCD. These results indicate that climacostol possesses interesting antitumor and pro-apoptotic activities and encourage further *in vivo* investigations to assess its potential use in cancer chemotherapy.

S – 03A -Functional analyses of the tetraspanin 6 membrane protein during *Trichomonas vaginalis* migration and pathogenesis

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The unicellular parasitic *Trichomonas vaginalis* is the causative agent of trichomoniasis, the most prevalent non-viral sexually transmitted disease in humans. *T. vaginalis* is an obligate extracellular pathogen and thus proteins on the surface of the parasite are likely to play critical roles in host-parasite interactions and pathogenesis. Here, we report the identification and functional characterization of *T. vaginalis* tetraspanin-6 (TSP6). TSP6 is member of the tetraspanin family, which comprises a large superfamily of cell surface-associated membrane proteins that has been implicated in fundamental biological processes, including cell adhesion, migration, fusion and proliferation in vertebrates. Our studies show that TSP6 targets to both the plasma membrane and the flagella of parasites cultured in the absence of host cells. The protein relocalizes during exposure to vaginal epithelial cells (VECs). Moreover, qPCR analysis of parasites exposed to VECs reveals a dual upregulation over time. The complex localization and expression pattern of TSP6 suggests that its intracellular trafficking and distribution must be tightly regulated during parasite pathogenesis. In order to evaluate TSP6 function, cell adhesion and migration assays were performed using parasites that over-express full length and a C-terminal truncated version of TSP6 (TSP6DCt). No effect was observed on parasites that over-express full length TSP6. In contrast a dominant negative effect was exerted by TSP6DCt. Exogenous expression of TSP6DCt significantly reduced migration through matrigel invasion chambers (a mimic of the extracellular matrix). No effect was observed in adhesion experiments when either full-length TSP6 or TSP6DCt was over-expressed. In addition, TSP6DCt does not relocalize upon exposure to VECs as observed for the full length protein. In conclusion, TSP6 appears to influence cellular migration, with the TSP6 tail being of particular importance in determining the “outside-in” signals that follow ligand engagement. The elucidation of determinants involved in the process of migration may reveal virulence factors and novel therapeutic targets to combat disease.

S - 03B - Trichomonads under stress: the pseudocyst strategy

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Tritrichomonas foetus is a flagellated protozoan parasite that causes trichomoniasis, a major sexually transmitted disease in cattle. *T. foetus* presents a simple life cycle. When grown in axenic cultures, the trophozoite is characterized by a teardrop shape, three anterior flagella and one recurrent flagellum. However, under stress conditions such as a decrease of nutrients, drugs or abrupt changes in temperature, this organism rounds up and forms pseudocysts. In this form the flagella are internalized, but no cyst wall surrounds the cell. These rounded organisms have been considered to be a degenerative form but, currently, it is believed that this form is reversible and represents a defense mechanism in unfavorable environmental conditions. Although the pseudocysts can be observed in several species of trichomonads, including *T. foetus* and *T. vaginalis*, their biological aspects are not fully appreciated by most parasitologists. Studies performed previously by our group have shown that *T. foetus* pseudocysts are capable to divide with characteristics different from those found in the pear-shaped parasites (Pereira-Neves et al., 2003). In addition, we also demonstrated that pseudocysts are able to adhere to host cells and the adhesion rate is higher than for the pear-shaped parasites. In addition, little is known about the behavior and role of pseudocysts in trichomoniasis. Consequently, questions concerning their biology, such as: (a) Are they degenerative forms? (b) Are these forms cytopathic? (c) Are pseudocysts able to endocytose? are under study by us. In an attempt to clarify the questions mentioned above, both pear-shaped cells and pseudocysts were submitted to endocytic activities using different endocytic tracers. The cytopathic effect of *T. foetus* on epithelial cells was explored comparing the behavior of pseudocysts and pear-shaped parasites under interaction with MDCK monolayers, an epithelial kidney canine cell. The conclusions were that: (1) Pseudocysts are not degenerative forms, since they are active and able to endocytose different tracers; (2) pseudocysts demonstrated a significantly higher endocytic ability when compared to the pear- shaped form; (3) *T. foetus* pseudocysts adhere to MDCK cells with much stronger avidity than the pear-shaped parasites; (4) Pseudocysts are more cytopathogenic since they exerted a higher cytopathic effect on a MDCK cell monolayer when compared to pear-shaped parasites and present a higher transformation to amoeboid form when in contact with host-cells. Furthermore, *T. foetus* pseudocysts undergo mitosis by a different process from that of pear-shaped parasites. Studies in course by our group have shown that under stress conditions, *T. foetus* pseudocysts proceed with nuclear and mastigont division without corresponding cytoplasmic division creating a polymastigont cell. When these cells are again under favorable environment, single organisms are seen budding out from the polymastigont cell.

Since *T. foetus* leads to considerable economic losses worldwide, thus, the discovery of an infective and more cytotoxic form will open new directions for research focused on treatment and knowledge of diseases caused by this parasite.

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S - 03C - Elucidation of cellular and molecular mechanisms powering *Entamoeba histolytica* motility during human amoebiasis

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Our project aims to understand the role of crawling cell motility in the mechanism sustaining the invasion of human tissues by the amoebic parasite *Entamoeba histolytica,* the agent of amoebiasis. Our specific objective is to dissect the mechanism activating *E. histolytica* motility leading to the human infection by i) determining how *E. histolytica* became a polarized cell during the infection and forms a pseudopodia and ii) deciphering the role of inflammatory molecules in parasite activation for motility. Amoeboid movement is a rapid motion characterized by flexible oscillatory cell shape changes with dynamic and multiple membrane protrusions and retractions. The challenges raised by parasite motility are closed related to those raised by motile immunocompetent cells, which like *E. histolytica*, undergoes directed motility in chemotatic environments. Specific regulations allow these cells to displace in different environments such as blood, mucus, epithelia and lymphatic circulation. The membrane of cells in amoeboid motion deforms at a high frequency resembling cells with their plasma membrane detaches from the cytoskeleton showing prolonged blebbing while spreading. Then, according to the environment properties, *E. histolytica* polarizes and protrude an unique membrane extension called pseudopodia. Imaging analysis of *E. histolytica* motility in diverse biological conditions allow to conclude that depending on the environment, *E. histolytica* displacements can be split in directed or random modes. Random displacements are observed in the absence of chemotactic stimulus, and their definition is essentially based on the statistical and geometric characteristics of cell trajectories: very short persistence length, isotropically distributed trajectories within a large variety of cell trajectories. A striking feature of *E. histolytica* motility is that random displacements occur together with very active blebbing activity. Direct motion occurs during chemotaxis, in which the persistence of trajectories is sustained and directionality is defined by the concentration of a chemical compound attracting the cell. We demonstrate that key elements of human inflammatory response during amoebiasis, such as the tumour necrosis factor and interlukine-8, are chemoattractant for the parasite.

Key references:

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S - 03D - DISRUPTION OF THE RNA INTERFERENCE PATHWAY ABOLISHES ANTIGENIC VARIATION IN THE INTESTINAL PARASITE *GIARDIA LAMBLIA*

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Giardia, a parasitic protozoan of humans, is a major source of waterborne diarrheal disease worldwide. *Giardia* is also an excellent system to study the evolution of cellular processes since it belongs to the earliest branch of the eukaryotic lineage. *Giardia* trophozoites undergo fundamental changes to survive outside the intestine of their host by differentiating into infective cysts. Encystation entails the synthesis, processing, transport, secretion and extracellular assembly of cyst wall components. To survive within the intestine, *Giardia* go through antigenic variation, a process by which the parasite continuously switches its major surface molecules, allowing the parasite to evade the host's immune response and produce chronic and recurrent infections. The main goal of our laboratory is to better understand the basic molecular mechanisms involved in *Giardia* adaptation and differentiation. Regarding antigenic variation, one variant-specific surface protein (VSP) is expressed on the surface of each trophozoite at a particular point in time, from a repertoire of approximately 190 *vsp* genes present in the parasite's genome. Here, we show that *vsp* expression is regulated by a mechanism that involves both RNA-dependent RNA polymerase (RdRP) and Dicer homologs, known components of the RNA interference (RNAi) pathway. Unlike *Plasmodium falciparum* and *Trypanosoma brucei*, where transcriptional silencing of non-expressed VAR and VSG genes occurs, *Giardia* clones transcribe many *vsp* mRNAs but only accumulate transcripts encoding the single surface antigen that is expressed on the parasite's surface. Detection of antisense RNAs corresponding to the silenced *vsp*s and small RNAs from the silenced but not for the expressed *vsp* implicate the RNAi pathway in antigenic variation. Additional findings show that an epigenetic process, involving particular histone modifications, play a role in the variable expression and, therefore, on the concentration of individual VSP transcripts, which, in turn, influence the selection of the VSP that can circumvent the silencing process and be expressed on the trophozoites surface. Remarkably, knockdown of Dicer or RdRP leads to a change from single to multiple VSP expression in individual trophozoites. These results demonstrate the involvement of a post-transcriptional gene silencing (PTGS) mechanism in regulating the expression of surface antigens in *Giardia*. Infection of experimental animals with these multiple VSPs-expressing trophozoites protect them to subsequent infections with individual clones, providing direct evidence regarding the importance of variable surface antigens of pathogens in the establishment of the infection and on evading the host immune response. Our results also indicate that the manipulation of the mechanisms of antigenic variation in *Giardia*, and potentially in other human parasites, could facilitate the development of vaccines against important pathogens.

Supported by CONICET, FONCYT, HHMI, and EU.

S – 04A - *Toxoplasma gondii*: Master of survival at the wildlife- domestic animal-human interface

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With global distribution and capacity to potentially infect all cell types in most warm blooded animals, *Toxoplasma gondii* is arguably the most successful zoonotic parasite in the world. Although the parasite's ability to undergo sexual multiplication in the feline intestine is not essential for the sustainability and expansion of the parasite population, it provides *T. gondii* with an opportunity for greater survival through genetic recombination and wider distribution in the environment with the dissemination of oocysts in the feces of the felid definitive hosts. The increasing popularity of cats as pets, and common practice of allowing these cats to deposit feces outside, as well as the maintenance of feral cat colonies provides ample opportunities for *T. gondii* oocysts to enter the environment and be transmitted to other animals, including humans and wildlife. Results will be presented from our National Science Foundation-funded Ecology of Infectious Disease research undertaken 2005-2009 to understand the ecological determinants of *T. gondii* transmission from wild and domestic terrestrial felids to the threatened southern sea otter population in coastal California. Detailed studies on sea otters and their prey have provided clues to potential sources of infection and the association of infection with dietary specialization which otters may have adapted to accommodate limited food resources. Surrogate microspheres which mimic the behavior of oocysts in aquatic habitats are also being utilized in transport studies to test the hypothesis that estuarine wetland degradation increases the transport of *T. gondii* oocysts from land to sea. The data collected in these transdisciplinary studies are being used to develop predictive models to evaluate the impact of changes in cat abundance, infection prevalence, habitat structure and prey selection, with the aim to guide management strategies to reduce the exposure of humans and sensitive wildlife species to *T. gondii.*

(Characters with spaces allowed are 2000 and this has 1996)

S - 04B - Animal reservoirs of human sleeping sickness: identification by multilocus genotyping

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Human infective African trypanosomes have been known to infect wild game and livestock since the early 1960's when a series of human volunteer experiments were undertaken. The epidemiology and role of non-human hosts in the spread and maintenance of the human disease is complicated by the existence of three morphologically identical sub-species of which two are human infective. This fact has stimulated research into methods for defining human infective sub-species and evaluating the role of the animal reservoirs in the maintenance of the disease in humans and the origins of outbreaks. The availability of the genome sequence of *T.brucei* has facilitated the development of molecular markers and these have been used to address a range of questions about the nature of the human infective parasites and the role of reservoir hosts. In this paper, we will review these developments and present our current state of knowledge about the evolution and diversity of these parasites. Specifically the following questions will be addressed: (1) How important is the animal reservoir? (2) Did human infectivity arise once or several times? (3) Does genetic exchange occur and how stable are the parasites over time? (4) Does the existence of an animal reservoir offer new approaches to control?

S - 04C - MOLECULAR EPIDEMIOLOGY OF DOMESTIC AND SYLVATIC *TRYPANOSOMA CRUZI* INFECTION IN THE GRAN CHACO REGION

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The structure of *Trypanosoma cruzi* transmission cycles includes a wide diversity of mammal hosts, triatomine bug species and parasite lineages. Domestic and sylvatic transmission cycles show different degrees of overlapping. Few such studies have been conducted in the Gran Chaco, a hyperendemic region for Chagas disease where *Triatoma infestans* is the main vector. Could the introduction of sylvatic *T. cruzi* parasites pose a threat to domestic transmission control efforts? As part of a regional study aimed at modeling the transmission dynamics and control of *T. cruzi* in the Gran Chaco, we assessed the distribution of *T. cruzi* lineages (identified by PCR strategies) in *Triatoma infestans*, domestic dogs, cats, humans and sylvatic mammals in the Argentinean Chaco. *T. cruzi* lineage II predominated among the 99 isolates characterized and lineage I among the six isolates obtained from sylvatic mammals. *T. cruzi* lineage IIe predominated in domestic habitats. Domestic and sylvatic cycles overlapped in our study area in Santiago del Estero in the late 1980s, when intense domestic transmission occurred, and still overlap at present though marginally. The household distribution of *T. cruzi* lineages showed that bugs, dogs and cats from a given house compound shared the same parasite lineage in most cases. This result lends further support to the importance of dogs and cats as domestic reservoir hosts of *T. cruzi*. The introduction of *T. cruzi* from sylvatic into domestic habitats would occur very rarely in the current epidemiological context of rural communities under sustained vector surveillance in the Argentine Chaco.
Supported by NIH, IRDC, TDR, UBA.

S - 04D - ZOONOTIC BABESIAS: IS THERE AN AFRICAN CONNECTION?

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Babesia parasites have been known to science for 120 years, but the first human cases were recorded only 50 years ago. Today, human babesiosis is regarded as an emerging disease. Traditionally, *Babesia* species were described on morphology and host. It is now realised that this gave an incomplete picture; molecular characterisation is revealing that the single species of the past are frequently species complexes, which are not necessarily closely related. This stresses the importance of research in this field, especially in characterising the babesias of wildlife, which could pose potential threats to humans. Although tick-borne diseases are rife among African livestock and wildlife, confirmed human babesiosis cases are extremely rare on that continent. The distribution of human babesiosis and malaria seem to be mutually exclusive, and most human babesiosis cases are reported from the temperate regions. Is this a true reflection of the situation, or are human babesiosis cases generally misdiagnosed as malaria in malaria-endemic areas?
Supported by the National Research Foundation of South Africa.

S – 05A - WHAT GEOSTATISTICS CAN TELL US ABOUT THE ECOLOGY OF CILIATES AND OTHER PROTISTS?

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Protists, including benthic foraminifera and diatoms, planktonic ciliates, soil ciliates and amoebae, are patchy in their distributions. As the spatial distribution of organisms is well known to affect energy flux and food web structure in ecosystems, we must characterise patchiness of protists if we wish to assess their ecological function. Of course, the concept of patchiness can be directly or intuitively perceived in macroscopic organisms, but the patchiness of protists is often not so obvious. However, it can be described and analysed. Our goal in this presentation is to introduce the audience to a useful suit of methods that characterise patchiness: geostatistics, originally developed to evaluate the distribution of minerals for the mining industry, was first applied to ecological problems in the 1980s. In an ecological context, geostatistics is a suite of methods that quantify the spatial patterns of ecological phenomena and provide error-estimates associated with these predictions – we will introduce these in a user-friendly fashion. Then we will illustrate how, using these tools, our work over the last 10 years has characterised the presence and extent of patches focusing on abundance, biomass, production, and biodiversity of protists in a broad range of aquatic ecosystems. Then, we will show how we have used these measures of patchiness of biota in conjunction with measurements of abiotic factors (e.g. water fluxes, seasonal changes) to predict the ecological driving forces that cause patchiness. To this end we have combined geostatistics, multiple regression analysis, and principal component analysis to explain why patches might occur. Finally, we will suggest that the next step is to move towards an approach that assesses the underlying processes that generate the spatial structure in protists communities through analysis of the spatial pattern itself.

S - 05B - REASSESSING THE ROLE OF CILIATES AND OTHER PROTISTS IN THE DIETS OF MARINE FISH LARVAE

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For more than 100 years it has been known that fish larvae consume protists, and there is a growing awareness that they may be important in the diet of pelagic marine fish larvae. Despite this, fish ecologists and fisheries scientists remain reluctant to include protists when assessing the food web dynamics that regulate the survival and recruitment of larval fish. Although not intended as an exhaustive review of the subject, this presentation considers why researchers do not pay more attention to protists in larval fish diets and how we might correct this oversight. To support the contention that this link must be explored in more detail, we briefly review three areas of study that have long recognised the importance of protists in fish larvae diets: the amateur aquarium trade, the commercial aquaculture industry, and experimental research on fish. We next consider the consequences of not directly linking protists and larval fish in food web models, by reviewing the literature and making back of the envelope calculations as to their importance. Finally, by assessing the scientific mindsets that have led to this oversight we offer suggestions for future study and recommendations for developing methodologies to best establish the impact of fish larval grazing on protists. Our ultimate aim is to indicate that this link is important and encourage researchers to pursue it by the proper use of existing methods and the application of new technologies.
Supported by the Royal Society.

S - 05C - PERITRICHS AS EPIBIONTS ON AQUATIC INVERTEBRATES

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Epibiotic relationships are a widespread phenomenon in marine, estuarine and freshwater environments, and include diverse epibiont organisms such as bacteria, protists, rotifers, and barnacles. Despite its wide occurrence, epibiosis is still poorly known regarding its consequences, advantages, and disadvantages for host and epibiont. Among protists, the ciliates in the Sub-Class Peritrichia are commonly found living as epibionts in aquatic invertebrates, especially crustaceans and mollusks. Surveys performed in the United States and in Brazil demonstrated that the peritrich genera *Zoothamnium*, *Epistylis*, and *Opercularia* are the most commonly found as epibionts on crustaceans and mollusks. In this presentation I will give a general overview about the epibiotic relationship between aquatic invertebrates and peritrichs emphasizing aspects of the life cycle of the ciliate epibiont.

S - 05D - ECOLOGICAL IMPLICATIONS OF PARASITISM IN CILIATES AND OTHER PROTISTS

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Heterotrophic and phototrophic protists are infected by a wide variety of pathogens including viruses, bacteria, and eukaryotic microbes. The importance of the pathogens to the ecology of host organism, or the structure and function of microbial food webs has gained increasing attention over the past several decades. Among the better studied hosts are phototrophic species that form algal blooms in freshwater and marine ecosystems. Lytic viruses are now recognized as major causes of phytoplankton mortality with the potential to cause population level changes on time scales of hours to days. Likewise, epizootics produced by chytrid fungi, Perkinsozoa, heterotrophic microflagellates, and parasitic dinoflagellates have been linked to mass mortality of hosts leading to suppression or retardation of phytoplankton blooms and selective effects on species composition leading to successional changes in phytoplankton communities. Much less effort has been expended to understand the importance of parasitism to the ecology of heterotrophic protists. Nonetheless, parasitic dinoflagellates have been shown to cause mortality of ciliate microzooplankton equivalent to grazing pressure of dominant macrozooplankton. Recent work in estuarine systems on the east coast of North America has shown widespread occurrence of parasitic dinoflagellates in many ciliate taxa. In some instances, different parasites compete for host resource, with co-infection occurring in the same host individual. In other settings, different parasites are restricted to a given host species. Parasite induced mortality may alter the ciliate population structure by influencing relative abundance of taxa, causing a shift in the size of individuals in a host population, and inducing production of dormant stages by host species.

S – 06A - The new eukaryotic systematics and its implication for the evolution of membrane trafficking

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Our understanding of eukaryotic relationships has changed dramatically in the past 15 years. A more complete understanding of sequence evolution, an appreciation of the value of ultrastructural information and the advent of large-scale multigene datasets drawn from protist genomics has unseated the old paradigm based on ssu rDNA gene phylogenies. Instead the diversity of eukaryotes appears divided into 6 eukaryotic supergroups, with recent analyses providing crucial resolution amongst them at the highest level. Globally important parasites, as well as organisms formerly thought to be primitive eukaryotes, are spread across the eukaryotic tree. This new phylogeny of eukaryotes provides important context for interpreting parasitological and evolutionary cell biological data, as illustrated by reconstruction of the membrane-trafficking machinery present in the last common eukaryotic ancestor. Instead of a primitive or simple set of endomembrane machinery, the ancestral eukaryote appears to have possessed a sophisticated cellular trafficking system. New data, from several critical free-living relatives of parasitic protists including *Naegleria gruberi* and *Guillardia theta* will be presented elaborating on this complexity addressing both the overall trafficking machinery and specific analyses of the ArfGAP gene family. Supported by a start-up grant from the University of Alberta.

S - 06B - MITOCHONDRION-DERIVED GENES AND ORGANELLES IN FREE-LIVING ANAEROBIC PROTISTS.

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More than a dozen independent protist lineages are adapted to life in low oxygen environments and, as a result, possess modified mitochondria. These ‘mitochondria-related organelles’ (MROs) have been best studied in parasitic taxa such as the trichomonads, entamoebae, diplomonads, chytrid fungi, *Blastocystis* and Microsporidia. From these studies, two broad categories of MROs have emerged: hydrogenosomes and mitosomes. Yet, studies of the diversity of free-living anaerobic protists in the environment have revealed many possibly ‘new’ MROs exist in this organisms. Here I focus on current advances in understanding the MROs of three free-living protists: the amoebozoan *Mastigamoeba balamuthi*, the heteroloboseid *Sawyeria marylandensis* and the jakobid *Andalucia incarcerata*. Expressed sequence tag studies and followup immunocytochemical studies indicate that while there are conserved core pathways/systems observed in most MROs, there are a variety of auxiliary functions of these organelles. The functional spectrum of MROs is continuous and significantly overlaps with classical aerobic mitochondria.
Supported by the Canadian Institutes for Health Research and a grant to CGEB from the Tula Foundation.

S - 06C - *Chromera velia* – an extraordinary missing link

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Most parasitic Apicomplexans, including *Plasmodium*, *Toxoplasma*, *Eimeria* and other medically important members, contain a highly reduced plastid termed the apicoplast. Due to the reduction of its genome, it was so far impossible to convincingly trace its evolutionary origin. *Chromera velia* is a marine protist associated with corals, which has been recently shown to share the common origin with the Apicomplexa. We demonstrate that *C. velia* also shares features known only from the plastids of dinoflagellates, such as plastid gene minicircles. However, contrary to the peridinin-type dinoflagellates, the plastid of *C. velia* contains, in addition to minicircles, a large plastid linear molecule of about 120 kb. Moreover, we demonstrate that the only gene found so far in the mitochondrial genome of *C. velia*, cytochrome oxidase subunit 1, is also found on various minicircles. Both organellar genomes show unique features as well as synapomorphies with the dinoflagellates and/or apicomplexans.

S - 06D - THE GENOME OF NAEGLERIA GRUBERI ILLUMINATES EARLY EUKARYOTIC VERSATILITY

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Comparisons between genome sequences from diverse free-living eukaryotic lineages can illuminate core features of contemporary and ancient eukaryotes. We sequenced the 41 Mbp genome of the free-living and heterotrophic protist, the amoeboflagellate *Naegleria gruberi*. The single-celled *Naegleria* transitions between amoeboid and flagellate forms and belongs to a diverse, exotic and little-understood eukaryotic lineage – the Heterolobosea – that is well placed phylogenetically for uncovering early eukaryotic complexity. Features shared between *Naegleria* and any other major eukaryotic group likely existed in their common ancestor, permitting inferences about early eukaryotic evolution. Genome sequencing efforts have focused primarily on opisthokonts and plants, or parasitic protists. This limited sampling of eukaryotic diversity has left gaps in our understanding of most major eukaryotic lineages. This is the first genome from a member of the Heterolobosea, and one of the few genomes from a free-living heterotrophic protist. We assembled the *N. gruberi* genome from ~8-fold redundant coverage of random paired-end shotgun sequence using genomic DNA prepared from an axenic, asexual culture of the NEG-M strain (ATCC 30224). Over 99% of 38,211 ESTs align to the draft assembly. The genome's 15,727 predicted genes encode an unprecedented metabolic flexibility including aerobic respiration and anaerobic metabolism with concomitant hydrogen production, a finding with fundamental implications for mitochondrial evolution. *Naegleria*'s amoeboid nature allowed us to create the first catalog of genes associated with amoeboid motility via phylogenetic profiling. Our genomic analysis, the first of a free-living species from JEH (jakobids, euglenozoa, heteroloboseans), substantially extends the idea that early eukaryotes possessed complex trafficking, cytoskeletal, metabolic, signalling, and regulatory modules and emphasizes subsequent losses, particularly in parasitic lineages. These extensive capabilities were required by the long-extinct common ancestor and are still needed for *Naegleria*'s versatile cellular behavior.

S - 06E - Progress towards a genome sequence for the free-living Kinetoplastid *Bodo saltans*.

Andrew Jackson
Wellcome Trust Sanger Institute

B. saltans is a free-living, Kinetoplastid flagellate common in freshwater systems throughout the world. It is also among the most closely related free-living Kinetoplastids to the parasitic trypanosomatids, which include human pathogens such as *Trypanosoma brucei* (African sleeping sickness), *T. cruzi* (Chagas disease) and *Leishmania* spp. (Leishmaniasis). Since 2005, we have had access to completed genome sequences for these parasitic trypanosomatids and these have demonstrated that, while they have many fundamental commonalities at the genome level, those aspects specifically associated with disease are generally species-specific and appear mutually exclusive. We have chosen to resolve the genome sequence of *B. saltans*, to establish a free-living 'outgroup' with which to base comparative analyses of genome content and function. It is hoped that this will resolve many of the enigmatic features of trypanosomatid genomes and provide a full account of their similarities and differences in terms of gene gain and loss, contrasting ancestry and innovation. Overall, we anticipate that, together, the *B. saltans* and trypanosomatid genome data set will idfentify the genomic changes accompanying the evolution of parasitism in this phylum. Here, we present preliminary findings from the *B. saltans* genome, its gene content and gene order relative to *T. brucei* and *L. major*, and report the latest progress in this on-going project.

S - 06F - WHAT DO *CARPEDIEMONAS* AND LIKE ORGANISMS TELL US ABOUT THE EVOLUTION OF PARASITIC DIPLOMONADS?

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Diplomonads (Diplomonadida) are a predominantly parasitic group of protists that is remarkable for having simplified and/or reduced endomembrane systems and mitochondrial organelles - the latter in the form of 'mitosomes'. In addition to their significance as pathogens, for more than two decades diplomonads have held a position of unique importance in accounts of the origin and evolution of eukaryotic cells. Until recently, however, the immediate phylogenetic affinities of diplomonads had received little attention, and thus our understanding of the evolution of diplomonads themselves has been limited. This is now changing through the ubiquity of molecular phylogenetics, and expanded efforts to culture poorly known flagellates, especially free-living forms. Two groups are traditionally considered as close relatives of diplomonads - retortamonads and enteromonads - both also predominantly parasites or commensals. Molecular phylogenies support these affinities, although enteromonads prove to fall polyphyletically within the diplomonad clade. Relatively recently it was established through multiple gene phylogenies as well as comparative morphology that a small free-living flagellate called *Carpediemonas membranifera* was also related to diplomonads and retortamonads. Within the last five years ~20 additional isolates of '*Carpediemonas*-like' organisms have been cultured, and these divide into five additional major clades, including *Dysnectes*, *Hicanonectes*, and others still unnamed. All of these *Carpediemonas*-like organisms are free-living marine anaerobes/microaerophiles, and most have 'excavate'-type feeding grooves, as well as other cytoskeletal similarities. Wherever studied they have conspicuous but cristae-lacking mitochondrial organelles that more closely resemble the hydrogenosomes of parabasalids than the mitosomes of diplomonads. Molecular phylogenies confirm the affinities of these organisms with diplomonads. However, the clades are highly distinct from one another on SSUrRNA gene trees, and ongoing work using multiple molecular markers aims to establish the relationships amongst these lineages, to determine whether they form a single clade, or whether they actually represent a series of branches at the base of diplomonads. Irrespective of the history within the diplomonad-retortamonad clade, *Carpediemonas* and like organisms pinpoint the origin of diplomonads among free-living bacterivores that already possessed atypical mitochondrial organelles of some form, but otherwise are similar to mitochondrion-bearing flagellates such as jakobids.

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S – 07A - PROTEOMIC INSIGHTS INTO THE STRUCTURE AND FUNCTION OF THE TRYPANOSOME FLAGELLUM.

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The 9+2 microtubule axoneme of eukaryotic flagella and cilia represents one of the most iconic structures built by eukaryotic cells and has been highly conserved through eukaryotic evolution. Our proteomic analyses have identified several hundred trypanosome flagellum proteins and these datasets, allied to the availability of tractable reverse genetics tools, have helped establish *Trypanosoma brucei* as a popular model for studying eukaryotic flagellar biology. Since many aspects of human health are dependent upon the assembly of functional flagella/cilia studies on trypanosome flagella can also provide important mechanistic insights into inherited human diseases characterized by defective cilia the so-called ciliopathies. Of course *T. brucei* is also a pathogen of significant medical and veterinary importance, causing devastating disease in humans and other animals in sub-Saharan Africa. There is little hope of a vaccine and a desperate need for modern drug therapies. Interestingly, we have shown that bloodstream trypanosomes are exquisitely sensitive to perturbations in flagellum function and since flagellar motility is essential for cytokinesis the trypanosome flagellum has been identified as a novel drug target in African sleeping sickness. In my talk I will provide an overview of our proteomic studies on the trypanosome flagellum, as well as present data derived from recent, and ongoing, studies that shed light on the recruitment and targeting of trypanosome proteins to the flagellum compartment.
Supported by the Biotechnology and Biological Sciences Research Council.

S - 07B - CHARACTERIZATON OF MESSENGER RNA-ASSOCIATED PROTEINS IN *TRYPANOSOMA CRUZI*: POSTTRANSCRIPTIONAL REGULATORS OF GENE EXPRESSION?

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In trypanosomatids, the regulation of gene expression is principally posttranscriptional. In eukaryotes, mRNAs processed in the nucleus may be transported to the cytoplasm, where their expression may be modulated by association with specific sets of proteins, forming messenger ribonucleoprotein complexes (mRNPs). The mRNAs forming these mRNP complexes may be translated (polysomal mRNPs) or stored in the cytoplasm (polysome-free mRNPs), forming RNA granules given different names according to the proteins with which they are associated (e.g., P-bodies, stress granules). We have previously provided evidence for the existence of stable mRNAs not associated with polysomes. We have also characterized TcDhh1, a DEAD box RNA helicase considered to be a marker of P-bodies. Other groups have provided evidence for the presence of stress granules in *T.cruzi*. We investigated the composition of these granules further, by carrying out mass spectrometry characterization of the proteins of the mRNPs immunoprecipitated with anti-TcDhh1 antiserum. We also used a ribonomic approach to identify the RNAs associated with the immunoprecipitated complexes. We found that TcDhh1-associated mRNAs were regulated in a stage-specific manner and that these mRNAs were present in smaller amounts in epimastigotes than in parasites at other developmental stages. This suggests that TcDhh1 granules are specialized structures playing an important role in the regulation of gene expression in *T.cruzi*.
We investigated the dynamic association between proteins and mRNAs, by using poly(T) beads to purify the mRNP complexes from polysomal and postpolysomal fractions from exponentially growing epimastigotes and epimastigotes subjected to nutritional stress. A mass spectrometry-based comparison of protein contents led to the identification of 542 RNA-binding proteins. Some of these proteins (24) were found in all fractions, but several proteins exclusive to individual fractions were also observed, facilitating a more complete analysis of *T. cruzi* mRNP composition. The association of specific sets of proteins with mRNAs may play a key role in the cytoplasmic regulation of gene expression in *T.cruzi*.
Most of the work described here was carried out by Fabiola Holetz, Lysangela Ronalte Alves, Alejandro Correa and Andrea Rodrigues Ávila. This research received financial support from CNPq and PAPES-FIOCRUZ.

S - 07D – PROTEOMICS PLATFORMS FOR STUDYING THE EMERGING AND OPPORTUNISTIC PROTISTS

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Proteomics is currently an area of great interest. In the area of functional genomics, it is an array of complex technologies that incorporates protein separation methods, mass spectrometry and bioinformatics on a massive scale. Proteomics has potential applications in diagnosing emerging infectious agents as it provides an efficient view of entire genomes' expressions and provides new insights into gene function, disease pathophysiology, disease classification and drug resistance. Different proteomics platforms have been used, from gel-based to liquid chromatography (LC)-based separations of proteins and peptides, alongside the corresponding detection by mass spectrometry (MS). Due to their sensitivity, specificity and short analysis time, these methods have become the preferred approach to detect and identify compounds otherwise difficult to analyze by other methods. We describe here a combination of top-down and bottom-up proteomics approaches applied by our group to the study of protists, with special emphasis on the proteome of human isolates of the emerging and opportunistic protists such as microsporidia, *Criptosporidium* and free-living amoebae. As an initial approach, we conducted gel-based proteomics (2-DE /MS) to study protein expression of two human microsporidian isolates, *Encephalitozoon intestinalis* (CDC V:307) and *Brachiola algerae* (CDC V:404) during their multiplication within Vero monkey kidney (E6) cells. Microsporidial parasites survive and develop inside the host cells and during their merogonic multiplication and sporogonic differentiation they display different patterns of protein expression and we detected biomarkers associated with spore maturation proteins. A second approach was a top-down whole organism biomarker discovery for rapid identification of biomarkers using an emerging versatile technique based on matrix-assisted laser desorption-ionization mass spectrometry (MALDI-TOF MS). Protists belonging to different genera and species were analyzed and unique profiles were determined. We then applied bottom-up gel-independent shotgun proteomics approaches based on LC-MS/MS studies as the next step for biomarker identification of *E. cuniculi* isolates. Even when studying a protist with a small genome (2.9 million base pair), however, the number of proteins in the proteome of *E.cuniculi* exceeds by far the number of genes in their condensed genome. The complexity of their proteome is due to sequence polymorphisms, posttranslational modifications and other protein-processing mechanisms. In addition, a proteome's proteins span a concentration range that exceeds the dynamic range of any single analytical method or instrument. Of the many fractionation technologies available, we applied three: affinity purification, LC-based and GeLC-based separation. The different fractions were digested and the resulting peptides were analyzed by MS and Tandem-MS using both a Waters nanoLC-QTOF Premier and an Applied Biosystems 4800 MALDI-TOF/TOF instrument. QTOF expression data were analyzed using the ProteinLynx Global Server, and label-free quantitative data was obtained. MS/MS data were searched against the entire NCBI database using the Mascot search algorithm. Peptides matched with low confidence were verified manually and the searches were combined and processed by Scaffold (Proteome Software Inc.) to generate a list of valid proteins. Several common and unique biomarkers were detected among the *E. cuniculi* isolates studied, including proteins that were immunoprecipitated and other proteins affiliated to the different cell components, such as the polar tube, spore wall and ribosomal proteins. The biological application and validation of the proteome study begins here. In summary, a comprehensive proteomics analysis was conducted using a combination of sample preparation methods, including gel-based and gel-independent platforms, qualitative and quantitative mass spectrometric approaches and bioinformatics tools to study the proteome of emerging and opportunistic protists. Even with the limitation of the current methods we believe that the proteomics and mass spectrometry applications provide another way of analyzing protists and will profoundly improve the diagnosis, prognosis, treatment and prevention of the diseases caused by these organisms.

S – 08A - THE RELICT PLASTID OF MALARIA PARASITES: EVOLUTIONARY ORIGIN, FUNCTION & THERAPEUTIC POTENTIAL

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The apicoplast (a relict plastid of malaria parasites) has emerged as a promising target for new antimalarials. Apicoplasts are indispensable but their exact function remains uncertain. To understand more about the apicoplast we assembled a predicted organelle proteome. Our current estimates identify more that 500 apicoplast proteins, which represents about 10% of the parasite genome, engaged in this compartment. These apicoplast proteins comprise complete pathways for fatty acid and isoprenoid biosynthesis plus a partial set of haem synthesis enzymes. We believe these anabolic pathways are essential to parasite survival because end products are exported from the apicoplast for use elsewhere in the parasite cell. Our current focus is to verify the biochemical activities for these pathways and to understand how this anabolism is fuelled. We have identified and characterized apicoplast membrane transporters that likely import triose phosphates plus apicoplast modifying enzymes that convert these sugars into substrates for fatty acid and isoprenoid biosynthesis. Numerous apicoplast enzymes for these pathways, and additional apicoplast housekeeping activities, are excellent drug targets.
Suported by: ARC, NHMRC, HHMI

S - 08B - Using *Toxoplasma* genetics to dissect the biology of the apicoplast

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Apicomplexa are an important group of eukaryotic pathogens that includes the causative agents of malaria, toxoplasmosis and cryptosporidiosis. In the absence of effective vaccines, management of these diseases rests heavily on drug treatment. However, treatment remains challenging due to limited efficacy and the rapid emergence of drug resistance. New drugs with novel modes of action are urgently needed. One of the prime targets for new therapies is the apicoplast. The apicoplast is derived from a red algal endosymbiont that was acquired by secondary endosymbiosis. The organelle is surrounded by four delimiting membranes, a tell tale of its complex evolutionary history. We use the power of genetic experimentation in *Toxoplasma* to unravel the function and biology of this fascinating organelle. We have constructed a series of conditional null mutants to pinpoint the truly critical metabolic function of the apicoplast and to identify the most promising targets for drug design. Taking a broader biological perspective we have also studied the cellular mechanisms critical to endosymbiosis. Host and endosymbiont have to interact in numerous ways to establish the exchange of metabolites and to ensure coordinated development and replication. We have identified key elements of the molecular machineries that enable import of proteins into the apicoplast and coordinate endosymbiont fission and segregation during host cell division.

S - 08C - Reservosomes, intriguing organelles from *Trypanosoma cruzi*

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Reservosomes are unique organelles that have a pivotal role in the life cycle of *Trypanosoma cruzi*. They were named for their unusual capacity to accumulate all of the macromolecules that are ingested by the parasite through an endocytic process. Although their main function is to store macromolecules, reservosomes also concentrate lysosomal hydrolases. Two lysosomal hydrolases have been well-characterised in *T. cruzi*: cruzipain and serine carboxypeptidase. Cruzipain, a cysteine proteinase, is considered a fundamental virulence factor for *T. cruzi* during parasite host cell invasion and for intracellular survival. Additionally, reservosomes concentrate the cruzipain natural inhibitor chagasin, suggesting that there is an endogenous modulation of cruzipain activity. Serine carboxypeptidase catalyses the hydrolysis of the carboxyterminal bond in peptides and proteins. Recently, *T. cruzi* autophagic processes were investigated in parasites under nutritional stress. Autophagy seems to be essential for parasite differentiation and survival. It has been proposed that the reservosome functions as an important regulator of protein concentrations and organelles during the metacyclogenesis process. Also, reservosome morphology is abnormal in epimastigotes after a long period of starvation. Each epimastigote form has several reservosomes, mainly in the posterior region of the cell. These organelles are surrounded by a unit membrane, with a mean diameter of 0.6 μm; it is usually globular but may appear asymmetrical. Ultrastructural cytochemical studies have shown that the electron-dense portion of the reservosome matrix is mainly composed of proteins while the electron-lucent inclusions are likely to be lipid. Initially, the presence of inner membranes was controversial. The existence of inner vesicles in HRP-loaded reservosomes was reported by ultrastructural cytochemistry and by freeze-fracture. Guided by the morphology of late endosomes in mammalian cells, the *T. cruzi* storage organelles were first designated as multivesicular bodies. Recently, using different TEM approaches, our group performed a detailed description of reservosome morphology. We demonstrated the presence of internal vesicles both in isolated reservosomes and in situ; we also observed long membrane profiles transversing the reservosome lumen. The inner vesicles, always present in low levels, do not have access to endocytosed macromolecules. Their nature remains to be determined. Another noticeable structure that we observed was a rod-shaped electron-lucent structure bound by a membrane monolayer. The large amount of lipids in the organelle may cause heterogeneous arrangements of the membrane and lead to differentiated lipid regions, which could be functional. Reservosomes were found to be comparable to mammalian late endosomes (pre-lysosomes), with a pH of 6.0, the presence of acid hydrolases and the lack of a lysosomal molecular marker. Typically, the pH of endocytic compartments is kept low by the action of V-type (vacuolar) proton ATPases. Surprisingly, the acidification of reservosomes, as well as of the entire epimastigote endocytic pathway, is a result of the activity of P-type proton ATPases; this phenomenon is unique among all eukaryotic cells. A P-type proton pump has been characterised in plants, yeast and, recently, in trypanosomatids. In the case of *T. cruzi*, two tandemly arranged genes, *TcHA1* and *TcHA2*, encode two pumps: TcHA1 is present in the plasma membrane and endocytic pathway, and TcHA2 acidifies reservosomes exclusively. Due to their absence in mammals and presence in trypanosomatids, the P-type H^+-ATPases may be a potential chemotherapy target against trypanosomiasis.

Reservosomes can be isolated in a purified subcellular fraction. The first biochemical analysis showed that reservosomes accumulate lipids; the protein/lipid ratio of the purified fraction is 1:1, while the ratio in whole parasite extracts is 2:1. Cholesteryl ester and ergosterol are massively concentrated in this organelle. Few reservosomal resident proteins have been described so far. To obtain a protein profile, understand its function and identify molecular marker candidates, we have recently performed a subcellular proteomic analysis of a purified reservosome fraction and total reservosome membrane using liquid chromatography coupled to mass spectrometry (LC-MS/MS). We identified around 700 proteins with predicted or unknown functions. The presence of previously characterised proteins was confirmed, such as cruzipain, serine carboxypeptidase, P-type H^+-ATPase isoforms and an ABC transporter. A P-glycoprotein was also found. A similar transporter was recently suggested to function in heme uptake through the epimastigote plasma membrane, as its fast internalization is impaired by the typical inhibitors. In untreated control parasites, heme concentrates in reservosomes a few minutes after entry. Also, proteins involved in signal transduction and lipid metabolism were found in the reservosome proteome. Endosomal integral membrane proteins and proteins involved in membrane trafficking, especially small GTPases from the Rab family (Rab 1, Rab2b, Rab 7 and Rab18), were also detected. TcRab7 is a small GTPase homologue of the mammalian late endosome marker Rab 7, previously found by immunocytochemistry to be localised at the Golgi complex, rather than reservosomes. The discovery of TcRAb7 in the reservosome proteome may indicate that there is trafficking between these two organelles.

Reservosomes have been described as an exclusive structure of epimastigote forms. While lipid and protein uptake have never been demonstrated in either trypomastigotes or amastigotes, intracellular organelles that share many reservosomal features were recently described in the *T. cruzi* mammalian stages. Like reservosomes, they are concentrated in the parasite's posterior region; they accumulate cruzipain, its natural inhibitor chagasin and serine carboxypetidase. They are acidic and have the P-type H^+-ATPase. Interestingly, rod-shaped electron-lucent lipid bodies, similar to those that were recently characterised in the reservosome lumen, were also found in trypomastigote and amastigote hydrolase-rich compartments.

Collectively, these results indicate that these compartments are closely related. Nonetheless, they differ from reservosomes in the ability to store external macromolecules. Because of the low internal pH and accumulation of lysosomal hydrolases, we have proposed that epimastigote reservosomes and trypomastigotes and amastigotes organelles be considered lysosomal-related organelles (LROs), a group of organelles that share fundamental properties with mammalian lysosomes.

In conclusion, reservosomes have been implicated mainly in macromolecule storage; however, a complex role for these organelles in the *T. cruzi* life cycle has been demonstrated by its pivotal function in digestive, autophagic and recycling processes. Furthermore, an improved understanding of the key genes and proteins in the *T. cruzi* genome and reservosome proteome, respectively, as well as additional high resolution electron microscopy techniques, may allow us to gain insight into the endocytic pathway that exists in this peculiar cell biology model. The recent identification in the *T. cruzi* infective stages of organelles that share characteristics with reservosomes, organelles that are so dissimilar from mammalian cell compartments, may provide new potential chemotherapy targets for the treatment of Chagas's disease.
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S - 08D - ACIDOCALCISOMES AND POLYPHOSPHATE (POLY P) IN PROTISTS

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Acidocalcisomes are acidic electron-dense organelles, rich in pyrophosphate and polyphosphate (poly P) complexed with calcium and other cations. While its matrix contains enzymes related to poly P metabolism, the membrane of the acidocalcisomes has a number of pumps (Ca^{2+}-ATPase, V-H^+-ATPase, H^+-PPase), exchangers (Na^+/H^+, Ca^{2+}/H^+), and at least one channel (aquaporin) [1]. Acidocalcisomes are present in both prokaryotes and eukaryotes and are an important storage of cations and phosphorus. They also play an important role in osmoregulation and interact with the contractile vacuole complex in a number of eukaryotic microbes [1]. In parasitic protists acidocalcisomes possess enzymes that are absent from their mammalian counterparts and could be potential targets for chemotherapy [2]. Acidocalcisomes resemble lysosome-related organelles (LRO) from mammalian cells in many of their properties. They share similar morphological characteristics, acidic properties, phosphorus contents, and a system for targeting of their membrane proteins through adaptor complex-3 (AP-3). Storage of phosphate and heavy metals may represent the ancestral physiological function of acidocalcisomes, with cation and pH homeostasis and osmoregulatory functions derived following the divergence of prokaryotes and eukaryotes. Poly P is present in every cell in nature and is highly abundant in acidocalcisomes. It plays essential roles in the virulence of major pathogens such as those causing dysentery, tuberculosis, and anthrax; in apoptosis; in the proliferative aspects of cancer; and in osteoporosis and aging. By manipulating the expression of the genes involved in pyrophosphate and polyphosphate metabolism and the cellular levels of their products, we are creating phenotypes that are providing clues to the role of pyrophosphate and poly P in the physiology and development of protists. Overexpression of a soluble vacuolar pyrophosphatase resulted in a 3-fold increase in long-chain poly P in *Trypanosoma cruzi* while RNA interference (RNAi) experiments of this enzyme in *T. brucei* resulted in a significant decrease in long-chain poly P suggesting that the enzyme is directly or indirectly involved in long-chain poly P synthesis. On the other hand, RNAi experiments of the vacuolar transporter chaperones 1 and 4 of *T. brucei* resulted in a significant decrease in short-chain poly P, in agreement with the suggested role of the VTC complex as a poly P synthase in yeasts.
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S – 09A - Endosymbiosis in trypanosomatids offers new clues on the eukaryotic cell evolution

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Some trypanosomatids harbor a symbiotic bacterium, which maintains a close association with the host, constituting an excellent model to study organelle origin and cellular evolution. Both partners co-evolve in a mutualistic relationship characterized by intensive metabolic exchanges; the endosymbiont contains enzymes and metabolic precursors that complete essential biosynthetic pathways of the host protozoa, as the urea cycle and hemin production. Conversely, the symbiont is capable of obtaining part of the required energetic molecules from the host glycosomes. The symbiont is enclosed by two unit membranes, like Gram-negative bacteria, however the peptidoglycan layer is reduced and the septum is absent during the cellular division. There was controversy about the origin of the outer membrane: some authors considered that it derives from the host cell membrane, while others claimed for its prokaryotic nature. Recently, we identified a porin-like channel, in the endosymbiont envelope, suggesting that the outer membrane of the trypanosomatid endosymbiont presents bacterial origin. Lipid analyses of purified endosymbionts indicated a complete absence of sterols and a phospholipid composition different from that of mitochondria or whole protozoan cells. Phosphatidylcholine (PC) is a major component of the endosymbiont envelope, as also described for eubacteria, which are closely associated with eukaryotes. Symbionts are capable of synthesizing phospholipids after isolation from the host cell, but in this case the major phospholipid produced was phosphatidylethanolamine, suggesting that the symbiotic bacterium may obtain part of its PC, or even PC precursors, from the host trypanosomatid. Another interesting point is the coordinated division between the symbiont and the host protozoan structures. It is well known that in trypanosomatids, cell division involves morphological changes and requires coordinated replication and segregation of the nucleus, kinetoplast and flagellum. In endosymbiont-containing trypanosomatids this process is more complex, since each daughter cell carries only one symbiotic bacterium, indicating that the prokaryote must replicate synchronically with the host protozoan. In cells containing the endosymbiont, the bacterium divides independently of the appearance of the new flagellum, always before the kinetoplast and the nucleus segregation. In addition, the endosymbiont remains associated with the host cell nucleus, presenting various shapes during the host cell cycle. The systematic sequencing of DNA from the *Crithidia deanei* endosymbiont estimates that the genome presents only 850 kb, indicating genomic shrinkage in relation to *Bordetella parapertussis*, the ancestral bacterium. Furthermore, according to 16S ribosomal DNA sequences, endosymbionts of trypanosomatids protozoa are identical, being classified in the ß division of Proteobacteria. This suggests that a single event in nature gave rise to all endosymbiont-bearing trypanosomatids, recapitulating the process that led to the formation of mitochondrion in eukaryotic cells.

S - 09B - Lateral gene transfer, genome evolution and endosymbiosis.

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During the last 10 years, our view of spread of genes between unrelated organisms through horizontal DNA transfer has gone all the way from a curious but unlikely phenomenon to a major evolutionary force with profound influence in the diversification of life. Current evidence shows that lateral gene transfer is very frequent among prokaryotes, where genes are exchanged even across the Eubacteria-Archaea domain boundary greatly contributing to the astonishing metabolic diversity of prokaryotes. In eukaryotes the prevalence of lateral gene transfer and the nature of the mechanisms involved in the process are less known, but the rapidly increasing wealth of genomic information suggests that the origin and evolution of nucleated organisms has also been strongly influenced by gene exchange. Genome data show that many genes present in nuclear genomes have been horizontally acquired from bacteria, archaea and unrelated eukaryotes. This presentation will examine current evidence for the involvement of lateral gene transfer in the evolution of eukaryotes and their genomes. The relationships between genome evolution, lateral gene transfer and symbiosis in dinoflagellates was examined by analysing molecular data from the cosmopolitan colourless flagellate *Oxyrrhis marina*. The genome of this phagotrophic protist contains genes representing typically plastidial metabolic pathways such as isoprenoid and amino acid synthesis and also genes of bacterial origin. Among the latter, a remarkable example is the presence of a bacterial Proteorhodopsin, a protein involved in an alternative energy-generating mechanisms known to be horizontally spread among diverse bacteria which has been adopted also by some dinoflagellates. New data on the origin, characteristics, localization and possible role of this protein will be discussed. The data considered here show that the biology of *Oxyrrhis marina* has been profoundly influenced by the acquisition of genes from diverse sources, clearly illustrating the importance of these processes for the evolution of eukaryotes.

S - 09C - INFECTION OF *HOLOSPORA* AND ACQUISITION OF STRESS RESISTANCE OF THE HOST *PARAMECIUM*

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Holospora species are endonuclear symbionts of the ciliate *Paramecium* species and belong to alfa-proteobacteria. Infectious forms of this bacterium shows distinctive structure, one half of which contains the cytoplasm and the other half of a periplasmic lumen with an electron-translucent invasion tip. To date, nine species have been described. All show species-specificity and nucleus-specificity in their habitats. They cannot grow outside the host cell with ordinary culture media. Indirect immunofluorescence microscopy with a monoclonal antibody (mAb) specific for the host vacuolar-type ATPase (V-ATPase) showed that the infectious form (10–15 μm long) engulfed into the host digestive vacuole (DV) disrupts the DV membrane to appear the host cytoplasm. In the presence of the V-ATPase inhibitors, concanamycin A and concanamycin B, both the acidification of the DV and the bacterial escape from the DV were inhibited completely. The bacterium appeared in the host cytoplasm migrates to their target nucleus, distinguishes two kinds of the host nuclear envelope and invates into the target nucleus with this invasion tip ahead but not with the other tip. To investigate the underlying molecular mechanism of the infection, a mAb against the invasionl tip-specific 89-kDa protein of *H. obtusa* was developed and identified the gene. The deduced amino acid sequence carries two actin-binding motifs near the N-terminal. Indirect immunofluorescence microscopy with the mAbs of the 89-kDa protein and *P. caudatum* actin shows that during escape from the host DV, the 89-kDa proteins translocates to the outside of the tip triggered by low pH of the DV. In the host cytoplasm, bacteria keep the 89-kDa proteins outside the tip and the host actins accumulate around the exposed 89-kDa proteins. When the bacterium invades the macronucleus, the 89-kDa proteins and actins are left behind at the entry point of the nuclear envelope. The actin polymerization inhibitor, latrunculin B, inhibits the bacterial infection. These results show that the 89-kDa proteins and the host actin play a role in *Holospora*'s escape from the host DV, migration through the host cytoplasm and the invasion into the target nucleus. Bacterial recognition of their target nuclei is controlled by an affinity between lipopolysaccharides (LPSs) of the outer membrane of the infectious form and an unknown receptor substance of the target nuclear envelope. These results also demonstrate that *Holospora* arrives to its target nucleus without wrapping with membranes derived from the host cell because the bacterium distinguishes its target nuclear envelope by direct binding between their LPSs and nuclear envelopes. Differential display reverse transcribed PCR showed that *H. obtusa* alters multiple gene expression of the host after establishing endosymbiosis. For example, hsp70 and 60 genes are enhanced by infection of *Holospora*, and *Holopsora* secrets GroEL homologs to the host cell. Thus, *Holospora*-bearing *Paramecium* cell acquires various stress resistances. This suggests that *Paramecium* cells become adapted to unsuitable environments for their habitations through with *Holospora* species. Therefore, their interaction is mutualistic rather than commensal if the host cell is in a stressful environment. However, the molecular mechanism for the symbiont-induced alteration of the host's gene expression remains unknown. A periplasmic 63-kDa protein of *H. obtusa* might be one cause for induction of the alteration of the host's gene expression, because this protein is secreted into the host macronucleus, not only from the invaded bacteria in early infection but also from the infectious forms differentiated from the reproductive forms in the macronucleus. Furthermore, deduced amino acid sequence from a gene encoding the 63-kDa protein shows that this protein involves predicted two DNA-binding motifs. Supported by JSPS (No. 17405020).

S - 09D - Metabolic Control between the Symbiotic *Chlorella* and the Host *Paramecium*

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Metabolic control, including the transfer of materials between a host and symbiont, is important for understanding symbiotic relationships. However, sugars, mainly maltose, are the only confirmed class of material transferred from symbionts to *Paramecium bursaria*. An axenic Japanese *Chlorella* symbiont, which had been thought to be difficult to isolate from *P. bursaria* and maintain in culture, was found to be unable to utilize nitrate. The lack of nitrate reductase activity in the symbiont (F36-ZK) suggests that this feature of F36-ZK is an extreme example of an adaptation to the symbiotic milieu. F36-ZK possesses unique physiological features compared to free-living *Chlorella*. One of them is constitutive amino acid transport, which is usually lacking in free-living *Chlorella*, although amino acid transport is inducible in some free-living strains. Based on this characteristic, F36-ZK is thought to receive amino acids from its host. In the course of study of the features of transport, it was found that the ability for transport was affected by some compounds in the surroundings. Calcium ions suppress this ability, while sugars including glucose improve it depending on their concentration. The mechanistic details of these effects are unknown; however, this feature implied the possibility of metabolic control by the host using common intracellular materials. Symbiotic *Chlorella* cells are enclosed individually by a bilayered membrane in the host cell, which is called a perialgal vacuole, and thus the amino acid transport ability of the symbiont could be controlled by the conditions in the tiny space between the inner membrane of the vacuole and the symbiont cell. Furthermore, transfer of materials between the symbiont and the host achieved through the vacuolar membrane; therefore, the permeability of this membrane should also be an important limiting factor in the transfer. Based on the results of a trial to identify the amino acid supplied from the host, arginine is the most plausible candidate, because it can be transferred through the membrane and can support the growth of the symbiont in reinfection experiments. The other characteristic feature of F36-ZK is its photosynthetic carbon fixation. The pH dependence of carbon fixation by F36-ZK and free-living *Chlorella vulgaris* showed opposing tendencies, and the enhancement of carbon fixation at alkaline pH seems to be a notable feature of F36-ZK. Carbon fixation by an F36-ZK cell suspension in sodium phosphate buffer is increased by addition of host extract. The principle of the host extract was studied and three divalent cations, calcium, potassium, and magnesium, were identified as the active substances. In the presence of each cation alone, little enhancement of carbon fixation by F36-ZK was observed. However, when a second cation was added, algal carbon fixation approximately doubled. Carbon fixation was enhanced by the three-cation mixture to the same degree as the host extract. F36-ZK releases maltose at acidic pH like other symbiotic *Chlorella* and produces maltose from starch using β-amylase even in the light. Thus, carbon fixation and the release of photosynthate could also be controlled by the concentration of cations and by pH, respectively, in the perialgal vacuole. F36-ZK possesses unique features, and some of these differ from previously reported symbiotic algal characteristics. However, the control systems between host and symbiont of Japanese *P. bursaria* could be very similar to those of other *P. bursaria*. Further putative method of metabolic control between the symbiont and the host will also be discussed.

S – 10A - Apicomplexan gliding motility: zoom in on the "Glideosome"

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Gliding motility is a unique attribute of the members of Apicomplexans that is essential for the parasite migration across biological barriers, active host cell invasion and egress from infected cells. This substrate-dependent mode of locomotion is powered by the glideosome, a machinery that involves the concerted action of secretory adhesins, a myosin motor, factors regulating actin dynamics and proteases. During invasion, complexes of soluble and transmembrane micronemes proteins (MICs) are discharged at the apical pole of the parasite where some of them act as adhesins and bind to host cell receptors. The relocalization of these complexes to the posterior pole of the parasite is powered by the parasite actomyosin system and eventually the MICs are released from the parasite surface by the action of parasite proteases. The timing, duration and orientation of gliding motility are tightly regulated to assure the successful establishment of infection. Assembly of the glideosome is controlled by posttranslational modifications of its components while polymerzation of actin is mediated locally by the action of two formins.

S - 10B - Constructing and managing a mobile flagellum: the challenge of trypanosomes in the tsetse fly

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African trypanosomes are flagellated protists responsible for sleeping sickness. They alternate between mammalian hosts and tsetse flies, where they undergo a series of complex steps of differentiation and proliferation. At least 9 different stages can be recognised morphologically especially based on the length and the position of the flagellum. This organelle is the most conspicuous feature of the parasite and drives cell motility. Recent proteomic analysis revealed that at least 330 proteins are present in the flagellum, including several dynein motors found at defined substructures termed outer and inner dynein arms. We wish to understand (1) the mode of assembly of the flagellum, (2) its role in cell motility and (3) how do these parameters evolve during the parasite cycle.

The flagellum is constructed by intraflagellar transport (IFT), a dynamic process where kinesin motors transport particles to the distal tip where they are remodelled and recycled towards the base of the flagellum via dynein motors. IFT likely transports axoneme proteins to the distal tip for assembly. We recently identified a novel leucine-rich protein (LRC50) that controls the formation of outer dynein arms in the trypanosome flagellum. In its absence, cells assemble flagella of normal length but show altered motility and absence of certain outer dynein arms. LRC50 localises to the cytoplasm but it does not participate to IFT and neither does it appear to be a structural component of the flagellum. Western blot analysis showed that in the absence of LRC50, one component of the outer dynein arm was no longer associated to the flagellum but was mostly found in the cytoplasm. We propose that LRC50 could control the loading of dynein arm precursors to the IFT machinery.

Videomicroscopy analysis revealed that trypanosomes swim with their flagellum leading, using a propulsive wave that propagates from tip to base at high frequency but with low amplitude. Reverse genetics analysis demonstrated that this motility is driven by outer dynein arm action. Cultured cells are also able to switch to a reverse wave from base to tip with a markedly higher amplitude and lower frequency, leading to cell re-orientation but little or no backward swimming. This motility is maintained in the absence of outer dynein arms resulting in cells that twist but do not show net forward movement. These two types of flagellar movements were observed *in vivo* in trypanosomes infecting the midgut of the tsetse fly, with spectacular alternation between propulsive and reverse wave, resulting in fast but erratic progression within the peritrophic space in both directions. At later stages of differentiation, the length of the flagellum increases up to 30 µm and the cell body becomes thinner. These mesocyclic parasites show accelerated speed and directional motility with almost exclusively propulsive waves. As cell density is high at this point, apparent colony behaviour was observed. These parasites differentiate to the long epimastigote stage where they look like spermatozoa and give birth to a short epimastigote cell with a much shorter flagellum (less than 10 µm). Remarkably, the formation of this short flagellum is accompanied by a spectacular asymmetric division and by extensive remodelling of the cytoskeleton. Finally, parasites migrate to the salivary gland where they anchor to the epithelium via their flagellum whose membrane produces extensive elongation. Remarkably, the flagellum remains motile and the attached cells vibrate constantly. Propagation in the fly of parasites expressing a fluorescent flagellar protein now opens up the possibility of direct observation in live insects.

These data reveal the importance and the versatility of the flagellum during the trypanosome parasite cycle.

S - 10C - Encystation in *Giardia lamblia*: no reason to get stressed

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Giardia lacks a classical Golgi and can only produce rudimentary N-linked core glycans. This is consistent with the absence of identifiable factors involved in N-glycan-dependent quality control of protein folding and degradation in the endoplasmatic reticulum (ER). ER quality control and systems which facilitate proper folding of newly synthesized secreted proteins comprise factors involved in triggering the unfolded protein response (UPR) which results in upregulation of the transcription of >300 genes in higher eukaryotes. The core systems of the UPR are conserved from yeast to mammals but only very few homologs could be identified in the Giardia Genome Database (GGD). Most important, key ER resident sensors/transducers (e.g. Ire1), or the central trans acting activator of UPR gene expression (HAC1) are absent. In the context of the systematic minimization of cellular systems during Giardia evolution this raised the question how Giardia deals with acute ER stress induced for example by changing conditions in the gut lumen. Encystation of trophozoites is triggered by environmental cues and entails complete remodelling of the parasite surface. This stage-differentiation process involves synthesis, folding and export of vast amounts of cyst wall proteins (CWPs) from the ER to specialized compartments termed encystation-specific vesicles. To make the link between differentiation and quality control mechanisms we investigated if encystation, which also has a distinct transcriptionally regulated component, begins as a stress response or, conversely, if the large amount of CWPs in the ER induces folding stress and UPR.

In this work we used transcriptional profiling with whole genome microarrays to identify genes which are upregulated in response to redox stress affecting the secretory pathway, or to an unspecific stress (40^{o}C). For the former we mimicked overload of the ER with misfolded protein by treating trophozoites with non-lethal concentrations of the reducing agent dithiothreitol (DTT). For comparison with the transcriptional response during encystation, we analyzed expression profiles of parasites induced to differentiate for 45 min, 3 and 7 hours.

Analysis of the stress response induced by DTT revealed a limited set of ~30 mRNAs that were significantly upregulated after 30 minutes. The regulation of this set of “early” genes was moderately dose-dependent and overlapped only in one case with mRNAs upregulated in response to heat stress. To determine the dynamics of the redox stress response we performed time course experiments over 2 hours. We find 1) a defined transcriptional response; however, 2) unlike the yeast UPR, which is constant over time, the giardial stress response is organized as two distinct “waves” of upregulated transcripts, and 3) the total number of upregulated genes is only one fifth of the yeast set of UPR genes. Our analyses also showed that the sets of differentially transcribed genes during encystation or under redox stress do not overlap.
Taken together, the data reveal a distinct capacity to respond to environmental insults in a very specific way. However, despite the upregulation of a core set of chaperones and protein disulfide isomerases the datasets point to a fundamentally different organization of the giardial stress response when compared with the classical UPR that is not easily explained by reductive evolution alone. In addition, we find no evidence that encystation induces protein folding stress despite the massive influx of cyst wall proteins into the ER.

S - 10D - Live on an intracellular island: The peculiar case of *P. falciparum*, a parasitic freebooter *par excellence*.

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The malaria parasite, *P. falciparum* is an interesting model system in which to study protein transport and trafficking processes. In addition to it's possession of a tertiary endosymbiotic plastid (referred to as the apicoplast), to which proteins must be conveyed, the peculiar choice of host cell (the mature human erythrocyte) presents the parasite with several challenges which it must solve to allow for successful intra-cellular development and multiplication. Here, we present our most recent data on protein transport from the parasite to the host erythrocyte. We reveal that parasite proteins destined for the host cell cytosol are subjected to a rate-limiting protein unfolding barrier which must be overcome prior to translocation across the membrane of the parasitophorous vacuole. The data is discussed in relation to the nature of the proposed vacuolar protein conducting channel (VPCC).
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S – 11A - *LEISHMANIA*/HIV CO-INFECTION AND INFECTIONS BY LOWER TRYPANOSOMATIDS

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The evolution of HIV/AIDS pandemic over the last decades has had its effect on the natural history of leishmaniases, both in clinical and epidemiological aspects. Indeed, leishmaniasis is considered, after toxoplasmosis, the most common opportunistic protozoan in AIDS patients. During the 80-90s´ decades, due to the overlapping of endemic leishmaniasis with epidemic AIDS, the Mediterranean Basin became the paradigm of *Leishmania*/HIV co-infection. Currently, both the ruralization of AIDS together with the urbanization of leishmaniasis are creating new opportunities for the emergence of new co-infection cases. Thus there is a growing report of HIV-related leishmanaisis in the Indian subcontinent, East Africa and Latin America. HIV/AIDS-related leishmaniasis may occur either as a newly acquired infection or as a reactivation of a latent infection; being this latter disturbing in endemic areas where asymptomatic infection with visceral or cutaneous *Leishmania* species can occur. Both infectious agents share target cells. *Leishmania* infects and multiplies inside the macrophages, and HIV can also invade and replicate in these. *Leishmania* can increases HIV replication principally due to chronic activation of the immune system, which is one of the key determinants of the HIV-associated condition. On the other hand HIV also causes an inhibition of the proliferative response against *Leishmania*, favouring the spread of the parasite. Therefore, in HIV+ individuals leishmaniasis promotes the development of AIDS definitory conditions and its clinical progression, reducing their life expectancy. In terms of transmission, though the geographical distribution of leishmaniasis is restricted to the distribution of the sand fly vector, HIV infection can modify the traditional zoonotic/anthroponotic transmission patterns. Factors as: i) lower therapeutic success rates, ii) high level of relapses, and iii) higher parasitaemia in HIV+ patients, as well as atypical manifestations that hinder the diagnosis, mean that these patients can increase the number of human reservoirs in areas where the transmission is anthroponotic. On the other hand, these same characteristics may help to create anthroponotic transmission foci in areas where transmission is traditionally zoonotic. Other non-human monoxenus trypanosomatids have been identified in human infections causing cutaneous and diffuse cutaneous lesions and visceral disease. Most of the cases have been related to HIV/AIDS, and where initially classified as *Leishmania* infections. When identification was addressed usually *Herpetomonas*, *Crithidia* or *Leptomonas* were the suspects. However in most cases there was no positive identification neither of the protozoa nor of the possible route of infection.
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S - 11B - What is the relationship between *Cryptosporidium* and its host?

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The ability to culture *Cryptosporidium in vitro* has confirmed the long held belief from molecular phylogenetic studies that *Cryptosporidium* is not a coccidian but is more closely related to gregarine protozoa. This understanding has helped in the interpretation of morphological studies at both the light and ultrastructural levels on several species of *Cryptopsoridium*, including the zoonotic form *C. parvum*. These studies have demonstrated the existence of previously undescribed developmental stages in the life cycle of *Cryptopsoridium*, and have revealed an extended extracellular phase of development both *in vitro* and *in vivo*. The relationship of *Cryptosporidium* with the host cell appears tenuous and it is not clear whether *Cryptosporidum* is capable of progressing through its entire developmental cycle extracellularly in a host and/or cell culture. It is also not understood whether extracellular stages can invade cells or if they represent rudimentary stages of an ancestral life cycle. Interaction with the host cell is very similar to what is seen in gregarines including an extracytoplasmic location and connection to the host cell via a myzocytosis-like feeding mechanism. However, *Cryptosporidium* appears to differ by inducing the host cell to overlay it with the host cell apical membrane. These new findings are discussed in the context of both phylogenetic and host-parasite relationships.

S - 11C - Exploring Novel Targeting Strategies for Opportunistic Protozoal Pathogens

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In the last decade, major strides have been made in antiretroviral therapies for treatment of AIDS due to HIV infection. Currently, opportunistic infections are the primary cause of suffering and death in individuals with AIDS. Many of these infections are produced by parasites that rarely affect individuals who are not immunocompromised. Unfortunately, successful combination therapies against the HIV-1 virus still leave most patients susceptible to opportunistic parasitic infections. Among the most prevalent parasitic infections are due to the protozoal pathogens, Cryptosporidium and Toxoplasma. There are few drugs available to treat these parasitic infections. The drugs that are available suffer from a lack of selectivity, resulting in host toxicity, untoward side effects, and ineffectiveness due to development of resistance. Thus there is a need to identify novel targeting strategies that may ultimately lead to therapeutics that are more selective and less toxic. Our mechanistic and structural studies focus on unique bifunctional enzymes present only in parasites that may serve as a potential target for the discovery of novel anti-parasitic drugs for treatment of opportunistic infections in AIDS patients.

Two enzymes crucial for DNA synthesis and one-carbon transfers are thymidylate synthase (TS) and dihydrofolate reductase (DHFR). In many protozoan parasites, these two catalytic activities are located on a single polypeptide chain to form a bifunctional thymidylate synthase/dihydrofolate reductase (TS-DHFR) enzyme. In contrast, in mammalian species, the TS and DFHR activities occur on separate monofunctional enzymes. While there has been considerable effort directed toward active site inhibitors, it is the unique functional nature of the linker regions and the non-active site regions of the bifunctional TS/DHFR parasitic enzymes that may offer a novel directions for inhibition and treatment of these parasites. A considerable amount of mechanistic information is available for the human monofunctional TS and DHFR enzymes and each enzyme has been successfully targeted with the anticancer drugs, 5-fluorouracil and methotrexate, respectively. Much less is known about the bifunctional enzymes from protozoal parasites such as Cryptosporidium and Toxoplasma that are AIDS pathogens. Preliminary studies from our laboratory using a multidisciplinary strategy including mechanistic and computational studies, structural analysis, coupled with synthesis and cellular studies suggest there are unique features of the bifunctional enzymes that are essential to function. A long-term goal of this research is to take advantage of the unique differences between parasitic and human enzymes and develop novel antiparasitic drugs for the treatment of opportunistic parasitic infections.

S - 11D - RECENT ADVANCES IN RESEARCH ON OPPORTUNISTIC MICROSPORIDIA

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The microsporidia are a phylum of eukaryotic spore forming obligate intracellular parasites consisting of over 1200 reported species infecting all major animal groups. They exhibit varying degrees of host specificity from those infecting a narrow host range to some infecting both invertebrates and vertebrates. For over 100 years, the microsporidia have been primarily known for their infections in insects and fish. However, it was not until the 1970's that they were documented as human parasites. Their significance as human-infecting organisms became apparent when HIV infections created a large population of immune-compromised individuals. Subsequently, microsporidial infections in other immune compromising situations including organ transplants and chemotherapy were recognized. Among the approximately one dozen different microsporidia infecting humans, virtually every tissue of the body has been reported to support their growth. Additionally, the eye serves both as a site of infection in immune competent individuals and as a portal of entry for dissemination in immune suppressed individuals. Microsporidial spores are the diagnostic structure for this group. They are the only extracellular stage in the life cycle and they are very resistant in the environment. The spores range in size from 1–20 µm but the human infecting species range from 1-4µm with the two most common species being 1 µm. They are of single cell origin, possess a thick resistant spore coat and contain a sporoplasm surrounded by a coiled single polar filament attached to the anterior of the spore. The polar filament is everted (becoming a tube) in the germination process, allowing the passage of the infective sporoplasm through it, resulting in host cells being infected by "inoculation". Microsporidial growth in host cell culture has been a crucial tool for the study of their development. Most of the human- infecting microsporidia have been maintained in cell culture which has provided a means to study anti-microsporidial agents, provide organisms for basic research and provide for the visualization/ localization of various labeled antibodies for the study of microsporidial structures. The lack of a continuous culture system for *Enterocytozoon bieneusi* (one of the two most common human infecting microsporidia) has greatly hampered its research.

As obligate intracellular parasites, the microsporidia, have evolved as well adapted and specialized organisms which are highly-diverged (degenerate), with a life style incorporating several unique features. A Golgi is present that has evolved a specialized function in the formation of the polar filament and a morphology resembling a vesicular mass. The microsporidia were considered amitochondriate until recently when it was discovered that more than a dozen genes encoding mitochondrion-derived proteins have been identified and localized to a body now identified as a mitosome. The microsporidia are eukaryotic but have prokaryotic sized ribosomes. Centrioles, a usual feature of eukaryotes, are lacking in the microsporidia. During karyokinesis, the nuclear envelope remains intact and the spindle is intranuclear. Phylogenetic analysis of their β-tubulin genes suggests that the microsporidia are related to the fungi. "The fungal nature of microsporidia indicates that microsporidia have undergone severe selective reduction permeating every level of their biology: From cell structures to metabolism, and from genomics to gene structure, microsporidia are reduced" (Keeling, P.J. and Fast, N.M. 2002). Several genome projects are in progress for different microsporidia. The smallest eukaryotic genome (2.3 Mb) known is that of the microsporidium, *Encephalitozoon intestinalis.* The largest microsporidial genome is that of *Glugea atherinae* (19.5Mb). Both morphology and molecular biology are currently in use for taxonomic purposes to define both genera and families. Consequently, depending on ones' approach, different taxonomic schemes have been generated. The time is approaching when a unified and integrated taxonomic system will be developed.

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S – 12A - Neutrophil-dendric cell interactions in leishmaniasis

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Neutrophils are rapidly recruited to sites of *Leishmania major* infection in the skin and represent the first infected cell type early in the response. However, PMN are short-lived and undergo apoptosis at the inflammatory site. We investigated whether dendritic cells (DCs) might sense and interact with PMN in the skin, and how this contact might modulate their function as antigen presenting cells. Neutrophil depletion resulted in a striking enhancement of the proliferative response of adoptively transferred OT-I cells following needle injection or sand fly transmission of *L. major* transgenic parasites expressing OVA. Using lysozyme-GFP mice injected in the skin with RFP-expressing *L. major* to obtain sort-purified, infected and uninfected neutrophils, there was preferential uptake of the parasitized neutrophils by DC following reinjection in the ear dermis. A difference that correlated with the enhanced apoptotic status of the infected neutrophils, as determined by annexin V staining. When RFP-*L. major* metacyclic promastigotes were injected directly, the majority of the infected, RFP+ DC recovered from the skin were found to harbor markers of PMN, suggesting that the normal early encounter of DC with *L. major* is in the context of infected neutrophils. Interestingly, the number of infected, RFP+ DCs was the same after *in vivo* infection in neutrophil depleted or undepleted mice. However, the expression of maturation markers on infected DCs from depleted mice was more pronounced than infected DCs from undepleted mice. Furthermore, using *L. major* expressing both, RFP and OVA, the infected DCs from depleted mice were more efficient at activating OT-I cells ex vivo, as measured by IFN-gamma release. These data demonstrate both a novel mechanism by which *Leishmania* parasites can induce immune dysregulation and a previously unrecognized role of infected PMN during the immune response to *L. major*.

S - 12B - Intra-cellular signaling in *Leishmania*-infected cells : Parasite protein trafficking in infected cells and engagement of endogenous signaling pathways.

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Infection with *Leishmania* parasites causes diverse disease presentations in humans including cutaneous lesions, mucocutaneous lesions and visceral disease. These parasites live in cells that are usually in an inflammatory milieu. To survive in that environment *Leishmania* modulate their host cell's responses to multiple stimuli; however, the mechanisms by which they achieve this are still poorly understood. It is possible that infection engages host signaling pathways that mediate suppression of other pathways. The PI3K signaling pathway is emerging as an important pathway that is engaged by *Leishmania* parasites to prolong the survival of the infected cell and block the production of IL-12 in addition to other proinflammatory mediators. Given that parasites can reside in cells for many days, engagement of signaling pathways that are vital for parasite survival has to be sustained. We provide evidence that the downstream intermediate, AKT, in the PI3K signaling pathway is recruited to the parasitophorous vacuole (PV) and activated there, which implies that the machinery for generating the lipid second messengers for PI3K signaling is present at the PV membrane. It is possible that molecules elaborated by the parasite within the PV might target the PI3K signaling pathway or other signaling pathways in the infected cell. However, evidence that *Leishmania* parasites elaborate molecules that target host cell functions in the PV or in the host cell cytosol and nucleus is limited. We have implemented the *i*n *v*ivo *i*nduced *a*ntigen *t*echnology (IVIAT) to identify parasite molecules that are preferentially expressed in infected cells. These studies show that there is a subset of parasite molecules that are preferentially expressed in infected cells. They traffic out of the PV into the host cell cytosol and nucleus. The targets of these molecules are currently being sort. Taken together, *Leishmania* parasites condition the infected cell by releasing putative effector molecules into the cell and also by engaging signaling pathways that exert a dominant suppressive effect on other signaling pathways.

S - 12C - Are *Leishmania braziliensis* metallopeptidases responsible for a variety of disease phenotypes?

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Leishmania (Viannia) braziliensis, a parasite autochthonous of the American continent, is the main etiological agent of American Tegumentary Leishmaniasis (ATL). The disease encompasses a broad spectrum of clinical manifestations ranging from self-healing cutaneous lesions to disseminated forms, with mucosal damage. Previous works of our group showed that *L. (V.) braziliensis* strains isolated from patients with cutaneous, disseminated and mucosal forms of ATL display different profiles of metallopeptidase activities. We also showed that the zymographic profiles remain unaltered during prolonged *in vitro* culture, suggesting that the proteolytic activity pattern is a stable phenotypic characteristic of these parasites. Following these results, we investigated the cellular localization of these molecules and their relation to the major surface peptidase (gp63) of *Leishmania*. Flow cytometry and fluorescence microscopy analyses corroborated the presence of metallopeptidases with homologous domains to gp63 in the parasites and revealed differences in the expression level of such molecules among the isolates. The cellular distribution of metallopeptidases, assessed by confocal analysis, showed the existence of intracellular metallopeptidases with homologous domains to gp63, predominantly located near the flagellar pocket. During interaction of macrophages with mucosal, disseminated and cutaneous isolates of *L. (V.) braziliensis* it was observed a complex profile of proteolytic activities composed of the sum of activities from macrophages and intracellular parasites. Use of 1,10-phenantroline, a specific metallopeptidase inhibitor, abrogated all enzymatic activities. When the proteolytic profile of *Leishmania*-macrophage interaction was compared with the proteolytic profile from promastigote´s extracts it was observed that intracellular amastigotes express some of the main metallopeptidase activities observed in promastigote forms. Using optical and scanning electron microscopies it was observed that *L. (V.) braziliensis* mucosal, disseminated and cutaneous isolates exhibited different abilities to infect macrophages. Finally, use of 1,10-phenantroline during interaction affected the invasion and multiplication abilities of mucosal isolate.
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S - 12D - The role of surface glycoconjugates in *Leishmania* virulence

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The surface of *Leishmania* parasites is covered by a complex array of glycoconjugates, including abundant GPI-anchored molecules such as lipophosphoglycan (LPG), gp63, and proteophosphoglycans (PPGs), and their membranes differ from other eukaryotes in containing abundant levels of plasmalogens and ergosterol as well as inositolphosphorylceramide (IPC). The overlapping structural and biophysical properties of these elements complicate the dissection of their roles, and thus we have applied forward and reverse genetics as well as genomic approaches to this question. Forward genetic studies of LPG have identified a number of biosynthetic genes affecting LPG alone or in combination with other glycoconjugates, one example being the specifically LPG-deficient *lpg1-* mutants of *L. major* or *L. donovani*. Studies of this mutant have confirmed some but not all proposed LPG functions, establishing the role of LPG in mediating binding and survival in some but not all sand fly hosts, confirmed the role of LPG in complement and oxidant resistance in the initial stages of mammalian infection, but surprisingly have shown that transient inhibition of phagolysosomal fusion is not required for macrophage survival in the absence of an oxidative burst. As expected *lpg1-* amastigotes are normal as this parasite stage does not express LPG.

Studies of mutants lacking the *LPG2* GDP-Man transporter and deficient in all phosphoglycans (PGs) have proven especially informative. In *L. major lpg2-* parasites show a 'persistence without pathology' phenotype, while in contrast, *L. mexicana* mutants show only defects typical of LPG deficiency (complement sensitivity); recent studies suggest *L. donovani* most closely resembles *L. major* (Gaur *et a*l, *Exptl. Parasitol*., 2009) Interestingly, an *L. major* mutant lacking the UDP-Gal transporters *LPG5A* and *LPG5B*, and similarly lacking all PGs, resembles the *lpg1-* mutant in showing defects only in promastigote but not amastigote virulence (Capul *et al J. Biol Chem*. and *Infection & Immunity*, 2008). As the known PG and *LPG2*-dependent glycoconjugate repertoire is identical in all three *Leishmania* species, these findings argue for another *LPG2*-dependent function in *L. major* and *L. donovani.* Systematic genetic and glycomic studies may thus be required to identify the 'missing' and presumably non-abundant *LPG2*-dependent glycoconjugate. Other studies underway are focusing on the metabolism and roles of IPC metabolism in *Leishmania,* based on studies of mutants in genes controlling key steps of biosynthesis and/or degradation.

We recently developed a system for regulatable expression of LPG and potentially other glycoconjugates (da Silva *et al*, *Proc. Natl. Acad. Sci. USA* 2009). This incorporates a protein based approach where a conditionally active 'destabilization domain' ('dd', a mutated mammalian FK506 binding protein) is fused to the protein of interest. In the absence of appropriate ligands (Shld1-, rapamycin or FK506), the 'dd' is unfolded and directs the fusion protein for degradation in the proteasome; in the presence of ligand, the 'dd' folds properly and the fusion protein is stabilized. We used this system to obtain regulated LPG expression in *L. major*, by generating 'knock-in' parasites fusing the 'dd' to the enzyme UDP-galactopyranosyl mutase (UGM), required for the synthesis of galactosylfuranose in the LPG core (Beverley *et al, Euk. Cell* 2005). The levels of LPG expression in the dd-UGM knock-in can be controlled readily and quantitatively, and in the fully 'on' or 'off' states yields parasites whose surface closely resembles that of WT or LPG-deficient parasites. This system shows great promise for the study of LPG and other glycoconjugates during the infectious cycle *in vivo*, as well as many other *Leishmania* proteins.