Biologia Celular- Cell Biology

BC01 - REDESCRIPTION OF *TINTINNOPSIS PARVULA* JÖRGENSEN, 1912 (CILIOPHORA, SPIROTRICHEA, TINTINNINA), INCLUDING ITS PECULIAR LORICA ULTRASTRUCTURE

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The majority of tintinnid species (Ciliophora, Spirotrichea, Tintinnina) was described, using merely lorica features. Since the considerable phenotypic plasticity of the lorica is known today, cell features are investigated to contribute to the establishment of a natural tintinnid classification. *Tintinnopsis parvula* Jörgensen, 1912 has apparently a cosmopolitan distribution in marine and brackish coastal waters. The species is redescribed from material collected in coastal waters of the Irish Sea near the Isle of Man (Great Britain), using live observation, protargol impregnation, and scanning electron microscopy. The species has an agglomerated obconical lorica 38–60 × 24–31 µm in size with a slightly narrowed collar 18–23 µm wide and up to 19 µm long. The somatic ciliary pattern is of the most complex type, viz., it comprises a ventral, dorsal, and posterior kinety as well as a right, left, and lateral ciliary field. The oral primordium develops apparently apokinetally posterior to the lateral ciliary field. In cultures without particles that can be agglutinated, *Tintinnopsis parvula* generates a hyaline lorica whose thin wall consists of an irregular network of fibres 0.04–0.3 µm thick and a few attached or interwoven particles of biotic and abiotic origin. In congeners, three further structures of the lorica matrix were documented by transmission electron micrographs: (i) a solid inner and outer layer enclosing an alveoli layer, (ii) a single solid layer, and (iii) several discontinuous layers of irregularly arranged alveoli. Thus, the matrix ultrastructure might represent a promising feature for a reliable subdivision of the huge genus *Tintinnopsis* Stein, 1867 in the future.
Supported by the Austrian Science Foundation (FWF; Project P20461-B17).

BC02 - CAUDAL CIRRI ARE PRESENT IN *PLEUROTRICHA LANCEOLATA* (EHRENBERG, 1835) STEIN, 1859 (CILIOPHORA, SPIROTRICHEA)

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Absence of caudal cirri has been considered an apomorphic character state in the assessment of phenotypic phylogenetic relationships of the Oxytrichidae. A close relationship of *Pleurotricha* to *Histriculus* has been inferred on the basis of this feature. At least one species originally assigned to *Pleurotricha* has been transferred to *Coniculostomum* due to the presence of caudal cirri. Gupta transferred *Allotricha curdsi*, a species with caudal cirri, to *Pleurotricha* and emended the genus diagnosis of *Pleurotricha* to include presence of caudal cirri. The state of this important character for the genus *Pleurotricha* remained unresolved. The infraciliature, morphometrics, ontogenesis, cyst morphology and a partial 18S rDNA gene sequence of cultured specimens of a of *Pleuroticha lanceolata* from a semiterrestrial habitat in Idaho, USA were studied using standard *in vivo* observation, silver impregnation techniques and molecular methods. The identification of *Pleurotricha lanceolata* was confirmed on the basis its morphologic, morphometric, ontogenetic features, partial 18S rDNA sequence and resting cyst morphology. The three caudal cirri arise from the posterior ends of dorsal kineties 1, 2 and 4, conforming to the typical dorsal morphogenetic pattern of oxytrichids. The marginal cirral rows are nonconfluent posteriorly. The current study documents the presence of caudal cirri, the plesiomorphic character state for oxytrichids, in the type species, *Pleurotricha lanceolata*. A close phylogenteic relationship between *Pleurotricha* and *Histriculus* based on the absence of caudal cirri is not supported. A redescription of *Pleurotricha grandis* is required to assess the presence or absence of caudal cirri in this species. Because *Coniculostomum indica* differs significantly in macronuclear morphology and the morphology of its dorsal kineties from members of the genus *Pleurotricha*, its systematic position remains uncertain. The findings of this study validate the emended diagnosis of *Pleurotricha* to include the presence of caudal cirri.
Supported by the Boise State University College of Arts and Sciences.

BC03 - EFFECT OF IRON ON *TRICHOMONAS VAGINALIS* HYDROGENOSOMES: PROTEOMIC AND TRANSCRIPTOMIC STUDIES

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Trichomonas vaginalis is a sexually transmitted pathogen of humans harboring mitochondria-related organelles called hydrogenosomes. The main function of hydrogenosomes is the synthesis of ATP by substrate level phosphorylation and the formation of iron-sulphur clusters. It was also suggested that hydrogenosomes are involved in amino acid metabolism. Iron has been reported to modulates the differential expression of trichomonads surface antigens, virulence factors and expression of several Fe-S and non Fe-S hydrogenosomal proteins. . The analysis of *T. vaginalis* genome sequence, revealed a number of genes coding for proteins with putative hydrogenosomal localization function of which is unknown including a number of hypothetical proteins. In order to investigate effect of iron on proteomic level, we developed and optimized procedures for quantitative analysis of hydrogenosomal proteomes. The organelles were separated by differential and Optiprep-sucrose gradient centrifugation using cell lysate from trichomonads grown under different iron levels. The proteins were labeled by iTRAQ and after tryptic digestion, the peptides were separated by high resolution nano reversed-phase high-performance liquid chromatography coupled with MALDI TOF/TOF (LC/MS-MS). Altogether we identified 528 proteins from which 72 were predicted to localize in hydrogenosomes. Under iron-rich and iron-restricted conditions, 49 and 258 proteins were upregulated, respectively with 2,0 fold-change. Higher protein level under iron-rich conditions was particularly observed for proteins involved in substrate catabolism and ATP synthesis, while pathway mediated formation of iron–sulfur cluster assembly was upregulated under iron restricted conditions. The proteomic data were corroborated by transcriptomic analysis using the cDNA microarray containing 4950 genes, including 65 genes coding for putative hydrogenosomal proteins. Our results indicate that iron plays a key role in the regulation of *T. vaginalis* gene expression.

BC04 - *Paramecium tetraurelia* as a model for Ca^{2+} signaling and signal downregulation in protozoa

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Ca^{2+} signals govern many processes, also in protozoa, from free-living forms to parasites (some of which require a Ca^{2+} signal for host cell penetration). Our group was focusing on Ca^{2+}-dependency of exocytosis of dense-core secretory vesicles (trichocysts) in *Paramecium* cells, as well as on ciliary beat regulation. We faced the problem of highly mobile cells, refusing the uptake of fluorochrome esters, and rapid fluorochrome sequestration. We injected fluorochromes, and combined stimulation eventually with rapid confocal Ca^{2+} imaging. We also measured Ca^{2+}/calmodulin-activated currents over the cell membrane to monitor subplasmalemmal Ca^{2+} currents. This was complemented by quench-flow stimulation, from 30 ms on, followed by structural EM analysis and a protocol retaining Ca^{2+} for energy-dispersive x-ray microanalysis. All this occurred within ~30 ms, slightly below the apparent halftime of synchronous exocytosis whose kinetics depends of extracellular Ca^{2+}, $[Ca^{2+}]_o$, although the mechanism of Ca^{2+} signaling during exo-endocytosis involves a store-operated Ca^{2+} influx. The stores are the “alveolar sacs”, flat compartments tightly attached to the cell membrane (just like “inner membrane complexes” in related parasites, *Toxoplasma* and *Plasmodium*). We have characterized only recently their rather unorthodox Ca^{2+}-release channels (see Ladenburger et al. 2009).

While the signal sweeps into the cell, it is rapidly bound to the infraciliary lattice, a cortical meshwork containing the Ca^{2+}-binding protein, centrin (see Sehring et al. 2009). Knock-out cells show its high Ca^{2+} buffering capacity. Further on, Ca^{2+} is downregulated as follows. As Ca^{2+}-pumps in the cell membrane and in the cortical stores proved rather slow, we also envisaged the osmoregulatory system (ORS, known to release Ca^{2+}). Its H^+-ATPase can drive an antiport system, as the downregulation of Ca^{2+} can be inhibited by concanamycin A (work in progress). The IP_3 receptors we found in the ORS my serve fine-tuning and their expression depends on $[Ca^{2+}]_o$. A number of additional unorthodox Ca^{2+}-release channels currently under investigation may deserve some interest also for some of the protozoan parasites.

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BC05 - INFECTION PROCESS OF SYMBIOTIC ALGAE TO THE ALGAE-FREE *PARAMECIUM BURSARIA*

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Each symbiotic *Chlorella* sp. of the ciliate *Paramecium bursaria* is enclosed in a perialgal vacuole (PV) derived from the host digestive vacuole (DV). Algae-free paramecia and symbiotic algae still have an ability to grow independently and can be experimentally reinfected by mixing with each other. This phenomenon provides an excellent opportunity to elucidate cell-to-cell interactions between the protozoa and algae during establishment of the secondary endosymbiosis. However, the detailed algal infection process had been unclear. Using pulse-label of the alga-free paramecia with the isolated symbiotic algae and chase method, we found 4 important cytological events needed to establish endosymbiosis. (1) Three minutes after mixing, some algae show resistance to the host lysosomal enzymes in the DVs even if the digested ones are coexisted. This is not an inherent property because it occurs even in the cloned symbiotic algae. The algal fate does not depend on the cell cycle stage or location in the DV, and does not need algal protein synthesis. (2) Thirty minutes after mixing, the alga starts to escape from the DVs by budding of the membrane into the cytoplasm. (3) Within 15 minutes after the escape, vacuole enclosing one green alga differentiates to the PV from the DV, which gives protection from the lysosomal fusion. (4) After that, the alga localizes in the primary lysosome-less host cell surface by an affinity of the PV to unknown structures of the host. At about 24 hours after mixing, the alga increases by cell division and establishes endosymbiosis. Infection experiments with infection-capable and -incapable algae indicate that the infectivity is based on their ability to localize beneath the host surface after escaping from the DVs. This algal infection process is different from those of other symbiotic or parasitic organisms to their hosts so far known. Supported by JSPS.

BC06 - *Tritrichomonas foetus*: budding from multinucleated pseudocysts

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Tritrichomonas foetus is a flagellated protozoan parasite that causes bovine trichomoniasis, a major sexually transmitted disease in cattle. This parasite presents a simple life cycle, exhibiting only the trophozoitic form, which is characterized by a pear-shaped body. Under unfavorable environmental conditions, the *T. foetus* trophozoites can round up and internalize their flagella forming pseudocysts. For many years, pseudocysts were considered to be a degenerated form. Currently, it is believed that this form is reversible and that its formation represents a defense mechanism to unfavorable environmental conditions. There are still several open questions concerning pseudocysts´ biology, such as: is this form capable to divide? Thus, in the present study, complementary techniques, such as, immunofluorescence, videomicroscopy and transmission electron microscopy were used in order to better understand the pseudocyst mitosis and compare it with our previous knowledge of the division process in pear-shaped parasites. Here we show that during pseudocyst mitosis the cell proceeds with duplication cytoskeletal and mastigont structures; the pole-to-nucleus spindle is responsible for the karyokinesis and not the axostyle as occurs in the pear-form; nucleus division occurs but without the corresponding cytoplasm division. Thus, giant multinucleated cells which present many mastigont structures are formed. These polymastigont/multinucleated cells are maintained when the cells are under stress conditions. When environmental conditions become favorable, the flagella are externalized and new organisms, one by one, gradually bud from the multinucleated cell. Thus, we concluded that pseudocysts are capable to divide with characteristics different from those found in the pear-shaped parasites.
Supported by AUSU, CAPES, CNPq, FAPERJ and PRONEX.

BC07 - EXPLOITATION OF MEMBRANE MICRODOMAINS BY *TOXOPLASMA GONDII*

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Membrane “rafts” are small and dynamic regions significantly enriched in cholesterol, glycosphingolipds, sphingomyelin, GPI-anchored proteins and unique integral membrane proteins. The exploitation of rafts by intracellular pathogens may facilitate invasion. *Toxoplasma gondii* is an active pathogen with respect to its mode of invasion and intracellular residence. The vast majority of host cell proteins do not pass beyond the moving junction into the parasitophorous vacuole membrane (PVM) that surrounds the parasite, but many components of PVM are also constituents of host cell membrane microdomains. We evaluated the participation of microdomains in invasion of *T. gondii* into LLC-MK2 and murine macrophages through transient depletion of host cells cholesterol with either methyl-beta-cyclodextrin (MβCD) [final concentrations of 5, 10 and 20 mM for 30 min before interaction]; or Filipin [final concentrations of 1, 3 and 6 nM for 30 min before interaction]. Reversibility for MβCD was tested by posterior addition of 20% fetal bovine serum in 199 medium. After interaction (50 parasites per cell), samples were processed for light microscopy and adhesion and internalization indexes on cells treated with MβCD significantly diminished in relation to controls. At 20 mM of MβCD, inhibition of internalization reached 80%, moreover with macrophages the inhibition was higher, reaching almost 100%. Treatment with MβCD followed by cholesterol reposition before interaction with *T. gondii*, completely reverted inhibition in macrophages, but this reversibility was not observed in LLC-MK2. Treatment with Filipin did not interfere in interactions with LLC-MK2 cells. This can be due to formation of aggregates of cholesterol instead of its depletion. Taken together, these results indicate that internalization of *T. gondii* in professional phagocytes as macrophages may include different pathways in relation to non professional phagocytes as LLC-MK2 cells.
Supported by CNPq, CAPES and FAPERJ

BC08 - GOLGI COMPLEX PARTICIPATES IN THE CYST WALL ASSEMBLY IN *ACANTHAMOEBA* SPP.

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Differentiation into resistant stages (encystation; sporulation) is a common reaction to unfavourable conditions of the environment in many different microorganisms. Highly resistant cell wall or coat formation and/or decrease of the metabolism are among many other changes organisms undergo in order to protect themselves. Cell surfaces of “vulnerable” and resistant forms differ in composition: new material is usually synthesised and deposited on cell surface to form a protective external layer. Trophozoites of *Acanthamoeba* spp. differentiate into cysts with double-layered wall. The process of the cyst wall assembly can be subdivided into two distinct phases: exocyst (outer mostly proteinaceous layer) formation as the first phase and endocyst (inner cellulotic layer) formation as the second phase. We used staining with fluorescent brightener Calcofluor white to follow the process of cellulose/other polysaccharide synthesis/deposition after induction of the encystation in vitro. Calcofluor positive vesicles were detectable in the cytoplasm of encysting trophozoites shortly after induction. These vesicles were subsequently transported to cell surface during the phase of exocyst assembly. To confirm Golgi origin of these vesicles, encystation trophozoites were exposed to concentration range of Brefeldin A. Decrease in the number of trophozoites with Calcofluor-positive vesicles after the treatment in contrast to its increase in the control group, suggests Golgi participation in the cyst wall assembly. To further confirm these findings, double staining with either, antibody against Golgi specific protein or fluorescent ceramides was used. Our results strongly indicate the involvement of Golgi transportation machinery in the process of cyst wall layers assembly. However further studies are needed in order to characterize the polysaccharide content of the vesicles in detail.
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BC09 - Amoeboid Motility (AM) and Flagellar Motility (FM): Dialing into cellular motility using comparative genomics

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Flagellar and amoeboid motility are the two most common forms of locomotion used by protists. *Naegleria gruberi* is known for its remarkable ability to metamorphose from amoebae into streamlined biflagellates that swim a hundred times faster than amoebae crawl. This rapid (<1.5 h) change begins with the cessation of amoeboid movement and actin synthesis, followed by the regulated synthesis of tubulin and other flagellar components, and *de novo* assembly of an entire cytoplasmic microtubule cytoskeleton, including canonical basal bodies and 9+2 flagella. Using the newly sequenced *Naegleria* genome and phylogenetic profiling, we have identified protein families conserved only in eukaryotes with flagellar motility (Flagellar-Motility associated genes; FMs) or amoeboid motility (Amoeboid-Motility associated genes; AMs). *Naegleria*'s 182 FM gene families are consistent with typical eukaryotic flagellar structure, and also include proteins required for flagellar beating, intraflagellar transport, and 36 novel flagellar-associated genes. The 63 AMs include genes known to be involved in amoeboid motility, membrane differentiation, and 19 novel genes. Using the awesome power of *Naegleria* differentiation, we further validated the AM and FM gene sets via microarray analysis. Nearly 80% of FMs are transcriptionally induced at least 2-fold (with p-values < 0.01, with multiple testing correction), consistent with their function in flagella. Similarly, 60% of AMs are repressed during the transition from the amoeboid state. Finally, we used the timing of induction to subdivide FMs into subsets enriched in axonemal proteins (induced late) and basal body proteins (induced early). This set includes 38 potential basal body genes, including 8 novel genes that are conserved across eukaryotes. We are currently using human cell assays to functionally characterize these candidate basal body genes.
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BC10 - Interaction of *Leishmania amazonensis*-macrophage: effects of different inhibitors on the adhesion and internalization process

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Leishmaniasis comprises a large spectrum of tropical diseases caused by parasites from the *Leishmania* genus. *Leishmania* gets into the host's macrophage via phagocytosis mechanisms and transforms from a promastigote form to an amastigote form inside the parasitophorous vacuole. Here, we analyzed some aspects of the interaction of *Leishmania amazonensis* promastigotes with murine macrophages using inhibitors of the phagocytosis. Dynasore is a small molecule that inhibits de GTPase function of dynamin, which are involved in the clathrin-dependent phagocytosis. Amiloride is one inhibitor of the macropinocytosis, which is a form of endocytosis characterized by a cell surface ruffling. Wortmannin is an inhibitor of the phosphoinositide 3-kinase (PI3K), which is implicated in the completion of macropinocytosis. We observed in control cells that after 5 minutes of interaction the majority of the parasites were adhered to the macrophage by the cellular body. In addition, vacuoles containing promastigotes were already observed. After 2 hours of interaction, the promastigotes were adhered mostly by the flagellum and there were several amastigotes inside the parasitophorous vacuoles. Scanning electron microscopy showed that the macrophages emit large ruffling of membranes to engulf the promastigotes by the cell body and the flagellar tip. Pre-treatment of macrophages with Dynasore firstly induced an inhibition of the adhesion in concentrations upper than 40 µM, and after 45 min of interaction, the internalization of the parasites was also inhibited, even at a concentration of 20 µM. Amiloride and Wortmannin also inhibited the internalization of *L. amazonensis* promastigotes in concentrations of 25 µM and 10 nm, respectively. Taken together, these results indicate that the process of internalization of *L. amazonensis* promastigotes by murine macrophages involves several endocytic mechanisms with the participation of dynamin, PI 3-kinase, and macropinocytosis.
Supported by CAPES, FAPERJ and CNPq.

BC11 - *LEISHMANIA MAJOR*, BUT NOT *LEISHMANIA AMAZONENSIS*, INDUCES REACTIVE OXYGEN INTERMEDIATES PRODUCTION BY CBA/J MICE MACROPHAGES

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CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*, indicating an important role for macrophages on determination of *Leishmania* infection outcome. It is well known that some *Leishmania* sp. are destroyed by reactive oxygen intermediates (ROI) inside macrophages, although some *Leishmania* sp. could also inhibit ROI production by those cells. Previously we demonstrated that *L. major* infection induces higher hydrogen peroxide (H_2O_2) production than *L. amazonensis* on CBA/J infected macrophages. We hypothesized that the infection outcome in CBA/J macrophages is related to ROI production early after *L. major* and *L. amazonensis* interaction with these cells. Using a high sensitive approach based on photon counts of lucigenin/luminol-enhanced chemiluminescence, we detected superoxide (O_2^-) and H_2O_2 production early after phagocytosis and on established *L. major* or *L. amazonensis* infection. It was observed that *L. major* induced O_2^- production, while *L. amazonensis* internalization occurred in absence of O_2^- production by those cells. The O_2^- production during *L. major*-macrophage interaction was due to NADPH oxidase function, since it was abolished by apocyanin treatment. In addition, *L. major* also induces higher levels of H_2O_2 production by CBA/J macrophages when compared to *L. amazonensis*-infected cells. After 6 and 24h after infection, *L. major* and *L. amazonensis* induced similar levels of O_2^- production in CBA/J macrophages. These results suggest that CBA/J macrophages control *L. major* infection by a mechanism dependent on ROI production by host cell. Current experiments goals to evaluate the mechanisms related to ROI effects on the reduction of *L. major* parasitism inside macrophages and the mechanism of *L. amazonensis* blockage of CBA/J macrophages' killing mechanisms. Supported by CAPES and FAPESB ET64/2004; 5742/2006.

BC12 - Chromatin changes caused by overexpression of anti-silencing function 1 (Asf 1) in *Trypanosoma brucei*

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Asf 1 (anti-silencing function 1) is one of the most conserved histone chaperones among all the eukaryotes. It's known that this protein has a bromodomain that interacts with the histone heterodimer H3/H4 and controls the nucleosome assembly. For this reason Asf 1 is consequently is involved in DNA replication, transcription, DNA repair and silencing/anti-silencing. As *Trypanosoma brucei* the agent of African Trypanosomiasis modulates transcription of some genes by changing the chromatin structure, we studied the role of Asf in this parasite. In a previous study, we have found that RNAi for Asf 1 causes cell growth arrest. Here we asked whether an excess of this protein could affect *T. brucei* growth. Asf 1 was cloned in the vector pLew100 and used to transform 29-13 procyclic and 90-13 bloodstream lines. Upon Asf 1 expression induction with tetracycline, we observed no effect on cellular growth and cell cycle. However, Asf 1 overexpression caused a significant increase in the nucleus size, probably due to alterations in the chromatin structure. We will present results showing what these changes are and how they affect the gene expression.

BC13 - Macropinocytosis: *Trypanosoma cruzi* new entry pathway into peritoneal macrophages?

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Several intracellular pathogens are internalized by host cells via multiple endocytic pathways. *Trypanosoma cruzi*, the ethiological agent of Chagas disease, may not be different. Evidences indicate that *T.cruzi* entry may occur by endocytosis/phagocytosis or by an active manner. Recently studies described that several pathogens such as *Salmonella* and *Shigella* can entry into host cells by a macropinocytosis mechanism, although this process was initially considered an endocitic process where cells internalized only large amounts of solutes. Our previous results, using scanning electron microscopy and transmission electron microscopy showed that trypomastigotes can entry in peritoneal macrophages through macropinosome-like structures, formed from cell surface ruffles resembling the micropinocytosis process. To investigate if a macropinocytosis-like process would take place during *T.cruzi* entry into macrophages, we used amiloride, a selective inhibitor of macropinocytosis and membrane ruffling. When peritoneal macrophages were treated with crescent amiloride concentrations and then allowed to interact with the three developmental stages of *T.cruzi,* we observed a drastic reduction in the entry process of all developmental forms. By field emission scanning electron microscopy, we observed that parasites remained only attached to host cell plasma membrane. Trypomastigotes and epimastigotes presented part of their bodies recovered by a large part of host cell plasma membrane, like an initial formation of macropinosomes, after two hours of infection. This process, when observed in control experiments, showed, in the most part of interaction events, parasites recovered by a cup-like structure originated from macrophage plasma membrane until they are completely internalized. By transmission electron microscopy we observed that internalized parasites were found in the cell periphery. Together, these results suggest that *T.cruzi* can also entry in peritoneal macrophage by a macripinocytosis pathway.
Supported by CNPq, CAPES and Faperj

BC14 - Differential Roles for G-Protein Coupled Endothelin Receptor Subtypes (ETbR and ETaR) during the Temporal Progression of Human Smooth Muscle Cell Invasion by *Trypanosoma cruzi*

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The internalization of *Trypanosoma cruzi* trypomastigotes by non-phagocytic host cells is the final outcome of a complex communication process, forged by intimate host-parasite adhesive interactions and reciprocal signaling events. In several models, productive infection depends on lysosomal fusion to the nascent parasitophorous vacuole. Classical studies indicated that $[Ca^{2+}]_i$-dependent intracellular responses transduced by G-protein coupled receptors (GPCR) can drive lysosomal exocytosis and fusion to the plasma membrane, ultimately allowing for the endocytic internalization of the parasite. Bradykinin receptors were the first example of a GPCR with defined pharmacological specificity that drive parasite internalization by cardiovascular cells. We now report that TCT (Dm28c) can also explore the endothelin (ET) pathway to invade human smooth muscle cells (hSMC), endothelial cells (HUVECs) and mouse cardiomyocytes through the triggering of ET receptors (subtypes ETaR and ETbR). Interaction assays performed with either CHO-ETbR or CHO-ETaR revealed that both types of GPCRs promote parasite uptake. Addition of ETbR (BQ788) or ETaR antagonists (BQ123), selectively reduced invasion of CHO-ETbR or CHO-ETaR, respectively. We then used these pharmacological tools to study the outcome of parasite interaction with hosts cells that naturally express ETRs. Our data showed that TCT invade hSMC and mouse cardiomyocytes through the signaling of both ETbR and ETaR whereas invasion of HUVECs is exclusively dependent on ETbR pathway. Given evidences that intracellular retention signals are required to consolidate infection we then checked whether ETRs could be involved in such processes. These studies revealed that ETbR is exclusively involved in host cell penetration, while ETaR controls parasite retention in the infected hSMC. Additional studies are required to determine whether vacuole-associated *T. cruzi* are able to convert big-endothelin into bioactive ETR agonists during the complex dynamics of the invasion process.
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BC15 - Flagellar surface structure of Protozoa by Atomic Force Microscopy

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In many different cells, motility is often mediated by flagellar beating. Protozoa have a high and coordinated motility which contributes to make the parasites members of this group extremely pathogenic to mammalian hosts. Understanding the structural aspects of the flagellum may be important to an identification of novel targets for therapeutic intervention. Our group has used Atomic Force Microscopy (AFM) to examine the ultrastructure of *Trypanosoma cruzi*, obtaining additional information about the sub-structural organization of the flagellum. AFM images could reveal a periodically organized structure along the flagellar furrow. This furrow was oriented through the major flagellar axis; moreover the nature and function of this structure are unknown. We can speculate that this channel, in some way, delimitate the two distinct domains of the flagellar membrane, the axoneme and the Paraflagellar Rod (PFR). To test this hypothesis, we decided to analyze different protozoa, some presenting and others not presenting the PFR structure. We analyzed *Trypanosoma cruzi, Trypanosoma brucei* and *Herpetomonas megaseliae,* which show the PFR structure, and *Giardia lamblia* and *Chritidia deanei* which do not present the PFR or only a very reduced one (*C. deanei*). Analysis of AFM images of *T. cruzi, T. brucei* and *H. megaseliae* flagella demonstrated similar and equivalent furrow along the main axis of the flagellum. In contrast, we could not observe the flagellar furrow in *G. lamblia* and *C. deanei*. Altogether, our results confirm an association between the flagellar furrow and the presence of the PFR.
Supported by CAPES, CNPq and FAPERJ.

BC16 - TRANSCRIPTOMIC ALTERATIONS IN *TRYPANOSOMA CRUZI*-INFECTED CARDIAC MYOCYTES

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Trypanosoma cruzi infection is a major cause of cardiomyopathy. Gene profiling studies of hearts from infected mice have revealed prominent changes in expression of numerous genes within many functional pathways. This variety of transcriptomic changes in infected mice raises the question of the extent to which gene expression alterations in whole hearts are due to changes in cardiac myocytes or to other cells. We employed oligonucleotide arrays to examine cultures of infected cardiac myocytes 48hr post-infection. Statistical comparison of gene expression levels of 2,258 unigenes in four independent cultures of infected and uninfected myocytes detected significant > 1.5 fold changes in 221 (8.8%) of the sampled genes. Major categories of affected genes included those involved in immune response, extracellular matrix and cell adhesion. These findings on infected cardiac myocytes in culture reveal that changes in gene expression that have been described in the heart in Chagas disease are the consequence of both direct infection of the myocytes and changes resulting from presence of other cell types in the myocardium.
Supported by: CNPq, FAPERJ, CAPES, National Institutes of Health (NIH) and Fogarty International Training Grant.

BC17 - *TRYPANOSOMA CRUZI* INDUCE BLOOD CELLS TO RELEASE MICROVESICLES THAT INHIBIT COMPLEMENT LYSIS AND PROMOTE CELL INVASION

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During infection of mammals, the insect-derived trypomastigote metacyclic forms of *Trypanosoma cruzi* have to escape lysis by the complement system and invade host cells. We have detected that *T. cruzi* trypomastigote metacyclic forms induce the release of microvesicles (MVs) from monocytic (THP-1) and lymphocytic (Jurkat) cells. MVs originate from the cell plasma membrane, present asymmetric distribution of phosphatidylserine, and are between 200 to 500 nm. The release of MVs by monocytic cells after *T. cruzi* stimulus is Ca^{2+}-dependent and is inhibited by 5 mM EGTA, 1.5 μM thapsigargin, and 100 nM wortmannin. We have investigated the role of MVs in the parasite complement lysis and invasion in eukaryotic cells. Firstly, complement lysis of *T. cruzi* epimastigote forms by human serum was inhibited by MVs in a dose-dependent manner. MVs bind to C3, but rather than inhibiting C3b deposition on the parasite surface, they inhibited further cleavage of C3b to iC3b. Addition of exogenous C3 restored the parasite lysis in the presence of MVs. We also detected an increase in the amount of C2a bound to the parasite surface proportional to MV concentration, and a slow dissociation of C2a in the presence of MVs, indicating that MVs could be interacting with, and stabilizing, C3 convertase. Monocyte- and lymphocyte-derived MVs increased invasion of *T. cruzi* in Vero and HeLa cells. The MV's effect on *T. cruzi* invasion was inhibited by pre-incubating MVs with anti-TGF-β antibodies or pre-incubating the cells with SB-431542, a TGF-β receptor kinase inhibitor. Also, TGF-β was detected in MVs as measured by ELISA and flow cytometry. The roles of both MVs and TGF-β in *T. cruzi* invasion are under investigation. These results suggest that *T. cruzi* induce microvesiculation in blood cells that contributes to parasite success in the early stage of infection.
Supported by CNPq, Fiocruz and FAPERJ.

BC18 - EFFECTS OF ACRIFLAVINE ON TRYPANOSOMATIDS: ULTRASTRUCTURAL, BIOCHEMICAL AND MOLECULAR APPROACHES

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The Trypanosomatidae family comprises flagellated protozoa which present an unusual structure, the kinetoplast, which contains the mitochondrial DNA (kDNA) arranged in catenated maxicircles and minicircles. This unique organization of the kDNA network and the susceptibility to a great variety of compounds, make this structure a potential target to chemotherapy. The kDNA presents different arrangements that vary according to species and stage of development, being also modified by the presence of an endosymbiont. In this work we analysed the effects of acriflavine, a DNA intercalating agent, in trypanosomatid growth, kinetoplast ultrastructure, minicircle configuration and O_2 consumption. In order to analyze the effect of acriflavine on proliferation of *Trypanosoma cruzi* epimastigotes and *Blastocrithidia culicis*, cells were cultivated with different drug concentrations (1 to 50 μg/ml). After each 12 and 24 hours, part of the culture was removed for cell counting or for processing to transmission electron microscopy. Ultrastructural analysis and the TdT technique were employed to verify the acriflavine effect on kDNA distribution. Molecular approaches were used to evaluate minicircles configuration after drug treatment. For this purpose, total protozoa DNA (control and treated cells) was digested with nuclease and submitted to the Southern blot technique using the Universal Minicircle Sequence (UMS) labeled with γATP^{32} as probe. Our results showed that acriflavine promoted a dose-dependent inhibitory effect on the cell proliferation and generated dyskinetoplastic protozoa on both species. Ultrastructural analysis showed that the acriflavine treatment promoted a drastic ultrastructural modification in the kinetoplast, which changed kDNA distribution and compactation. Biochemical approaches showed the acriflavine ability to decrease the protozoa oxygen rate consumption, indicating that the energetic metabolism of mitochondrion was affected in treated cells. Molecular data suggest that acriflavine induces minicircle linearization in *T. cruzi* and *B. culicis*.
Supported by CNPq, FAPERJ and CAPES.

BC19 - EXPLORING SIGNALING EVENTS SURROUNDING EXTRACELLULAR AMASTIGOTE INVASION PROCESSES OF *TRYPANOSOMA CRUZI*

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Amastigotes which are generated by the extracellular differentiation of trypomastigotes are referred to as Extracellular Amastigotes (EA) and are able to invade cultured cells. EA of the G strain promptly aggregate actin filaments by attaching to dorsal microvilli of HeLa cells and, as a result, cup-like structures are formed underneath the parasite. EA is therefore dependent on host actin filaments polymerization to invade cells. EA invasion can be easily detected by several techniques, such as freeze-fracture replicas of recently-infected HeLa cells. However, signaling events surrounding these processes are still obscure. In the present study, we aim to examine these events and EA invasion features by focusing on the following molecules: cortactin, ezrin, Protein Kinase D (PKD) and a set of kinase inhibitors. Cortactin has emerged as a key signaling protein in cellular processes such as endocytosis and tumor invasion. The ability of cortactin to interact with and alter the cortical actin network is central to its role in regulating these processes. Ezrin is characterized by an N-terminal FERM domain and a C-terminal actin-binding domain. Once activated, ezrin dissociates and acts as a plasma membrane-cytoskeletal linker and thereby affects a variety of cellular activities, such as actin cytoskeleton regulation, control of cell shape, cellular adherence and migration, and the modulation of intracellular signaling pathways. It has been recently demonstrated that cortactin is a substrate of PKD phosphorylation *in vivo* and it also colocalizes with ezrin. Hela, Vero and CHO cells were transfected with the following markers- cortactin, ezrin, RhoA and PKD GFP-vectors- infected with EA and examined for the acquisition of these markers. Cells were previously treated with kinase (PKC, MAP kinase, Src, PI3K and Rho) inhibitors and control cells were left untreated. A number of initial assays have yielded encouraging results. For example, PKD is recruited to sites of actin remodeling at the leading edge of EA invasion, which also recruits cortactin. Co-localization of PKD and cortactin may be an indication that PKD plays a role in cytoskeletal reorganization. PKC–PKD signaling cascade is crucial to PKD function in cells. PI3K seems to interfere with neither ezrin nor cortactin recruitment. Here, we propose a signaling pathway model of EA entry. This pathway includes the above mentioned kinases as upstream molecules.
Supported by: FAPESP.

BC20 - NEGLECTED THOUGH ENABLING AN ENDLESS LIFE: THE RESTING CYSTS OF PROTISTS

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Many protists can produce a dormant stage, the resting cyst, when the environmental conditions become unfavourable or dangerous. Indeed, it is the resting cyst which makes possible the endless life of protists! Thus, it is difficult to understand why morphologic, physiologic, and ecological research on resting cysts has ceased almost completely. Looking at the great morphologic, ontogenetic, and histochemical diversity, I am convinced that resting cysts are the key to some main ecological questions, for instance, why a certain species is active at a certain time and under certain conditions. In the lecture, I shall highlight some of the diversity of the ciliate resting cysts, emphasizing the so-called pericyst, i.e., the most distal layer, which is often highly complex and contains, inter alia, scale-like structures (“lepidosomes”). The function of the pericyst, which is lost when the cell excysts, is a true mystery when considered under an energetic aspect because, as yet, a specific physiological or ecological function is not recognizable. For the ecologists, I shall show that certain ciliates produce cyst wall precursors during the trophic stage, possibly with the intention to be able to encyst rapidly if necessary. Further, I shall show some cases where encysting ciliates “enslave” food vacuoles to finish the cyst wall after actual encystment and/or to divide within the cyst. Physiologists might be surprised by the complex morphology of the cyst’s stage products, i.e., of the glycoproteins. Supported by the Austrian Science Foundation, FWF project P-19699-B17.

BC21 - CRYPTOSPORIDIA: EPICELLULAR PARASITES EMBRACED BY THE HOST CELL MEMBRANE

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The development of two gastric cryptosporidia, *Cryptosporidium muris* from experimentally infected rodents (*Mastomys natalensis*) and *Cryptosporidium fragile* from naturally infected toads (*Duttaphrynus melanostictus*), were studied using transmission and high resolution field emission scanning electron microscopy. Observations on these evolutionarily distinct species, from a mammal and an amphibian respectively, allowed us to map ultrastructural aspects of the cryptosporidian invasion process along with the origin and architecture of parasitophorous sac, and to generalize our observations for gastric cryptosporidia. Both species exhibited a comparable strategy of host cell invasion, in which contact between the invading zoite and the host cell induced recruitment of the microvillar membrane. The recruited part of the host cell membrane lost its microvillous appearance and formed a tight-fitting elastic membrane fold, encircling the contact zone between zoite and host cell, which gradually rose along the zoite, subsequently forming the parasitophorous sac. Thus, cryptosporidia do not penetrate under the host cell plasma membrane, nor do they come into close contact with the host cell cytoplasm. The only exception is a region of early tunnel connection between the parasite and the host cell; nevertheless, even this transient connection disappears when the feeder organelle forms and becomes limited by a dense line separating it from the filamentous projection, i.e. from the parasitophorous sac of host cell origin. The parasite remains attached to the host cell surface, only enveloped by the host cell membrane folds. Therefore, the term epicellular more accurately defines the host compartment within which cryptosporidia reside than does the term intracellular-extracytoplasmic. Further analyses revealed the existence of pore-like structures in the surface of the parasitophorous sac. As these structures were also seen in older developmental stages, they are not a priori considered initial non-fused areas before the rims of membrane folds completely coalesce.
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BC22 - The actin cytoskeleton of *Giardia intestinalis*

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Giardia intestinalis is a flagellated intestinal parasite that causes diarrheal disease in a wide variety of hosts including over 100 million people each year. A diplomonad, it is thought to belong to the earliest diverging eukaryotic lineage, and is much more divergent from yeast than yeast is from man. Thus, Giardia is a valuable model organism for gaining key insights into early eukaryotic cell evolution. Giardia has a complex microtubule cytoskeleton including basal bodies and axonemes and novel structures such as the ventral disc, an organelle involved in parasite attachment to the intestinal wall of the host. Although the majority of the components of the microtubule cytoskeleton can be identified in the giardial genome, no members of the canonical actin cytoskeleton can be identified except for actin itself (58% identity on average), and three divergent actin related proteins (ARPs). Missing from the giardial genome are myosin motors, Arp2/3 and other proteins needed for actin nucleation, proteins that regulate actin polymerization such as profilin, and actin cross linking proteins. Our objective is to characterize the divergent giardial actin cytoskeleton which we expect to provide a unique perspective on actin cell biology and evolution. Phylogenetic analysis reveals that all three giardial ARPs cluster with either Arp8 or Arp9 and GFP fusions to these ARPs localize to the nuclei suggesting they function in chromatin remodeling and not filament formation. We have generated an antibody against giardial actin that labels unique actin structures of unknown function as well as the nuclei, cell cortex, and axonemes. Disrupting actin with Morpholinos results in a number of interesting phenotypes including cytokinesis and polarity defects; we are currently assessing the role actin plays in giardial endocytosis. Importantly, the defects observed in the actin Morpholino studies demonstrate that actin has multiple functions in *Giardia.* The presence of discreet asymmetrically localized actin structures suggests the presence of actin binding proteins to specifically recruit actin to these structures. Therefore, we are performing a two-hybrid screen using giardial actin as the bait to identify actin binding proteins in Giardia. Supported by NSF and NIH.

BC23 - THE SUMO-CONJUGATING SYSTEM IN *GIARDIA LAMBLIA*

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Reversible post-translational modifications of proteins have critical roles in many cellular processes owing to their ability to cause rapid changes in functions of pre-existing proteins and sucellular structures. Sumoylation, a reversible post-translational modification of proteins by the small ubiquitin-related modifiers (SUMOs), is crucial in a variety of biological processes, including transcriptional regulation, signaling transduction, cell cycle progression and differentiation. SUMO proteins are highly conserved across eukaryotes and consist of four components in mammals, SUMO-1, SUMO-2, SUMO-3, and SUMO-4. There is only one SUMO gene SMT3 in budding yeast, while there exist at least eight SUMO paralogs in plants. In parasites, protein sumoylation has been described in *Plasmodium falciparum* and in *Toxoplasma gondii*. Here, we report that the sumoylation system is present in *Giardia lamblia* by means of the conserved components, including a single SUMO gene, the enzymes required for SUMO processing and ligation and the SUMO proteases for the conjugate disassembly. After amplification by PCR, all of the components of the sumoylation pathway were used to transfect *Giardia lamblia* trophozoites and the localization of each one was determined. In order to find sumoylation substrates, immunoblotting experiments using anti-SUMO mAb were performed. We could identify the enzyme arginine deiminase as a sumoylated protein. The ability of anti-SUMO mAb to immunoprecipitate the 85 kDa band of gADI confirmed the gADI-SUMO interaction. Proteins modified by SUMO could alter their sub-cellular localization, activity or stability. We found that during differentiation of the parasite mainly the sumoylated form of gADI translocate to the nucleus, inducing the downregulation of the encystation process. Overall, the SUMO conjugation system appears to be a functionally pathway for protein modification in *Giardia lamblia* with initial data indication that it may plays a role during cyst development.
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BC24 - THE UNIQUE MITOCHONDRION RELATED ORGANELLE OF *BLASTOCYSTIS*

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Blastocystis sp. is a unicellular human intestinal parasite that belongs to the stramenopiles, a diverse group that also comprises brown algae, diatoms, and other protistan eukaryotes with classical aerobic mitochondria. *Blastocystis* is a strict anaerobe and it was suggested that it might possess hydrogenosomes, anaerobic ATP-generating organelles related to mitochondria. However, in contrast to typical hydrogenosomes the *Blastocystis* organelles contain a genome, which has recently been sequenced. In addition, analyses of >12,000 expressed sequence tags (ESTs) from a *Blastocystis* cDNA library have identified 115 putative mitochondrial and hydrogenosomal proteins. Amongst these, [FeFe]-hydrogenase, a hydrogenosomal marker protein was previously shown to be localized to the mitochondrion-related organelles (MROs). Along with the previously published data, preliminary results from bioinformatic analyses, complementation and cellular localisation studies demonstrate that these organelles also have functional canonical mitochondrial pathways for amino acid metabolism, iron-sulphur cluster biogenesis, mitochondrial protein import and an incomplete tricarboxylic acid cycle. Collectively, these data suggest that the *Blastocystis* MROs are metabolic intermediates between mitochondria and hydrogenosomes. Consequently, these data highlight the essential functions of mitochondria conserved across diverse eukaryotic lineages as well as illuminate the metabolic plasticity of these fundamental organelles.
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BC25 - Membrane domains and boundaries at the host-parasite interface: influences of the cytoskeleton on the flagellar pocket of African trypanosomes

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A key feature of immune evasion for African trypanosomes is the functional specialization of the surface membrane at the base of the flagellum to an internal region known as the flagellar pocket (FP) – the sole site of endo- and exocytosis in bloodstream-form *Trypanosoma brucei*. By localising many of its invariant endocytosis receptors in the FP protected environment, the parasite is able to keep them sheltered from aspects of host recognition. The FP membrane is contiguous with those of the cell body and the flagellum, but is biochemically distinct. The structural features that maintain this individuality are not known. Moreover, we lack a clear understanding of how extracellular components gain access to the FP lumen (which is an essential prerequisite to uptake), and how the organelle is organized to perform its multiple associated functions. Here, we used electron tomography and freeze-fracture to define domains and boundaries on the trypanosome surface membranes, and their association with distinct cytoskeletal features. We show that FP membrane is largely homogenous and uniformly competent for endocytosis. However, when endocytosis is blocked, receptor-mediated and fluid-phase endocytic markers accumulate specifically on membrane associated with four specialised microtubules found in the FP region. These microtubules traverse boundaries and are associated with a continuous channel that connects the FP lumen to the extracellular space. The accumulation of endocytic markers in this channel suggests that it is the major transport route into the FP.
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BC26 - B-1 CELLS ARE HIGHLY SUSCEPTIBLE TO *TRYPANOSOMA CRUZI* INFECTION

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Establishment of *T. cruzi* infection is critically dependent on host cell invasion by metacyclic trypomastigotes. Different mammalian cell types are susceptible to metacyclic form invasion at varying degrees. B-1 cells, which are distinct from B-2 and constitute the main source of B cell-derived interleukin 10 (IL-10), have never been investigated as target for *T. cruzi* infection. In this study we examined the ability of metacyclic forms (CL strain) to enter B-1b cells, which proliferate in primary cultures of normal adherent mouse peritoneal cells and transform into a novel type of mononuclear phagocytes. Assays were performed by incubating parasites with B-1b cells from C57/BL6 mice, at 1:1 parasite:cell ratio. At 1h, 24, 48, 72, 96 and 120 h, the cells were fixed and stained. After 1h, ~60% of the cells was infected and in most cells with more than one internalized parasite. By 72 h infection, B1-b cells were full of intracellular amastigotes and by 96 h not only differentiation to trypomastigotes, but also free parasites in the supernatant, were detectable. The number of trypomastigotes in the supernatant of 120 h culture of B-1b cells was high. Invasion assays with HeLa cells, as well as with bone marrow-derived macrophages, were performed. After 1 h incubation, the number of macrophages invaded by parasites was ~30%, whereas the percentage of infected HeLa cells barely reached 1%. Even at 10:1 parasite:cell ratio, the parasite entry into HeLa cells was lower, being in the 20-30% range. To determine whether IL-10 contributed to the susceptibility of B-1b cells, experiments were performed with B-1b cells from IL-10 knock out (IL-10KO) C57/BL6 mice. No difference in infection between wild type and IL-10KO cells was observed. Among others, one marked difference between B-1b and HeLa cells was the high intensity of labeling of B-1b cells with lysosomal marker LAMP-1.
Work supported by FAPESP and CNPq.

BC27 - MEMBRANE RAFTS AND LYSOSOMES DURING *TRYPANOSOMA CRUZI* INVASION IN PRIMARY CARDIOMYOCYTES

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Trypanosoma cruzi, the etiological agent of Chagas´Disease, can invade a wide variety of cell types, varying from phagocytic to non-phagocytic cells. The parasitic invasion of the latter occurs through two convergent paths: host membrane invagination with subsequent lysosomal fusion or direct lysosomal fusion at the parasite attachment site. The interaction between parasite and host cell membrane is then one of the critical steps to the establishment of a well succeeded invasion. Among the non-phagocytic host cells we can stand out the infection of cardiomyocytes, which may consequently lead to the development of Chagas cardiomyopathy. Recently, it has been shown that cholesterol and cell microdomains might influence parasite-host cell membrane interaction and invasion in fibroblasts. In the present work we investigated whether specific regions of host plasma membrane enriched in cholesterol and sphingolipids, known as *membrane rafts*, are involved in the cardiomyocyte entrance process by *T. cruzi*. We also investigated the participation of the membrane rafts in lysosome fusion during *T. cruzi* entry. Cardiomyocytes were treated with the MβCD, a drug which can deplete cellular cholesterol content. HγCD, an inactive cyclodextrin was used as control. As observed for fibroblasts, MβCD treatment at different concentrations (5, 10 and 15mM) decreased *T. cruzi* invasion in a dose dependent manner without interfering with cell viability, showing that cholesterol is important for efficient *T. cruzi* entry. Alternatively, cell treatment with HγCD did not interfere with parasite invasion process. On the other hand, upon treatment, it was observed an increase in the percentage of recently internalized parasites enveloped by lysosomal membrane. Decreased entry with increased lysosome association may have been occurred due to non-regulated/directed fusion of lysosomes with the plasma membrane. This process is now under investigation.
Supported by CNPq and WHO.

BC28 - THE TRUE DIVERSITY OF DEVESCOVINID FLAGELLATES (PARABASALIA) IN THE TERMITE *INCISITERMES MARGINIPENNIS*

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In our study we revised the diversity of devescovinid flagellates in the termite *Incisitermes marginipennis.* More than 40 years ago, ten species of devescovinid flagellates were described in the gut content of this termite (Pérez-Reyes and López-Ochoterena, 1965). Based on light microscopic examinations, the flagellates were classified into the two genera *Devescovina* and *Metadevescovina*. On the one hand, it is unclear whether the genus *Metadevescovina* is a true genus or just a synonym for *Devescovina*. On the other hand, Kirby (1945) found only two instead of 10 devescovinid species in the same termite. Therefore, we thoroughly reinvestigated the composition of the devescovinid species in *I. marginipennis*. We combined the light microscopic study (live preparations, Protargol-staining, immunofluorescence of tubulin, DAPI) with the first ultrastructural investigation of the genus *Metadevescovina* (SEM, TEM) and with molecular phylogenetic analysis of the small subunit rRNA genes of the gut flagellates. Our results show that *I. marginipennis* contains only one single species of devescovinid flagellates, *Metadevescovina modica*. The microscopic analyses of devescovinid individuals showed only slight variations, e.g. in size and the path of the parabasal body. The genetic analyses proved the existence of one species of *Metadevescovina* (comprising three closely related phylotypes with sequence similarity >99.4%) and the absence of a *Devescovina* sequence. Monophyly of the cluster and the dense colonization with spirochetal epibionts corroborate the validity of the genus *Metadevescovina* and allow its differentiation from other devescovinid flagellates.
Supported by the DFG.

BC29 - PHYLOGENETIC POSITION OF THE CILIATE GENUS *EPICLINTES* (CILIOPHORA, STICHOTRICHIA) WITH A DESCRIPTION OF THE MORPHOLOGY AND MORPHOGENESIS OF *EPICLINTES AURICULARIS RARISETUS* NOV. SUBSPEC.

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The morphology and morphogenesis of the marine ciliate *Epiclintes auricularis rarisetus* nov. subspec., collected from northern China, were studied in live and protargol-impregnated specimens. This isolate can be recognized by its elongate, contractile and tripartite body with a size of 200-400 × 20-40 μm in vivo, 24-70 macronuclear nodules, about 30 ventrally located adoral membranelles, 2-3 frontal and 10-18 transverse cirri, 8-9 fronto-midventral rows, ca. 22-31 left and 35-54 right marginal cirri, 3 dorsal kineties, of which kineties 1 and 3 link together anteriorly. This form demonstrates several features rarely, if ever, found in urostylids, e.g. an absence of frontoterminal cirri and, during morphogenesis, the partial replacement of the parental adoral zone of membranelles, the formation de novo of the oral primordium and of the anlagen for the marginal rows and dorsal kineties, and the contribution of almost all FVT anlagen to the formation of the transverse cirri. In addition, its morphogenesis appears to differ in several points of detail from that of *E. auricularis auricularis* nov. stat., based on published data. These remarkable morphological and morphogenetic traits suggest the systematic position of *Epiclintes* within the Urostylida is questionable. In order to investigate this problem, the SSU rRNA gene was sequenced in *Epiclintes auricularis auricularis* and *E. auricularis rarisetus*, and phylogenetic trees constructed. The results show that the two taxa form a branch that is peripheral to all other typical stichotrichs. Within the Stichotrichia, two monotypes of *Epiclintes* branch off first in all analyses performed with high BI and moderate MP supporting value (100% BI, 58%MP) while low ML and NJ supporting value (<50%). Based on both morphological/morphogenetic and molecular data, *Epiclintes* might represent a unique taxon at the rank of suborder within the Urostylida.
Supported by the NSFC (project nos. 30870264, 30570236) and the Darwin Initiative (project no. 14-015).

BC30 - TAXONOMY OF FOUR MARINE CILIATES, *EUPLOTES SINICA* SP. N., *EUPLOTES PARABALTEATUS* SP. N., *EUPLOTES ORIENTALIS* SP. N. AND *EUPLOTES RAIKOVI* AGAMALIEV, 1966 (PROTISTA; CILIOPHORA; EUPLOTIDA)

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The living morphology, infraciliature and silverline system of four marine *Euplotes,* i.e. *E. sinica* sp. n., *E. parabalteatus* sp. n., *E. orientalis* sp. n. and *E. raikovi* Agamaliev, 1966, isolated from the sand beach near Qingdao, China, were investigated. Among these forms, *E. sinica* is characterized by having conspicuous dorsal ridges, single marginal cirrus and silverline system of double-*patella*-I type. Both *E. parabalteatus* and *E. orientalis* are extremely small forms (only ca. 35 μm long) and have fewer adoral membranelles, however, the former lacks ridges and reduced cirri and possesses double-*eurystomus*-type silverline system, the latter has conspicuous ridges and 2 reduced cirri with silverline system of double-*patella*-I type. *E. raikovi* differs in the presence of 8 frontoventral (including one reduced cirrus), single marginal cirrus and silverline system of double-patella-I type. The small subunit rRNA genes from these species were newly sequenced. Comparisons with similar congeners clearly support the identification based on the morphological studies. Meanwhile, sequences-based phylogenetic trees show the distinct positions of these species within the genus *Euplotes,* locating in two major clades or branching as two peripheral taxa respectively.
Supported by NSFC (project no. 30570236) and a grant from the Center of Excellence in Biodiversity, King Saud University.

BC31 - SCANNING ELECTRON MICROSCOPIC STUDY OF MEDITERRANEAN TINTINNID LORICAE (CILIOPHORA, SPIROTRICHEA, TINTINNINA)

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Usually, tintinnid loricae were investigated only by means of a light microscope. Therefore, a scanning electron microscopic study was preformed on the loricae of four species from the families Codonellidae, Codonellopsidae, and Rhabdonellidae collected in the Mediterranean Sea near Villefranche-sur-mer (France). This site is the type locality of *Codonella aspera* Kofoid & Campbell, 1929. The ultrastructure of its lorica and the peculiar closing apparatus is demonstrated for the first time. The agglomerated lorica is about 94 µm long and urceolate, viz., it comprises a globular bowl and an obconical collar. The agglutinated particles are apparently incrustrated by a successive secretion of distinct homogenous matrix layers. *Stenosemella ventricosa* (Claparède & Lachmann, 1858) Jörgensen, 1924 is the type of the genus. Its lorica is about 87 µm long and consists of an agglomerated obovate bowl and a short hyaline collar with some minute ellipsoidal windows. Thus, the genus *Luminella* Kofoid & Campbell, 1939 established for *Stenosemella*-like tintinnids with collar windows is considered a synonym. The lorica of *Codonellopsis schabi* Brandt, 1906 is about 85 µm long and langeniform, viz., it is composed of an agglomerated globular bowl and a hyaline cylindroidal collar with several spirals and some small windows. Based on the material from the type locality, the lorica of *Rhabdonella spiralis* (Fol, 1881) Brandt, 1906, the type of the genus, was ultrastructurally studied for the first time. The lorica is about 315 µm long and elongate obconical. Minute circular windows are scattered between the sinistrally spiralled and anastomosing ribs of the outer lorica surface; the alveoli of the wall are, however, only recognizable in the light microscope. The present findings contribute together with cytological studies to the establishment of a natural tintinnid classification.

Supported by the Austrian Science Foundation (FWF; Project P20461-B17).

BC32 - *Trypanosoma dionisii* mucin-like surface glycoproteins are implicated in mammalian cell invasion

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T. dionisii, a parasite from bats, which is closely related to *T. cruzi*, the agent of Chagas' disease, invades cultured mammalian cells but does not infect humans. This may be due to the susceptibility of *T. dionisii* metacyclic forms to human complement-mediated lysis. We have found that *T. dionisii* metacyclic forms express a protein of ~82 kDa, apparently unrelated to *T. cruzi* surface glycoprotein gp82 implicated in target cell entry, in addition to molecules related to *T. cruzi* mucin-like gp35/50 glycoproteins. Invasion assays using human epithelial HeLa cells showed that the entry of *T. dionisii* metacyclic forms is significantly inhibited by gp35/50 glicoproteínas purified from *T. dionisii* or *T. cruzi* strains CL and G. *T. dionisii* mucins were not recognized by monoclonal antibodies 2B10 or 10D8 directed to distinct carbohydrate epitopes of *T. cruzi* gp35/50 molecules, but reacted with polyclonal antibodies to CL strain gp35/50. Experiments to identify the components of the signaling cascades induced during *T. dionisii* invasion of HeLa cells suggested the involvement of parasite protein tyrosine kinase (PTK), as well as of phosphoinositide (PI)-3 kinase, and Ca^{2+} mobilization from acidocalciosomes. Host cell PTK and protein kinase C, but not PI-3 kinase, appear to be activated during *T. dionisii* internalization. The questions related to energy requirement by parasites for host cell invasion are also under investigation.

Supported by FAPESP,CNPq and CAPES

BC33 - EUGREGARINE TROPHOZOITE DETACHMENT FROM THE HOST EPITHELIUM VIA EPIMERITE RETRACTION: FICTION OR FACT?

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Eugregarines represent a diverse group of apicomplexans parasitizing invertebrates. Their sporozoites generally develop into epicellular trophozoites attached to the host epithelium by a specialized attachment organelle called the epimerite and their attachment strategy is similar to those in cryptosporidia. The architecture of the epimerite with associated structures and the mechanism of trophozoite detachment from the host epithelium were studied in *Gregarina polymorpha* in the intestine of *Tenebrio molitor* larvae, using electron and immunofluorescence microscopy. Here we propose a new hypothesis on the mechanism of trophozoite detachment from the host epithelium based on epimerite retraction back into the protomerite, instead of the commonly accepted theory describing gradual epimerite constriction and subsequent separation facilitated by the contractility of osmiophilic ring (membrane fusion site). This conclusion is based on observations on several eugregarine species in which: (i) epimerite appears as a dynamic structure whose shape dramatically changes depending on whether it is embedded into the epithelium or not; (ii) we never observed any remains of recently discarded epimerite in host epithelium; (iii) the epimerite exhibits significant decrease and shrinking at the end of trophozoite development; (iv) there is obvious accumulation of filamentous actin at the epimerite base and in the protomerite apical end, and a patch accumulation in the protomerite of maturing and mature trophozoites, suggesting the presence of contractile elements; (v) the cortical zone and area of the membrane fusion site are structures rich in myosin; (vi) there is no real fusion between the host and epimerite plasma membranes along their interface; (vii) a new plasma membrane would have to form at the protomerite apical end to cover damaged area after eventual epimerite separation; (viii) the protomerite top, formerly bearing the epimerite, is covered by an intact, smooth plasma membrane lacking signs of any damage.
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BC34 - Lipid Analysis of Reservosomes from *Trypanosoma cruzi*

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Reservosomes are lysosome-related organelles from *Trypanosoma cruzi* epimastigotes that store high amounts of lipids and proteins as a result of endocytosis. Reservosome-like structures were identified in trypomastigotes and amastigotes. Cruzipain, serine carboxipeptidase, lipases and mannosidases constitute a set of enzymes that control the macromolecule digestion. In addition, reservosomes present internal membranes and vesicles whose origin is unknown. A reservosomal ABCA1 transporter and lipid inclusions that tend to form large rectangular plaques surrounded by a phospholipid monolayer were reported. This fastidious shape was only observed in foam cells cholesterol crystals or wax ester inclusions from *Acinetobacter sp*. In this work, we analyzed the lipid composition of reservosomes using subcellular fractionation associated to lipidomic as well as microscopy analysis. In this way, epimastigotes were cultivated in LIT medium supplemented with low (1%, 5%), 10% (control) or high (20, 50%) percentage of fetal calf serum (FCS). LIT supplemented with FCS led to lipid accumulation in the parasite in a dose-dependent manner, as shown by Nile Red staining followed by fluorimetric measurement. Electron microscopy analysis showed the absence of lipid inclusions and homotypic reservosome fusion in epimastigotes cultivated in low FCS concentration. Abundant sword-shaped lipid profiles were observed crossing the organelle in high serum concentration cultures. When lipid-full epimastigotes were incubated in serum-free medium, lipid inclusions disappeared suggesting that the parasite is able to metabolize the inclusions. Gas chromatography-mass spectrometry analysis revealed that isolated reservosomes present a high content of cholesterol and cholesteryl esters, followed by ergosterol and squalene. The fatty acids are mainly composed by palmitate, followed by oleic or miristate. Inositolphosphorylceramides, glyceroinositols, glycerophosphoglycerols, sphingomyelin or phosphatidylcholine are the major class of phospholipids present in our analysis. Glycolipid analysis is in course. Reservosomes may represent a multipurpose-organelle in lipid storing and distribution contributing to the epimastigote integrated metabolism.
Supported by: CNPq, FAPERJ

BC35 - Changes in cell wall properties of symbiotic *Chlorella* during symbiosis in *Paramecium bursaria*

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Morphological and chemical properties of the cell wall of the symbiotic *Chlorella* strain PB-SW1C1 were examined in either free-living cells or those re-infected into apo-symbiotic host cells of *Paramecium bursaria* PB-SW1. The cell wall of free-living *Chlorella* stained with Calcofluor white M2R, which is a fluorescent dye that stains β-D-glucopyranose polysaccharides. Cell walls of symbiotic *Chlorella* that had just been isolated by mechanically disrupting both the *P. bursaria* plasma membrane and the perialgal membrane with a microneedle, did not stain with Calcofluor. Thickness of the cell wall was the same whether the *Chlorella* was free-living or symbiotic, and was about 20 nm. The outer surface of the cell wall of the symbiotic *Chlorella* had a fluffy appearance, while that of the free-living *Chlorella* was smooth. These results indicate that the cell wall of *Chlorella* changes in both structure and chemical properties with the establishment of the *Chlorella-Paramecium* symbiosis.

BC36 - Morphology and notes on morphogenesis during cell division of a new species within the genus *Deviata* (Protozoa, Ciliophora)

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This study aims at describing the morphology and certain morphogenetic stages of a new ciliate species from Argentina within the genus *Deviata*. Samples were taken from a temporary freshwater pond near the city of Dolores, Buenos Aires Province. Cultures were made by adding table water and a wheat grain to the samples in Petri dishes. Ciliates were studied alive and after silver impregnation with Protargol. *Deviata* sp. measures 130-180 µm x 45-70 µm *in vivo* and its cytoplasm appears dark grey because of the presence of refractive globules. The species possesses 8-9 long cirral rows on the right and 9-13 on the left of the buccal cavity, and 3 long dorsal rows of dikinetids. The adoral zone is composed of 39-48 membranelles; paroral extends beyond the distal end of the endoral membrane. There are 4 macronuclear nodules and usually 2 micronuclei. A single contractile vacuole is located equatorially on the left body margin. During the morphogenesis of cell division the anlage 4 for the proter develops from disaggregated cirri from row R4. The anlage 4 for the opisthe develops probably from the oral primordium and from disaggregated cirri from row R5. This new species mainly differs morphologically from its congeners in having a high number of cirral rows, the 3 long dorsal rows of dikinetids, and a high number of adoral membranelles. Concerning the morphogenesis during cell division, the two right-most ventral rows of cirri replicate as in *D. abbrevescens* Eigner, 1995, but the development of anlagen 4 is different in both species.
Supported by CONICET and UNLP.

BC37 - Differential Roles for G-Protein Coupled Endothelin Receptor Subtypes (ETbR and ETaR) during the Temporal Progression of Human Smooth Muscle Cell Invasion by *Trypanosoma cruzi*

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The internalization of *Trypanosoma cruzi* trypomastigotes by non-phagocytic host cells is the final outcome of a complex communication process, forged by intimate host-parasite adhesive interactions and reciprocal signaling events. In several models, productive infection depends on lysosomal fusion to the nascent parasitophorous vacuole. Classical studies indicated that $[Ca^{2+}]_i$-dependent intracellular responses transduced by G-protein coupled receptors (GPCR) can drive lysosomal exocytosis and fusion to the plasma membrane, ultimately allowing for the endocytic internalization of the parasite. Bradykinin receptors were the first example of a GPCR with defined pharmacological specificity that drive parasite internalization by cardiovascular cells. We now report that TCT (Dm28c) can also explore the endothelin (ET) pathway to invade human smooth muscle cells (hSMC), endothelial cells (HUVECs) and mouse cardiomyocytes through the triggering of ET receptors (subtypes ETaR and ETbR). Interaction assays performed with either CHO-ETbR or CHO-ETaR revealed that both types of GPCRs promote parasite uptake. Addition of ETbR (BQ788) or ETaR antagonists (BQ123), selectively reduced invasion of CHO-ETbR or CHO-ETaR, respectively. We then used these pharmacological tools to study the outcome of parasite interaction with hosts cells that naturally express ETRs. Our data showed that TCT invade hSMC and mouse cardiomyocytes through the signaling of both ETbR and ETaR whereas invasion of HUVECs is exclusively dependent on ETbR pathway. Given evidences that intracellular retention signals are required to consolidate infection we then checked whether ETRs could be involved in such processes. These studies revealed that ETbR is exclusively involved in host cell penetration, while ETaR controls parasite retention in the infected hSMC. Additional studies are required to determine whether vacuole-associated *T. cruzi* are able to convert big-endothelin into bioactive ETR agonists during the complex dynamics of the invasion process. Supported by: FAPERJ, CNPq (Instituto Nacional de Ciência e Tecnologia em Biologia Estrutural e Bioimagem).

BC38 - A NEW GENUS AND SPECIES IN THE FAMILY DELTOPYLIDAE (CILIOPHORA, HYMENOSTOMATIA), A HISTOPHAGOUS FRESHWATER TETRAHYMENID CILIATE FROM IDAHO (NORTHWEST U.S.A.): MORPHOLOGY, ONTOGENESIS AND MOLECULAR PHYLOGENY.

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A new tetrahymenid ciliate was discovered in a freshwater canal in Boise, Idaho, U.S.A. Morphology, ontogeny and molecular phylogeny were studied by standard methods. The ontogeny and histophagous mode of nutrition are similar to those of *Deltopylum rhabdoides* Fauré-Fremiet and Mugard, 1946. The new ciliate is distinguished morphologically by its disc-shaped caudal ciliary complex and formation of two forms of resting cyst, one form bearing tangled tubular or cylindrical lepidosomes. The silverline pattern is tetrahymenid. Sequences for the nuclear-coded small subunit ribosomal RNA (SSrRNA) gene and the mitochondrial cytochrome oxidase subunit 1 (COI) gene, place it basal within the order Tetrahymenida, well separated from members of the family Tetrahymenidae (*Lambornella, Tetrahymena*) and also from other tetrahymenids (*Colpidium, Dexiostoma, Glaucoma*). The genetic divergence between this species and other genera of the order Tetrahymenida are large enough to suggest placement in a separate family. This corroborates the morphological data, since the elaborate caudal ciliary complex and the lepidosome-covered resting cyst of this species are not found in *Deltopylum* or other Tetrahymenidae. This new genus and *Deltopylum*, histophagous genera sharing a number of morphologic features and a polytomic mode of division, are placed in the resurrected tetrahymenid family, Deltopylidae Song and Wilbert, 1989.
Supported, in part, through funding to the Canadian Barcode of Life Network from Genome Canada through the Genomics Institute and the Boise State University College of Arts and Sciences.

BC39 - COMBINED DATA SETS OF MORPHOLOGY AND SSU rRNA GENE SEQUENCE SUGGEST THAT THE NEW GENUS *EURYSTOMA* N. GEN. REPRESENTS A NEW FAMILY EURYSTOMATIDAE N. FAM. (CILIOPHORA, SCUTICOCILIATIA), WITH DESCRIPTION OF A NEW SPECIES *EURYSTOMA SINICUM* N. SP.

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Recently, an undescribed marine ciliate was isolated from China sea. Investigation of its morphology and infraciliature revealed it as an undescribed species representing a new genus and family, *Eurystoma* n. gen., type of Eurystomatidae n. fam. The new family is defined by a close-set, apically positioned oral membranelles and a dominant buccal field that is surrounded by an almost completely circular paroral membrane, which are unique in all known scuticociliates. The new genus is defined by having a small oral membranelle 1 (M1), bipartite M2, and well-developed M3; a body surface faintly sculptured with a silverline system in a quadrangular, reticulate pattern; and a cytostome located at the anterior 1/3 of a large buccal field. The type species of the new genus, *E. sinicum* n. sp., is defined mainly by the combination of a conspicuously flattened body, several caudal cilia, extremely long cilia associated with the buccal apparatus, and a contractile vacuole located subcaudally. Analyses of sequences of the small subunit rRNA genes of the new species and other ciliates produced trees with similar topologies that strongly support a clade containing *Eurystoma* and *Cyclidium*, which is associated with a clade containing all pleuronematids but basal to it. Within its clade, *Eurystoma* is always basal to *Cyclidium*. The great divergence in both buccal and somatic ciliature between *Eurystoma* and all other known scuticociliates supports the establishment of a new family for *Eurystoma*.
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BC40 - SECRETORY ORGANELLES THROUGH INTRACELLULAR CYCLE OF *TOXOPLASMA GONDII*

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Tachyzoites of *Toxoplasma* successful active invasion is a quick, dynamic process resulting from coordinated action of apical complex structures and the sequential secretion of specialized organelles: rhoptries, micronemes and dense granules. These organelles have a crucial role in invasion and survival, our goals was to analyze the distribution of these organelles along intracellular cycle that lasts average 48 hours. We have tracked secretory organelles at different times after invasion, employing transmission electron microscopy (TEM) including 3-D reconstruction from serial sections and stereology. Tachyzoites of RH strain kept in mice and monolayers of LLC-MK2, as host cells in a 10:1 parasite-host cell ratio. Interaction was for 2-5 min, 2h, 7h and 24h at 37 ^{0}C, for a follow up of the sequence. Observation was made on a ZEISS 900 at 80kV or Jeol 1200 (TEM). IMOD software was used for 3 D reconstruction and ITEM software for stereology. A 3-D models of an invading tachyzoites showed that the host cell membrane made contact with the lateral portion of its cell body and that the conoid did not touch the host cell membrane. Micronemes were observed regularly disposed around the conoid just under the sub-pellicular microtubules. Stereology of extracellular tachyzoites showed that rhoptries filled 3,03% of total cell volume, and dense granules 5,78%, while in intracelullars tachyzoites with 7 hours of interaction rhoptries filled 4,02% of total cell volume, and dense granules 2,89% in this time of infection the parasites are in endodiogeny. Considering that at that time the first round of division is usually being accomplished and dense granules secretion is in progress, in order to build the intravacuolar network, these numbers suggest that organelle numbers and relative volume varies along the intracellular cell cycle. However PTA staining had the same pattern in extra and intracellular tachyzoites.
Supported by CNPq and FAPERJ

BC41 - Immunolocalization of BILBO1 protein in *Trypanosoma cruzi* and *Leishmania major*

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The flagellar pocket (FP) is the unique site of endocytosis and exocytosis in *Trypanosoma brucei*. During cell division, FP duplication is tightly coordinated to flagellum biogenesis and segregation. The new flagellum appears in exits the lumen of the FP at a constricted site called the flagellar pocket collar (FPC).
Recently, a new protein, BILBO1, was described (Bonhivers et al., PLoS Biol. 6:e105, 2008) as a component of the cytoskeletal framework that it is essential to both collar and flagellar pocket formation, but not to basal body duplication and flagellum growth. Searching at BLAST searches of the GeneDB databases, identified eight orthologs of the *bilbo1* gene were identified inin kinetoplastid parasites, among them *Leishmania major* and *Trypanosoma cruzi*.
In order to investigate if these orthologs are expressed and to characterize where their products are localized in these two parasites, a polyclonal antibody raised against *T. brucei* BILBO1 was used in immunofluorescence and western blot studies. Two protein bands were recognized on *T. cruzi* epimastigote whole extract, coherent with the presence of two ortholog genes, while on *L. major* promastigote extract several proteins of a broad molecular mass range were recognized. The existence of post-translational processing is under investigation.
The T. brucei Anti-BILBOBILBO1 polyclonal stained strongly the flagellar pocket of both *T. cruzi* epimastigotes and *L. major* promastigotes. Dividing *T. cruzi* epimastigotes depicted similar BILBOBILBO1 staining in the new and the old flagellar pocket. Surprisingly, *T. cruzi* trypomastigote or amastigote flagellar pockets were not recognized. The antibody also bound along allthe flagellum length of both epimastigote and trypomastigote forms.
Immunoelectronmicroscopy localizationsImmuno-electron microscopy localization studies are being performed to precisely describe the structures recognized by BILBO antibody.
Supported by: FAPERJ, CNPq, CAPES.

BC42 - Speculations on the motility of the conoid of Toxoplasma gondii based on ultrastructural data

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The conoid is a small cone-shaped structure composed of spirally arranged microtubules that does not exist in all Apicomplexa. These microtubules are not formed by 13 protofilaments and there tubulins are arranged in an open comma shape. The conoid moves in and out of parasite's body during parasite gliding, adhesion and invasion of host cells. This process can be stimulated "in vitro" with calcium ionophore. However, besides its calcium dependence, little is know about the mechanics of motility of the conoid. We used Field Emission Scanning Electron Microscopy (FE-SEM) and Transmission Electron Microscopy (TEM) to analyze the fine structure of the conoid of tachyzoites extracted with detergent after calcium ionophore treatment. We tried to combine and confront the morphological observations with information on proteins previously identified as associated to the conoid to build a model for the motility of the conoid. By TEM, we observed the presence of bridges between the conoid and the posterior polar ring and between the anterior and apical polar rings. These bridges were also observed by FE-SEM. Globular structures that at the FE-SEM had a periodicity that matched the microtubules assembly covered by the outer surface of the conoidal microtubules correlate well to the electron dense matrix that was also observed by TEM. These structures could be microtubule associated motor proteins, previously localized at this site by immunocytochemical methods. We observed that the diameter of the organelle does not change as it moves up or down, and we hypothesize that its elevation can be associated to its partial rotation, following the spiral assembly of its fibers. It is our goal to combine these ultrastructural data to the presence of proteins like dynein and TgCAMs, already reported at this site.
Supported by CNPq, CAPES and FAPERJ

BC43 - Characterization of the components of the cyst matrix of *Toxoplasma gondii*

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Toxoplasma gondii is one of the most important clinical opportunistic pathogens for immunocompromised patients. In these individuals the reactivation of the infection from the breakdown of tissue cysts can lead to lethal encephalitis. Despite the key role played by the cyst in the persistence of the infection, the ultrastructure and biogenesis of this important life stage are poorly understood. In this work, we characterized the components of the cyst matrix in cysts isolated from brains of mice infected with Me 49 strain, processed for transmission electron microscopy and quick-freeze/freeze-fracture/deep-etching technique. In ultrathin sections the matrix presented a dense aspect, filled with filamentous material, diverse vesicles and tubules. The tubules were spread all over the cyst matrix and ranged from 30 to 50 nm in diameter. Some tubule profiles presented wider regions. Depicted by quick-freeze/freeze-fracture/deep-etching technique these tubules presented a smooth aspect on their surface. By cryo-immunocytochemistry these tubules were positively labeled with anti-GRA1, suggesting they could be the reminiscent intravacuolar network of the tachyzoite's parasitophorous vacuole. The cyst matrix was also filled with diverse vesicles. The smaller ones ranged from 50 to 140 nm in diameter and some presented a dense coat on their external surface, observed either in ultrathin sections and in quick-freezed/freeze-fractured/deep-etched metal replicas. Large vesicles ranging from 250 to 700 nm in diameter were also seen in the cyst matrix, their lumen presented electron dense material of similar granulosity to that found in the cyst wall. Post-fixation of the cysts with imidazole-buffered osmium tetroxide solution revealed vesicles containing insaturated lipid material in the vicinity of the cyst wall. When the cysts were processed for cytochemical detection of carbohydrates (Thierry technique), the interior of these vesicles was positively labeled, as well as the amylopectin granules found within the bradyzoites.
Supported by CNPq and FAPERJ.

BC44 - Ultrastructure and molecular phylogenetic position of a novel anaerobic stramenopile

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Molecular phylogenetic analysis of sequences derived from PCR surveys of natural environments is a useful technique for gaining insights into the diversity of uncultured/unidentified eukaryotes, and this approach has been especially important for understanding the genetic diversity of heterotrophic stramenopiles. Molecular surveys of marine biodiversity have demonstrated a large number of DNA sequence clades of uncultured/unidentified stramenopiles, such as MAST1-12 (i.e., "marine stramenopile" clades 1 -12), some of which represent diverse lineages recovered from anaerobic environments. In this study, we isolated a heterotrophic flagellate from anaerobic costal sediments collected in Cape Cod, MA (USA) and successfully established an anaerobic culture containing bacteria as a food source. Two flagella were inserted subapically on the cell, and a permanent bacterivorous cytostome was observed on the ventral side of the cell. The overall organization of the flagellar apparatus was characterized with serial TEM sections and was more similar to the apparatus found in anaerobic stramenopiles like bicosoecids and placidids than to proteromonads and opalinids. However, a detailed comparative analysis of the flagellar apparatus in this novel stramenopile also demonstrated significant differences with bicosoecids and placidids. Maximum Likelihood analyses of SSU rRNA gene sequences showed that this flagellate grouped strongly with early diverging, heterotrophic stramenopiles; however, a specific phylogenetic relationship with any known group of stramenopiles remained unclear, and the sequence from this flagellate did not cluster specifically with any of the MAST clades. Consequently, the flagellate cannot be classified with any taxonomic group of stramenopile proposed so far and must be treated as an anaerobic stramenopile *incertae sedis*. Our data also indicate that available environmental DNA surveys do not account for the full diversity of anaerobic stramenopiles, making investigations like ours necessary for improving our overall understanding of these ubiquitous flagellates.
Supported by Tula Foundation, NSERC and CIFAR.

BC45 - A novel, free-living parabasalid from Pacific mangrove sediments groups strongly with parasitic trichomonads as inferred from ultrastructural and molecular phylogenetic data.

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The Trichomonada consists mainly of parasitic lineages that infect both vertebrate and invertebrate hosts and possess non-canonical mitochondria associated with anaerobic lifestyles. Ultrastructural and molecular phylogenetic evidence indicate that this taxon is split into four main subgroups: the Monocercomonadidae, the Trichomonadidae, the Devescocinidae and the Colonymphidae. Only six species in four genera of trichomonads are known to be free-living flagellates that inhabit anaerobic environments: *Monotrichomonas carabina*, *Monotrichomonas* sp.*, Ditrichomonas honigbergi*, *Pseudotrichomonas keilini*, *Honigbergiella ruminantium,* and *Honigbergiella* sp. These free-living flagellates form a well-supported clade as inferred from phylogenetic analyses of SSU rRNA gene sequences and are classified as the Monocercomonadidae. We isolated a novel free-living trichomonad from mangrove sediments near Ishigaki Island, in the southern part of Japan, and investigated its ultrastructural characters and molecular phtylogenetic position based on SSU rRNA gene sequences. Our Maximum Likelihood tree demonstrated that this flagellate is separated from the monocercomonadid clade and instead grouped strongly within the Trichomonadidae, which also includes the human parasite, *Trichomonas vaginalis*. This free-living flagellate had several trichomonadid features, including two to three anterior flagella (depending on the life stage), a posterior flagellum with a lamellar-type undulating membrane, and an axostyle that protruded from the posterior end of the cell. Morphological and molecular evidence show that this flagellate is a novel free-living trichomonad that provides insights into the evolutionary history of both parasitic and free-living lifestyles within the Parabasalia.
Supported by the Tula Foundation, NSERC and CIFAR.

BC46 - ULTRASTRUCTURE OF THE PARASITOPHOROUS VACUOLE OF *TOXOPLASMA GONDII* BY FIELD EMISSION SCANNING ELECTRON MICROSCOPY

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Toxoplasma gondiii is an obligate intracellular protozoan that actively invades all types of nucleated cells of warm-blooded animals, including the man. During invasion a parasitophorous vacuole (PV) in which the parasite will reproduce is formed. This PV is constituted by selected lipids and proteins of the host cell plasma membrane, some of its proteins, and products of rhoptries secretion. Once inside it, dense granules secretion gives rise to an intravacuolar network of membranous tubules, previously shown by field emission scanning electron microscopy of dry cleaved cells to connect the parasites to each other and to the residual body and the PV surface. LLC-MK2 cell 24 hours post infection with tachyzoites of the RH strain were fixed, cryoprotected, cleaved and macerated for 7 days in 0.1% OsO_4 as proposed by Tanaka and observed with a Jeol-6340 FESEM. This method allowed more faithful images of the PV and its association with the intravacuolar network, clearly showing the fusion of some of the tubules with the inner surface of the PV, what was confirmed by transmission electron microscopy. On the other hand, fusion of the tubules and the parasites was never observed, indicating that the tubules do not give direct access to host cell cytoplasmic nutrients, as previously proposed. Besides it, in the late stage of intracellular development, pores of variable sizes but always with the rims decorated with spherical particles of uniform size were seen in some vacuoles, suggesting they could result from the secretion of the recently described microneme derived perforins. Cryo-fixed cells, which were cleaved inside the microscope chamber, reinforced the notion that these pores are not artifacts. Pores of the same nature were also observed in PVs present in the peritoneal exudate. These observations indicate that this technique may reveal new aspects of the intracellular organization of the PV.
Supported by CNPq.

BC47 - PHYLOGENETIC POSITION OF HALTERIIDA (ALVEOLATA, CILIOPHORA, SPIROTRICHEA) HYPOTHESIZED FROM COMBINED ANALYSES OF MORPHOLOGICAL AND MOLECULAR DATA

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One of the most intriguing issues in the systematics of Spirotrichea is the phylogeny of halteriids, which are classified either as oligotrichs or stichotrichs according to the analyzed data. Such discrepancy indicates conflict in signal from different genes and morphology, which were never analyzed simultaneously. We thus hypothesized the phylogeny of the major Spirotrichea groups based on combined parsimony analyses of morphological and molecular data, emphasizing the kinships of Halteriida. Sequences of α-tubulin from 16 species and 18S-rDNA from 25 were independently aligned in ClustalX, with gap opening/extension costs set according to alignment q-score optimization. Twenty one morphological characters were included and weighted according to approximate gap costs. Parsimony analyses were run in PAUP* using two different optimality criteria: tree length (steps) and Goloboff's fit exploring seven concavities (K=2–8). Nodal support was assessed from 1000 jackknife replicates. Character polarity was determined *a posteriori* using two species of Heterotrichea as outgroup. Transformations were inspected in WinClada under multiple optimization criteria. All tree searches consistently resulted in the same single tree. The disruptive effect of missing data from the lack of α-tubulin content in some terminals was minimal, since the exclusion of this partition did not affect branch support values significantly, which were generally > 50%, neither alter tree topology. *Protocruzia*, *Phacodinium* and *Kiitricha* diverged early in the Spirotrichea tree, corroborating recent molecular studies. The other terminals formed a major dichotomy. One clade comprised the euplotids + stichotrichs; and the other, *Gastrocirrhus* + oligotrichs. This later included Halteriida as a basal oligotrich branch (~87% support). Morphological transformations which resulted in halteriids + other oligotrichs clade were unambiguous, and consisted of enantiotropic morphogenesis acquisition and loss of fronto-ventral-transverse cirri. Most molecular synapomorphies uniting this clade, however, exhibited ambiguous optimization, and the 18S transformations were mostly homoplastic to stichotrichs. Financial support: CAPES.

BC48 - PHYLOGENETIC STUDY OF STICHOTRICHIA SMALL AND LYNN, 1985 (ALVEOLATA, CILIOPHORA, SPIROTRICHEA), BASED ON MORPHOLOGY

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The systematics of Stichotrichia is possibly one of the most problematic subjects in ciliate biology. So far, molecular phylogenies produced hypotheses which generally contradicted morphology-based classifications, albeit unfortunately, morphological analyses were scarcely published. This work aims to hypothesize the phylogeny of Stichotrichia, testing its monophyly and of the three major recognized orders (i.e. Sporadotrichida, Stichotrichida and Urostylida) based on simultaneous cladistic analyses of morphological data. A 120 species x 80 characters matrix was assembled from data present in the literature and analyzed in PAUP* 4b10 using parsimony with implied weights as optimality criterion (K=2–9). Polarity was determined *a posteriori* according to outgroup (5 Hypotrichia representatives) position, and Bremer index was used to measure clade stability. A strict consensus of the cladograms of minimum length and highest fit within the range of concavities explored was used to inspect the characters distribution. The results consistently corroborated the monophyly of Stichotrichia, placing *Discocephalus* as intermediate from Hypotrichia. However, the three major orders were polyphyletic. *Prodiscocephalus–Pseudoamphisiella, Epiclintes* spp., and *Amphisiella–Hemigastrostyla–Trachelostyla* branched early from the cladograms. Two main clades were formed within the core Stichotrichia. One contained most urostylids (including *Rigidothrix–Uroleptus*) at one branch, and most amphisiellids, gonostomatids and kahliellids at the other. The other clade included, *inter alia*, the oxytrichines and stylonychines, but also *Neokeronopsis* and *Pattersoniella*. Concerning the evolution of key characters, the midventral complex was a widespread plesiomorphic feature, modified in typical "6 FV-primordia" stichotrichs, but reversed independently at least in *Neokeronopsis*, *Pattersoniella* and *Anteholosticha heterocirrata*; and the fragmentation of the rightmost dorsal kinety was a synapomorphy of the expanded oxytrichine-stylonychine clade, that nevertheless, was lost various times in internal groups. Noteworthy, the results consistently indicated the natural history of stichotrichs is marked by plenty of reversions and convergences/parallelisms of features in most lineages. Supported by CAPES.

BC49 - The O_2 Consumption of *Crithidia deanei*: a trypanosomatid harboring a symbiont bacteria.

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Symbiosis in trypanosomatid protozoa is characterized by the presence of an obligatory intracellular bacterium, which co-evolves with the host protozoa through a mutualist relationship. Thus, the endosymbiosis in trypanosomatids constitutes an excellent model to study organelle origin and cellular evolution. In *Crithidia deanei*, the endosymbiont promotes morphological alterations in the protozoan and maintains intense metabolic changes with its hosts, by supplying amino acids, hemin and vitamines. Previous works have showed that the endosymbiont-bearing strain presents a lower generation time and a higher metabolic capacity, when compared to the cured strain. Thus, in the present work we compared the O_2 consumption of endosymbiont-containing and endosymbiont-free strains of *C. deanei*. Furthermore O_2 uptake by endosymbionts and mitochondria obtained after cell fractioning was also measured in an oxymeter. The mitochondrial metabolism of the symbiont-bearing strain was studied by using inhibitors to the distinct protein complexes of the respiratory chain. Data showed that oligomycin (0.5 – 16 µg/mL), an inhibitor of F_o F_1 ATP synthase, had no effect on O_2 uptake, indicating that a large portion of respiration is not coupled to the ATP synthesis. Conversely, use of 2.5 µM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), a proton ionophore that uncouples O_2 consumption from ATP synthesis, stimulated the O_2 uptake up in 30%, when compared to control cells. Cyanide, a complex IV inhibitor, was able to inhibit O_2 consumption up to saturation, after using higher concentrations (71.5 % of inhibition at 1.4 mM cyanide). It was detected a cyanide resistant respiration. The isolated mitochondria presented higher rates of O_2 consumption when compared to symbionts. Our next goal is to investigate the O_2 consumption by the aposymbiotic strain and to study the endosymbiont influence on the host respiration.
This work was supported by: CAPES, CNPq and FAPERJ.

BC50 - EFFECTS OF MILTEFOSINE ON PROLIFERATION, ULTRASTRUCTURE AND PHOSPHOLIPID COMPOSITION OF *CRITHIDIA DEANEI*, AN ENDOSYMBIONT-BEARING TRYPANOSOMATID

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Crithidia deanei is a trypanosomatid with co-evolves with an endosymbiotic bacterium through a mutualistic relationship. Our previous works have showed that the symbiont envelope presents phosphatidylcholine (PC) and that part of this phospholipid, is obtained from the host protozoan. In this study, we tested the effects of miltefosine on *C. deanei* proliferation, ultraestruture and phospholipid composition. It is well known that this drug has effects on cellular signaling and impairs the PC biosynthesis in mammalian cells and in lower eukaryotes. Our results showed a low effect of this drug on protozoan proliferation, when compared to other drugs that also target PC biosynthesis. Data obtained by transmission electron microscopy, showed ultrastructural effects of miltefosine on *C. deanei*, as plasma membrane shedding and blebbing, mitochondrial swelling and convolutions of the endosymbiont envelope. The use of ^{32}Pi as tracer, revealed that the protozoan phospholipid composition was affected by miltefosine: treatment for 24h with 10µM decreased the phosphatidylcholine, phosphatidylethanolamine (PE) and cardiolipin (CL) content. Interestingly, cell treatment with 25 µM of miltefosine for 36h induced phosphatidylinositol (PI) biosynthesis and enhanced PC, PE and CL contents. Prolonged treatment with 50 µM miltefosine maintained high levels of PI and the PC content was similar to control cells. Taken together, data suggest that in *C. deanei* miltefosine inhibits PC, PE and CL biosynthesis, but induces PI production. PI, which participates in signaling mechanisms, may somehow induce alternative pathways that maintain satisfactory levels of phospholipids during the miltefosine treatment. Mitochondria obtained from protozoa treated with 10 µM miltefosine, presented a higher decrease in PC and CL content, while isolated symbionts showed lower contents of PC, PE and PI, reinforcing the idea that an intensive metabolic exchange occurs between the host trypanosomatid and structures of symbiotic origin.
Supported by: CNPq, FAPERJ, CAPES.

BC51 - LOCALIZATION OF KINETOPLAST PROTEINS, KAP4 AND DNA POLYMERASE-B , DURING THE *TRYPANOSOMA CRUZI* CELL CYCLE

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Trypanosomatids present a typical cell division that involves the segregation of single copy and specialized structures, as the nucleus, the kinetoplast and the flagellum, in an equal mode to both daughter-cells. The kinetoplast is an enlarged portion of the trypanosomatid mitochondrion, which contains DNA (kDNA). This structure presents proteins associated to kinetoplast DNA (KAPs), which are related to the organization of the kDNA network and the DNA polymerase-β (polβ), which is involved in the replication process. Recent studies about the *Trypanosoma cruzi* cell cycle showed that during the G1 phase the protozoan presents just one nucleus, one kinetoplast and one flagellum. In S phase, the duplication of the nuclear and the kinetoplast genetic material takes place. In G2 phase, the kinetoplast segregates and then mitosis occurs. Thus, cytokinesis generates two identical daughter-cells. The main goal of this work was to verify the distribution of TcKAP4 and Tcpolβ during the G1, S, G2 phases and cytokinesis in *T. cruzi* epimastigote form. For this purpose we performed localization assays, by immunofluorescence and ultrastructural immunocytochemistry using antibodies raised against both *T. cruzi* proteins. Results showed that during the G1/S phase, KAP4 is mainly observed at the kinetoplast antipodal sites, whereas from G2 to cytokinesis labeling is seen dispersed through the kinetoplast. The Tcpolβ protein is strictly localized on the kinetoplast antipodal sites in G1/S protozoa, but as cell cycle continues, labeling is seen throughout the kDNA network. Taken together, data suggest that kinetoplast proteins present different localization according to the trypanosomatid cell cycle phases. Supported by: CNPq and FAPERJ

BC52 - CHARACTERIZATION OF PROTEINS OF GOLGI COMPLEX OF *TRITRICHOMONAS FOETUS*

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Tritrichomonas foetus is the causative agent of bovine trichomoniasis disease which a major cause of infertility and abortion leading to considerable economic losses in beef-producing areas of the world. Furthermore, *T. foetus* presents a particular cell structure such as an interesting presence of a well-developed Golgi complex that probably indicates a significant role, similar to that previously described in other cells. On the other hand, unlike the cells of higher eukaryotes, the Golgi of *T. foetus* does not break down into small vesicles during cell division or when it is under drugs treatment. Nevertheless, little is known about the resident-proteins composition of this organelle. In order to gain a better understanding of biochemical aspects of *T. foetus* Golgi complex, in present work we developed a panel of monoclonal antibodies from mice immunized with this organelle. One of these mAbs, the antibody anti-Golgi 20.3, was used to characterize the proteins of the Golgi complex. The specificity of the antibody to the Golgi complex was verified and the localization of Golgi complex was confirmed by labeling found in cells treated with the C_6NBD ceramide and immunocytochemistry. The monoclonal antibody 20.3 was also used in controls such as *Leishmania* and *Trypanosoma cruzi* and resulted in a perinuclear labeling, whereas in the MDCK cell line, the location was observed in the Golgi complex, indicating a conservation of these proteins. In addition, complementary techniques such as immunoprecipitation, immunoblotting and spectrometry mass were used to isolate and identify proteins recognized by antibody anti-Golgi 20.3. Two proteins with molecular weight of 60 kDa and 66 kDa were revealed. Database search of 66 kDa demonstrated similar amino acid sequence to found in five proteins of *Trichomonas vaginalis*. However, the manual sequencing and analysis by Q-TOF are still needed to confirm the data obtained by mass spectrometry.
Supported by AUSU, CAPES, CNPq, FAPERJ and PRONEX.

BC53 - LACK PROTEIN: A PUTATIVE HUMAN PLASMINOGEN RECEPTOR IN *LEISHMANIA MEXICANA*

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Leishmania mexicana binds the human serin-protease precursor Plasminogen (Plg) to its surface and little is known about the function this interaction plays in the host-pathogen relationship. We hypothesized this could be part of a strategy involved in the establishment and spreading into the mammal host skin similar to that of the endogenous membrane proteases (e.g. GP53). In *L. mexicana* AZV α-enolase accounts for ≈60% of the *Leishmania*-Plg interaction, the remaining 40% is bound by unknown receptor/s. Looking for such receptors a ligand blotting with Plg of a 2D gel-electrophoresis of the microsomal fraction of parasites showed a spot that subsequent mass-spectrometric analysis revealed as LACK (***L****eishmania* **A**ctivated **C**-**K**inase receptor). In order to assess if this protein could be a Plg receptor we cloned and expressed the LACK gene of *L. mexicana* AZV obtaining a soluble recombinant protein (LACKr) in ZYP-5052 medium and produced anti-LACKr serum in rabbit. We showed the presence of LACK in the cytosolic and microsomal fractions of parasites by Western blot and characterized the *in vitro* LACKr-Plg interaction with ELISA obtaining a Kd value of 1,2 □M around the physiologycal Plg concentration in human plasma. This interaction was inhibited by ε-aminocaproic acid suggesting lysine residues involved in the binding. These findings pinpoint LACK as a plausible Plg receptor although further research is needed to validate this function. If the *Leishmania*-Plg interaction plays an important role in the infectivity of *Leishmania*, the receptors involved could be good candidates for the development of vaccines or immunotherapy that may control and/or prevent leishmaniasis.
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BC54 -EVALUATION OF THE EFFECTIVENESS OF 1,2-PROPANEDIOL AS CRYOPROTECTOR FOR *Trypanosoma evansi*

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Trypanosoma evansi is a hemoparasite that causes the disease known as “Mal das cadeiras” or Surra in horses. It has been described in several animals such as capybara, small rodents, cattle and pig. The cultivation of the parasite in laboratory can be achieved by infections *in vivo* or *in vitro* (not efficient), being the cultivation *in vivo* the main method. However, after several transfer in mice laboratory the infectivity of the parasite decreases. To avoid changes in the infectivity and to reduce the use of animals, the cryopreservation is shown as low cost and viable alternative. This study compared the cryoprotectants, glycerol and 1-2 propanediol on infectivity in the host (parasitemia peak, pre-patent period and longevity). Twelve mice (*Mus musculus*) were used to form the glycerol group (GG) and 1-2 propanediol group (GP), both with five animals and control group (GC) with two animals. 5mL of blood containing 10^9 trypomastigotes/mL were stored in 5 cryotubes with glycerol and 5 cryotubes with 1, 2-propanediol. The cryoprotectants were used as a 10% solution. The sample was maintained for 5 minutes in liquid nitrogen and then stored by 60 hours at -196°C. Later it were kept per 5 minutes at 37°C and inoculated in its respective groups. All the animals groups received 0,3 mL of each sample. Every 12 hours the parameters were evaluated by direct smear of the tail. The results were analyzed by ANOVA and Tukey test. It was found that there was no statistical variation between groups GG and GP in the parameters, evaluated, however the 1-2 propanediol is easier to handle by its lower viscosity. The viability of the parasites after thawing and other cryoprotectants are being further evaluated.
Supported by: UDESC

BC55- Effect of drugs affecting sterols in *Trichomonas vaginalis*

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The flagellated protozoan parasite *Trichomonas vaginalis* is the etiologic agent of trichomoniasis, one of the most widespread sexually transmitted diseases (STDs) worldwide. In women, trichomoniasis causes adverse pregnancy outcomes, as pre-terms rupture of membranes, pre-term delivery, low-birth-weight infants, cervical cancer and also increases the transmission of HIV. Currently, the treatment of this disease consists in the use of metronidazole. However, this medicine presents side effects such as metallic taste, nausea and other health problems as disadvantages. In addition, during pregnancy this infection is exacerbated by hormonal effects, just when the use of metronidazole is not advised. Thus, the study of other drugs effects is extremely important in order to find a better medicine on the treatment of trichomoniasis. The aim of this study is to analyse the effect of new drugs that interfere in some metabolic pathways, such as the sterol byosinthesis. The drugs, BPQ-OH (3-(biphenyl-4-yl)-3-hydroxyquinuclidine) and 24(R,S,),25- Epiminolanosterol were tested in *T. vaginalis* at concentrations of 10 μM. 22,26 Azasterol (20- Piperidin-2-yl-5-pregnan-3_-20(R)- diol) at 5μM. The parasites were grown in TYM medium for 48h, and the drugs were added after 24h of culture growth. The drugs epiminolanosterol and BPQ-OH presented an antiproliferative effect in 32h of incubation. However, azasterol was effective only after 24h. We observed that epiminolanosterol presented a better antiproliferative effect when compared to the other drugs. Ultrastructural observations showed alterations in those trichomonas treated with the drugs, such as membrane *blebbling,* among others. Some cells internalized the flagella forming pseudocysts, as a sign of stress. By transmission electron microscopy, it was found that the hydrogenosomes were damaged. Autophagy was observed with the smooth endoplasmic reticulum surrounding damaged hydrogenosomes. In conclusion: all parasites were injured with all drugs here tested, but epiminolanosterol exhibited the better anti-profiliferative effect.
Supported by: AUSU, CAPES, CNPq, FAPERJ, PRONEX.

BC56 - Cell death of bovine oviduct cells after interaction with parasite *Tritrichomonas foetus*

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Tritrichomonas foetus is an extracellular parasite of the reproductive tract in cattle. The parasite is the causative agent of cattle trichomonosis, one of the most prevalent sexually transmitted diseases in cattle. In order to investigate the cytophatic effects of *T. foetus* in deeper parts of the reproductive tract, a bovine primary oviduct epithelial cell system (BOECs) was developed. Reproductive tracts were obtained from cows and the effect of co-incubation of *T. foetus* with BOECs in different times was analyzed by scanning, transmission and fluorescence microscopy. Viability tests were performed using colorimetric methods, *TUNEL* (*Terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling*), fluorescein diacetate, propidium iodide, JC-1 and annexin-V. The results reported here demonstrate that: (1) the *in vitro* oviduct epithelium has been shown to be useful in interaction experiments with *T. foetus*; (2) *T. foetus* adheres to the BOECs as single separate cells, and posteriorly the cells aggregate in large clusters; (3) the process of adhesion was initially by the posterior region of the cell where the axostyle protrudes, and forms filopodia, and digitopodia; (4) *T. foetus* provoked a severe damage to BOECs leaving imprints in the epithelial cells, wide intercellular spaces, and provoking large lesions in the epithelium; (5) the parasite provokes BOECs death by apoptosis and secondary necrosis. Our observations indicate the possibility that *T. foetus* can move up to oviduct and infertility in cows could be mediated by oviduct cells attack by *T. foetus.*
Supported by AUSU, CAPES, CNPq, FAPERJ AND PRONEX

BC57 - *NOVISTROMBIDIUM SINICUM* N. SP. AND *N. ORIENTALE* N. SP. (PROTOZOA: CILIOPHORA): TWO NEW OLIGOTRICH CILIATES FROM A MANGROVE WETLAND, SOUTH CHINA

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Ciliated protozoa are an important and abundant component of aquatic ecosystems. Our knowledge on their species inventory in mangrove wetland is still limited. During the faunistic surveys on ciliates in mangrove wetland (114°04′E; 22°37′N) of the South China, two new oligotrich ciliates, *Novistrombidium sinicum* n. sp. and *N. orientale* n. sp. were sampled. The morphology and infraciliature of them were studied from live and protargol-stained specimens. *Novistrombidium sinicum* is distinguished from its congeners by the combination of following characters: the ellipsoidal cell shape with prominent apical protrusion, extrusomes equidistantly arranged, three posterior directed thigmotactic membranelles, one ellipsoidal macronucleus, and the ventral kinety anteriorly terminated below the girdle kinety. The small sized *N. orientale* can be separated from its congeners by: the extrusomes equidistantly arranged, two posterior directed thigmotactic membranelles, one ellipsoidal macronucleus, the ventral kinety anteriorly terminated below the girdle kinety, and the number of dikinetids in ventral and girdle kineties.
Supported by NSFC (No. 30870280).

BC58 - AN IMPROVED CLASSIFICATION OF PLEUROSTOMATIDA BASED ON MORPHOLOGY AND MOLECULAR PHYLOGENY (CILIOPHORA, PROTOZOA)

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The taxonomy of pleurostomatid ciliates, especially the marine ones, is extremely confusing with the increasing of new taxa and due to the fact that many “known forms” are mostly insufficiently described before. In last few years, the morphology and infraciliature of 40 plus species belonging to 8 genera of Pleurostomatida have been investigated during our study on the biodiversity of marine ciliates in China seas. Based on the morphological characters combining the molecular data, an improved classification of pleurostomatids is proposed here. Four families were suggested mainly according to the arrangement of the right somatic kineties (RSK): (1) Apolitonotidae n. fam., the anterior end of RSK gradually shortened along 2-3 rows of leftmost RSK, including *Apolitonotus* n. gen.; (2) Kentrophyllidae n. fam., the middle RSK gradually shortened and usually forming one anterior and one posterior suture, including *Kentrophyllum* and *Epiphyllum*; (3) Amphileptidae Bütschli, 1889, the RSK forming one anterior suture, including *Amphileptus*, *Apoamphileptus* et al.; and 4) Litonotidae Kent, 1882, the anterior end of RSK gradually shortened along peroral kineties, including *Litonotus*, *Loxophyllm* et al. Molecular information based on the small subunit ribosomal RNA (SSU rRNA) gene sequences supports the monophyly of all these four families, which confirms in great deal the efficiency of our classification. The results indicate also that the arrangement of the right somatic kineties might reflect their evolutional status of pleurostomatid ciliates. In addition, four families branch out in the order of Apolitonotidae – Kentrophyllidae – Amphileptidae – Litonotidae in our phylogenetic trees.
Supported by the NSFC.

BC59 - EXPLORING THE NEW CILIATE WORLD IN THE LITTLE PONDS OF TANK BROMELIADS

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It is now well established that the little ponds formed by the coalescing leaves of bromeliads contain not only many endemic metazoans, such as insect larvae and amphibians, but also many specific ciliates. As yet, about 40 undescribed species have been discovered in circa 100 samples from Central and South America (some described in the literature cited below). Several of the species represent new genera and families, and some, which are very spectacular, will be shown in a film and by scanning micrographs. Ecologically, the most prominent feature of the tank bromeliad ciliate community is the occurrence of many species with the ability to switch from a mircostome, bacteria-feeding morph to a macrostome, phagotrophic morph. Very likely, this peculiarity evolved in reaction to the extreme competition occurring between protozoans and metazoans when the tanks dry out during the drier seasons. Several of the new species have close relatives in freshwater or soil, and few of the new genera are monotypic, showing a specific evolution within the bromeliad habitat. Further, morphological and molecular evolution seems to be decoupled in many tank ciliates, i.e, in spite of conspicuous morphologic and ontogenetic differences, the ribosomal gene sequences differ only slightly from relatives living in ordinary freshwater or soil. Supported by the Austrian Science Foundation, FWF project P20360-B17.

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BC60 - A MONOGRAPH OF THE DILEPTINA (CILIOPHORA, HAPTORIA): ALPHA-DIVERSITY AND PHYLOGENY

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Dileptids belong to the subclass Haptoria (Ciliophora, Litostomatea) and are characterized by a proboscis carrying a complex, unique ciliary pattern. The name-giving genus *Dileptus* has been used as a model organism in several studies on regeneration, regulation of the ciliary pattern, conjugation, and food acquisition. Over the years, this large genus has been split into six genera mainly on the base of the nuclear and ciliary pattern: *Dimacrocaryon*, *Monilicaryon*, *Rimaleptus*, *Pseudomonilicaryon*, and two new genera that will be described in our monograph. We have revised the whole suborder Dileptina and recognized three or five families, eleven genera, and seventy valid species, of which about half was described or re-described by the Salzburg ciliate researchers. The synonymy rate is rather low, that is, about 15% because most of the species are very distinct. The genus *Dileptus* plays an important role in understanding haptorid evolution because it branches basally in molecular phylogenies. However, such position, based on a single species sequenced, is not supported by morphological data. The formation of a *Spathidium*-like body and ciliary pattern during and after ontogenesis and conjugation suggests a close relationship of spathidiids and dileptids, but it is not known whether the dileptids evolved from a *Spathidium*-like ancestor by the formation of a proboscis or the spathidiids lost the proboscis of a *Dileptus*-like ancestor. Based on literature and new data, we suggest an evolutionary framework for the dileptids, using a Hennigian argumentation scheme with the nuclear and ciliary pattern as main features. In a further step, the Hennigian system will be combined with gene sequence data. Supported by the Austrian Science Foundation, FWF project P-19699-B17.

BC61 - NUCLEAR PROTEINS AND CHROMOSOME STRUCTURES OF THE ANCESTRAL DINOFLAGELLATE *OXYRRHIS MARINA*

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Dinoflagellates possess a quite peculiar nucleus. Chromosomes in dinoflagellates are permanently condensed throughout the whole cell cycle (ie, chromosomes are condensed even in interphase) and are divided by extra-nuclear microtubules running in cytoplasmic tunnels that penetrate the dividing nucleus. In addition, typical nuclear proteins such as histones, which are well conserved in eukaryotes, are not found in dinoflagellate nuclei. Some non-chromosomal nuclear proteins were identified, and models of the chromosomal structure were proposed. However, no chromosomal proteins were identified and molecular structure of dinoflagellate chromosomes is still not understood. To elucidate the architecture of dinoflagellate chromosomes, we focused on the nuclear protein in the most ancestral dinoflagellate *Oxyrrhis marina,* whose nuclear characteristics are considered as intermediate between those in typical dinoflagellates and those in common eukaryotes. Based on past studies, only one nuclear protein, namely Np23, was reported to be present in *O. marina*, but it has not been characterized well. In this study, we established the method for isolating *Oxyrrhis* nucleus and determined the partial amino acid sequences of Np23. We performed 3'RACE and obtained plural Np23 genes. Estimated amino acid sequence of Np23 did not include any typical domains that are known for other eukaryotic nuclear proteins. Thus, we conclude that Np23 is a novel nuclear protein in the nucleus of *O. marina*.

BC62 - CRYOPRESERVATION OF *TRYPANOSOMA EVANSI* CROMATOGRAPHED BY DEAE-CELLULOSE COLUMN

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Trypanosoma evansi is the world widest distributed trypanosomatid, present in all continents. This protozoan causes a disease know as "Surra" or "Mal das Cadeiras", witch affects many species, like horses, dogs, coates, small rodents, pigs, capybaras and bovines. In Brazil, this disease is endemic in Pantanal Matogrossense. Strain's maintenance in laboratory is done by passages on "in vivo" culture (using mice or rats), since there is not an effective culture medium described in literature. Cryopreservation is a way used to keep the parasite on laboratory. Besides preserving the protozoan for many years, this method keeps the original characteristics of *T. evansi* as found in nature, because the strain can be frozen right after its isolation of the infected host. However the processes of frozen and thawing results in damages to the parasite, as separation of the cytoplasm from the pellicle, occurrence of large vacuoles in the cytoplasm and karyoplasm, loss of cytoplasmatic ribosomes, membrane injuries, enlargement of the flagellar pocket and denaturation of chromatin. This work tested a method of cryopreservation using trypomastigote forms of *T. evansi* purified by chromatography in DEAE-Cellulose column eluated in PBS-glucose. Rats (*Rattus norvegicus*) was infected with cryopreservated and purified in DEAE-Cellulose samples, blood cryopreservated samples and non-cryopreservated samples. The evaluated parameters were longevity, pre-patent period and number of parasites of the samples before and after the cryopreservation and the chromatography. The parasite loss was 10 times greater in samples cryopreserved in blood than in chromatographed samples, showing a better survey rate of the parasites eluted in PBS-glucose, meanwhile the group infected with the purified samples had higher longevity and pre-patent period rates than others groups.

Supported by UDESC and UFSM

BC63 - Expression and subcellular localization of kinetoplast-associated proteins in the different developmental stages of *Trypanosoma cruzi*

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The kinetoplast DNA (kDNA) of trypanosomatids consists of an unusual arrangement of circular molecules catenated into a single network. The diameter of the isolated kDNA network is similar to that of the entire cell. However, within the kinetoplast matrix, the kDNA is highly condensed. The bulk of knowledge about the packaging, maintenance and replication of the kDNA network has come from studies of *C. fasciculata* and *T. brucei*. Little is known about the proteins associated with the kDNA of *T. cruzi*, a parasitic protozoon that shows distinct patterns of kDNA condensation during their complex morphogenetic development. KAPs (Kinetoplast-Associated Proteins) are candidate proteins for kDNA packaging and organization in trypanosomatids, since in *Crithidia fasciculata* they are capable of condensing the kDNA network *in vitro*. The KAPs of *C. fasciculata* (CfKAPs) are small basic proteins, which contains lysine- and alanine-rich domains. The trypanosomatid genomes sequenced to date have several sequences that share some degree of similarity with CfKAPs studied so far. In this work, we have analyzed KAP coding sequences in trypanosomatid genomes, organized them by means of phylogenetic and syntenic analyses and defined two *T.cruzi* KAP proteins, distinct from those originally described in *C. fasciculata*. Such proteins, TcKAP4 and TcKAP6, were cloned, expressed and antisera were generated against recombinant proteins. Imunolabeling assays revealed that the distribution of TcKAPs in different developmental stages of *T. cruzi* is related to the kinetoplast format: in disk-shaped structures, like those found in epimastigotes and amastigotes, proteins are seen dispersed through the kDNA network. Conversely, in rounded kinetoplasts, like those observed in intermediate forms and trypomastigotes, KAPs are mainly located at the kDNA periphery. Taken together, these data indicate that the kDNA rearrangement that takes place during the *T. cruzi* differentiation, is accompanied by TcKAPs redistribution within the kinetoplast.
Supported by FAPERJ and CNPq

BC64 - REDESCRIPTION OF *LICNOPHORA CHATTONI* VILLENEUVE-BRACHON, 1939 (CILIOPHORA, SPIROTRICHEA), EPIBIONT OF THE HYDROZOAN *ZYZZYZUS WARRENI* CALDER, 1988

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The poorly known species *Licnophora chattoni* is redescribed using modern microscopy techniques, and new observations on its morphology are provided, based on specimens living in association to the tentacles of the hydrozoan *Zyzzyzus warreni*, collected on the Segredo shore, located in São Sebastião (northern coast of São Paulo, Brazil). The specimens were analyzed *in vivo* under DIC, after silver impregnation (protargol), and transmission and electron microscopy preparations. *L. chattoni* has clear transparent cytoplasm, with numerous cortical granules. The body is subdivided in two distinct regions. The anterior, of ovate shape, is connected by a neck constriction to the posterior, which is the adhesive disc. It measures about 43 – 48µm long x 21-23 µm wide, and is dorso-ventrally compressed, with the dorsal surface convex. The adoral zone of membranelles has about 81 peristomial membranelles and about 20 infundibular membranelles. The paroral membrane has long cilia and extends from the anterior region of the body to the adhesive disc, within a groove. The adhesive disc is surrounded by a *velum* that covers four dikineties and a conspicuous row of dikinetids bearing long cilia, which are interrupted in the ventral surface, where two short kineties form a ventral field. One dorsal kinety is present around the neck constriction. The nucleus has one micronucleus and 11 -12 macronuclear fragments distributed within the body. The overall morphology of this species resembles *L. auerbachii*, differing mostly in the body dimensions, number of macronuclear fragments, and number of kineties in the ventral field. The ultrastructure of *L. chattoni* is also similar to that of *L. auerbachii*, characterized by Silva-Neto (1994, *Annales Sci. Natur., Zool.* Paris. (2)15: 49-62).
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BC65 - MORPHOLOGY OF *STEENSTRUPIELLA STEENSTRUPII* (CLAPARÈDE AND LACHMANN, 1858), (CILIOPHORA: CHOREOTRICHIDA) FROM THE COAST OF SÃO PAULO, BRAZIL

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Tintinnids are important components of the plankton, and are characterized by the presence of a lorica. We contribute to the knowledge of *Steenstripiella seenstrupii* by presenting new data on the lorica, and, for the first time, observations on the somatic ciliature of the cell. The ciliates were sampled from the waters of São Sebastião Channel (São Paulo) using 25µm plankton mesh, studied in vivo under DIC, after protargol impregnation and sacanning electron microscopy. They present hyaline lorica, measuring 140 – 150µm x 40 µm, elongate with funnel-shaped collar. The Aboral end is slightly round and closed, with a small invagination. The lorica also has three long thin fins. Seen from the oral side, the lorica is triangular. The cell is elongate and has a short pedunculus that attaches the organism in the inner wall of the lorica. The somatic ciliature is composed by 9 comb-shaped short kineties, with 6 – 10 kinetids each; a dense lateral ciliar field and ventral and dorsal kineties. It has 12 – 14 membranelles, 8 macronuclear segments measuring about 5 – 10µm, and one 2 – 3µm wide micronucleus located near the third anterior macronuclear segment, and another one near the fourth. One encysted cell was found within a lorica. The cells of *S. steenstrupii* were found to be very similar to those of *Ormosella achantharus* and *Amphorides* sp. Supported by CAPES, CNPq., FAPERJ and FAPESP.

BC66 - INFECTION OF MACROPHAGES BY *Trypanosoma cruzi*: EFFECTS OF TREATMENT WITH THE ENDOTHELIN RECEPTOR BLOCKER BOSENTAN

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Endothelins are involved in both the vascular dysfunction and the cardiomyopathy observed in patients with Chaga's disease. Previous studies from our group have suggested that treatment of cultured macrophages with Bosentan, a nonspecific blocker of endothelin receptors ET_A and ET_B, modifies the secretion pattern of these cells and diminishes their viability when in concentrations ranging from 10^{-4} to 10^{-7}M. Besides, this treatment increases the infection index and proliferation of amastigotes inside macrophages. In the present work we have analyzed if the macrophage response depends on the Bosentan concentration by testing it in the 10^{-5} to 10^{-9}M range. Treatment with Bosentan caused a drop of 15% (10^{-8}M) in the basal expression of Interleukin-10 (IL-10). In infected cells the treatment caused an increase of 16%(10^{-8}M) in the expression of Tumor Necrosis Factor Alpha (TNF-α) and of 11% (10^{-7}M) in the expression of IL-10. Endothelins seems to modulate the secretion of these molecules in macrophages in a dose-dependent manner. Moreover, the diminished cell viability resulting from the treatment with Bosentan doesn't reflect upon the production of cytokines and NO.
Supported by FAPEMIG and CNPq.

BC67 - *Giardia intestinalis*: Aphidicolin influence on trophozoites´ cell cycle

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Aphidicolin (APH), a reversible DNA polymerase inhibitor used to synchronize various cell lines in G1/S phase of cell cycle has been used recently also to synchronize bi-nucleate flagellate *Giardia intestinalis.* Progress in cell cycle studies of this common parasite of humans and other mammals infecting small intestine and causing diarrhea, is suffering mainly from the absence of an effective synchronization method. We analyzed *Giardia* cells exposed to APH by using several characteristics to find out to what extent the inhibitor influences individual cells. In agreement with previous reports, aphidicolin causes aligning *Giardia* trophozoites in G1/S phase according to DNA content of the cells as shown by flow cytometry analysis. On the contrary to this apparent synchronization, characteristics of cytoplasmic cycle indicate that the trophozoites do not stop at the G1/S phase. Morphological characteristics show that the treated trophozoites resemble cells from G2-phase or aging cultures (increasing cell size, median body in nearly 100% cells, significantly larger median bodies), which points to dissociation of nuclear and cytoplasmic cycles. Similar effect has been described also in some other inhibitors and cell lines. We also found that the exposure to aphidicolin results in phosphorylation of histone H2A. This *Giardia* protein contains the same SQ motif as histone variant H2AX in mammalian cells enabling phosphorylation of the histone, which is involved in signaling pathway triggered as a reaction to double strand breaks. These results suggest that behavior of *Giardia* trophozoites under the APH pressure resemble more than a synchronized population a reaction to the blocked replication and damaged DNA.

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BC68 - LYSOSOME EXOCYTOSIS: AN IMPORTANT EVENT DURING *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTE INVASION OF LAMP DEFICIENT CELLS

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Trypanosoma cruzi is an obligate intracellular organism in vertebrate hosts. Lysosomes are involved in parasite cell invasion and LAMPs (lysosome associated membrane proteins) are the most abundant glycoproteins of lysosome membranes. In addition, the parasite temporarily resides inside LAMP-containing phagolysosomes before escaping to the cytosol. This study is the first to investigate *T. cruzi* extracellular amastigotes (EA) invasion in LAMP-1 and LAMP-2 single knockout cells and in two different LAMP double-knockout clones. When compared to their respective wild type clones, EA showed higher infectivity in LAMP-2 and no difference was seen in LAMP-1 knockout cells. Similarly, EA invasion rate was higher for one of the double knockout clones but
not for the other. In order to determine the relative importance of LAMP-1 and LAMP-2 to EA infection, we used RNAi to knockdown the levels of these proteins in cells. The high rate of EA invasion in LAMP-2 single knockout cells, however, was not due to inhibition of LAMP-2 expression. Higher invasion rate correlated with higher lysosome exocytosis and with a premature lysosomal marker acquisition. These findings suggested that lysosome exocytosis is important to EA cell invasion. In addition, EA phagolysosome maturation in knockout cell lines differed from reported results, indicating that EA enter cells by means of a mechanism other than receptor-mediated phagocytosis.
Financial support: CNPq, CAPES, FAPESP.

BC69 - NEW DETAILS OF THE WHOLE LIFE CYCLE AND GAMETE MORPHOLOGY OF THE RED TIDE DINOFLAGELLATE *NOCTILUCA SCINTILLANS* (EHRENBERG) MCCARTNEY

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Noctiluca scintillans is an unarmored heterotrophic dinoflagellate that inhabits oceans all over the world, and is sometimes responsible for harmful red tides. Our previous phylogenetic analysis using two 8protein-coding genes revealed that *N. scintillans* is one of the most ancestral dinoflagellates as well as *Oxyrrhis marina*. Previously, we also demonstrated the complete life cycle of *N. scintillans.* In the course of the observations, we found that, unlike trophonts which keep few dinoflagellate-like characters, the gametes retain typical dinoflagellate characters: longitudinal and transverse grooves, and two flagella that differ in length and motion (but the extent of differentiation is low). In addition, an ornamental rod is running along the axoneme in the longer flagellum. These characteristics are common in core-dinoflagellates. Taking the ancestral position in molecular phylogeny and the gamete morphology in consideration, the gametes of *N. scintillans* can be regarded as a primitive dinoflagellate similar to the core-dinoflagellates. Based on these information, we discuss a possible early history of the dinoflagellate evolution: haploid core dinoflagellates evolved from noctilucid-like gametes of a diploid ancestor via neoteny. This scenario can also rationally explain the ploidy change from diploidy to haploidy in dinoflagellates.

BC70 - Metabolic enzymes are altered during *Giardia* Encystment

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We have shown that *Giardia* produces nitric oxide (NO) and have demonstrated the activity of a sedimentable nitric oxide synthase (NOS). In this study, we have cloned and expressed the NOS found in *Giardia* (gNOS), which apparently contains non-heme iron. gNOS (ORF 91252, *Giardia* database) is about half the size of other NOS proteins and only has the ferredoxin reductase-like domain as in human iNOS with a similarity index of 19. NO production might be the result of the activity of gNOS or gNOS plus other proteins or might be the result of a non-enzymatic process. We show that NO increases during the early stages of encystment. This increase in NO production is temporally linked to a decrease in oxygen uptake stimulated by exogenous glucose or metronidazole and also with the appearance of mRNA for key encystment proteins (Gnp and Cwp 2). Additionally, we have detected proposed nitrosylation sites in key proteins associated with encystment and energy production pathways. Standard NOS inhibitors markedly inhibit NO production and encystment. We also show that the activity of a glyceraldehyde 3 phosphate dehydrogenase (G3PDH) is detectable in non-encysting trophozoites (120 mU/mg protein) and in trophozoites induced to encyst for 6 h, but activity falls below the limits of detection at 12 or 24 h into encystment. We can induce a rapid (ca. 1 min) and significant reduction in this activity (by 31 %) by the addition of 10 μM nitrosylcysteine (an NO donor) to the enzyme in non-encysting cell lysates. Thus, we hypothesize that NO may play a role in directing the biochemical and cellular changes that occur during encystment via protein nitrosylation. Our data support this and may explain how carbohydrate metabolism switches from catabolic to biosynthetic during encystment.

BC71 - Bistability and *Giardia* Encystment

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Parasitic protozoa form cysts (encystment) often for survival during nutrient deprivation or other harmful environmental change and for transmission to different hosts. In vitro, we know that *Giardia* encyst when bile sequesters lipids necessary for this lipid auxotroph, and in vivo they encyst to infect new hosts. In vitro, encystment is observed at low levels even in cultures without exogenous bile. Prokaryotes and eukaryotes may elicit gene expression that involves activation and/or repression of developmentally regulated genes in response to environmental stress. This regulation is controlled by a master regulator, which promotes the bifurcation of the population into phenotypically distinct sub-populations that are genetically identical even when the stress is absent. Such populations are described as bistable. Bistability has not been assessed in protozoan encystment though it would seem an obvious bistable process since: 1) parasite survival depends upon the ability to form cysts for transmission regardless of external factors, and 2) the process, once induced, appears to go to completion regardless of whether the inducing stimulus remains. Using immunofluorescence, Western blots, and individual cell culturing, we show that trophozoites growing even without exogenous bile exhibit a basal level of encystment (~10%). Furthermore, when they were induced to encyst with bile for specific periods of time and then bile was removed, the levels of encystment were not significantly different from the encystment control levels (bile present throughout). These two observations suggests that cysts form at a basal level even without the bile stimulus and that the encystment pathway, once induced, goes to completion even if the induction signal is removed - typical properties of a bistable regulatory system. Such a system, once confirmed by additional experiments, would confirm encystment pathways as major targets for chemotherapy.

BC72 - INTERACTION BETWEEN *GIARDIA LAMBLIA* AND THE EPITHELIAL CELL LINE CACO-2

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Giardia lamblia, an important cause of diarrheal disease, resides in the small intestinal lumen in close apposition to epithelial cells. Since the disease mechanisms underlying giardiasis are poorly understood, elucidating the specific interactions of the parasite with the host epithelium is likely to provide clues to a better understanding of the pathogenesis. In this work, Caco-2 cells were used as an in vitro model to characterize the inherent pathomechanisms of *G. lamblia* infection. The transepithelial electrical resistance of Caco-2 monolayers was measured and permeability to ions increased about 20% in samples after interaction between parasite and host. This result suggests disruption of the monolayer paracellular barrier. Immunofluorescence assays, showed that the integrity of tight junction as analyzed by the staining pattern for the proteins ZO-1, ZO-2 and claudin-1 was affected after parasite attachment. Nevertheless, no significant changes were observed for the adherens junction proteins E-cadherin, β-catenin and the protein desmosomal 2/3-desmocollin. Using field emission scanning electron microscope (FESEM), we observed a kind of retraction of actin filaments in the microvillus region of Caco-2 during contact with *G. lamblia*. This change may be a host cell response to physics and chemicals stimuli when in contact to the parasite, causing a rearrangement of protein filaments. Moreover, we observed morphological alteration of the *G. lamblia* ventro-lateral flange pattern, since it appeared retracted when in contact to the microvilli of intestinal cells. Approximately 93% of analyzed parasites showed a hide flange when interacting with Caco-2, whereas only 32.33% showed this morphology when in contact to thin microscopic slide. This interaction differences to substrates by the parasite remain to be defined; however more studies are being performed aiming a better understanding of the mechanisms of *G. lamblia* interaction with epithelial cells.
Supported by CAPES, CNPq, Pronex/FAPERJ and FAPERJ.

BC73 - BLOCKING OF *TOXOPLASMA GONDII* INVASION BY DYNAMIN INHIBITOR

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Dynasore is a compound capable of blocking pinch off of nascent vesicles from plasma membrane by inhibiting the GTPase dynamin. Internalization of *Toxoplasma gondii* tachyzoites is described as an active process conducted by the parasite. During parasite invasion, as the host cell plasma membrane invaginates the formation of parasitophorous vacuole takes place. Dynasore was an efficient inhibitor of parasite invasion of LLC-MK2 cells not only after pretreatment of host cells, but also when the compound was added simultaneously with the parasites. Host cell pretreatment was successfully tested for reversibility. However pretreatment of parasites with Dynasore had no effect on invasion. Transmission electron microscopy showed most of the parasites outside host cells and a few ones in vacuoles close to the cell periphery. Scanning electron microscopy confirmed this observation, with several parasites seen adhered to the cell surface and a few only halfway into the host cell, indicating an impairment of infection. Taken together these data show that dynamin plays a key role in *T. gondii* invasion, reinforcing the hypothesis that this process does not depend solely on the parasite.
Supported by: CNPq

BC74 - Molecular and biochemical characterization of the pyruvate phosphate dikinase (PPDK) from *Trypanosoma cruzi*

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In Latin America, the morbidity and mortality associated to American Tripanosomiasis (Chagas disease), caused by *Trypanosoma cruzi*, is much higher than that associated to malaria, schistosomiasis or leishmaniasis. Chagas disease is distributed in America from south U.S. to south Argentina. At present, there are 18-20 million people infected with *T. cruzi*, and other 40 million at risk. After almost one century from the discovery of the causative agent of Chagas disease, there are no drugs or vaccines to cure or prevent the illness. Rational design of new drugs is in progress. Glycolytic enzymes have been studied as possible chemotherapeutic targets due to their particular characteristic of compartmentalization in the glycosome. The enzyme Pyruvate Phosphate Dikinase (PPDK) has been found in T. cruzis glycosomes associated to the membrane (inactive), located outwards, and in the matrix. PPDK acts detoxifying PPi, a product of synthesis pathways, and maintaining the energetic balance inside the organelle. We intend to characterize biochemically and molecularly both PPDKs from T. cruzi. The PPDK gene was cloned and the recombinant protein expressed and purified. Policlonal antibodies were produced. It kinetic constants were determined in the glycolitic sense, obtaining a Km of 16.9, 68 and 396 µM for AMP, PPi and PEP respectively, higher values than those reported for *T. brucei*, and similar to those reported for some C_3 plants. It activity is not significantly affected by DTT, but it is by KCl concentration and pH variations, and inhibited by divalent ions like Ca^{2+} and Mn^{2+}. It calculated molecular weight was of 100 kDa, and it predominant oligomerization state determined to be tetrameric, by molecular exclusion chromatography. It was identified in the glycosome by differential centrifugation and permeabilization with digitonin.
Supported by FONACIT

BC75 - INTESTINAL *TRYPANOSOMA CRUZI* INDUCED HISTOPATHOLOGICAL CHANGES IN ACUTE AND CHRONIC EXPERIMENTAL MURINE INFECTION

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The study of the experimental Chagas' disease may contribute to better explain the pathogenesis of megaesophagus and megacolon. Current models of chronic infection in mice are based in histological data collected early at the course of disease. The chronic, long term maintained mice for 15 months were studied in order to investigate aspects of plasticity of intestinal smooth muscle and enteric nerves. We evaluated histopathological changes of intestinal wall and enteric nervous system of *T. cruzi*-infected mice. A group of Swiss mice was infected with 50.000 trypomastigotes of Y strain. At 11º day post-infection a sub-group was sacrificed (acute phase) and other sub-group was treated with Benznidazol and sacrificed 15 months after infection (chronic phase). Age-pared non-infected mice were used as control. The entire extension of the colon was sampled in a swiss-roll for histopathology in morphometrics analysis and comparisons by ANOVA. In the acute phase all animals presented inflammatory lesions related to intense and diffuse parasitism of submucosa that presented enlarged when compared to controls. The infiltrating cells presented at muscular layer were positive for T cells markers. Intense degenerative and inflammatory changes correlated to increased reticular fiber quantification, associated to necrosis of muscle cells, especially in the distal colon. Most neurons and glial cells of the Meissner and Auerbach plexus were preserved but a decreased intermuscular nerve bundle density was detected. In the chronic phase the parasitism was insignificant, but we notice significant increased thickness of the colon wall and diffuse hypertrophy of individual muscle cell as well as increased collagen deposition indicating fibrosis repair in injured areas affected at acute phase. Mast cell were significantly increased in muscular layers. Meissner and Auerbach plexus architecture were focally affected, and a significant decrease of intermuscular nerve fibers were detected.
Supported by CNPq and FAPEMIG.

BC76 - FUNCTION OF MATING PHEROMONES, GAMONE 1 and 2, IN THE CILIATED PROTOZOA *BLEPHARISMA JAPONICUM*

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Most ciliated protozoa undergo conjugation under food-deprived conditions. In some species, substances that induce conjugation (mating substances, mating pheromones or gamones) have been identified. Conjugation of *Blepharisma japonicum* is characterized by a specific cell-cell interaction between complementary mating-type cells, I and II, that is induced by chemical substances called gamones. To elucidate how the gamones induce conjugating pair formation, we examined the effect of each gamone on the complementary mating-type cells, and showed that both gamones could induce behavioral and morphological changes in the complementary mating-type cells prior to the commitment for pair formation. We also showed that expressions of genes which may be involved in regulating the progression of conjugation were enhanced in the gamone-stimulated cells. These results suggest that a certain gamone-triggered signaling pathway is involved in the conjugation process in *B. japonicum*, by which expression of the conjugation-related genes is up-regulated, leading to the morphological changes and mating pair formation in the gamone-recipient cells. Gamone 1, which is secreted by type I cells and is the first glycoprotein discovered as a conjugation-inducing substance in ciliates, is specifically recognized by mating type II cells. We investigated whether the oligosaccharide attached to gamone 1 is indispensable for conjugation-inducing activity, by modifying the gamone 1 that lacks the oligosaccharide. We found that the gamone 1 without the oligosaccharide showed a much reduced activity, suggesting that the oligosaccharide attached to gamone 1 has an important role to exert the conjugation-inducing activity of gamone 1.
Supported by JSPS and Japan Society of Protozoology.

BC77 - MORPHOLOGY AND MORPHOGENESIS OF *CAMPANELLA UMBELLARIA* (CILIOPHORA: PERITRICHIA), A FRESHWATER CILIATE FROM SOUTHERN CHINA

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The morphology and morphogenesis of *Campanella umbellaria* (Linnaeus, 1758) Goldfuss, 1820, isolated from a freshwater pond of Hangzhou, China, were investigated using live observations, and protargol impregnation. Based on the Hangzhou populations, this species is characterized thus: cell *in vivo* about 180 × 125 μm, inverted bell –shaped with wide peristomial lip that are invariably reflected aborally; macronucleus C-shaped and transversely oriented; one contractile vacuole apically located; haplokinety and polykinety making 4 and 1/2 turns in their outer course, of which infundibular polykinety 1 and 2 with 3 equal rows of kinetosomes, polykinety 3 consists of more than 3 kinetosomes; Morphogenesis reveals the following characteristics: (1) the whole process of binary fission takes 1-1.5 hours to complete, which occurs once every 18 hours on average; (2) stomatogenesis commences with the dedifferentiation of germinal kinety. In the opisthe, infundibular polykineties 2 and 3 as well haplokinety originate from germinal kinety, while infundibular polykinety 1 from haplokinety. Two germinal kineties for both daughter cells appear almost at the same time; (3) micronucleus changes prior to the macronucleus, which acts in a usual way; (4) both the scopula and bubble cleave into two equal portions, then they become circle respectively along with the division of two buccal ciliary apparatus.
Supported by the NSFC (project no. 30670222) and State Key Laboratory of Freshwater Ecology and Biotechnology,Chinese Academy of Sciences (No.2009FB11).

BC78 - Ablation of TbTOR-like 1 protein of *Trypanosoma brucei* causes an elevation of polyphosphate levels in acidocalcisomes .

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TOR (target of rapamycin) is a kinase of the phosphatidylinositol kinase-related kinase (PIKK) family that controls cell growth in eukaryotes in response to nutrients, energy and growth factors alterations. Studies with mammalian cell and yeast signaling pathways have shown that nutrient starvation inhibits TOR activities, which results in G1 cell cycle arrest, and triggers a stress response program leading to blockage of translation initiation. The same stress response can be observed in cells treated with rapamycin, an immunosuppressant drug, which binds to FKBP12 prolyl-isomerase forming a complex with the TOR kinase. In trypanosomes two TOR orthologs (TbTOR1 and TbTOR2), and two other proteins with significant similarities to yeast or mammalian TORs (TbTOR-like 1 and TbTOR-like 2) were identified. To gain insight into the physiological role of the TbTOR-like 1 kinase, RNA interference experiments were carried out in *T. brucei* procyclic and bloodstream forms. Ablation of TbTOR-like 1 expression by RNA interference caused a significant increase in the amount of polyphosphate (poly-P) and pyrophosphate (PPi). The acidocalcisomes, known to store polyphosphate, appeared in large numbers and enriched in electrondense materials. At the same time, the cells showed a decreased response to hyposmotic stress. In addition, the cells delayed in S phase of the cell division cycle. The levels of TbTOR-like 1 were reduced after 2 days of RNAi and no protein were detected after 5 days of induction with tetracycline as observed in Western blot experiments. RNAi induction of TbTOR-like 1 for longer periods also results in growth arrest with giant, multinucleate and multiflagellated parasites. Based on these findings we suggest that TbTOR-like 1 may participate in the regulation of poly-P and PPi homeostasis, physiology and growth in *T. brucei*.
Supported by FAPESP and CNPq

BC79 - COMPARATIVE ANALYSIS OF THE ENZYME CONSTITUTIVE NITRIC OXIDE SYNTHASE (cNOS) IN DIFFERENTS SPECIES OF LEISHMANIA CAUSED OF CUTANEOUS LEISHMANIASIS

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American Tegumentary Leishmaniasis (ATL) is a parasitic disease, widely spread in most countries of Latin America, and caused by different species of the genus *Leishmania*. This protozoan is an obligate intracellular parasite that developed mechanisms to subvert the microbicidal activity of macrophages, such as inhibition of superoxide and nitric oxide (NO) production. In murine leishmaniasis, the nitric oxide plays a crucial role in the killing of parasites in vitro and in vivo. In this work, we analyzed the constitutive Oxido Nitric Synthase (cNOS) expression and NO production by three species of *Leishmania*, *Leishmania* (*Leishmania*) *amazonensis*, *Leishmania (Viannia) shawi e Leishmania (Viannia) braziliensis.* Promastigotes in stationary and logarithmic phase of growth were studied comparatively to evaluate NO production and cNOS activity. Parasites were maintained in RPMI 1640 medium supplemented with 10% of fetal bovine serum at 27°C. For detection of NADPH-diaphorasic activity, promastigotes were incubated with 1mM NADPH for 2 hours and analyzed in Interferential Microscopy (Axiophot Zeiss). Leishmania cNOS was identified in promastigotes using indirect Immunofluorescence assays, using polyclonal antibody anti-cNOS (Sigma) diluted 1:100 in PBS-BSA-Tw. The measure of the nitric oxide production was determinate in the supernatants of promastigotes cultures as nitrite form by adding Griess reagent. Immunofluorescence and cytochemistry assays showed that promastigotes of three *Leishmania* species are able to express cNOS. However, *L. braziliensis* had a strong reactivity when compared with *L. amazonensis and L. shawi*. By Nitrite measure was observed that the species were able to produce NO, but *L. braziliensis* interaction produced the highest amount of NO in comparison with the other species. In conclusion, a correlation between the expression of cNOS and NO production by distinct Leishmanias species and clinical manifestations suggest a possible down-regulation mechanism of the NO production by the host cell by the presence of parasites.
Supported by CNPq/UFPA.

BC80 - Amastigote forms of the parasite *Trypanosoma cruzi* are more resistant to nitric oxide than trypomastigote ones. An in vitro assay with sodium nitroprusside.

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Despite the myriad of molecules produced by the immune cells against *Trypanosoma cruzi*, including NO, this protozoan generally by pass the adversities and is able to establish the infection. Studies on the evasion mechanisms in different protozoans can supply important tracks to better understand the interaction of parasites with their hosts. Several hypotheses have been done on the ability of the *T. cruzi* to modulate the host cell response, however, this is the first time that the resistance of trypomastigote (both bloodstream and MK2 cell culture derived) and amastigote (MK2 cell culture derived) forms of *T. cruzi* to NO toxic effects were directly tested *in vitro*. Using a chemical NO donor, the sodium nitroprusside (SNP), we evaluated the effect of NO on *T. cruzi*, and demonstrated that NO is not such a straight trypanocidal molecule, at least not for the amastigote forms. At high dosages of released NO by SNP amastigotes were able to survive to the toxic effects of NO, showing that these multiplicative forms are far more resistant than trypomastigote ones. Additionally the trypomastigote forms seem to undergo into a differentiation process, assuming a round shape and losing its apparent flagellum. Our findings points to amastigotes as the more resistant life stage of *T.cruzi* regarding to NO, and also suggest that the oxidative stress might be related to the differentiation process of this protozoan parasite.Suported by CNPq

BC81 - POLYPHOSPHATE MOBILIZATION IN ACIDOCALCISOME-LIKE VESICLES IN *EUGLENA GRACILIS* CULTIVATED IN THE PRESENCE AND ABSENCE OF LIGHT.

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Acidocalcisomes are electron dense acidic organelles rich in calcium and phosphorus described in trypanosomatid parasites and several microorganisms. Different functions have been attributed to acidocalcisomes, such as the storage of calcium and other cations, pH homeostasis and osmoregulation. Phosphorous is usually stored in these organelles in the form of short and long chain polyphosphate. Poly P is a polyfunctional compound that plays a role in phosphate methabolism, energy reservation, cation sequestration, and a vital role in stress response and stationary-phase adaptation in many cells. *E. gracilis* is a photosynthetic free living unicellular protist that has an autotrophic behavior when cultivated in the presence of light and an heterotrophic behavior in the absence of light, where its chloroplasts reduces to proplastids. Our group has found structural and functional evidence for the presence of acidocalcisome-like organelles in *Euglena gracils.* As in a number of organisms a large amount of poly P is found in the acidocalcisomes, we hypothesize that polyphosphate accumulated within these organelles may play a role in energy metabolism in *Euglena gracilis* submitted to cultivation in the presence or absence of light. In this work, the mobilization of polyphosphate during the transition between autotrophic and heterotrophic states is described. X-ray microanalysis showed a substantial amount of oxygen and phosphorus, presumably in the form of poly P, in electron dense organelles that resemble acidocalcisomes. Polyphosphate extraction showed an increase in the levels of short chain poly P during the first four days of the transition between autotrophic and heterotrophic states. In contrast, the levels of long chain poly P decreased in the same period, reaching the steady state levels after four days of change in the illumination conditions. These results suggest that poly P, presumably stored within acidocalcisome-like structures, might have a functional role in the energy metabolism in these cells.
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BC82 - Inducible nitric oxide synthase degradation pathway in activated macrophages after *Toxoplasma gondii* infection

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Activated macrophages produce nitric oxide (NO), which is a microbicidal agent. This production is catalyzed by inducible nitric oxide synthase (iNOS), which uses arginine as substrate producing NO and citruline. It has been demonstrated that NO production is inhibited by *Toxoplasma gondii* infection, the agent that causes toxoplasmosis. This inhibition is caused by iNOS disappearance of only infected macrophages as visualized by immunofluorescence. Two main iNOS degradation pathways have been described: the proteasome and the calpaine pathway. This work aims the identification of the iNOS degradation pathway caused by *T. gondii* infection of activated macrophages. For this, J774-A1 macrophage cell line was cultured with Dulbecco's modified Eagle's medium supplemented with 5 % of fetal bovine serum. The *T. gondii* (RH strain) was maintained by peritoneal passages in Swiss mice. Macrophages were activated with lipopolysaccharide and Interferon-gamma for 24h, treated with lactacystine (proteasome inhibitor), or with calpeptine (calpaine inhibitor) and infected with the parasite. Coverslips were collected after 2, 6 and 24h to assay iNOS expression by immunofluorescence and the supernatant after 6 and 24h of interaction was evaluate to determine NO production. iNOS was also detected by Western blotting. iNOS was not visualized in macrophages infected with *T. gondii*. Macrophages treated with lactacystine infected with *T. gondii* showed iNOS expression. However, calpeptin treatment could not revert NO inhibition. These results indicate that *T. gondii* infection activates the proteasome pathway degrading iNOS.
Supported by: FAPERJ, CNPq, CAPES

BC83 - INFLAMMATORY MONONUCLEAR PHAGOCYTE IN LEISHMANIASIS: PHENOTYPE AND CELL MIGRATION

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Leishmania infection modulates integrin function in inflammatory phagocytes, affecting cell migration and parasite dissemination. The aim of this study is to identify inflammatory phagocytes populations susceptible to *Leishmania* infection and the potential changes in their migratory capabilities after infection. We defined the kinetics of phagocyte migration from the peritoneum to the draining lymph node after thioglycollate or *Leishmania* stimuli using injection and tracking experiments, combined with immunohistochemistry and flow cytometry. We observed that thioglycollate-elicited inflammatory mononuclear phagocytes expressed CD11b (78±6%), F4/80 (65±9%) and CD11c (28±15%). Among these cells, 64% were CD11b+F4/80+ and 9±5% were CD11b+CD11c+. In the draining lymph node 98% of the cells had a myeloid-macrophagic (CD11b+ and F4/80+) phenotype. Myeloid dendritic cells (CD11c+/CD11b+) accounted for 28% of the emigrated cells. Furthermore, while in the peritoneum most of the dendritic cells (91%) had a CD11c+/MHC-II^{low} (immature phenotype), most of the dendritic cells emigrated to the lymph node (82%) had a CD11b/MHC^{high} (mature phenotype). Among the peritoneal phagocytes the proportion of infected cells were higher among the CD11b+ (>70%) than among the CD11b- (~30%) cell populations. The CD11c+/CD11b+ phagocytes presented the highest infection ratio (77+22%) among the CD11b+ cells. Among the CD11c+ *Leishmania*-infected phagocytes, 28% also expressed MHC-II, and this expression was high in 6% of these cells. The migration model used herein allows a high number of cells to be examined and isolated for further studies. Hence, we are now investigating the pattern of adhesion molecule expression in these cell populations and their capability to transport *Leishmania* to the draining lymph node. Supported by CNPq and FAPESB

BC84 - INFECTION WITH LEISHMANIA MODLATES HUMAN MONOCYTE AND HUMAN MACROPHAGE ADHESION TO CONNECTIVE MATRIX COMPONENTS

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In a previous study we showed that infection with Leishmania reduces the adherence of mice peritoneal inflammatory macrophages and of human peripheral blood monocytes to the connective tissue. The Leishmania-induced decrease in adherence of mice inflammatory macrophages to the connective tissue was due to impairment in beta-1 integrin function. In this work we explore this phenomenon using human peripheral blood monocytes and human macrophages differentiated in vitro from peripheral blood monocytes. In both human monocyte and macrophages the adherence to connective tissue is divalent cation dependent, suggesting integrin participation in the process. Infection with Leishmania decreases the adherence of both monocytes (59% inhibition, $P<0.0001$) and macrophages (85% inhibition, $P<0.05$) to the connective tissue and to connective matrix components: adherence to fibronectin decreased 96% in monocytes ($P<0.05$) and 79% ($P<0.05$) in macrophage after Leishmania infection, and adherence to collagen decreased 91% in monocytes and 82% in macrophage after Leishmania infection. Incubation with leishmania lysate decreased macrophage (43% inhibition, $P<0.05$) but not monocyte adherence to connective tissue. Our data confirms our previous studies using murine cells. We are now examining the profile of adhesion molecule expression by these infected cells and the potential interference by inflammatory cytokines in the adhesive change induced by Leishmania infection. Financial support: CNPq and FAPESB.

BC85 - GENE SILENCING DISCLOSES MULTIPLE, BUT WIDELY SPECIFIC FUNCTIONAL ROLES OF THE ACTIN4 SUBFAMILY IN *PARAMECIUM TETRAURELIA* CELLS

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Paramecium cells possess more actin isoforms than most other cells. The actin4 subfamily consists of two paralogs which show less than 50% identity to the other isoforms. We have previously localized actin4 isoforms to several structures in *Paramecium tetraurelia* cells, i.e., cleavage furrow, nascent food vacuoles, oral apparatus, cilia and cell surface. In the present study, we performed gene silencing experiments under conditions appropriate to knock down selectively actin4 isoforms. We found a remarkable coherence between actin4 silencing and disturbance of nuclear development of both, micro- and macronucleus, paralleled by reduced cell division. Over longer silencing periods, actin4 silencing entailed reduced phagocytic activity paralleled by accumulation of “acidosomes” (reported as derivatives of early endosomes) near the cytopharynx where they normally fuse with phagosomes. In addition, near the cell surface, extensively elaborated misshaped early endosomes (“terminal cisternae”) occurred and trichocyst exocytosis was impaired. The structural/functional alterations we see mainly concern subcellular components to which actin4 has been localized, with the additional observation of an effect on karyokinesis. In essence, actin4 exerts pleiotropic effects at widely different sites of the *Paramecium* cell and cannot be substituted by any other isoform of the large number of actins occurring in these cells.
Supported by DFG.

BC86 - EFFECT OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) ON THE ARGINASE ACTIVITY OF *LEISHMANIA (VIANNIA) BRAZILIENSIS, LEISHMANIA (LEISHMANIA) AMAZONENSIS* AND *LEISHMANIA (LEISHMANIA) CHAGASI*

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Leishmaniasis is caused by various species of Leishmania protozoa that determine different clinical forms, ranging from self-healing skin ulcers to disfiguring mucosal lesions and fatal visceral disease. Parasite and/or host factors that determine different tissue localization of the parasite and diverse clinical presentations are not known. Some evidences indicate that parasite-related factors are important for these differences. The initial phase of parasite-host interaction is crucial for the resolution or establishment of the disease where different factors participate including growth factors. IGF-I is a systemic hormone with pleiotropic effects and present in the skin and inside macrophages, it is one of the first factors encountered by *Leishmania* promastigotes when injected into the skin. We reported previously that IGF-I increase arginase expression and activity in *L. (L.) amazonensis* strain (Vendrame et al, SJI, 66:287, 2007). The arginase inductions play an important role in the *Leishmania*-host interaction, being important for parasite survival. The objective of this study is to analyze the effect of IGF-I on the arginase activity in different strain of *Leishmania*: *Leishmania (V.) braziliensis, Leishmania (L.) amazonensis* and *Leishmania (L.) chagasi.* For this purpose, $1x10^7$ promastigotes of different species of *Leishmania* on stationary phase of growth were stimulated with IGF-I (50ng/mL) or maintained without IGF-I (control). We studied arginase activity by urea detection. The arginase activity (Mu arginase/10^7 parasites) were 8.8 ± 0.4 (control) and 9.9 ± 0.2 (with IGF-I stimulus) in *L. (V.) braziliensis,* 19.3 ± 0.6 (control) and 28.4 ± 0.7 (with IGF-I) in *L. (L.) amazonensis* and 32.4 ± 1.3 (control) and 19.4 ± 0.6 (with IGF-I) in *L. (L.) chagasi.* These results showed differences in the arginase activity amongst the strains analyzed already at basal condition and further with IGF-I stimulus. The data suggest that it might be associated with the modulation of disease progression in these distinct clinical forms of disease. **Supported by LIM/38 (HC-FMUSP) and FAPESP.**

BC87 - ENOLASE - PLASMINOGEN INTERACTION IN *Trypanosoma cruzi*

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Chagas disease is a widely distributed parasitic disease caused by *Trypanosoma cruzi.* Among the 16 to 18 million people affected in Latin America, there is an increasing number of deaths and cases of premature disability. After a century of its discovery, vector control in endemic areas and prevention of infection via blood supply have become the only ways to combat transmission. Many proteins participate in the complex process of host cell infection by *T. cruzi,* but only a few of them have been identified experimentally. The glycoprotein Plasminogen (Plg) is the zymogen of the serine protease plasmin (Plm), which is a key enzyme in the host's fibrinolytic pathway. The acquired Plm activity promotes dissemination and pathogen crossing through basement membranes. Many eukaryotic surface molecules interact with Plg, and specific receptors have been described previously. Lysine or lysine analogs such as ε-aminocaproic acid (ε-ACA) mimic COOH-terminal lysine and thereby inhibit the interaction. Our research group has shown that recombinant *T. cruzi* enolase (rTcENO) bound human Plg in a dose-dependent manner mediated by lysine residues. In the present study, we determine that the dissociation constant (K_d) of plasminogen to the surface of immobilized *T. cruzi* trypomastigotes is 84,5 nM and that ε-ACA blocks the association, indicating that lysines mediate the interaction. We have identified a membrane-bound α-enolase on *T. cruzi* trypomastigotes surface. This classic plasminogen receptor could mediate Plg binding to *T. cruzi*. Inmunopathological studies of affected organs in infected experimental murine model immunized with rTcENO support the importance of blocking this receptor, suggesting a central role for *T. cruzi*-Plg interaction on disease evolution. Substantial decrease in the quantity of circulating blood trypomastigotes and mortality of immunized animals infected with lethal doses of parasites give sustention to this hypothesis.
Supported by CDCHT-ULA.

BC88 - BONE MARROW CELL THERAPY AMELIORATES AND REVERSES CHAGASIC CARDIOMYOPATHY IN A MOUSE MODEL

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Chronic chagasic cardiomyopathy, which is caused by the protozoan *Trypanosoma cruzi,* is a major cause of heart failure in Latin America. It is a disease for which effective treatment in its advanced clinical forms is lacking. We have previously shown that bone marrow mononuclear cell (BMC) transplantation is effective in reducing inflammation and fibrosis in them mouse model of Chagas disease. The present study used magnetic resonance imaging to assess changes in the cardiac morphology of infected mice after therapy with BMCs. Serial imaging of the BMC-treated mice revealed regression of the right ventricular dilatation typically observed in the chagasic mouse model.
Supported by: CNPq, FAPERJ, National Institutes of Health (NIH) and Fogarty International Training Grant.

BC89 - CHARACTERIZATION OF A PHOTOLYASE/CRYPTOCHROME PROTEIN FROM *TRYPANOSOMA CRUZI*

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Many eukariotic and prokariotic organisms have photoreceptor proteins in which proteins belonging to the Phtolyase/Cryptochrome family are included. These latter proteins are flavoproteins that, in the case of photolyases, have the capacity to light-drive repair of DNA that has been damaged by UV. Cryptochromes share structural similarity to DNA photolyase but lack photolyase activity. The genome databases of Trypanosomatids (*Trypanosoma spp and Leishmania spp.*) report presence of proteins of the Phtolyase/Cryptochrome family; two sequences in *T. brucei* and *Leishmania spp*. and only one in *T. cruzi*. In this work we cloned and expressed in *Escherichia coli* the Photolyase/Cryptpchrome of *T.cruzi* as a first step toward understanding the function of these proteins in trypanosomatids. The recombinant protein resulted to be insoluble and was used to produce antibodies against this protein. Cell fractionation by differential centrifugation indicated that the Photolyase/Cryptochrome is located at the mitochondrion. Immunofluorescence studies showed that this protein is associated to kinetoplast and is fully expressed by the epimastigote but not by the trypomastigote form. In epimastigotes, this protein is more expressed in parasites from exponential phase during the growth curve. A phylogenetic analysis suggests that this protein is specific of Trypanosomatids. However, some conserved residues of cryptochromes type Dash, also called single stranded photolyase, are present. Due its association with the kinetoplast, this protein might be involved in DNA reparation or transcriptional regulation.

BC90 - ELEMENTAL COMPOSITION AND STRUCTURE OF ACIDOCALCISOMES IN *PLASMODIUM CHABAUDI*

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Acidocalcisomes are acidic calcium-storage organelles characterized by their acidic nature, high density, and high content of oxygen, phosphorus, calcium, and other elements. They were first characterized in trypanosomatids and subsequently found in a wide range of microorganisms, including apicomplexan parasites such as *Toxoplasma gondii* and *Plasmodium* species. Acidocalcisomes are involved in numerous biological functions such as pH homeostasis, functional control of the intracellular concentration of several ions, polyphosphate metabolism and osmoregulation, all mechanisms that involve mobilization and transport of ions and osmolytes in the cell. During the course of infection, which involves different hosts, *Plasmodium* parasites face extreme variations on the physicochemical properties of the extracellular environment and, therefore, it is likely possible that Plasmodium acidocalcisomes might regulate the adaptation of the parasite to the new environment. In this regard, *Plasmodium chabaudi* is an attractive model for the study of the biogenesis of the acidocalcisomes because a synchronized life cycle, controlled by circadian period, can be obtained in mouse models, producing bleeds enriched in specific developmental forms of the parasite. Electron microscopy observation of thin sections showed the presence of electron dense vacuoles resembling acidocalcisomes. X-ray microanalysis showed high amounts of phosphorus and calcium in these organelles. Erythrocytes infected with *Plasmodium chabaudi* (AJ strain) incubated with the weak base Acridine Orange showed the acidic nature of several internal compartments with size and distribution similar to those described for the acidocalcisomes. A similar stain was also observed in isolated parasites. Immunolabeling of infected erythrocytes with polyclonal antibodies against an *A. thaliana* V-H^+-pyrophosphatase also showed an intense fluorescence in cytoplasmatic organelles with similar distribution to the acidic electron-dense vacuoles. Altogether, the results suggest that *Plasmodium chabaudi* present a pool of organelles with acidocalcisome characteristics. The mechanisms underlying the physiological role of these organelles in the different developmental forms of *P. chabaudi* deserves further investigation.
Supported by CNPq and FAPERJ.

BC91 - IDENTIFICATION OF THRAUSTOCHYTRID STRAINS ISOLATED FROM ARGENTINEAN TEMPERATE AND COLD ENVIRONMENTS

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Thraustochytrids (Labyrinthulomycetes, Heterokonta) are common marine microorganisms that have attracted much attention in last years as polyunsaturated fatty acids (PUFAs) producers. Their taxonomical identification at genus level is nowadays under revision as recent studies demonstrated that traditional morphological features (generally assessed using the multiple baiting technique) do not correlate with phylogenetic lineages. In this context, the complete characterization and taxonomical identification of new thraustochytrids isolations fatally require of both biochemical and molecular analysis. Even so, the adjudication of a given isolation to currently recognized taxa is not always an easy task, which suggests the existence of an underlying, unresolved diversity within this group of organisms. The aim of this work is to present the characterization of thirty thraustochytrids isolations from Argentinean temperate and cold environments as a preliminary attempt for their taxonomical identification. Observations on morphology and development on pollen baits suggest that they belong either to the genera *Schizochytrium* (*sensu lato*), *Ulkenia* (*sensu lato*) and *Thraustochytrium*. Evidence that species of each genus could be identified by their colony morphology, which remains constant in different culture media, is presented. Even so, their ability to grow in six different culture media was also investigated, finding that similar strains show similar nutritional requirements. Both, PUFAs profiles and 18S RNA ribosomal genes sequencing have been already established for some Argentinean isolations and are in course for the remaining ones. Integration of the results based on morphological, nutritional, biochemical and molecular studies will allow us not only approaching Argentinean thraustochytrids diversity but the global knowledge of these organisms.

BC92 - Protein import into hydrogenosomes of *Trichomonas vaginalis* involves both N-terminal and internal targeting signals – a case study of thioredoxin reductases

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The parabasalian flagellate *Trichomonas vaginalis* harbours mitochondria-related organelles of anaerobic ATP synthesis, hydrogenosomes, which harbor oxygen-sensitive enzymes essential to its pyruvate metabolism. In the human urogenital tract *T. vaginalis* is, however, regularly exposed to low oxygen concentrations and therefore must possess antioxidant systems protecting the organellar environment against the detrimental effects of molecular oxygen and reactive oxygen species. We have identified two closely related hydrogenosomal thioredoxin reductases, TrxRs, the hitherto missing component of a thioredoxin-linked hydrogenosomal antioxidant system. One of the two hydrogenosomal TrxR isoforms, TrxRh1, carried an N-terminal extension resembling known hydrogenosomal targeting signals. Expression of hemagglutinin-tagged TrxRh1 in transfected *T. vaginalis* cells revealed that its N-terminal extension was necessary to import the protein into the organelles. The second hydrogenosomal TrxR isoform, TrxRh2, had no N-terminal targeting signal, but was efficiently targeted to hydrogenosomes nonetheless. N-terminal presequences from hydrogenosomal proteins with known processing sites, the alpha subunit of succinyl-CoA synthetase (SCS□) and pyruvate:ferredoxin oxidoreductase A, were investigated for their ability to direct mature TrxRh1 to hydrogenosomes. Neither presequence directed TrxRh1 to hydrogenosomes, indicating that neither extention is, by itself, sufficient for hydrogenosomal targeting. Moreover, SCS□ lacking its N-terminal extension was efficiently imported into hydrogenosomes, indicating that this extension is not required for import of this major hydrogenosomal protein. The finding that some hydrogenosomal enzymes require N-terminal signals for import, but that in others the N-terminal extension is not necessary for targeting, indicate the presence of additional targeting signals within the mature subunit of several hydrogenosome-localized proteins.

Supported by the DFG

BC93 - *Giardia* Lipoprotein Receptor-related Protein: a novel LDLR member family in a primitive eukaryotic cell.

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The LDLR is the founding member of a family of seven structurally related transmembrane proteins (Lipoprotein Receptor-related Protein 1 –LRP-1, 1b, megalin/LRP2, LDL receptor, very low-density lipoprotein receptor, MEGF7/LRP4, LRP8/apolipoprotein E receptor2). In a GGD survey, we identified a protein that shares a cysteine-rich N-terminal domain with LRP1 and LRP1b and named gLRP for ***G**iardia* **L**ipoprotein **R**eceptor-related **P**rotein. This is a 116 kDa predicted type I membrane protein that possesses a signal peptide, a 20 aa transmembrane domain (from aa 1009 to 1029), and a cytoplasmic tail of 41 aa at the C-terminus. Interestingly, the cysteine-rich domain has been shown to be important for LDL binding and LDL internalization by endocytosis. Using confocal microscopy, we determined that the HA-tagged gLRP colocalized with LDL at the plasma membrane and in the lysosome-like peripheral vacuoles (PVs). Surface localization of gLRP was confirmed using TIRFM. Furthermore, epifluorescence microscopy demonstrated that gLRP was also present in the nuclei. The nuclear localization of gLRP implies the participation of this receptor in cell signaling, as has been extensively described for the LRP family. In addition, similar to LRP1 and LRP1b, gLRP contains a potential FXNPXY-type internalization signal (FNSPTY) within its cytoplasmic tail. By YTH and co-IPP assays, we found that gLRP directly bind to the medium subunit of *Giardia* adaptor protein 2. Because no LRP-like adaptor proteins that contain a PTB domain, such as ARH or Dab, have been found in *Giardia*, we hypothesized that gAP2 alone has the ability to direct gLRP to the PVs. These results suggest that gLRP is involved in the internalization of cholesterol from LDL via a regulated AP2-clathrin-dependent pathway. We can conclude that the delivery of specific proteins to the lysosome occurs in this parasite, with the peculiarity that the machinery involved in the process seems to be very simple. Supported by 1R01TW0072450, NIH (USA), PIP6563, CONICET (Argentina), and PICT20221, FONCyT (Argentina).

BC94 - Effect of Bromoenol lactone on the secretory and endocytic pathways in *Leishmania*: Traffic of GPI- anchored molecules

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Structural and functional aspects of the endocytic/exocytic pathway in Trypanosomatids have shown to be significantly different when compared with mammalian cells. Although the structure and composition of many intracellular compartments in *Trypanosoma* and *Leishmania* have been elucidated, the mechanism that controls their fusion remains uncharacterized. We have been investigating the Bromoenol lactone (BEL) effect, an irreversible inhibitor of Ca(2+)-independent PLA2 (iPLA2), in the traffic of *Leishmania amazonensis* promastigotes intracellular molecules. Parasites, grown for 72h in Schneider medium, were incubated for 1h with 2.5 µM BEL and analyzed by transmission electron microscopy, flow cytometry and SDS-PAGE. BEL reduced in 22% the amount of proteins secreted/excreted in promastigote culture supernatant. SDS-PAGE analysis of the promastigotas molecules released after 3 h incubation in culture medium without serum, showed a polypeptides panel ranging from 46 to 104 kDa. The same pattern was observed after BEL treatment. The relative intensities of the reactive bands were estimated by scanning densitometry. Preliminary analysis indicated a reduction of approximately 38% in two bands with 48 and 63KDa, after BEL treatment. The released materials in control cells and after BEL incubation will be investigate by imnunobloting, using different antibodies against *Leishmania.* Preliminary results obtained with the immunocytochemical localization of GP63 after treatment, indicated an increase in the amount of gold markers associated to the multivesicular tubule. Treated parasites incubated for 1h in the presence of BSA-TRITC and analyzed by flow cytometry, showed an inhibition of approximately 40% in the fluid phase endocytic activity. The effects of BEL in the kinetics of compartments fusion using immunofluorescent markers are under investigation. The results indicate that iPLA2 can be involved in the fusion control of endocytic/ exocyitic compartments in *L. amazonensis*.
Supported by CNPq, CAPES, FAPERJ, Pronex

BC95 - MORPHOLOGICAL CHARACTERIZATION OF *EUTINTINNUS TUBULOSUS* (OSTENFELD, 1889) (CILIOPHORA: SPIROTRICHA) FROM A TROPICAL EUTROPHIC BAY

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Tintinnids are loricate ciliates, important constituents in planktonic environments mostly in marine waters, which are components of the marine microbial loop. Historically, identification and systematic schemes for tintinnine ciliates have emphasized lorica structure and virtually ignored zooid morphology. Recent studies have shown considerable diversity in oral and somatic ciliation among tintinnine taxa. The main objective of the research was to provide a morphological characterization of a Brazilian strain of *Eutintinnus tubulosus*. We contribute to the knowledge of *E. tubulosus* morphology by presenting new data on the lorica, and for the first time, observations on the oral and somatic ciliation. Ciliates were sampled from Guanabara Bay, Rio de Janeiro, Brazil, using Van Dorn bottle, studied *in vivo* under DIC and after protargol impregnation. *Eutintinnus tubulosus* was encountered sporadically from June and September, 2005 and January, 2006, and was observed in Guanabara Bay in surface waters above 20°C. The ciliates presented cylindrical hyaline lorica opened in oral and aboral portion [length mean 89.5 µm (86-92 µm), oral diameter mean 18.8 µm (18-20 µm)]. On average, the oral ciliature were composed of 1 paraoral membrane, 18 peristomial and 3 infundibular membranelles. The oral polykinetid were composed of three rows of kinetosomes that were similar in length. The somatic ciliature were composed of 20 somatic kineties (12 ventral and 8 dorsal) displayed in heterogeneous groups. The macronucleus consisted of four moniliphormis macronuclear nodules connected by isthmus from 4 to 10 µm length. Two spherical micronuclei (2µm in diameter) were observed with the former close to the first macronuclei nodule and the latter located between the third and forth macronuclei nodule. We observe four stages of stomatogenesis of this ciliate.
Supported by CAPES, CNPq and FAPERJ.

BC96 - MEASUREMENTS OF REACTIVE SPECIES OF OXYGEN FROM RECRUITED INFLAMMATORY CELLS IN MICE EAR DERMAL LEAFLETS

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CBA/J mice are resistant to *Leishmania major* (Lm) and susceptible to *Leishmania amazonensis* (La). In this work we measured the production of ROS (Reactive Oxygen Species) in a novel way. Our aim was to evaluate the role of ROS produced by inflammatory neutrophils (PMN) in the initial stages of infection. Ears leaflets (dorsal and ventral) obtained from infected mice were used throughout the experiment, as well as non-infected controls. The production of ROS was measured by secondary excitation using lucigenin for the NADPH oxidase (NOX) product superoxide (O_2^-), and luminol for products of myeloperoxidase (MPO) mainly, in this case, hypochlorous acid (HOCl). The role of these enzymes in the production of these ROS, was confirmed by the addition of inhibitors – Diphenyliodonium for NOX and azide for MPO. The measurements were performed using a photon counter of great sensitivity specially developed. CBA/J mice were infected by injection in both ears with 5 $X10^5$ promastigotes of La or Lm. One hour after infection mice were euthanized and the two leaflets separated with the dermal face down towards the photomultiplier tube, on a 35mm Petri dish containing 1ml D-MEM. In a parallel experiment, the same measurements were performed in PMN obtained from the abdominal cavity of mice submitted to intra peritoneal injections of La or Lm. PMN in RPMI, were reinfected *in vitro* with La or Lm (10:1). The results shows that the production of ROS reflects differences in susceptibility to La/Lm. Higher production of ROS were observed in both, ears leaflets, and PMN infected with Lm. The higher ROS production was the same independent of their origin – MPO or NOX.

BC97 - Nucleolar Relocalization of mRNAs and RNA Binding Proteins Induced by Transcriptional Stress and Severe Heat Shock in Trypanosoma cruzi

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TcSR62 is a RNA Binding Protein (RBP) from T. cruzi belonging to the SR-related RBP family. SR and SR-related proteins have multiple roles in mRNA metabolism, particularly as regulators of splicing. TcSR62 localizes mainly to the nucleus in a speckled pattern. We have recently found that TcSR62 is involved in mRNA metabolism.
In this work, we found that TcSR62, as well as other nuclear (TcPTB) or cytoplasmic (TcPABP) RBPs, were relocalized to the nucleolus in parasites submitted to transcriptional stress. We then found that RBPs nucleolar relocalization was a specific response which could only take place in the replicating stages of the parasites epimastigote and the intracellular amastigote but not in the infective and non-replicating trypomastigote stage. We also evaluated by RNA-FISH analysis, the effect of transcriptional arrest or severe heat shock treatment upon the localization pattern of the mRNA population. Surprisingly, both treatments resulted in the accumulation of bulk mRNA in the nucleolus. Further analysis showed that mRNA and RBPs accumulation was mediated by an active mechanism modulated by the phosphorylation status of the parasite. In order to study a possible role of TcSR62 in such stress response, over-expression of truncated versions of TcSR62 are currently being evaluated. These data might imply that *T. cruzi* has evolved a particular pathway either to deal with transcriptional stress by sequestering several main actors involved in gene expression into the nucleolus or, alternatively, RBPs and mRNAs have a nucleolar phase before reaching their "normal" localization, thus suggesting that in this ancient organism the nucleolus may also play a role in mRNA metabolism.
Supported by CONICET and Agencia Nacional de Promoción Científica.

BC98 - COMPARATIVE EVALUATION OF HISTOLOGICAL ALTERATIONS IN LYMPH NODES OF DOGS INFECTED WITH *Trypanosoma cruzi* Be62 OR Be78 STRAIN IN THE ACUTE PHASE OF CHAGAS DISEASE

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Trypanosoma cruzi is a protozoan parasite transmitted in both Central and South America by a triatomine vectors among a large variety of mammalian species, including humans and dogs. To understand the parasite infection damages in lymphoid organs we studied the essential histological alterations in lymph nodes of the dogs infected with two different *T. cruzi* strain during the acute phase of the Chagas disease. All dogs were experimentally infected with 2000 metacyclic trypomastigotes by conjunctiva route. Twenty nine lymph nodes of animals infected with the Be62 and twenty six lymph nodes of animals infected with the Be78 strain were collected during euthanasia. All lymph nodes were fixed in buffer formalin and processed to histopathological analyses. Four micrometers paraffin sections were obtained, H&E stained and analyzed by optical microscopy. The presence or absence of thickness and inflammation capsule, the cortical follicular hyperplasia, the hypertrophy and hyperplasia of macrophages in the cords and medullar sinus, edema, congestion, hemossiderosis and hemorrhagic process were evaluated. The hyperplasia and hypertrophy of the nodes and follicles of cortical area and cords and medullar sinus were the most frequents alterations on the Be62 than Be78 infection. On a other hand, the thickness and presence of capsule and subcapsular sinus inflammatory reaction were more usual in Be78 strain infection. Other studied alterations cannot be observed. The general histopathological picture of lymph nodes infected with the Be62 was characterized by diffuse inflammatory reaction composed by plasmocells, macrophages and lymphocytes mainly observed in the medullar cords and the medullar sinus. To Be78 infection lymph nodes presented an inflammatory reaction of lower intensity and easily observed in capsule and subcapsular sinus. Parasite tissue load and is being performed and the results will be associated with the present results in order to assess mechanisms of the histological lymphoid damage in the Chagas disease.
Sponsors: FAPEMIG, CNPq and UFOP

BC99 - ANALYSIS OF INTERACTION FROM MURINE MACROPHAGES WITH SUBPOPULATION OF *Toxoplasma gondii* THAT EXPOSE OR NO PHOSPHATIDILSERINE

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Phosphatidylserine (PS) exposure is a main event that indicates apoptosis. This exposure is fundamental for the Transforming Growth Factor-beta1 (TGF-b1) signaling that induced an anti-inflammatory response during phagocytosis of apoptotic cells. Some protozoan parasites expose PS, inhibiting macrophage inflammatory activity by mimicking the uptake of apoptotic cells. Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. Activated macrophages control *T. gondii* growth by nitric oxide (NO) production. However, *T. gondii* active invasion inhibits NO production, allowing parasite persistence. Our group showed that the mechanism used by *T. gondii* to inhibit NO production persisting in activated macrophages is similar to what Leishmania uses depending on PS exposure. In this work were realized interactions with isolated population of *T. gondii* and murine macrophages for analysis of penetration mechanism and survival of parasite. For this, *T. gondii* PS+ and PS- subpopulation were separated with annexin V conjugated magnetic beads. Results obtained by flow cytometry confirmed the efficiency of the mechanism of isolation of subpopulations. After the isolation interactions were realized for 1, 24 and 48 hours with macrophages. Data obtained from the evaluated of nitrite in culture medium collected during the interaction shows a significant decrease in nitric oxide production from the interaction of macrophages with PS + subpopulation of *T. gondii* on interactions realized with the total population of parasites and the PS-subpopulation. Results by Scanning Electron Microscopy shown that PS+ population of *T. gondii* invade macrophages by active penetration, but PS- population invades by a phagocytic mechanisms. The infection index and multiplication parameters shown that *T. gondii* PS+ as PS-, were slow when compared with the total population of parasite. The results suggest that PS+ and PS- population of *T. gondii* do invade macrophages with different forms and the growth of parasite depend on the total population.

BC100 - INVESTIGATION OF THE ASF1 (*Anti Silencing Factor 1)* IN THE CONTROL OF THE GENE EXPRESSION IN *LEISHMANIA MAJOR*

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Anti Silencing Factor 1 (ASF1) is a histone chaperone that contributes to the histone deposition during nucleosome assembly in newly replicated DNA. In addition, it has been shown that ASF1 is involved in the cellular response to DNA damage and transcriptional silencing, interacting with different proteins. We have previously identified a putative ASF1 in *L. major.* Previous functional studies indicate that also in trypanosomatids this putative ASF1 seems to be involved in the chromatin packaging and in DNA damage protection. In an attempt to evaluate the involvement of ASF1 in the control of gene expression in *Leishmania major*, we analyzed the proteome of a *L. major* transfectant that overexpresses ASF1 (*Lm[pXNeo-ASF1]*) by comparison with the one of a control line (*Lm[pXNeo)]*). We analyzed the protein profile differences of each line by two-dimensional gel electrophoresis. We rescued a group of differentially expressed proteins to be identified by mass spectrometry. Based on the fact that ASF1 controls the expression of genes present at the chromosome ends in yeast, we decided to use Real Time PCR to investigate a possible role of LmASF1 in the control of expression of Leishmania genes close to telomeres, which allowed confirming the modification of the level of expression of some of these genes when LmASF1 is overexpressed. In addition, with the objective to confirm the role of LmASF1 in DNA repair processes we submitted *Lm[pXNeo-ASF1]* and *Lm[pXNeo]*, to gamma irradiation (500Gy); the results indicate that the transfectant overexpressing LmASF1 recovers from the damage differently from control line. This set of complementary approaches allows understanding what the roles of ASF1 in Leishmania parasites are.
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BC101 - A PRELIMINARY INVESTIGATION ON THE ROLE PLAYED BY SERINE PROTEASES IN THE DIFFERENTIATION PROCESS OF THE PROTOZOAN *ACANTHAMOEBA POLYPHAGA*

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Acanthamoeba represents a cosmopolitan genus of free-living amoebas which are potentially pathogenic to humans. *Acanthamoeba* infections are difficult to treat, especially due to the ability of the protozoan to fast change its evolutive form from a metabolic active trophozoite to a resistant cyst stage. The cyst possesses a double-layered wall resistant to most of anti-amoebic drugs yet tested. *Acanthamoeba polyphaga* trophozoytes are able to secrete and release proteases, predominantly the serine ones which in turn, play important roles in both cytoadhesion and cytotoxicity exerted by the protozoan. Recently it was demonstrated that serine proteases are also involved in the trophozoite-to-cyst conversion in *A. castellanii.* Experiments were here designed to investigate the role played by serine proteases in the differentiation of *A. polyphaga.* Initially, zymographic assays were carried out by using secretions collected from *A. polyphaga* trophozoites which have been treated or not with AEBSF, a well known serine protease inhibitor. Viability assays were also performed in order to find the amount of AEBSF that could be used without causing amoebae damage. 2 x 10^5 trophozoites.ml^{-1} were inoculated in encystment medium supplemented or not with AEBSF. Cyst formation was dramatically reduced among trophozoites that have been found incubated in AEBSF-supplemented medium, suggesting participation of serine proteases in such encystment process of *A. polyphaga*. In contrast, incubation of trophozoites in the presence of the alcohol soluble serine protease inhibitor PMSF results in the inhibition of the progression of the encystment process. Interestingly, such inhibition in the progression of the encystment process was observed at similar levels when the PMSF solvents, as ethanol or isopropanol, were assayed. The last results clearly show that alcoholic solvents may greatly influence the differentiation process of *A. polyphaga*, at least *in vitro*.
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BC102 - A morphological investigation of the interaction between *Entamoeba histolytica* and tridimensional molecular networks made of type I collagen

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Studies focusing the *in vitro* interaction between parasitic protozoa and ECM molecules underlie that the phenomenon includes signal exchanges from ECM to parasites and from parasites to ECM. Signals from ECM to parasites are of mechanochemical origin since each one of chemistry, architecture, and mechanics concerning networks formed by ECM molecules influence the general behavior of most of the yet investigated eukaryotic cells. *In vivo* parasites are facing mechanical forces when found trapped into a molecular mesh. By using scanning electron microscopy we here investigate the interaction between trophozoytes of *E. histolytica* and bidimensional (2D) or tridimensional (3D) matrices made of collagen I (COL). The trophozoytes (strain HM1:IMSS) were allowed to interact with 1,5 (*low density*) or 3,0 (*high density*) mg.ml^{-1} COL and leaded to polymerization for 1 h at 37^{o}C. Trophozoytes were allowed to interact with 3D (*invasion assays*) or 2D (*spreading assays*) for 24h and 48h. Alternatively, trophozoytes were poured in contact with COL under polymerization (*interaction assays*).The ability of *E. histolytica* to remodel COL matrices was followed by scanning electron microscopy (conventional and field emission modes). Observations carried out at conventional electron microscopy revealed that the trophozoytes induced a profound architectural rearrangement of the networks. As observed during interaction assays, such rearrangement is usually originated at physical contacts established by trophozoytes with some COL fibrils. At field emission scanning electron microscopy we observed thin cytoplasmic projections emerging from trophozoytes surface contacting the COL films made at 2D. Such cytoplasmic expansions were not observed among parasites during interaction assays. By using videomicroscopy we followed the parasite behavior during invasion assays. As expected, most of parasites interacting with low density matrices presented a high migration rate comparatively to those interacting with high density matrices.
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BC103 - Morphological and morphometrical characterization of trypanosomes found in armoured Catfish

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The purpose of this study was to characterize the morphology and morphometry of the developmental forms of trypanosomes found in armoured catfishes. Host fishes were collected from Rio Pomba (21º21'07"S, 43º02'49" O) Guarani City, MG, Brazil. The fishes were anesthetized and blood samples were obtained by cardiac puncture and analyzed for the presence of hemoparasites. The heart, liver, spleen and kidney were used to make organ impressions. The leeches were removed from fishes, relaxed in ethanol 5%, dissected and the proboscis, crop and gut were removed and prepared thin smears. Morphometric characterization was made in 20 parasites of each developmental forms. The prevalence was 95% and the parasitemia was 1.1 parasites/cm^2. Trypanosomes were detected in stained organ impressions, most commonly in kidney where 1.4 parasites/cm^2 were observed. The trypanosomes exhibit pleomorphism and the morphological analyses indicated there were four morphospecies separeted according to their morphological variations. Morphospecies 1 presents long and broad body, with conspicuous undulated membrane, and free flagellum. Morphospecies 2 presents long and slender body with pointed extremities, conspicuous undulated membrane, and free flagellum. Some individuals presented numerous vacuoles along the body extension. Morphospecies 3 presents long and striate body with more variable posterior end shape, often tending to be wider than related long forms, conspicuous undulated membrane, and free flagellum. Morphospecies 4 with short and slender body, conspicuous undulated membrane, and free flagellum. Epimastigotes and trypomastigotes were detected in the crop and gut of leeches. These parasites are shorter than blood tripomastigotes and presented elongated and slender body, conspicuous undulated membrane, and free flagellum. Another studies will be conducted for complementary characterization of these trypanosomes.
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BC104 - Purification of *L. (L.) amazonensis* infected macrophage phagolysosomes and the arginine transport

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Leishmania (L.) amazonensis at the mammal host infects macrophages escaping from the defense system and surviving in that hostile environment. To generate a system to study the defense mechanisms, we standardized a protocol to purify the phagolysosome, an organelle formed when the phagosome, containing the parasite, fuses with a lysosome. After infection of J774 murine macrophages with *L. (L.) amazonensis* mutant transfected with pX-GFP*,* we did controlled lyses and used flow cytometry with sorting to isolate the organelles containing green *L. (L.) amazonensis.* After fractionation, we evaluated the purity of organelles by flow cytometry in conjunction with confocal laser microscopy. The organelle membrane integrity was also confirmed by acid pH labeling with Neutral Red and the metabolic viability was confirmed using the MTT cytotoxicity test. The data show that we isolated viable organelles in a sufficient number to allow studies such as amino acid transport. To study arginine transport system in the phagolysosome, we started by the characterization of the transporter coding gene in *L.(L.) amazonensis.* Using *L. (L.) donovani* arginine transporter sequence, we built a probe and screened a cosmid library of *L. (L.) amazonensis*. One cosmid was selected and its DNA was sub-cloned into pGEM plasmid and the positive clone was used for sequencing. The obtained sequence presents 91% of identity and 95% of similarity with the *L. (L.) donovani* transporter. We also performed a reverse transcription from total RNA of *L. (L.) amazonensis* to map the 5' UTR and 3' UTR. We obtained two different sequences for 5'UTR, confirmed by two transcripts in Northern blot. The analysis of genomic sequence data confirms the presence of two copies in a genomic cluster. The next step is to see if the transporter present in the phagolysosome is from the host or from the parasite.
Supported by FAPESP and CNPq.

BC105 - *Toxoplasma gondii* negatively modules the cadherin expression in skeletal muscle cells inhibiting the in vitro myogenesis

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The apicomplexans, including *Toxoplasma gondii,* are a large and diverse group of parasitic protozoa that essentially lead obligate intracellular lifestyles. Mouse primary culture of skeletal muscle cells (SkMC) was employed as a model for experimental toxoplasmosis studies. In this work, it was analyzed the infective capacity of *T. gondii* tachyzoites in myoblasts and myotubes. Our results showed that after 24 h of interaction, myoblasts (61%) were more susceptible to *T. gondii* infection than myotubes (38%), suggesting differences in the ability of tachyzoites to invade these two cell types in the culture during the SkMC differentiation. The second aim was to verify the influence of *T. gondii* infection on SkMC myogenesis. We observed that even with a relation of only 1:1 (parasite-cell host) after 24 h of interaction, the infection was of only 43% leading to inhibition of 75% on the myogenesis process. The modulation of cadherin expression during *T. gondii*-SkMC interaction was investigated. Initialy, we demonstrated the cadherin localized at the contact areas between myoblasts and myotubes during the myogenesis process by confocal microscopy. SkMC infected with *T. gondii* analyzed by immunofluorescent and immunoblotting assays after 3, 12, 24 and 48 h of interaction showed that after 24 and 48 occured a reduction of 39% and 55% respectively in expression of cadherin protein, leading the inhibition of the cell membrane fusion process. By PCR assays we analyzed the regulation of M-cadherin (M for muscle) by mRNA levels in SkMC in the presence and absence of infection by *T. gondii.* Our data demonstrated reduction the M-cadherin mRNA expression after 3h of interaction being higher after 24 h. These data corrobore the suggestion of that the *T. gondii* is able negatively to modulate the cadherin expression, interfering molecularly with the surface of host cell, inhibiting the membranes fusion and consequently affect the myogenesis process.
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BC106 - IDENTIFYING MICROBIAL DIVERSITY IN THE BOVINE RUMEN: LINKING SEQUENCE WITH MORPHOLOGY

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Microorganisms within the rumen include bacteria, fungi, archaea, and protists. Of the rumen protists, the ciliates are the most abundant and well characterized. Rumen ciliates were initially described using microscopic examination with traditional taxonomic approaches, yet culture-independent approaches are more sensitive methods to identify and quantify microbial diversity. To characterize more thoroughly the diversity of this system, classical morphological features of rumen protists need to be linked to environmental sequence data. For this study, eukaryote or ciliate-specific fluorescent oligonucleotide ssu 18s rDNA probes were used in whole cell, rRNA-targeted, fluorescent in situ hybridization (whole cell FISH) in combination with anti-cytoskeletal immunostaining. This permitted us to link ciliate morphology with ssu rRNA sequence in freshly fixed rumen fluid. This work revealed a cytoplasmic distribution of ssu 18s rDNA probes in both non-ciliated eukaryotes and ciliates, and a variety of previously uncharacterized eukaryotes. Cytoskeletal immunostaining illustrated intact cilia, flagella, and internal microtubules. This is the first time FISH and immunostaining were used in succession to characterize the eukaryotes in the rumen or any uncultivated sample. Future use of this powerful technique permits us to both identify and characterize uncultivated protists and couple classical microscopic morphological imaging with sequence-based identification in the rumen.
Supported by NIH

BC107 - *Trypanosoma cruzi* alters connexin43 mRNA in mouse cardiac myocytes

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Cardiac remodeling (CR) is characterized by changes in myocardial structures in response to mechanical overload or cardiac injury. CR can be adaptive or can result from phenotypic modifications such as fibrosis, during clinical conditions that can alter myocardial gene expression. Chagas' disease, caused by the protozoan *Trypanosoma cruz*i, is endemic in Latin America affecting 16-18 million people while 90 million are at risk of infection. This disease causes the chagasic cardiomyopathy, a progressive disease where patients present myocarditis, fibrosis and myocardial hypertrophy. The correct expression of gap junction proteins (connexins) in the heart is required for proper function of the tissue, since the channels formed by these proteins directly connect neighboring cells, allowing intercellular transmission of current carrying ions and signaling second messenger molecules. We previously demonstrated that infection of cardiac myocytes with the Y strain of *Trypanosoma cruzi* altered connexin43 levels both at 1 and 72 hours post infection (hpi). When mice were infected with this parasite, Cx43 protein levels were decreased at the acute phase (11dpi). In the present study we evaluated whether this infection affects cardiac Cx43 mRNA. For this purpose, cultured cardiac myocytes were infected with *T. cruzi* and total RNA was harvested at 1 and 72(hpi). Samples were subjected to RT-PCR analyses and observed that there was no significant alterations in Cx43 mRNA at 1hpi. However there was a significant decrease (21%, ANOVA) in Cx43 bands when normalized to control (18S) bands. These observations indicate that the increase in Cx43 protein expression at 1hpi is not related to mRNA changes, but could rather be associated to changes in protein degradation through lysosomes. Also, the decrease in Cx43 mRNA at 72hpi is consistent with the 61% decrease in the levels of this protein during the infection and could be directly correlate. These observations indicate that the infection deeply alters Cx43 expression in cultured CMs, which may contribute to the pathogenesis of the dysrhythmias that accompanies *T. cruzi* infection. Supported by: CNPq, PAPES-FIOCRUZ and Fogarty Training Grant.

BC108 - Are determinants of cell entry and virulence synonymous for American trypanosomes?

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Not all of those which have contracted the protozoan parasite *Trypanosoma cruzi* will go on to manifest the symptoms of Chagas disease. Although there is little evidence for spontaneous cure of what is generally held to be a life-long infection, there is a profound heterogeneity in the pathologies arising. This stems in part from the genotype of the parasite, and in part from the genotype and immune status of the infected host. A close cousin of *T. cruzi*, *Trypanosoma rangeli* is also infectious, causing life-long infections in humans, but it is completely apathogenic. Our investigations of *T. cruzi* surface determinants using beads and *T. rangeli* as vehicles have demonstrated the critical importance of trans-sialidase (TS) and trans-sialidase like (TSL) proteins in host cell entry. We have shown that while both active and enzymically inactive TS proteins induce actin-dependent, caveolin-mediated endocytosis, enhanced uptake requiring G-protein mediated signalling is critically dependent of transsialidase activity. Our complementary use of heterologous expression for surface determinants including TS, TSLs and cruzipain in *T. rangeli* has led to the elucidation of the requirements for *T. cruzi* uptake and targeting to the autophagous and lysosomal compartments of the host cell which in turn dictate the parasites potential for survival and propogation in epithelial cells with important translational implications.

BC109 - IS *PRODISCOCEPHALUS* A HYPOTRICH CILIATE? A PHYLOGENETIC ANALYSIS BASED ON MORPHOGENETIC AND 18S rRNA GENE SEQUENCE DATA (CILIOPHORA: SPIROTRICHEA)

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The *Prodiscocephalus*-like ciliates, or discocephalines, are traditionally considered to be euplotid hypotrichs but whose precise systematic position has long been uncertain, mainly because of the paucity of morphogenetic data, with only two discocephalines having been investigated, and a complete absence of molecular data. In the present study the cortical development of *Prodiscocephalus borrori* was observed during binary division. Five features were observed that are characteristic of stichotrichs: (1) the oral primordium in the opisthe occurs *de novo* on the cell surface; (2) the undulating membrane in the proter is derived from the parental structure; (3) there are >5 FTV-cirral anlagen; (4) the two marginal rows form intrakinetally; (5) the dorsal anlagen are formed in two groups. By contrast, only two features are typical of euplotid hypotrichs, i.e. (1) several caudal cirri are formed from the rightmost DK-anlagen; (2) the FVT-cirral anlagen are formed in the primary mode, indicating that the discocephalines are more closely related to the stichotrichs than to the euplotids. Based on a combination of morphological and morphogenetic data, a phylogenetic tree was constructed which also suggests that the discocephalines group within the stichotrichs and separate from the euplotids. In addition, the complete small subunit rRNA (SS rRNA) gene of *P. borrori* was sequenced and analyzed. In the resulting SS rRNA tree, *P. borrori* is sister to the Stichotrichia-Oligotrichia-Choreotrichia assemblage, albeit with low bootstrap support, and separate from the euplotids. These data suggest that the discocephalines should be considered as a distinct group, at the rank of order or even subclass, within the class Spirotrichea Bütschli, 1889.
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