[October, 2008-10-27 – 16h20 - REAL ROOM]

SPC - 100 years of *Toxoplasma gondii*: contribution of the Brazilian science to a better knowledge of its structure and interaction with host cells.

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Scientific activity in Brazil has given important contributions to the present day knowledge on basic aspects of the biology, cultivation and structural organization of the pathogenic protozoan *Toxoplasma gondii* and its interaction with host cells. Certainly the very first important contribution was the initial description of the protozoan by Alfonso Splendore, published in Italian in the Revista da Sociedade Científica de São Paulo (The Journal of the São Paulo Scientific Society) in 1908. He carefully examined rabbits with symptoms of what he considered a new disease. Histopathological examinations led to the description of an intracellular and extracellular *corpuscoli* measuring about 5-8 X 2,5-4 μm, which corresponded to what we now know as tachyzoites; cystic forms with diameters varying from 8 to 40 μm were also reported. In the same year the same microorganism was described by Nicolle and Manceaux in a rodent captured in Tunis. A second important contribution was made by Guimarães and Meyer, in 1942 that established and described in detail a method to grow *T. gondii* in tissue cultures using a classical technique where small fragments of chick embryo tissues were kept on a drop of plasma supplemented with embryo extract. Also important were the first observations of the inner structure of the protozoan made by Meyer and co-workers in 1956 by transmission electron microscopy, when several cytoplasmic structures localized in its anterior portion, which correspond to the rhoptries and micronemes, were found. Subsequently, transmission electron microscopy of negatively stained cells was used to analyze the cytoskeleton of *T. gondii*, resulting in the initial description of the three-dimensional array of the sub-pellicular microtubules, the organization of the conoid and the suggestion that this structure, made of rings and microtubules, could change its shape and length. Later, in the 1980's, by using the freeze-fracture technique, specializations of the inner membrane complex that revealed its relation with the sub-pellicular microtubules, were described. More recently, several Brazilian groups have analyzed in detail basic aspects of the early interaction of the protozoan with the host cell, such as the involvement of protein phosphorylation during invasion and egress, transfer of host cell surface components to the protozoan surface, genesis of the parasitophorous vacuole and the organization of a complex tubular network inside it, especially visible in its three-dimensional organization by field emission scanning electron microscopy. The use of different cell types to host infection has shown that trophoblast cells are very susceptible to *T. gondii,* even in the presence of interferon-gamma. Another evasion strategy of *T. gondii* was described when active invasion of activated macrophages by tachyzoites inhibited nitric oxide production due to the disappearance of inducible nitric oxide synthase. In this process, a TGF-beta response triggered by the exposure of phosphatidylserine by the tachyzoites was described. Recently, some aspects of the sexual cycle of *T. gondii* have been reproduced *in vitro* using primary cultures of cat enterocytes. The study of egress of the protozoan from the cell has also received attention. We believe that the contribution of Brazilian science to knowledge on *T. gondii* biology is not over and will continue to flourish in years to come.

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CC - STUDIES ON GENE EXPRESSION REGULATION IN *LEISHMANIA* PARASITES

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The trypanosomatids genes are organized in long polycistronic arrays and monocistronic mRNAs are created by a process known as *trans*-splicing. The genetic organization of these ancient eukaryotes imposes diverse mechanisms to modulate gene expression as compared to other eukaryotes. We are interested in understanding some of these mechanisms in the human protozoan parasite *Leishmania.* We worked on the comparative analysis of *Leishmania major*, *Leishmania infantum* and *Leishmania braziliensis* genomes. This comparison revealed some interesting features of the genomes such as the presence of intact transposable elements and a putative RNAi machinery exclusively in *L. braziliensis*. In addition, the conservation of coding sequences contrasts with divergent intergenic regions, which turn into a fertile ground for the identification of regulatory elements. We used comparative genomics to identify such putative regulatory sequences. A pipeline was developed to identify *in silico* conserved elements present in noncoding regions of the three *Leishmania* species and more than 70 conserved elements were found. We identified some proteins which interact with one of these conserved elements and we are currently investigating their role. Other routes used by the parasite to control gene expression are also under investigation; one of them is focused on noncoding RNAs (ncRNA). The ectopic expression of a putative ncRNA, the *ODD3* gene, impairs growth rate of promastigotes in culture and drastically alters the cell morphology. A putative target to *ODD3* has been identified *in silico* and its transcript level is affected by *ODD3*. The co-transfection of this target into *L. major ODD3* transfectants led to partial reversion of the *ODD3* phenotype. The phenomenon is under investigation to confirm interactions and to evaluate the role of *ODD3* primary and secondary structure on the phenotype of transfectants. Other studies on the control of gene expression mechanisms of *Leishmania* include the investigation of the 5' and 3' untranslated regions (UTR) of glycosomal phosphoglycerate kinase (PGKC) in the control of the expression of this enzyme. We observed that both upstream and downstream sequences are involved in the maintenance of a low copy number of the plasmid and low levels of episomal transcripts. Both PGKC UTRs are also needed to confer high stability to its mRNA. By investigating different pathways and putative regulatory elements we intend to contribute for a better understanding of the regulation of gene expression in *Leishmania*.

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