MINI-CONFERENCES

[November, 2007-11-05 - 17h20 - ROOM A]

MC01 - Biosafety and Obscurantism

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Brazilian authorities are presently attempting to consolidate a set of norms that regulate the construction, cultivation, production, manipulation, transport, transfer, import, export, storage, research, commercialization, consumption, environmental release and disposal of genetic modified organisms (GMO) and their products. The implementation of these guidelines must follow the advances in the areas of biosafety and biotechnology aiming to protect the environment and human and animal health, in all cases obeying the precautionary principle for environmental protection (Art. 1^o, Biosafety Law 11,105/2005).

GMO's are popularly known as transgenic organisms since in the majority of cases the gene of a given organism is inserted into the genome of another organism that acquires a specific advantage.

Brazil abides to the rules of the Biodiversity Convention and the Cartagena Protocol, which adopt the precautionary principle, a novel concept that appeared in the legal systems of some European countries in reaction to environmental disasters due to the use of science - derived technologies. The precautionary principle is subject to many interpretations since its phraseology is not precise and used frequently in frank opposition to the foundations of scientific thought. The most commonly used definition reads: 'Whenever there is a threat to biodiversity, lack of full scientific certainty should not postpone measures to prevent the threat'. This is often interpreted as no new technology ought to be developed and no new productive activity should take place unless unequivocal scientific evidence proves that no harm to health or environment will result. So, the lack of scientific certainty is related to the threat. It does not matter whether the majority of scientists do not think there is a threat. It is sufficient that a minority thinks there is a threat for judges to argue that there is no scientific certainty and, thus, transgenic crops should be prohibited. That, in a nutshell, is the situation that the Brazilian biotechnology sector finds itself in today: agronomy, ecology and molecular biology have been substituted by judges and the courts. The precautionary principle is a Trojan horse for the introduction of obscurantism because it is anti-scientific. It requires absolute certainty, which can never be ascertained by science.

The campaigns against the use of GMO's - mainly in Europe and exported to Brazil - led by organizations of appreciable economic power have bred in the average citizen a diffuse fear of transgenic technologies that is difficult to counteract. It is difficult to employ reasoning, which requires at least some general scientific knowledge on, for instance, DNA chemistry or protein digestion, not accessible to the average citizen, to counter superficial and irresponsible statements targeted to incite strong emotional reactions, such as fear, in the general public. It is interesting to notice, however, that the average citizen is not concerned whether the cheese they eat is prepared with transgenic chymosin nor whether insulin, growth hormone, blood coagulation factors or vaccines against hepatitis B, HPV, dengue or avian flu, are produced or not by GMO's. Campaigns and fears are centered exclusively on food and feed from large plantations - soya, corn, rice, tomato, potato, beans - and cotton.

The rejection of biotechnology has no scientific or rational basis, and Brazilians are not taking full advantage of a potentially powerful tool for participating in future world-wide agricultural developments and advances. Part of the rejection is due to the fear that few international corporations may hold the power to control crop markets. This is foolish because it does not take into account the dynamics of market competition or the possibility of autochthonous development.

Non-governmental organizations, some financed from abroad, are responsible for the anti-transgenic mood presently at large. How would they, or the public they try to influence, react when Brazilian private or state-owned companies ask to commercialize transgenic sugar-cane or eucalyptus for the production of ethanol or pulp for paper production, respectively, with enormous potential economic benefits?

Walter Colli
President of the Technical National Committee of Biosafety (CTNBio)

[November, 2007-11-06 - 11h00 - ROOM B]

MC03 - Mammalian cell invasion by *Trypanosoma cruzi* infective forms

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Trypanosoma cruzi occurs as different strains or isolates that may be grouped in two major phylogenetic lineages: *T. cruzi* I associated to the sylvatic cycle and *T. cruzi* II, linked to the human disease. In the mammalian host the parasite has to invade cells and many studies implicated the flagellated trypomastigotes in this process. Several parasite surface components and few host cell receptors with which they interact have been identified. Our work mainly focused on how amastigotes, usually found growing in the cytoplasm, can invade mammalian cells with infectivities comparable to that of trypomastigotes. We found differences in cellular responses induced by amastigotes and trypomastigotes regarding cytoskeletal components and actin-rich projections. Extracellularly generated amastigotes (EA) of *T. cruzi* I strains may display greater infectivity than metacyclic

trypomastigotes (MT) towards cultured cell lines as well as target cells that have modified expression of different classes of cellular components. We also observed that EA of *T. cruzi* I strains were more infective towards cultured cells than parasites of *T. cruzi* II group, a behavior opposite to that of the classic trypomastigote forms. We then hypothesized that the expression of the carbohydrate epitope defined by Mab 1D9 on Ssp-4, a major amastigote surface antigen, was related to the greater infectivity of *T. cruzi* I amastigotes, particularly of the G strain. By examining *T. cruzi* II isolates from chagasic patients with distinct pathologies, we then verified that, although carbohydrate epitopes defined by Mabs 1D9 and 2B7 were involved in EA invasion, their abundance on the surface of the parasite was not directly related to their infectivity. In the search of additional parasite components that might be related to cell invasion, a DNA microarray approach has been undertaken by screening components that, being more abundant in G strain amastigotes, in comparison to the less infective CL strain parasites, could be associated to this phenotype. A hypothetical protein of 21 kDa with a signal peptide was selected and cloned. P21 is secreted by amastigotes and is ubiquitous among *T. cruzi* strains and developmental stages. Although the recombinant protein (His_6-P21) binds in a dose-dependent manner to cells, it does not trigger Ca^{2+} transients. Both His_6-P21 and an anti- His_6-P21 Mab inhibit cell invasion by G strain parasites [C.V. Silva et al., in preparation]. We are currently examining the potential signaling pathways that we envisaged are triggered by secreted P21 at the local micro-environment after attachment of the amastigote forms to the host cell.
In another approach aiming at understanding the role of cholesterol and membrane rafts in mammalian cell invasion by *T. cruzi* infective forms, we used the following tools: the cholesterol depleting compound methyl-β-cyclodextrin, filipin a cholesterol-binding antibiotic and cholera toxin subunit B (CTX-B) that binds to ganglioside GM1, a sphingolipid enriched in membrane rafts. Depletion of host cell cholesterol decreased both MT and EA invasion (of either G or CL strain), that were restored by replacing cholesterol. MT and to a lesser extent EA ergosterol removal also reduced their infectivity. Cholesterol recruitment to the sites of EA and MT invasion was disclosed by filipin labeling recently-infected cells. Moreover, CTX-B not only inhibited cell invasion by both infective forms but was also found in parasitophorous vacuoles (PV) of recently-internalized parasites, thus unveiling that both cholesterol and membrane rafts participate in mammalian cell invasion by *T. cruzi* .
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[November, 2007-11-06 - 11h30 - ROOM A]

MC04 - INTRA-SPECIES POLYMORPHISMS OF LIPOPHOSPHOGLYCAN STRUCTURE AND GEOGRAPHICAL DISTRIBUTION OF LEISHMANIA MAJOR

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Leishmania parasites utilize distinct strategies to survive and develop in the sand fly midgut, many involving the major parasite surface adhesin lipophosphoglycan (LPG). Modifications of the basic LPG phosphoglycan (repeating Gal-Man-P units; n = 15-30) backbone by side chain sugars play important roles in parasite survival in the sand fly and in vector competency. For example, in L. major (Friedlin V1 strain), the side chains were previously shown to consist primarily of one or two beta 1,3-galactose residues, which serve as binding ligands for Phlebotomus papatasi midgut galectins and survival. While inter-species variations in LPG side chain sugar substitutions have been extensively investigated, the significance of intra-species variations of side chain galactosylation of LPGs from L. major has only been minimally defined. L. major isolates from distinct geographical origins were extracted, purified, and analyzed to determine the extent and importance of beta1,3-Gal side chain substitution. The analyses showed that LPG repeat units derived from sub-Saharan Africa isolates are predominately unsubstituted, whereas those from north Africa and the Middle East are mainly mono- and di-galactosylated. Two isolates from Iran and Russia were found to have poly-galactosylated LPG repeat units. Varying the spectrum of side chain sugars may play an important role in the intra-species specificity of vectorial competence and transmission that is observed in nature.

[November, 2007-11-06 - 11h30 - ROOM B]

MC05 - The crucial role of glycosome turnover during trypanosome differentiation.

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In trypanosomatids, most glycolytic enzymes are sequestered in peroxisomes designated glycosomes. The function of glycosomes and the selective advantage that led to the compartmentation of glycolysis have been a matter of discussion for many years. We hypothesized that this metabolic compartmentation might be important for increasing the adaptability of the parasite to differentiation-related changes of environmental conditions. Whereas glycolytic enzymes constitute nearly the entire glycosomal protein content in bloodstream-form Trypanosoma brucei, they are quantitatively less important in procyclic insect-stage trypanosomes where the organelles also contain many other enzymatic systems. Also

in yeasts, the enzymatic content of peroxisomes may vary considerably and the biogenesis and degradation of these organelles are regulated according to the nutritional conditions. The peroxisome population is heterogenous; old peroxisomes, no longer appropriate for altered conditions, are degraded by an autophagy-like process, called 'pexophagy", while peroxisomes with a different metabolic repertoire are synthesized, enabling the organism to cope efficiently with the changed environment. To test if similar, regulated processes occur in trypanosomes, we followed the turnover of glycosomes and glycosomal enzymes during the differentiation of a pleiomorphic T. brucei strain. To this end, an experimental model system was developed in which trypanosomes were allowed to differentiate efficiently from the long-slender (LS) to the short-stumpy (SS) bloodstream-form, and subsequently from the SS to the procyclic (PC) insect form. By immunofluorescence microscopy it was shown that, during the LS-SS transition, glycosomes had a small but significant tendency to co-localize with the lysosome, indicative of glycosome degradation by pexophagy. Within minutes after triggering the differentiation of SS to PC trypanosomes, a further large increase in lysosomal volume and colocalisation was observed, lasting for about 1 h before decreasing again. These results have been corroborated by electron microscopy. Moreover, the occurrence of pexophagy was confirmed using this latter method by showing that glycosomes became surrounded by the lysosomal membrane and were engulfed, and that the lysosomal volume could be labelled with an antiserum against the glycosomal matrix enzyme aldolase. Furthermore, by quantitative PCR and Western blotting the alterations in expression of various enzymes were followed during differentiation. The transcript levels were normalized against the mRNA levels of actin, the protein levels against that of the life-cycle regulator TbZFP3; both actin and ZFP3 are known to be constitutively expressed. At the transcript level, only small variations were found for the glycosomal enzymes ALD and pyruvate-phosphate dikinase (PPDK), the mitochondrial acetate:succinate-CoA transferase, the cytosolic phosphoglycerate kinase (PGK-B) and pyruvate kinase (PYK) and the autophagy protein ATG8. In contrast, at the protein level it was shown that PPDK remained constant during the LS-SS transition and increased during the SS-PC differentiation; the glycolytic enzymes ALD and PYK gradually decreased during both the LS-SS and SS-PC transition, and ASCT increased during the first 15 min of the SS-PC transition. Together, these data show that no major changes occurred in the transcript levels of all these metabolic enzymes, but that considerable degradation of protein and new synthesis took place, and that the turnover of glycosomal enzymes involved pexophagy of the organelles. In parallel, a search in the genome databases of T. brucei, T. cruzi and Leishmania major against 40 yeast and mammalian autophagy-related genes confirmed the presence of orthologues of known autophagy-related genes (ATG), suggesting a functional but highly streamlined version of the process in these parasites. Moreover, sufficient molecular machinery was predicted to confirm that glycosome turnover can occur by a process homologous to pexophagy in yeasts.

[November, 2007-11-06 - 11h30 - ROOM C]

MC06 - The assembly of iron-sulfur clusters in *Trypanosoma brucei*

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Trypanosoma brucei, the causative agent of human sleeping sickness, is characterized by a number of unique cellular features. Since methods of reverse genetics are available for this flagellate, it can now be considered a model protist. Iron-sulfur (Fe-S) clusters are ancient and ubiquitous cofactors of proteins that are involved in a variety of biological functions, including enzyme catalysis, electron transport and gene expression. Nevertheless, little is known about how Fe-S clusters are assembled in *T. brucei*. So far, by means of RNA interference, we have down-regulated several evolutionary highly conserved components of the pathway, such as cysteine desulfurase IscS, metallochaperone IscU, frataxin, ferredoxin, and IscA. With the exception of IscA, all are essential for the parasite and their down-regulation results in reduced activity of the marker Fe-S enzyme aconitase in both the mitochondrion and cytosol. Moreover, interfering with these genes also decreased the activity of succinate dehydrogenase and fumarase, affected membrane potential of the mitochondrion and general oxygen consumption. This supports the hypothesis that the mitochondrion plays a fundamental and evolutionary conserved role in cellular Fe-S cluster assembly throughout the eukaryotes. Interestingly, we have rescued the frataxin know-down in *T. brucei* with its homologue from the hydrogenosome of *Trichomonas vaginalis* containing the hydrogenosome-targeting signal peptide. Further analyses of this rescue and the various RNAi knockdowns are under way.

[November, 2007-11-07 - 11h00 - ROOM A]

MC07 - Post-transcriptional regulation of stage-specific mRNAs in *Trypanosoma brucei*: a new function for an ancient protein superfamily?

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Kinetoplastids rely heavily on post-transcriptional mechanisms in order to regulate gene expression. This is particularly well documented for *Trypanosoma brucei*, which uses

elements in the 3‘ untranslated regions of mRNAs to regulate the expression of surface glycoproteins during its life cycle. Approximately 200 trypanosome genes are annotated as encoding putative RNA-binding proteins. To date, however, there is not a single example of an RNA-binding protein that regulates expression of specific mRNAs in *T. brucei*. When trypanosomes are taken up during a blood meal by the tsetse fly, they rapidly synthesise a new coat consisting of two classes of surface proteins known as EP and GPEET procyclins. This mixed coat persists for a few days, after which GPEET is repressed and EP1 and EP3 become the only detectable procyclins covering parasites in the tsetse fly midgut. We have previously shown that the 3‘ untranslated region of GPEET mRNA contains an element of 25 nucleotides that acts as a sensor of metabolites such as glycerol and glucose in the trypanosome‘s exterior milieu and is also responsible for developmental regulation in the tsetse fly. This element forms part of a central stem-loop structure (the LII domain) and has been shown to destabilise GPEET mRNA in late procyclic forms. Proteins in cytoplasmic extracts form three complexes with synthetic GPEET LII, but not with the closely related sequence from EP1 mRNA, providing an assay for specific binding activity. When cytoplasmic extracts were subjected to three sequential purification steps - heparin chromatography, monoQ chromatography and gel filtration - active fractions enriched for GPEET RNA-binding proteins were obtained. Following this procedure, N-terminal peptide sequences were obtained for one protein. Although not annotated as an RNA-binding protein, it is one of four proteins in *T. brucei* that contain a putative nucleic acid binding region known as an Alba domain. Based on this feature the protein was named TbAlba1. Members of the Alba superfamily have been shown to be associated with chromosomal DNA in Archaea and are components of RNAse P in eukaryotes. Knockdown of TbAlba1 by RNA interference caused the disappearance of one of the three RNA-binding complexes and a 90% reduction in steady state levels of GPEET mRNA. Purification of complexes containing N-terminally tagged TbAlba1 led to the identification of two other Alba proteins. The fourth Alba protein was identified in the enriched fractions, but has not (yet) been shown to interact with TbAlba1. RNA interference experiments are currently underway in order to determine whether these proteins also affect the stability or translation of GPEET mRNA. Proteins containing Alba domains are also encoded in the genomes of *T. cruzi* and *Leishmania*. Taken together, these findings suggest a new function for members of the Alba superfamily and open up avenues of research that have implications for gene regulation in many other organisms.

[November, 2007-11-07 - 11h00 - ROOM B]

MC08 - DNA recombination pathways, and their influence by mismatch repair, in *Trypanosoma brucei*

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DNA recombination functions universally in the maintenance of genome stability. The most prominent recombination pathways are homologous recombination and non-homologous end joining, which can perform specific functions in some organisms. One example of this is antigenic variation, a strategy for host immune evasion. In *Trypanosoma brucei*, antigenic variation occurs by periodic changes in its Variant Surface Glycoprotein (VSG) coat through the movement of *VSG* genes from an enormous silent archive into specialised expression sites. Genetic evidence indicates that this involves homologous recombination, though it is characterised by an unusually high rate of switching and by atypical substrate requirements. In addition, mutation of a number of enzymes of homologous recombination does not prevent *VSG switching*, merely impairs the process, indicating that other recombination pathways can act. We have used a number of approaches to characterise the recombination pathways used by *T. brucei*. We find that homologous recombination efficiency in *T. brucei* is strictly dependent on substrate length and is impeded by base mismatches, features shared by homologous recombination in all organisms characterised. Mismatch repair plays a crucial role in determining the efficiency of homologous recombination on relatively long substrates (150 bp and greater), but has much less influence on shorter substrates (25-150 bp). From this, we infer that 2 potentially distinct pathways of homologous recombination act in *T. brucei*. We have also searched for non-homologous end-joining in *T. brucei* both *in vivo* and *in vitro*, but have been unable to detect this form of recombination. Instead, a potentially prominent repair pathway that relies upon very short stretches of imperfect sequence homology (5-15 bp) acts. This reaction, microhomology mediated end-joining, represents a third pathway of *T. brucei* recombination.

[November, 2007-11-07 - 11h30 - ROOM A]

MC09 - Current themes in cytoplasmic gene expression control: Implications for protozoa

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In the cytoplasm, gene expression is largely regulated at the level of translation and RNA stability. I will review the current models of the translation initiation step, whereby the 5‘ m_7GpppG cap recruits the small ribosomal subunit to the mRNA to scan the 5‘ untranslated region, which is the point at which regulation is mostly exerted, and of the RNA decay pathway; as elucidated in model systems ranging from yeast to human cells in culture. Both pathways target the extremities of mRNA - the cap and the poly(A)-tail, and their binding factors. Interestingly, both are believed to occur in distinct cytoplasmic foci, called P(-rocessing) bodies, which contain several conserved RNA-binding proteins, the

cap-binding protein eIF4E, and RNA decay enzymes. Recent work also indicates that in human cells, microRNA-mediated down regulated of gene expression, in which the short 21 nt non-coding microRNAs anneal imperfectly to the 3‘ UTR (untranslated region) and either repress or degrade the target mRNA, also occurs in P-bodies[1,2]. I will then consider the implications of these findings to protozoa, which have several unusual modes of gene expression including polycistronic transcription, trans-splicing of a common leader region and cap4 formation. *Leishmania* and *Trypanosoma* possess families of translation initiation factors, only some members of which appear to be equivalent to canonical translation factors[3,4], and poly(A)-binding proteins. While they seem to lack microRNAs (as does yeast), *Trypanosoma* do contain some of the RNA-binding proteins associated with miRNP and/or P bodies, and their roles in regulating RNA metabolism in protozoa are currently being investigated.

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[November, 2007-11-07 - 11h30 - ROOM B]

MC10 - THE PENTOSE PHOSPHATE PATHWAY IN *TRYPANOSOMA CRUZI*.

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Trypanosoma cruzi is highly sensitive to oxidative stress caused by reactive oxygen species (ROS). Trypanothione (bis-glutathionyl-spermidine), which is used instead of glutathione to protect the cell against ROS, is kept reduced by trypanothione reductase, which utilizes NADPH. Although the parasite has enzymes like the NADP-linked glutamate dehydrogenase and malic enzyme, the major pathway for NADP reduction seems to be the pentose phosphate pathway (PPP). We have shown that the PPP is functional in *T. cruzi*, and that its seven enzymes are present in the four major stages in the parasite‘s biological cycle (1). We have cloned and expressed in Escherichia coli these enzymes as active proteins. Glucose 6-phosphate dehydrogenase (G6PDH), which controls glucose flux through the pathway by its response to the NADP/NADPH ratio, has an N-terminal extension of 37 residues, absent in the enzyme from mammals, which includes two Cys residues, and is inactivated by reducing agents such as DTT or GSH, as the G6PDHs from chloroplasts and cyanobacteria. The enzyme is induced up to 46-fold by hydrogen peroxide in metacyclics, a parasite form which is exposed to ROS (2). The T. cruzi 6-phosphogluconolactonase gene is present in the CL Brener clone as a single copy per haploid genome; its sequence predicts a PTS-1 C-terminal glycosomal targeting signal. The recombinant enzyme behaves as a monomer, with a molecular mass of 29 kDa. 6-phosphogluconate dehydrogenase is very unstable, but could be stabilized introducing two salt bridges (present in the *T. brucei* enzyme, and considered essential for dimerization) by site-directed mutagenesis (3). The ribose 5-phosphate isomerase belongs to Type B RPI, and genes encoding Type A RPIs, most frequent in eukaryotes, seem to be absent (4). Ribulose 5-phosphate epimerase is encoded by two genes; one of them predicts a PTS-1 glycosomal targeting signal (SHL) at the C terminus. Transaldolase, which is present as a single copy per haploid genome, presents at least three isoforms in epimastigotes. Transketolase is encoded by a single gene. The protein was shown to be a dimer with a MW of 146 kDa and is being characterized at present. All the enzymes of the PPP seem to have a major cytosolic component, although several of them have a secondary glycosomal localization, and also minor localizations in other organelles. Our results suggest that the oxidative branch of the PPP is essential for the protection of the parasite against oxidative stress. In addition, one of the enzymes of the non-oxidative branch is of prokaryotic type, and has no counterpart in the higher eukaryotic genomes sequenced until now. More studies, involving inhibitor kinetics and knock-out experiments, will be required to validate the PPP as a suitable target for the development of new drugs for the treatment of Chagas Disease.

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