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Session VI: Gene Expression

Gene expression profiling in *Trypanosoma brucei* based on microarray studies

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We are interested in the control of *T. brucei* mRNA abundance during differentiation. In the absence of a complete and annotated *T. brucei* genome, we have been working with a microarray comprising 24,567 different random 2kb genomic clones. These have been used to look for developmentally-regulated genes, and for transcripts induced or depleted following cold shock or alterations in the expression of various RNA-binding proteins. Extensive data are available for a comparison between bloodstream and procyclic forms, conducted with 927 and 427 strain trypanosomes, and with 427 strain polysomal RNA. In many cases, the degree of regulation seen with polysomes was higher than that using total RNA. Annotation of 450 clones is in progress. They include multiple representatives of known regulated genes: repetitive elements, expression-site-linked or VSG-like sequences, other characterised surface proteins, and metabolic genes. The limitations of the method were apparent - several spots containing segments of known regulated *loci* were detected in only one experiment. Conversely, several novel genes were "hit" more than once, confirming their regulation; overall at least 83% of the spots are probably correctly identified as differentially regulated. This dataset can serve as a basis for searches for regulatory elements.

Functional Genomics of *Trypanosoma cruzi*: from life cycle to transcriptome dynamics

Probst,C.M.¹, Pavoni,D.P.¹, Ávila,A.R.¹, Goes,V.M.¹, Picchi,G.¹, Sotomaior,V.¹, Correa,A.¹, Cardoso,J.¹, Passos,T.¹, Arauco,P.R.C.¹, Dallagiovanna,B.¹, Poersch,C.¹, Ozaki,L.S.², Xu,P.², Maloney,D.², Alves,J.², Wen,L.², Serrano,M.G.², Manque,P.A.², Carvalho,M.R.², Buck,G.A.², Fragoso,S.P.¹, Goldenberg,S.¹ and Krieger,M.A.¹

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Trypanosoma cruzi is the causative agent of Chagas disease and, as other kinetoplastida, has not been extensively studied by modern molecular biology techniques. Microarray analysis is a powerful tool to evaluate changes in mRNA levels in biological processes and to help clarify the control mechanisms of such events. To achieve these goals in the study of *T. cruzi* gene expression dynamics, we have constructed a microarray containing approximately 6,000 dsDNA probes and performed a functional genomics analysis on different biological conditions, as cell differentiation (metacyclogenesis, complete cell cycle), drug resistance, environment condition changes, drug responses and exogenous gene transfer. These studies aid our understanding of gene expression control, post transcriptional regulation and biological complexity. Together with an EST sequencing project done at our institutions, producing more than 6,000 cDNA sequences, these data are helping to clarify *T. cruzi* gene content, functional annotation of its transcripts and their integration in coordinated pathways. A better picture of *T. cruzi* biology is emerging, crucial for therapeutic purposes, but also for elucidating the peculiar biology of this parasite.

mRNA mobilization to polysomes and its role in gene expression regulation during *Trypanosoma cruzi* differentiation

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To improve our comprehension of the gene expression regulation during *T. cruzi* cellular differentiation we have done a systematic analysis of differentially expressed genes using a *T. cruzi* dsDNA microarray containing more than 6,000 probes. In order to evaluate gene specific RNA stability or, alternatively, selective polysomal mobilization, total and polysomal associated RNAs extracted from *T. cruzi* at different times of the differentiation process were used. The data strongly suggest that specific polysomal mobilization has an important role in *T. cruzi* gene expression regulation. Different sets of genes are mobilized to the polysomal fraction during the differentiation of the parasite even if the total mRNAs extracted from the same cell samples are very similar. Northern blot and quantitative RT-PCR, as well as, the identification of differentially expressed proteins by proteomic analysis corroborate the microarray data. The results indicate that selective recruitment of mRNA molecules to the translation machinery is an important mechanism of gene expression regulation during the life-cycle of *T. cruzi*.

The *Trypanosoma cruzi* Proteome

James Atwood¹, Brent Weatherly¹, Todd Minning¹, Cameron Cavola¹,
Becky Bundy¹, Fred Opperdoes², Ron Orlando¹ and **Rick Tarleton**¹
*University of Georgia*¹ and *Christian de Duve Institute of Cellular
Pathology*²

We have undertaken a whole organism, proteomic analysis of all four life cycle stages of *Trypanosoma cruzi*, the causative agent of Chagas disease. Peptides mapping to 2555 proteins in 1135 protein groups were identified, including up to 1097 genes initially annotated as "hypothetical", "putative", "probable" or "possible". Among these hypothetical genes are a newly identified family of related proteins that are predicted to be GPI-anchored and are only detected in the trypomastigote stage of the life cycle. Stage-specific expression of individual members of the trans-sialidase and retrotransposon hot spot (RHS) families of genes is also apparent in the *T. cruzi* proteome. Biochemically the proteome revealed few surprises; mitochondrial enzymes involved in energy metabolism, as well as those important for beta-oxidation of fatty acids are more prominent in amastigotes and metacyclic trypomastigotes than in trypomastigotes. This analysis complements the kinetoplastid genome annotation by confirming the production of a large number of hypothetical genes, many of which have orthologues in *T. brucei* and *Leishmania*, and by identifying additional gene products that were not predicted in the initial annotation of the *T. cruzi* genome.

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Quantitative analysis of global RNA and protein expression in *Leishmania* promastigotes and amastigotes

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Two approaches have been used to quantitate global RNA and protein expression in *Leishmania* promastigotes and amastigotes. DNA oligonucleotide microarrays representing 8160 *L. major* genes were produced using the data from the *L. major* genome database as of March, 2004. The microarrays (in triplicate) were hybridized with labeled probes synthesized from RNA from *L. major* log phase promastigotes or amastigotes isolated from lesions grown in BALB/c animals. Statistical methodology was used to quantitate expression profiles in the two different life stages. Analysis of stage specific expression showed that over 90% of the *L. major* genes were constitutively expressed. Using a 1.5 fold and greater expression ratio, 5% of promastigote genes and 3% lesion amastigote genes exhibited stage specific expression. The stage specific expression patterns will be correlated with functional pathways. To correlate gene expression with protein expression, the isotope-coded affinity tag (ICAT) technology coupled with mass spectrometry was used to quantitate the global expression of proteins in *L. mexicana* and *L. infantum* promastigotes and axenic amastigotes. Over 3700 peptides from *L. mexicana* and *L. infantum* were quantitatively analyzed and proteins were identified using the *L. major* genome database. There was a very high degree of correlation of the protein expression data to the RNA expression profiles. There was a similar level of stage specific protein expression, using a 5 fold and greater expression ratio, 5-6% of peptides derived from promastigotes and 5-8% from amastigotes exhibited stage specific expression. Comparative proteomics will be used to identify species specific expression profiles. The use of the new amino labeling reagents, iTRAQ (ABI), will also be discussed to quantitate the protein expressing profile of *L. infantum* membrane proteins.

Proteomic analyses in *Leishmania*

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The completed *Leishmania* genome is facilitating the use of global approaches to study gene/protein expression in *Leishmania*. Proteomic studies in *Leishmania* have so far concentrated on 2D gels and have been useful to find proteins that are regulated in a stage specific manner or that are implicated in drug resistance. New drug resistance genes such as the methionine adenosyltransferase in antifolate resistance were discovered in this manner. Several proteins were recently identified to be differentially expressed in *L. infantum* amastigote cells resistant to antimony. Nonetheless, several proteins, due either to their low amount or physical properties are not detected in 2D gels. To increase proteome representation we have developed fractionation protocols using i) ammonium sulphate precipitation, ii) size exclusion chromatography, iii) digitonin extraction, iv) and plasma membrane purification. Purified fractions of promastigotes/amastigotes or of sensitive and antimony resistant cells were compared by 2D gels and several proteins differentially expressed were observed and identified and found to be different than proteins characterized while using whole cell lysates. Fractionations have increased the depth of proteome analysis and have allowed the identification of new proteins that were not observed by conventional approaches.

Translational initiation in *Leishmania tarentolae* and *Phytomonas serpens* is strongly influenced by pre-ATG triplet

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The 5' and 3' untranslated regions (UTR) of the mRNAs are expected to influence the efficiency of translation initiation in kinetoplastid flagellates. Extensive database search showed that only a limited number of triplet combinations directly prefacing the ATG (pre-ATG) codon occur in the *Leishmania* spp. mRNAs. Therefore, we sought to examine to what extent the efficiency of mRNA translation is influenced by the pre-ATG triplet. To this end we constructed a library of pJBSegfp1.4sat expression vectors containing a gene coding for Enhanced Green Fluorescent Protein (EGFP) prefaced with randomized pre-ATG nucleotides. Sixty-four variants carrying unique pre-ATG triplets were integrated individually into the SSU rRNA locus of *L. tarentolae*, and resulting stable clones were assessed for levels of EGFP expression. The expression levels were quantified by Western blotting using the anti-EGFP antibody and by directly measuring the fluorescence of EGFP protein in living cells. We reproducibly found a surprisingly strong influence of the pre-ATG codons on the level of protein expression spreading over more than 20-folds. In order to understand the degree of evolutionary conservation of the observed pre-ATG codon effect we transformed *Phytomonas serpens*, a trypanosomatid parasitizing plants, with the above described constructs. The pattern of translation mediated by individual pre-ATGs in this flagellate was very similar to that observed in *L. tarentolae* suggesting a conserved mechanism of translation initiation site selection in kinetoplastid flagellates. The possibilities of comparing the obtained data with the microarray studies will be discussed.