

COMUNICAÇÃO

THE SECOND BURITI WORKSHOP ON TRANSFECTION OF PARASITES

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The development of new technologies for introducing exogenous DNA into parasites has allowed the study of protein function, mechanisms of gene expression and its regulation, drug resistance and designing of new potential chemotherapeutic targets. The use of these modern technologies was the main objective of the Second Buriti Workshop on Transfection of DNA into Parasites, that took place at the Laboratory for Multidisciplinary Research on Chagas Disease, Faculty of Health Sciences of the University of Brasília, from the 1st to 15th of July, 1995. The Workshop included thirteen investigators from various Brazilian and foreign research institutions, and an equal number of students that were selected from the candidate applicants from São Paulo, Minas Gerais, Goiás and Pernambuco, and the Federal District.

The theoretical aspects of the Workshop were introduced by Nancy R. Sturm (Department of Microbiology and Immunology, UCLA School of Medicine), who discussed the different methods of transfection along with requirements for electroporation and the various experimental conditions defined in the Workshop protocols. The speaker also discussed some peculiar biological features of kinetoplastid protozoa such as minixion gene array, polyadenylation and codon usage, and polycistronic sequences and maturation of mRNA.

John M. Kelly (London School of Tropical Medicine) reported on the vector he designed for transfection of *Trypanosoma cruzi* and *Leishmania* sp. This vector is based on the plasmid pBluescript and contains two copies

of the GAPDH gene, and the neomycin phosphotransferase gene as a selected marker. The vector characteristics include episomal replication and transformation of parasites resulting in expression (1% to 10% of total cellular protein) of recombinant proteins. The transfected phenotype can be maintained through the entire *T. cruzi* life cycle in the prolonged absence of drug selection. The pTEX vector has been used to study the functional aspects of cruzipain and trypanothione reductase, putative targets for chemotherapy^{5,7}.

David A. Campbell (Department of Microbiology and Immunology, UCLA School of Medicine) and David M. Engman (Department of Pathology, Northwestern University School of Medicine, Chicago) discussed several methods for analysing transfected cells using DNA, RNA and protein analysis. After studying several molecular biology methodologies, the students could see the importance of the particular biological features of these organisms. For example, trypanosomatid protein coding genes are expressed as operons with 5' capping, trans-splicing mechanism, and 3' polyadenylation. The discussion led to the methods of adding an epitope tag at the carboxyl-terminal end of the recombinant protein and determining its subcellular localization.

Elbio Rech (Cenargen, Brasília, BR) reported on the biolistic principles of transfection, exemplified by the "gene gun". The microbombardment process uses helium gas at high pressure and DNA-coated particles. The particles coated with DNA reach 1500km/h and reach all parts of the cell, including the organelles. This procedure is very useful in plant cells since their cell walls do not permit the entrance of exogenous DNA by other method of transfection (e.g., lipofection or precipitation with calcium phosphate). The potential for immunization using the gene gun is demonstrated by the successful vaccination against foot and mouth disease in cattle⁷.

Angela Cruz (Faculdade de Medicina de Ribeirão Preto, SP) spoke about gene targeting

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and genome analysis. Using electroporation techniques she obtained integration and replacement of the DHFR-TS gene of *Leishmania major*. Success was obtained using a non-virulent cell line but, when she attempted double knock out of the genes of a virulent cell line, the parasite became either tetraploid or aneuploid to avoid loss of the gene. Among the transfectants, she saw homologous recombination, random integrations and multiple recombination. With the aid of a cosmid library thousands of recombinants were analyzed by an improved methodology^{1,2,6}.

Lucille Floeter-Winter (Departamento de Parasitologia, ICB, USP) spoke on the use of transfection to study gene promoters. The analysis of transient transfection of *T. cruzi* and *Leishmania* sp. with different constructs containing sequential deletions upstream from the RNA pol I gene, she identified sequences containing promoters of gene expression. The promoter regions are very specific, since they did not function for *Chritidia fasciculata* and in some strains of *T. cruzi*.

Bianca Zingales (Instituto de Química, USP) showed that sera from Chagas heart disease patients cross-reacted with the recombinant B13 protein from *T. cruzi* and with the myosin from cardiac muscle cells. Of interest, these proteins share an epitope of 6 or 7 aminoacids. Further characterization of this epitope is being undertaken.

Experimental work. The second Buriti Workshop concentrated on how to design a successful transfection experiment using electroporation techniques. Seminars and practical exercises demonstrated the steps required for electroporation, such as testing the parameters of the electroporation machine, knowledge of the cell types used in the experiments (drug resistance, growth curve, etc.), and confirmation of results using negative and positive controls.

Characterization of the transfected cells was undertaken using genomic DNA digestion and hybridization by DNA dot and southern blot to demonstrate the presence of the transfected gene of interest and also, the nature of the transfected DNA (episomal or integrated) and an estimative of copy number present in the cell. RNA analysis by northern blot detected mRNA with specific probe thus showing that the exogenous gene was transcribed. The experiments showed how expression of the

recombinant protein was regulated. Further, protein analysis of reporter genes showed the vectors used work equally well with *Leishmania tarentolae*, *C. fasciculata* and *T. cruzi*. The vectors and the reporter genes, therefore, contain the signals these kinetoplastid protozoan parasites need for expression.

In brief, during two weeks instructors and students that assisted the Workshop designed and performed experiments using transfection procedures. Transfection of parasites has been used to study protein function, mechanisms of gene expression and regulation (e.g., promoter sequences), and gene products involved in virulence and drug resistance. The parasites that were electroporated and transfected (*L. tarentolae*, *C. fasciculata* and *T. cruzi*) served as tools for optimization of the working conditions. The experiments led the students to carry on DNA and RNA analyses. For instance, the students demonstrated the presence of the luciferase exogenous gene in transfected *T. cruzi* cells. Further, recombinant proteins were shown by Western blot and immunocytochemistry assays. The recombinant mHSP70 19E10 and FCB9 9E10 were localized at the kinetoplast and flagellum, respectively, of transfected *T. cruzi* cells, using 9E10 epitope tag antibody.

The Second Buriti Workshop gave the students the opportunity to perform electroporation of parasites and advanced molecular biological, biochemical and immunological techniques for characterization of gene expression in transfected cells. The applications of these techniques will be fundamental in developing new research tools to investigate the kinetoplastid protozoan parasites that cause various endemic diseases in the New World.

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