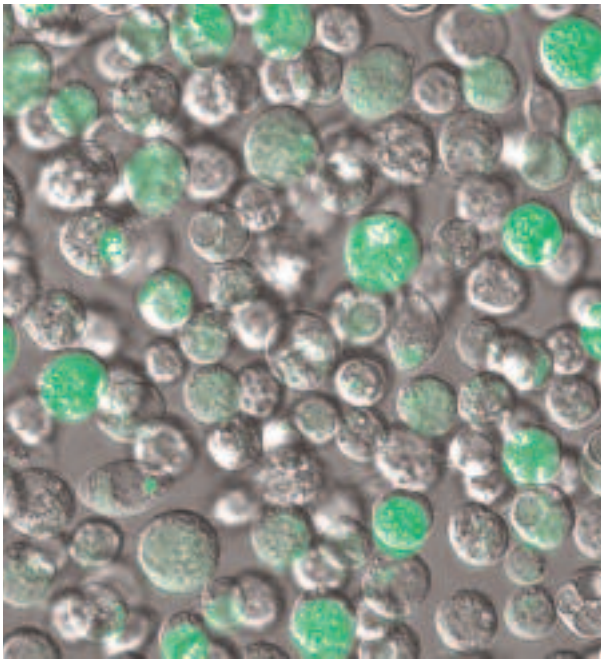
HETEROLOGUS GENE EXPRESSION IN DIPTERAN CELLS: MOLECULAR BIOLOGY AND BIOPROCESS ENGINEERING

Carlos Augusto PEREIRA

Butantan Institute



Drosophila melanogaster *cells expressing the rabies virus glycoprotein upon gene transfection*

The project assembles a multidisciplinary group constituted by biologists and chemical engineers from 5 different institutions in São Paulo State. The aims of the project are: the cloning of the rabies virus glycoprotein (RVGP) gene in expression vectors for the preparation of Schneider 2 (S2) *Drosophila melanogaster* cells stably transformed and expressing this gene, the optimization of a bioprocess for the production of the antigen in bioreactors, and the evaluation of its antigenic and immunogenic properties.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Plasmid vectors for S2 cells were constructed, characterized and used for cell transfection. S2 cells transfected with these vectors showed a high RVGP production (3 µg/10^7 cells) with enough quality to induce the antibody synthesis in immunized animals. We have formulated different S2 cell culture media and demonstrated that hemolymph and its fractions were capable of promoting cell growth and heterologous protein production. Optimized cell growth and metabolism have been obtained in bioreactors by controlling the cell sensitivity to hydrodynamic stress, the cell death and the RVGP production at the cell membrane. The RVGP synthesis parallels the cell growth and can be modulated. A higher hydrodynamic stress in bioreactors favors the recombinant protein production and the combination of this one with the medium formulation and alterations of the cell membrane are involved in a higher RVGP production. The S2 cell respiration, the dissolved oxygen concentration and the kLa of different systems of oxygen transfer were optimized for a higer RVGP production. A protocol for recombinant protein production by S2 cells was established and purification processes were developed. The RVGP produced was monitored by ELISA, flow cytometry, qPCR, confocal microscopy and western-blotting. *In vivo* assays showed a good immune response of mice to the RVGP. Quantitative and qualitative results of this project reinforce the good potential of S2 cells as a substrate for gene expression.

MAIN PUBLICATIONS

Yokomizo AY, Jorge SAC, Astray RM, Fernandes I, Ribeiro OG, Horton DSPQ, Tonso A, Tordo N, Pereira CA. 2006. Rabies virus glycoprotein expression in *Drosophila* S2 cells. I. Functional recombinant protein in stable co-transfected cell line. *Biotechnol. J*. **2**:102-109.

Santos MG, Jorge SAC , Brillet K, Pereira CA. 2007. Improving heterologous protein expression in transfected *Drosophila* S2 cells as assessed by EGFP expression. *Cytotechnology*. **54**:15-24.

Mendonça RZ, Oliveira EC, Pereira CA, Lebrun I. 2007. Effect of bioactive peptides isolated from yeastolate, lactalbumin and NZCase in the insect cell growth. *Bioprocess Biosyst Eng*. **30**:157-164.

Galesi ALL, Batista FRX, Mendonca RZ, Pereira CA, Moraes AM. 2007. Design of culture media for *Drosophila melanogaster* S2 cells producing recombinant G glycoprotein from rabies virus. In: *Cell Technology for Cell Products*, R. Smith (ed.), Springer, 403-413.

Galesi ALL, Pereira CA, Moraes AM. 2007. Culture of transgenic *Drosophila melanogaster* Schneider 2 cells in serum-free media based on TC100 basal medium. *Biotechnology Journal*. **2**:1399-1407.

Astray RM, Augusto E, Yokomizo AY, Pereira CA. 2007. Analytical approach for extraction of recombinant membrane viral glycoprotein from stably transfected *Drosophila melanogaster* cells. *Biotechnology Journal*. **3**: 98-103.

Swiech K, Silva CS, Arantes MK, Santos AS, Astray RM, Pereira CA, Suazo CAT. 2007. Characterization of growth and metabolism of *Drosophila melanogaster* cells transfected with the rabies virus glycoprotein gene. *Biotechnol. Appl. Biochem*. DOI:10.1042/BA20060148

Carlos Augusto PEREIRA

Instituto Butantan
Laboratório de Imunologia Viral
Avenida Vital Brasil, 1500 – Butantã
05503-900 – São Paulo, SP – Brasil

+55-11-3726-7222 ext. 2105
grugel@butantan.gov.br