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## 1994 ICGEB ACTIVITY REPORT

**Project title:** Oncogenes and anti-oncogenes in cell proliferation control.

**Principal Investigator:** Mari Cleide Sogayar Armelin

**Unido contract No.:** 93/267

**ICGEB Ref No.:** CRP/BRA 93-02

**Keywords:** Cell proliferation control / Oncogenes / Tumor suppressor genes / Malignant transformation / Tumor virus / cDNA cloning / Expression in bacterial and baculovirus systems.

### **Abstract:**

Controlled cell proliferation results from the concerted action of 2 types of gene products coded by, respectively, oncogenes and anti-oncogenes (also called tumor suppressor genes, TSG). Tumor growth (malignancies) result from deregulated expression of these same gene classes.

Cellular oncogenes (proto-oncogenes) code for peptide growth factors, their receptors, mediators and effectors. Several labs. have shown that platelet-derived growth factor (PDGF), signals cell division by changing the cell's transcriptional program and inducing a set of immediate-early genes among which are the nuclear proto-oncogenes *fos*, *jun*, and *myc* and the interleukin like genes JE and KC. We postulated that cell transformation by DNA and RNA tumor viruses could be effected through deregulation of proto-oncogenes' expression by the viral oncoproteins.

We previously showed that over-expression of simian sarcoma virus *v-sis* or polioma virus middle T (MT) leads to deregulated expression of several PDGF primary response genes. In addition to MT transfectant cell lines we are presently using transformation-defective MT cell lines to probe into the role played by cellular products in the malignant phenotype induced by the viral oncogene.

Glucocorticoid hormones display anti-tumor and anti-inflammatory activities and are known to modulate the mitogenic activity of peptide growth factors. On the other hand, the action of glucocorticoids has been shown to be modulated by *fos* and *jun* gene products.

Interaction of glucocorticoid hormones with their receptors lead to transcriptional modulation of several cellular genes. Some years ago we described a dramatic phenotypic reversion (from transformed to normal) caused by glucocorticoids in the C6/ST1 hypersensitive variant but not in the hormone-resistant C6/P7 variant. This cellular model system is extremely useful to study the molecular basis for the phenotypic reversion phenomenon.

Using differential hybridization of a cDNA library constructed from hydrocortisone-treated ST1 cultures, we recently isolated 4 cDNA sequences that are induced by the hormone in glucocorticoid-hypersensitive ST1 cells but not in hormone-resistant P7 cells. Other experimental strategies are being adopted to optimize the search for glucocorticoid-regulated cDNA sequences. The actual role of these genes in the phenotypic reversion will be investigated by over-expression of appropriate mammalian vectors containing these cDNAs in P7 cells.

A program to express and produce cellular proteins and polyclonal antisera was established in the lab. Bacterial expression systems are being used to produce proteins of the Fos and Jun families, in addition to the JE protein. The baculovirus expression system was established and has been used to express the mouse c-Fos protein and the human prolactin hormone. High titer antisera to Fos and Jun proteins are also being generated. This opens the way to generate antisera to glucocorticoid-regulated gene products.

**Background:**

Two types of gene products are associated, on one hand, with the control of cell division and, on the other, with uncontrolled growth that results in tumors, namely: oncogenes and tumor suppressor genes. However, while unscheduled or excessive oncogenes expression is related to tumor growth, it is the inactivation or lack of tumor suppressor products that is responsible for several hereditary tumors.

For the past 2 decades we have directed our research efforts towards understanding the molecular basis for controlled cell growth, malignant transformation induced by tumor viruses and the anti-tumor effects of glucocorticoid hormones.

We have mainly focused two cellular model systems, namely: a) Balb-3T3 normal fibroblasts transformed by viral oncogenes from RNA (SSV) or DNA (polyoma) viruses; b) rat C6 glioma cell variants that are either hyper-sensitive to glucocorticoids (ST1) or unresponsive to the hormone (P7 cells).

Transfectant cell lines overexpressing v-sis or Py-MT were generated and examined for the pattern of PDGF-early response genes. The results indicate that viral oncoproteins subvert the cell's transcriptional program. This idea is being tested using polyomavirus transformation-defective mutant cell lines generated in Dr. Thomas Roberts lab.

We previously showed that C6/ST1 cells revert from a transformed, tumoral phenotype to normal, under glucocorticoid treatment, whereas the C6/P7 variant does not respond to hormone treatment. In order to probe into the molecular basis for this phenotypic reversion, we set out to clone the glucocorticoid-regulated genes from hormone-treated ST1 cells. Four cDNA sequences have already been isolated and characterized. Other cDNA libraries are being constructed and screened. Subtraction hybridization and differential display are also being used to extend the panel of hormone-regulated genes.

**Objectives:**

- a) Analysis of the role played by PDGF-primary response genes in polyomavirus-induced cell transformation;
- b) Cloning and characterization of cDNA sequences regulated by glucocorticoid hormones in C6/ST1 rat glioma cells;
- c) Expression of growth control genes in bacterial and baculovirus systems;
- d) Generation of polyclonal antisera to recombinant proteins produced in bacterial and baculovirus systems.

**Work Progress:**

a) Analysis of the role played by PDGF-primary response genes in polyomavirus middle T (PyMT)-induced cell transformation: The data previously published by our lab., using PyMT transfectant cell lines, strongly suggested a role for several PDGF primary response genes (*c-myc*, *JE*, *fos* and *jun*) in polyomavirus cell transformation. In order to further probe into the role of these gene products, we are also using transformation-defective Balb-3T3 cell lines generated in Dr. Thomas Roberts' lab. with retroviral constructs containing mutant PyMT cDNAs (deletions and point mutations) (Morgan et al, 1988, *J. Virol.* 62, 3407-3414; Drucker & Roberts, 1991, *Nucl. Ac. Res.* 19, 6855-6861). Analysis of the expression of the PDGF-inducible genes, by immunoprecipitation, immunofluorescence and gel retardation assays, and comparison with the cell lines' transformation potential (measured by the ability to form colonies in agarose suspension and tumorigenic activity), led us to conclude that: a) overexpression of both mutant and wild type MT causes upregulation of *c-jun* and high basal levels of the c-Jun protein; b) MT (mutant and wild type) overexpression leads to high basal levels of the c-Fos protein but this protein is hypo-phosphorylated when compared with serum-induced parental A31

cells; c) wild type MT, but not mutant MT, downregulates the AP-1 binding activity but no correlation was found between the level of AP-1 binding activity and the tumorigenic potential.

**b) Cloning and characterization of cDNA sequences regulated by glucocorticoid hormones in C6/ST1 rat glioma cells:** A dramatic phenotypic reversion (from transformed to normal) is caused by glucocorticoids in the hyper-responsive C6/ST1 cell line but no effect is observed in the unresponsive C6/P7 variant. We are using differential hybridization to isolate and glucocorticoid-regulated cDNA sequences. A cDNA library was generated using poly a plus RNA from C6/ST1 cells treated with hydrocortisone plus cycloheximide for 5h. Differential screening led to the isolation of 4 different cDNA sequences (3 cellular and 1 retroviral) that are induced by the hormone. DNA sequencing allowed us to identify all 4 sequences as corresponding to rat metallothioneins 1 and 2 (clones C27 and C41), acidic glycoprotein (C36) and MLV env (clone CV2). Northern blot analysis confirmed the induction of these sequences by glucocorticoids. These cDNAs will be subcloned into mammalian expression vectors to transfect C6/P7 cells to investigate the possibility of transferring the ability to undergo the phenotypic transition.

**c) Expression of growth control genes in bacterial and baculovirus systems:** We are using the bacterial pEX and pGEX systems to express several members of the Fos and Jun protein families and the Rb tumor suppressor gene. cFos, Fra-1, Fra-2, cJun, JunB, JunD and p105Rb have been successfully expressed, purified and used to inoculate rabbits to generate antisera. The BacPak baculovirus system was set up to express human peptide hormones and the JE interleukin-like glycoprotein. Human prolactin is being produced with high yields (75mg/l culture, measured by radioimmuno assay). The alfa subunit of human FSH (follicle stimulating hormone) was obtained by reverse PCR and will also be subcloned into

the BacPak vector. Mouse JE was successfully subcloned and is currently being characterized.

**d) Generation of polyclonal antisera to recombinant proteins produced in bacterial and baculovirus systems.** The Fos, Jun and Rb proteins expressed in bacterial systems (see above) have been purified, characterized by Western blot analysis and used to inoculate rabbits to generate polyclonal antisera. The titers are being determined by immunofluorescence and ELISA.

### **Publications:**

#### **Articles:**

38. VALENTINI, S.R., CORVELLO, C.M. & ARMELIN, M.C.S. (1994) - cDNA cloning and expression of growth control genes. *Ciência e Cultura*, **45**, 223-230.
39. VALENTINI, S.R., OLIVEIRA, M.L.S., SASAHARA, R.M. & ARMELIN, M.C.S. (1994) - Glucocorticoid-regulated genes in transformed to normal phenotypic reversion. *Braz. J. Med. Biol. Res.* **27**, 541-546.
40. CORVELLO, C. M., BRAVO, R. & ARMELIN, M.C.S. (1994) - Isolation and characterization of c-fos recombinant baculovirus. *Braz. J. Med. Biol. Res.* **27**, 2365-2370.
41. VALENTINI, S. R. & ARMELIN, M. C. S. (1994) - Cloning of glucocorticoid-regulated genes in C6/ST1 transformed to normal phenotypic reversion. (Submitted to publication).

#### **Abstracts in International Meetings:**

25. ARMELIN, M. C. S., VALENTINI, S. R., CORVELLO, C. M. & SASAHARA, R. M. (1994) - Cloning and expression of genes related with growth control and neoplasia. Second Summer Southern Symposium, Santiago, Chile. p67-70.

26. OLIVEIRA, M. L. S., DRUKER, B. J. & ARMELIN, M. C. S. (1994) - Expression of PDGF primary response genes in wild type and mutant polyomavirus middle T overexpressing cells. Second Summer Southern Symposium, Santiago, Chile. p107.
27. VALENTINI, S. R. & ARMELIN, M. C. S. (1994) - Regulation of gene expression by glucocorticoid hormones in transformed to normal phenotypic reversion. Second Summer Southern Symposium, Santiago, Chile. p113.
28. VALENTINI, S. R. & ARMELIN, M. C. S. (1994) - Glucocorticoid-regulated genes detected by differential hybridization in a rat glioma cell line. 85th Annual Meeting of the American Association for Cancer Research, San Francisco, California, USA.

#### **Abstracts in Local Meetings:**

59. OLIVEIRA, M. L. S., VALENTINI, S.R., VENTURA, A., DRUCKER, B. J. & ARMELIN, M. C. S. (1994) Mutant polyomavirus middle T (MT) cell lines in the study of MT-induced cell transformation. XXIII Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, Caxambu, Minas Gerais.
60. VALENTINI, S. R., OLIVEIRA, M. L. S., SASAHARA, R. M. & ARMELIN, M. C. S. (1994) Molecular bases for the transformed to normal phenotypic reversion induced by glucocorticoid hormones in rat glioma cells. XXIII Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, Caxambu, Minas Gerais.
61. ABREU, P. A. E., ARMELIN, M. C. S. & HO, P. L. (1994) Fibroblast growth factor and cyclic AMP induce the expression of *fos* and *jun* proto-oncogenes in PC12 cells. XXIII Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, Caxambu, Minas Gerais.
62. MORTENSEN, C., CORVELLO, C. M., DIAS, L. E. M., BARTOLINI, P. & ARMELIN, M. C. S. (1994) Baculovirus system as an alternative to express human pituitary hormones. XXIII Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, Caxambu, Minas Gerais.



63. VALENTINI, S. R. & ARMELIN, M. C. S. (1994) Cloning of differentially expressed genes involved in glucocorticoid-induced phenotypic reversion of C6 glioma cells. XXIII Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, Caxambu, Minas Gerais.
64. MORTENSEN, C., CORVELLO, C. M., DIAS, L. E. M., BARTOLINI, P. & ARMELIN, M. C. S. (1994) Expressão de hormônios pituitários humanos em sistema de baculovirus. IV Congresso Brasileiro de Biomedicina, São Paulo, SP.
65. OLIVEIRA, M. L. S., ORTIS, F., CORDEIRO, S. Z. & Armelin, M. C. S. (1994) Cytoskeleton and extracellular matrix organization in mutant and wild type polyomavirus middle T-transformed Balb-3T3 cell lines. III Simposio Brasileiro sobre Matriz Extracelular (SIMEC), Angra dos Reis, RJ.

#### **Networking:**

Collaborations have been established with several laboratories from this and other countries. From this Biochemistry Department: Drs. Ana Maria Carmona and Sergio Verjovski de Almeida. From other Departments of this University: Drs. Fabio Nunes and Vera de Araujo (Dentistry School). from other research institutions: Drs. Paulo lee Ho (Butantan Institute) and Ricardo Brentani (Ludwig Institute, São Paulo branch). From other countries: Drs. Jorge Allende and Catherine Conneily, University of Chile Medical School, Santiago), Dr. Francisco Barrantes (Bahia Blanca, Argentina), Drs Thomas Roberts and Brian Drucker (Dana Farber Cancer Institute, Boston, USA).

One graduate student (Luciana Cruz) came from Dr. Allende's lab. to spend 2 months working on a ICGEB collaborative project (No. 92/056). One recently graduated student (Dr. Sandro Valentini) presented his thesis work at the AACR (American Association for Cancer Research) meeting in San Francisco, California,

USA in april and a graduate student (Regina Maki Sasahara) spent 3 months in Dr. Ruth Sager's lab. at the Dana Farber Cancer Institute, Boston, Mass., USA). Another graduate student (João Marcos Mercado) took a CABBIO (Brazil-Argentina Center for Biotechnology) course in Buenos Aires, Argentina in sept./october. Several students presented their work at local scientific meetings.

The principal investigator (Mari Sogayar Armelin): a) taught two 2 weeks practical graduate courses/workshops: "Control of gene expression in cell proliferation and differentiation" (in may) with the participation of Dr. Peter Geiduschek (University of California San Diego, Molecular Genetics, Biology Department) and "Cloning and expression of heterologous genes using baculovirus vectors as a biotechnological tool" (in november) with the participation of Drs. Linda King and Robert Possee (from Oxford, England) and Francisco Barrantes (Bahia Blanca, Argentina). The latter was a CABBIO international course; b) organized and chaired the Symposium "Molecular basis of gene expression" in the XXIII Meeting of the Brazilian Society for Biochemistry and Molecular Biology (14-17 may, Caxambu, Minas Gerais); c) participated in a CABBIO course taught by Dr. Barrantes in Bahia Blanca, Argentina, in october, d) organized, chaired and presented a Round Table on "Medical applications of molecular biology techniques", at the São Paulo Academy of Sciences meeting held in october (26-28); e) participated, as a lecturer, in the University Extension course on "Principles of the PCR technique and its applications to clinical diagnosis".



To be filled by ICGEB		To be filled by the Affiliated Centre	
Budgets as per original proposal		Summary of expenditures *	
1) Capital equipment	US\$ .....	1) Capital equipment	US\$ .....
2) consumables	US\$ 5,000.00	2) consumables	US\$ 5,940.00
3) training	US\$ 1,500.00	3) training	US\$ 1,014.31
4) literature	US\$ 500.00	4) literature	US\$ .....
5) miscellaneous	US\$ .....	5) miscellaneous	US\$ .....
<b>TOTAL GRANT</b>	<b>US\$ 7,000.00</b>	<b>TOTAL</b>	<b>US\$ 6,954.31</b>

Consumables:

Check No. 20778 (5/7/1994) Intermetra / BANESPA S. A. US\$ 3,380.00  
 20781 (3/9/1994) Belcap Intern. Corpora US\$ 64.00  
 20781 (1/12/1994) Unidas Com. Exterior LTDA. US\$ 2,496.00

Sub-Total US\$ 5,940,00

Education/Training/Literature

Check No. 18298 (13/5/1994) Raven Press US\$ 107.31  
 18300 (21/6/1994) American Assoc. Adv. Sciences US\$ 187.00  
 20775 (21/6/1994) American Assoc. Cancer Res. US\$ 190.00  
 20776 (21/6/1994) Current Protocols Mol. Biol. US\$ 170.00  
 20777 (27/6/1994) Biotechniques US\$ 220.00  
 20781 (6/12/1994) Regina Maki Sasahara US\$ 140.00

Sub-Total US\$ 1,014.31

TOTAL US\$ 6,954.31

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