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International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

14p. table

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Collaborative Research Programme

TERMINAL EVALUATION REPORT

UNIDO contract # 92/056

ICGEB ref. #: CRP/ CHI91-02

Project initiation: July 1992

Project termination: June 1995

International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

Collaborative Research Programme

TERMINAL EVALUATION REPORT

Part 1

Title of Project

Protein Phosphorylation as a regulatory mechanism for cellular proliferation

Keywords: Protein Kinases, casein kinase 1, casein kinase 2, cDNA cloning, site-directed mutagenesis, *Xenopus laevis*, *Brachydanio rerio*, NIH 3T3 Cells.

UNIDO contract #	**ICGEB ref. #: CRP/**
Project initiation:	**Project termination:**
Principal Investigator's name:	

Affiliate Centre mail address : National Committee for Biotechnology
CONICYT - Chilean National Committee of Science and Technology (DATI)
International Assistance
Canadá 308 - Santiago - Chile
Telephone no. (56 2) 274 45 37 Ex.2220 **Telex no.**
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Abstract:

The principle objective of the project was the characterization at the molecular level of two prominent nuclear protein kinases which have been postulated to participate in the control of cell proliferation. These kinases, casein kinase 1 and 2, have been shown to phosphorylate several key proteins involved in nucleic acid synthesis, cell division and oncogenic transformation. Using the model system of the amphibian oocyte, zebrafish embryos and a combination of protein chemistry and molecular biology techniques, significant progress was achieved in gaining new knowledge related to these kinases.

cDNAs coding for α and β subunits of *X.laevis* CK2 have been cloned, sequenced and expressed in *E.coli*. The properties of the reconstituted, recombinant enzyme have been studied. CK2α subunit has been shown to bind single or double stranded DNA and this interaction is abolished by the presence of the β subunit. Nine mutant subunits have been prepared (by site directed mutagenesis) and several have been characterized.

The cDNAs of α, β and α' have also been cloned from zebrafish and their expression during early embryogenesis has been analyzed by in situ hybridization, showing differences in their distribution in nervous tissues. Expression of CK2 α subunit during embryogenesis of *X.laevis* was also studied. Specific synthetic peptides were used to analyze substrate specificities and for the preparation of antibodies. The stable transformation of 3T3 cells with the CK2α gene has been achieved.

The cDNAs coding for CK1 of *X.laevis* and human have been cloned and expressed in bacteria. It has been demonstrated that CK1 is a dual specificity kinase, phosphorylating both ser/thr as well as tyrosine (in the synthetic peptide polyGluTyr,4:1). Human CK1 gene was localized on chromosome 13q13 by fluorescent in situ hybridization.

OBJECTIVES/METHODOLOGY
(proposed at the time of the submission of the research proposal)

The general objective of the project is to understand the role of some nuclear protein kinases in the control of cellular proliferation. More specifically, the project will concentrate on three enzymes found in *X.laevis* oocytes: casein kinase I, casein kinase II, and the equivalent of the CDC-2 kinase, enzymes that have been shown to phosphorylate some key proteins involved in nucleic acid synthesis cell division and in oncogenic transformation.

1. **Structure and Expression of the Genes coding for the *X.laevis* casein kinase I, casein kinase II and CDC-2 kinase.** The structure of the genes coding for these three important kinases will be studied by cloning and sequencing. cDNA clones will be isolated by screening and oocyte cDNA library using synthetic oligonucleotide probes and by the PCR technique. The β subunit has been postulated to have a regulatory function. It is of special interest to determine the autophosphorylation site on this subunit. In the case of the casein kinase I, an enzyme whose gene has not yet been cloned in any other species, the cloning will require purification of the enzyme, digestion of the protein and microsequencing of some of its peptides in order to design specific oligonucleotide probes.
Genomic clones of the genes of these same three enzymes will also be isolated in order to study the intron-exon structure of their genes and to establish whether some heterogeneity may arise from alternate splicing of the mRNAs. Differential expression will be studied in oocytes during different stages of oogenesis and embriogenesis.

2. **Synthetic peptides as model substrates, pseudo substrate inhibitors, and antigenic epitopes of *X.laevis* protein kinases.** In the case of CK II, we will follow up our observation that tyrosine residues present in clusters of acidic aminoacids greatly enhance the inhibitory capacity of pseudo substrate peptides. The relative position of the tyrosine residues with respect to the acidic residues will be explored. The relevance of these experiments lies in the observation that protein sequences containing tyrosyl residues in acidic clusters are excellent substrates for tyrosine kinases.
In the case of casein kinase 1, our initial experiments will concentrate on the possible effect of tyrosine and phosphotyrosine on the position of the phosphorylated serine in synthetic peptides. This study may provide a link between tyrosine kinases and ser/thr kinases, providing insights into the cascade of events in the proliferation signal pathway.

3. **Site-directed mutagenesis of protein kinases** will be used to modify selectively some of the regions of the pretin kinases that are considered important for their activity and regulation.
Some of the sequences which could be studied by site-directed mutagenesis could be the sequence: **R K L G R G K Y S E V F** of the subunit of CK II which differs from the consensus **G X G X X G X V X** found in all ATP-binding protein kinases.

4. **Relation of protein kinases to cellular transformation and to the products of oncogenes.** There is solid experimental evidence that links two of the kinases that we plan to study, casein kinase II and CDC-2 kinase, to cellular proliferation and to oncogenic proteins. Initially we plan to assay the activity of these two kinases in 3T3 cells that are transformed by different oncogenes that have been shown to be phosphorylated by either CK II or CDC-2 kinases (for example: SV40 large T antigen, myc, src and myb). The activity would be measured in cell extracts at different times after the cells have been transformed by the oncogenes or combination of oncogenes. Subsequently, experiments will be run to test whether the oncogene protein products are being phosphorylated by these enzymes. Microinjection of peptide inhibitors of the kinases and of "antisense" oligonucleotides for the mRNAs coding for these enzymes will also be studied.

These experiments will be run in collaboration with Dr. Mari Armelin of the Institute of Chemistry of the University of Sao Paulo, who has considerable expertise in cellular transformation with oncogenes.

RESULTS
(compare against the set objectives)

Objective 1 - Cloning and expression of protein kinases.

The main objectives relating to cloning, expression and regulation of specific protein kinases have been achieved during the grant period. cDNAs coding for CK1 and the two subunits of casein kinase II from *X.laevis* have been cloned, sequenced and expressed in bacteria. The α, α' and β subunits of CK2 from Zebrafish and the CK1 gene from human have also been cloned.
The purified recombinant proteins from *X.laevis* are active and have characteristics similar to the native enzymes. Genomic clones have also been obtained and partially sequenced for the CK2α gene, showing the presence of introns which indicate the presence of two homologous but different genes. A cdc-2 like gene was also cloned from *X.laevis*, however, this study was discontinued in view of the fact that several other groups published simultaneously similar clones.

The expression of CK2 in *X.laevis* was studied by determining the level of mRNA, protein and enzymatic activity of the α and β subunits during oogenesis, where significant increases were noted up through stage 6. In situ hybridization using antisense α and β RNAs showed that both genes are expressed in early embryos. Similar studies performed with zebrafish embryos show temporal and tissue specific expression of both α and β, with the β subunit present at relatively higher concentrations in developing neural tube and optic vesicles. YAC genomic clones containing the human CK1 sequence were used for fluorescent in situ hybridization to localize the gene in chromosome 13q13.

The recombinant *X.laevis* CK1 kinase activity has characteristics similar to the native enzyme isolated from other tissues. However, we have recently observed that CK1 is able to phosphorylate tyrosine residues in the synthetic substrate polyGluTyr 4.1 as well as the expected serine and threonine residues of other substrates. This interesting observation puts CK1 in the class of the rare "dual-specificity" kinase. (see publications Nº 1,3,4,5,6,10).

Objective 2 - Effects of Synthetic Peptides

Using synthetic peptides of known sequences, it was shown that CK2 discriminates the positon of tyrosine residues present in these inhibitor peptides containing clusters of glutamic acid residues. A preference was seen for tyrosine residues located in the N-terminal region. The recombinant wild type α subunit was shown to be less sensitive to inhibitor peptides whereas a mutated CK2α subunit $\alpha E^{75}E^{76}$ retained the sensitivity seen with the holoenzyme CK2 $\alpha_2\beta_2$.

Synthetic peptide substrates were also studied and it was observed that tyrosine residues also had positional effects on phosphorylating activity. For example, a synthetic substrate $(R)_3$ EYEETEEEE showed a two-fold higher relative catalytic efficiency as compared to a similar peptide in which phenylalanine replaced the tyrosine (Y) residue.

Studies with polyglutamylated derivators of folic acid including the drug methotrexate demonstrated that these compounds are efficient inhibitors of CK2 and the nature of both the aromatic group as well as the length of the acidic substitution affect the efficiency of inhibition. Again, the recognition of these compounds is largely dependent on the β subunit.
(See publications Nº 2,4).

Objective 3 - Site directed mutagenesis

The studies directed toward understanding the structure/fun[illegible]on relationships were approached by the preparation of mutants of both CK2 α and β [illegible]bunits.

Five different mutants of CK2α were prepared by PCR and sequenced. These included alterations in the nucleotide binding domain, an autophosphorylation site, a histidine repeat segment and a putative nuclear translocation sequence. The cDNAs of these mutants were subcloned and expressed in bacteria. The properties of three of the mutants have been studied and this work is still in progress. The $\alpha E^{[illegible]}E^{[illegible]}$ mutant was shown to have altered properties in relation to substrate and polyanion binding. An $\alpha F^{50}G^{51}$ mutant (nucleotide binding mutant) has altered catalytic properties.

The CK2 β subunit was mutated in 9 different positions and each mutant was sequenced, expressed and analyzed in relation to CK2α activation (kinase activity) and interaction (sucrose gradients). An interesting effect was observed with $\beta P^{58} \rightarrow A^{58}$ in the alteration of an attenuator effect exerted by adjacent acidic residues. Several of the mutants showed altered stability and capacity to activate α subunit. A deletion mutation (37 carboxy-terminal amino acids) drastically reduced the affinity of β for α subunit but retained the capacity to activate the kinase.
(See publications Nº 4,8,9,10).

Objective 4 - Role of Kinases in cells transformed by Oncogenes

This area of research was carried out in collaboration with Dr. Mari Armelin, Sao Paulo, Brazil. Dr. Pilar Carvallo and the graduate student Ms. Luciana Oliveira have carried out studies during three two to three month visits to the Armelin laboratory.

Using the cell line NIH 3T3, transfection was achieved using the CK2α subunit gene subcloned in the eukaryotic expression vector pMEX which contains the inducible MMSV promoter region and the S40 polyA signal sequence. Efforts to obtain stable clones containing the α gene were recently successful. Various stable transfected clones were obtained. PCR experiments have shown that the entire α gene is inserted in at least one cloned cell line. Parallel experiments were carried out with a mutated α subunit gene ($\alpha E^{[illegible]}E^{[illegible]}$) and again positive clones were obtained. The stable expression of this mutant in these cells is of considerable interest since the transport of the α subunit to the nucleus may be altered by this mutation. Studies are in progress related to the restriction mapping, copy number, and expression levels of CK2α in these cell lines.

Work plan and time schedule
(originally envisaged)

The First Year

1- **Cloning and expression of protein kinases**
Cloning and sequencing of the cDNAs coding for the α and β subunits of casein kinase II will be completed. Cloning and sequencing of CDC-2 and related kinases will be attempted. The purification of casein kinase I will be carried out.

2- **Synthetic peptides as substrates, inhibitors, and antigenic epitopes**
A study of the specificity of interaction of CK-II with tyrosine containing acidic peptides will be completed and the specificity of phosphorylation of CDC-2 of synthetic peptides will be tested. Likewise pseudosubstrates with analogous sequences will be tested.
Peptides will be synthesized to make antibodies for immunoprecipitation purposes.

3- **Site-Directed Mutagenesis** expression in bacteria of the α subunit of CK-II and the CDC-2-like kinase will be studied.

4- **Role of kinases in cells transformed by oncogenes** The activity of CK II and CDC-2 kinases would be measured during the initial stages of cellular transformation.

The Second Year

1- **Cloning and expression of protein kinases**
The cloning of casein kinase I will be initiated. The expression of the CK-II subunits and of the CDC-2 kinase during oogenesis and early development will be studied by Northern blot and by PCR amplification of mRNAs. Cloning of genomic DNA coding for CK-II and CDC-2 subunits will be initiated.

2- **Synthetic peptides** Synthetic peptides that are optimal and specific substrates will be designed and tested. Peptide inhibitors of CK-II, CK-I and CDC-2 kinases will be synthesized and tested.

3- **Site-Directed Mutagenesis** Specific putative functional sites of CK-II will be mutated and the effect of those mutations will be tested.

4- **Role of kinases in cells transformed by oncogenes** Phosphorylation of oncogene proteins by CK-II and CDC-2 in cells transformed by oncogenes.

The Third Year

1- **Cloning and expression of protein kinases** Cloning of genomic DNA coding for CK-I. Determination of the regulatory [illegible] that control the transcription of the genes of the 3 kinases will be studied.

2- **Synthetic peptides**
Peptides will be synthesized with sequences analogous to those in the β subunit of CK-II. These peptides will be tested for capacity to replace the β subunit or to compete with its binding to the α and α' subunits.

3- **Site-Directed Mutagenesis**
Site directed mutagenesis of the CDC-2 kinase and the CK-I kinase will be attempted with the objective of describing their functional and regulatory domains.

4- **Role of kinases in cell transformation by oncogenes**
Transformed cells will be injected with "antisense" RNA and with specific peptide inhibitors of the kinases in order to test the effect of these compounds on the transformation process.

Work plan and time schedule

(actual)

roject landmarks, duration of individual tasks (use bar charts); evaluation criteria (publications, atents, services, training)

YEAR 1	YEAR 2	YEAR 3	PUBLICATIONS REF.
ASEIN KINASE 2 (CK2) FROM *X. LAEVIS*			
LONING/EXPRESSION *X. LAEVIS* CK2 α AND β			1
CHARACTERIZATION OF RECOMBINANT CK2 α,β			3,4,9
SITE DIRECTED MUTAGENESIS: STUDIES WITH MUTANTES			7-10
TUDIES WITH MODEL PEPTIDES/FOLYL DERIVATES			2
	EXPRESSION OF *X. LAEVIS* IN OOGENESIS		11
	EXPRESSION OF *X. LAEVIS* IN TRANSFORMED CELLS		
K2 FROM ZEBRAFISH			
LONING/EXPRESSION ZEBRAFISH CK2 α α' β			6,7
	EXPRESSION OF CK2 α, β IN ZEBRAFISH EMBRYOS		6
		CHARACTERIZATION OF ZEBRAFISH CK2 α' SUBUNIT	
ASEIN KINASE 1 (CK1) FROM *X. LAEVIS* AND HUMAN			
LONING/EXPRESSION *X. LAEVIS* AND HUMAN CK1			<
	BIOCHEMICAL CHARACTERIZATION OF CK1		
		LOCATION IN HUMAN CHROMOSOMES	5

RAINING

CGEB POSTDOCTORAL FELLOW J.L. DANIOTTI, IN ALLENDE LAB.

OCTORAL THESIS:

------ M.V. HINRICHS CK2β CLONING AND SUBUNIT PROPERTIES----------------------

----------------------------V. WILHELM CK2α EXPRESSION DURING OOGENESIS AND EMBRYOGENESIS--------------

---- R. TELLEZ EFFECTS OF SYNTHETIC PEPTIDES ON CK2 ACTIVITY ---

---------- C. TAPIA CLONING, EXPRESSION AND CHARACTERISTICS OF CK1 -------------

ASTERS THESIS:

--L. OLIVEIRA EXPRESSION OF CK2 α AND β MUTANTS IN CULTIVED CELLS ----------

--P. ROJAS EXPRESSION OF CK2 β SUBUNIT DURING OOGENESIS

Publications Generated by the Project (years 1992-1995):

1. Jedlicki, A., Hinrichs, M.V., Allende, C.C., and Allende, J.E. (1992) The cDNAs coding for the alpha and beta subunits of *Xenopus laevis* casein kinase II. FEBS Lett. 297, 280-284.
2. Téllez, R., Allende, C.C., and Allende, J.E. (1992) Folylpolyglutamate Analogs can inhibit casein kinase II from *Xenopus laevis*. FEBS Lett. 308, 113-115.
3. Gatica, M., Hinrichs, M.V., Jedlicki, A. Allende, C.C., and Allende, J.E. (1993) Effect of metal ions on the activity of casein kinase II from *Xenopus laevis*. FEBS Lett. 315, 173-177.
4. Hinrichs, M.V., Jedlicki, A., Téllez, R., Pongor, S., Gatica, M., Allende, C.C., and Allende, J.E. (1993) Activity of recombinant *alpha*-subunits and *beta*-subunits of casein kinase II from *Xenopus laevis*. Biochemistry 32(28), 7310-7316.
5. Tapia, C., Featherstone, T., Gómez, C., Tailon-Miller, P., Allende, C.C. and Allende, J.E.(1994).Cloning and chromosomal localization of the gene coding for human protein kinase CK1. FEBS Lett. 349, 307-312.
6. Daniotti, J.L., Allende, M.L., Weinberg. E.S. and Allende, J.E. (1994) Cloning and expression of genes coding for protein kinase CK2 α and β subunits in zebrasfish (*Danio rerio*). Cell.Mol.Biol.Res. 40 (5/6), 431-439.
7. Allende, J.E. and Allende, C.C. (1995). Protein kinase CK2: An enzyme with multiple functions and a puzzling regulation. Invited review. FASEB J. 9, 313-323 (86 Refs).
8. Gatica, M., Jedlicki, A., Allende, C.C. and Allende, J.E. (1994) Activity of the $E^{75}E^{76}$ mutant of the α subunit of Casein Kinase II from *Xenopus laevis*. FEBS Lett. 340, 249-254.
9. Gatica, M., Jacob, G., Allende, C.C. and Allende, J.E. (1995) DNA inhibits the catalytic activity of the alpha subunit of protein kinase CK2. Biochemistry 34, 122-127.
10. Hinrichs, M.V., Gatica, M., Allende, C.C. and Allende, J.E. (1995) Site-directed mutants of the Beta subunit of protein kinase CK2 demonstrate important role of Proline 58. FEBS Lett.(in press).
11: Wilhelm, V., Rojas, P., Gatica, M., Allende, C.C. and Allende, J.E. (1995) Expression of the subunits of protein kinase CK2 during oogenesis in *Xenopus laevis*. Eur.J. Biochem. (in press).

NETWORKING

As mentioned in our previous progress reports, the collaboration with Dr. Sándor Pongor from the Trieste Laboratory of the ICGEB has been extremely important in the design and synthesis of peptide substrates and inhibitors and also in the installation of a core facility for nucleic acid and peptide synthesis and microsequencing in the Chilean laboratory.
Dr. Raúl Aguirre, who received an ICGEB fellowship to the Pongor laboratory, is now the tecnical director of this facility.

The collaboration with Dr. Mari Armelin has enabled us to broaden our approach to the use of mammalian cells and recently we have achieved the cloning of stably transformed cell line carrying the CK2α gene. The training of Ms. Luciana Oliveira en cell culture techniques has been most beneficial. Dr. Armelin has visited this laboratory on two occasions and Dr. Pilar Carvallo spent six weeks in the Armelin lab, measuring CK2 activity in transformed cells.

The Allende's laboratory has provided post-doctoral training to an ICGEB fellow, Dr. José Luis Daniotti, from Argentina, who has worked in the isolation of the CKII clones from *Zebra* fish.

Dr. Lawrence Banks of the Trieste laboratory has recently requested the vector containing the cloned casein kinase 1 gene for use in his research. We have forwarded these clones.

PUBLICATIONS

1. Jedlicki, A., Hinrichs, M.V., Allende, C.C., and Allende, J.E. (1992) The cDNAs coding for the alpha and beta subunits of *Xenopus laevis* casein kinase II. FEBS Lett. 297, 280-284.
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4. Hinrichs, M.V., Jedlicki, A., Téllez, R., Pongor, S., Gatica, M., Allende, C.C., and Allende, J.E. (1993) Activity of recombinant *alpha*-subunits and *beta*-subunits of casein kinase II from *Xenopus laevis*. Biochemistry 32(28), 7310-7316.
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6. Daniotti, J.L., Allende, M.L., Weinberg. E.S. and Allende, J.E. (1994) Cloning and expression of genes coding for protein kinase CK2 α and β subunits in zebrafish (*Danio rerio*). Cell.Mol.Biol.Res. 40 (5/6), 431-439.
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11. Wilhelm, V., Rojas, P., Gatica, M., Allende, C.C. and Allende, J.E. (1995) Expression of the subunits of protein kinase CK2 during oogenesis in *Xenopus laevis*. Eur.J. Biochem. (in press).

Part 4

STATEMENT OF EXPENDITURES

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$ 11,070.00
2) consumables	US$	2) consumables	US$ 36,228.36
3) training	US$	3) training	US$ 15,245.94
4) literature	US$	4) literature	US$ 2,455.70
5) miscellaneous	US$	5) miscellaneous	US$ ......–......
TOTAL GRANT	US$......................	TOTAL	US$ 65,000.00

Please itemize the following budget categories (if applicable)

Capital equipment

1992-93	Savant Gel Pump	1,725.00
	Thermolyne Orbital Shaker-Incubator	4,117.00
	Thermolyne Vortex Maximix	158.00
		6,000.00
1993-94	Forma Scientific -80°C Freezer	5,070.00
1994-95	None	-
	TOTAL	11,070.00

Training (provide names, duration of training, host laboratory)

Travel Scientific Meetings

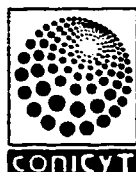
Year	Name	Duration	Host / Meeting	Amount
1992-93	Pilar Carvallo	2 months	Univ.Sao Paulo - Lab. M. Armelin - Brazil	US$ 550.00
	Marcelo Antonelli	1 week	Miami Summer Sympos., USA	US$ 1,449.32
	Maria Vict.Hinrichs	1 week	Cell Biology Congress, USA	US$ 450.00
	Rowena Tellez	1 week	Experiemtnal Biology Meeting, USA	US$ 1,045.00
1993-94	Terrence Featherstone, Washington Univ.USA - 2weeks - Santiago Lab.			US$ 1,000.00
	Luciana Oliveira	2 months	Univ.Sao Paulo -Lab. M. Armelin - Brazil	US$ 735.24
	Jorge E. Allende	1 week	FASEB Res.Conf./Protein Kinases - USA	US$ 1,413.00
	Jorge E. Allende	1 week	Ann.Meeting Soc.Biol. - Chile	US$ 260.00
1994-95	Luciana Oliveira	2 months	Univ.Sao Paulo - Lab. M. Armelin - Brazil	US$ 900.00
	Marcelo Antonelli	2 weeks	Cold Spring Harbor Lab. Embyol.Course-USA	US$ 2,412.00
	Jorge E. Allende	1 week	Ann.Meet. Soc. Biochem. Chile	US$ 181.38
	Students Wilhelm, Vignolo, Rojas	1 week	Ann. Meet.Soc.Biol. Chile	US$ 350.00
			Total Allende Lab	US$ 10,745.93
			Total Armelin Lab	US$ 4,500.00
			TOTAL	US$ 15,245.93

Literature
1993-1995

Annual Subscriptions CELL, Proceedings National Acad.Sci, Nature, Current Biology

TOTAL US$ 2,455.70

* Please do not send invoices, receipts etc.; these should be kept by the Affiliated Centre for future reference and sent to ICGEB upon request.



UNIDO STATEMENT OF ACCOUNTS DATE : AUGUST 08 1995

CONTRACT : Nº 92 / 056

INSTITUTION : UNIVERSIDAD DE CHILE

PROYECT TITLE : PROTEIN PHOSPHORYLATION AS A REGULATORY MECHANISM FOR CELLULAR PROLIFERATION.

PROYECTS LEADER : DR. JORGE E. ALLENDE

REPORT FOR THE PERIOD STARTING : FEBRUARY 1992 ENDING : JUNE 1995

I N C O M E : U.S.$. 65.000.00

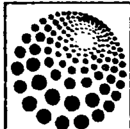
UNIDO FUNDS RECEIVED (DATE) MARCH 06 1992/JUNE 15 1993/JULY 21 1994

INTEREST EARNED : 0

TOTAL INCOME: U.S.$. 65.000,00

E X P E N D I T U R E

I T E M	ALLOCATED IN US.$.	RECEIVED IN US.$.	EXPENDITURE IN US.$.	ITEM BALANCE BROUGHT FWD.
SMALL EQUIPEMENT	11,000.00	11,000.00	11,070.00	(70.00)
CONSUMABLES & CHEMICALS				
SANTIAGO GROUP	35,000.00	35,000.00	32,684.06	2,315.94
[illegible]			[illegible]	
[illegible]			[illegible]	
SAO PAULO GROUP	4,000.00	4,000.00	6,000.00	(2,000.00)
EDUCATION & TRAINING				
[illegible]				
SANTIAGO GROUP	12,000.00	12,000.00	10,745.94	1,254.06
[illegible]			[illegible]	
[illegible]			[illegible]	
SAO PAULO GROUP	3,000.00	3,000.00	4,500.00	(1,500.00)
T O T A L	**65,000.00**	**65 000.00**	**65 000.00**	**0.00**



UNIDO　　　STATEMENT OF ACCOUNTS　　　DATE : AUGUST 08 1995

CONTRACT : Nº 92 / 056

INSTITUTION : UNIVERSIDAD DE CHILE

PROYECT TITLE : PROTEIN PHOSPHORYLATION AS A REGULATORY MECHANISM FOR CELLULAR PROLIFERATION.

PROYECTS LEADER : DR. JORGE E. ALLENDE

REPORT FOR THE PERIOD STARTING : JULY 1994　　　ENDING : JUNE 1995
YEAR 3

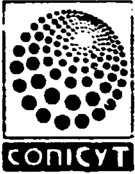
INCOME : U.S.$. 17.000,00

UNIDO FUNDS RECEIVED : DATE : JULY 21 1994
INTEREST EARNED : 0

TOTAL INCOME: U.S.$. 17.000,00

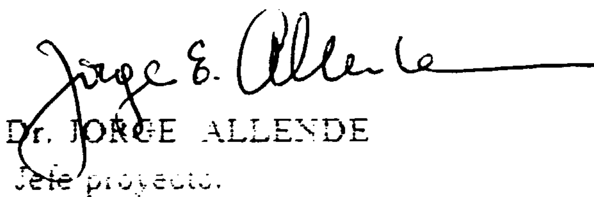
EXPENDITURE

ITEM	ALLOCATED IN US.$.	RECEIVED IN US.$.	EXPENDITURE IN US.$.	ITEM BALANCE BROUGHT FWD.
SMALL EQUIPEMENT	0.00	0.00	0.00	0.00
CONSUMABLES & CHEMICALS				
SANTIAGO GROUP	10.000.00	10.000.00	9.662.39	337.61
Chemicals			[illegible]	
Miscellaneous			[illegible]	
SAO PAULO GROUP	2,000.00	2.000.00	2.000.00	0.00
EDUCATION & TRAINING				
[illegible]				
SANTIAGO GROUP	3,500.00	3,500.00	3,843.38	(343.38)
Airplane Tickets			[illegible]	
[illegible]			[illegible]	
SAO PAULO GROUP	1,500.00	1,500.00	1.500.00	0.00
TOTAL	**17,000.00**	**17,000.00**	**17.005.77**	**(5.77)**

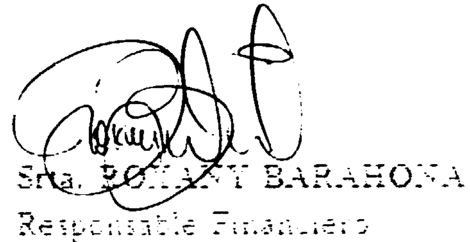


Certificamos que los costes arriba indicados se derivan de los recursos que ha sido preciso emplear para la realización de los trabajos previstos en el Contrato y que dichos costes son reales y corresponden a la definición de costes autorizados incluido en el Contrato y que se ha obtenido las autorizaciones pertinentes.

Certificamos que los ajustes por los motivos que fuere, hayan debido realizarse respecto a los estados de costes anteriormente presentados, han sido recogidos en la presente declaración.



Dr. JORGE ALLENDE
Jefe proyecto.



Srta. ROXANY BARAHONA
Responsable Financiero

European Journal of Biochemistry

Zürich, 26/06/95

Dr. Jorge E. Allende
Departamento de Bioquímica
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Universidad de Chile
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Casilla 70086
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Chile

Reference no.: 95-0896

Expression of the subunits of protein kinase CK2 during Oogenesis in Xenopus laevis

by

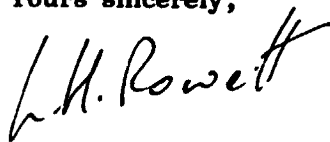
Wilhelm Vivian,
Rojas Patricio, Gatica Marta, Allende Catherine C., Allende Jorge E.

Editor: Girard

Dear Dr. Allende,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal. It is being copy-edited and will be forwarded to the publisher. You may expect to receive the proofs for correction in four to six weeks.

Yours sincerely,



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Editorial manager

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Prof.Jorge E.Allende
Departamento de Bioquimica
Universidad de Chile

May 16,1995

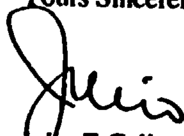
Re: Mns. JEC-3009

Dear Jorge:

Enclosed please find a referee's report on the article you submitted to FEBS Letters.We will be pleased to publish a revised version of your manuscript along the lines suggested by the referee.

Please complete the revisions as soon as possible so as to not delay publication.We need a diskette .

Yours Sincerely



Julio E.Celis
Professor

P.S. I have kept the figures.