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

Collaborative Research Programme

TERMINAL EVALUATION REPORT

UNIDO contract #

92/051/CW

ICGEB ref. #: CRP/ GRE 91-03

Project initiation:

Project termination:

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

Collaborative Research Programme

TERMINAL EVALUATION REPORT

Part 1

Title of Project	
Keywords: Structure and biological functions of a novel Eucaryotic Homologue of the DNA-A Replication Factor.	
UNIDO contract #	**ICGEB ref. #: CRP/**
Project initiation: 92/051 1992	**Project termination:** 1993
Principal Investigator's name: J. Papamatheakis	
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Abstract:	

We have cloned cDNAs that bind to sequences similar to the DNA-A motif encoding for a factor higly homologous to various procaryotic DNA-A proteins. Although the isolation was done from a mouse B lymphoid library on λgt11,various criteria and most strikinlgy, the identification of a second open reading frame located 3' to the DNA-A frame that is highly homologous to the procaryotic DNA-N polymerase , led us to the conlusion that this clone represents a contaminant of a yet unidentified bacterial DNA-A protein. Molecular phylogenetic analysis places this protein very closely to mycoplasmas.This study has allowed the localisation of the DNA binding domain of the protein within the 205 carboxyl treminal aminoacids.

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

Objectives at the time of submission were the sequence analysis of the various overlapping clones, the isolation and characterisation of a full length clone, and the characterisation of the recognition sequences involved in binding. Long term goals were the investigation of the biological effects of this protein and more specifically its function in either replication or transcription . To this end one would have to raise antibodies against the protein and use in vivo or in vitro systems ie tranfection and in vitro transcription - replication systems with DNA molecules, such as plasmids carrying the binding site.

The methodology proposed included various molecular biology techniques including , sequencing, clone mapping, electrophoretic mobility shift assays (EMSA) using the protein and various binding sites. The isolation of a full length clone would further allow the overexpression of the protein by transfection in various cellular backrounds in relation with studies on its effect on plasmids carrying the binding site. Another approach would be the study of its expression under different conditions i.e. in relation to the cell cycle and furthermore the isolation of the corresponding gene.

RESULTS
(compare against the set objectives)

We have analysed 3 cDNA clones isolated by their ability to bind to sequences related to the DNA-A consensus. Sequence analysis initially revealed that the clones are overlapping with identical 5' ends and variable 3´ ends. This analysis showed that the clones carry an open reading frame of 205 aminoacids.These sequences were found to be highly homologous (up to 55%) to the carboxyl end of various known procaryotic DNA-As.

We have carried out EMSA with 5 different oligonuleotides that contain mutations relative to the original probe used for the isolation and derived the consensus TG A/T T/G GATAAC that is in good agreement with other DNA-A binding sites.

In spite of repeated attempts, no other clone, longer at the 5' end was isolated from the library using probe screening. A single clone that appeared promising was found to have an internal EcoRI site and sequence analysis showed that was not related to the DNA-A proteins. Again, no further 5' upsteam clone was isolated using PCR. No clones ewr isolated from the probe screening of two independent mouse genomic libraries

Later on the 3' longer clones were sequenced completely and surprisingly a another reading frame was identified. This reading frame was highly homologous to the DNA-N procaryotic gene. This gene is situated in close proximity to the DNA-A gene in all known procaryotic cases. This result represents a major argument that the isolated clone from the mouse library , is not of mouse origin but rather of an unknown procaryote and therefore not useful for eucaryotic studies. We have carried out extensive phylogenetic analysis using the EMBL data base and defined that the closest known DNA-A sequence belongs to mycoplasma melitoli . It is therefore conluded that this sequence comes from a mycoplasma symbiote and may be interesting to further identify and study.

Part 3

Work plan and time schedule (originally envisaged)

1st year: Completion of structural and binding studies .

2nd year: Isolation of full length clone and characterisationand its expression.

Work plan and time schedule
(actual)

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

I. 1st and 2nd year : Structural and binding studies. Attempts to clone full length cDNA and or genomic clones for DNA-A.

II. Participation in the conference on "DNA replication " 1992 , Switzerland.

III. Under this grant two graduate students, a young Ph.D. and a techician were trained and worked in recombinant E. coli protein expression -isolation and handling.

NETWORKING

During the period of this grant we had the opportunity to collaborate and exchange ideas and material with the lab of Dr. Falaschi at ICGEB- Trieste.

PUBLICATIONS

A manuscript is under preparation.

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$ .....
2) consumables	US$	2) consumables	US$ 11.738
3) training	US$	3) training	US$ 32.588
4) literature	US$	4) literature	US$ .....
5) miscellaneous	US$	5) miscellaneous	US$ 1.162
TOTAL GRANT	US$.....	TOTAL	US$ 45.488

Please itemize the following budget categories (if applicable)

Capital equipment

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

Scarpelis G. Technician 1/1/92- 31/12/92 IMBB

Kretsovali N. Ph.D. 1/1/93 - 30/9/93 IMBB

Tsiotra Y. student 1/1/94 - 19/2/94 IMBB

Gregoriou M. student 1/1/93- 31/12/93 IMBB

Literature

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