GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 11/4/80

Project Title: Interaction of RNA Polymerase with DNA

Project No: G-41-B01

Project Director: Dr. Roger M. Wartell

Sponsor: DHEW/PHS/NIH - National Institute of Allergy and Infectious Diseases

Agreement Period: From 9/1/80 Until 8/31/81

Type Agreement: Grant No. 1R01AI16874-01

Amount: $56,044 Cost sharing: $2,803 (G-41-345)

Reports Required: Annual Progress Reports with Continuation applications; Terminal Progress Report upon Grant expiration

Sponsor Contact Person (s):

Technical Matters

(Program Official)
Irving P. DeLappe, PhD
Chief, Molecular Microbiology
and Parasitology Branch
NIAID
Bethesda, MD 20014
(301) 496-7114

Contractual Matters
(thru OCA)

Gary E. Thompson
Grants Management Officer
Grants Management Branch
EAP-NIAID
Bethesda, MD 20014
(301) 496-7075

Defense Priority Rating: None

Assigned to: Physics (School/Laboratory)

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CA-3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date February 23,1984

Project No. G-41-B01 School/~~XXX~~ Physics

Includes Subproject No.(s) NONE

Project Director(s) Dr. R.M. Wartell ~~XXXXX~~ / GIT

Sponsor DHEW/PHS/NIH - National Institute of Allergy and Infectious Diseases

Title "Interaction of RNA Ploymerase with DNA"

Effective Completion Date: 8/31/81 (Performance) 8/31/81 (Reports)

Grant/Contract Closeout Actions Remaining:

- [X] None
- [ ] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. N/A Continued by Project No. G-41-B02

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Form OCA 60:1028

G-4 E

SECTION IV

APPLICANT REPEAT GRANT NUMBER SHOWN ON PAGE 1	GRANT NUMBER G-41-B01/Wartell AI 16874-02	
SECTION IV—SUMMARY PROGRESS REPORT		
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
WARTELL, Roger A.	FROM	THROUGH
NAME OF ORGANIZATION		
School of Physics, Georgia Institute of Technology	Sept. 1, 1980	August 31, 1981

TITLE (Repeat title shown in Item 1 on first page)

Interaction of RMA Polymerase with DNA

1 List all publications, not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication.
2 Provide two reprints of publications not previously submitted to the awarding unit.
3 Progress Report (See instructions)

1. General Scientific Goals

The general scientific goals of the project have remained predominantly the same as originally proposed. We wish to study DNA restriction fragments containing transcription initiation regions and their interactions with RNA polymerase. An opportunity developed which has resulted in a study of a DNA restriction fragment containing a left-handed helical conformation.

2. Studies conducted

The first major goal was to obtain large quantities of two DNA restriction fragments 144 base pairs (bp.) and 64 bp. long containing the transcription initiation regions of the E. coli lactose operon. A 20 liter fermentor was built, and a high pressure Reverse Phase Chromatography system (RPC5) was developed. These systems were used to grow up and isolate plasmid DNA containing the inserted DNA fragments. Approximately 100 liters of cell strain pRMW 30 were grown, the plasmid DNA isolated and the two restriction fragments purified. Problems encountered in isolating plasmid DNA from frozen cell pastes were overcome. Approximately 1.5 mgs of the 144 bp.DNA and 0.7 mg of the 64 bp. DNA have been purified so far. Work is currently underway to digest 40 mg of pRMW 30 DNA (containing both fragments) and purify quantities of the fragments.

Raman spectroscopy was used to examine the vibrational spectra of the 144 bp. DNA in low salt solutions as well as a 95 bp. DNA which forms the central part of the 144 bp. DNA. In 0.01 M Na^+ and 0.1 M Na^+ salt solutions these DNAs are observed to maintain the "B" family right-handed duplex. The 95 bp. DNA was also examined in 4.5 M NaCl. It remains in the "B" type conformation although some changes in the Raman spectra are observed. A deconvolution program was developed to accurately measure intensities of overlapping Raman bands. A collaborative study was initiated with R. D. Wells and associates (Univ. of Wisconsin, Madison, Wisc.) on a 157 bp containing about 30 base pairs of the (dC-dG). (dC-dG) sequence on each end of the 95 bp DNA. Raman spectra were obtained of the 95 bp DNA, the 157 bp. DNA and the polymer poly (dG-dC) . poly (dG-dC) in high and low salt solutions. The data shows that in 4.5 M NaCl most if not all of the (dG-dC) ends are in the left-handed form, and much of the 95 bp. is no longer in the B type conformation.

One of the objectives of the project is to separate and purify the two strands of a short restriction fragment using alkaline RPC-5 chromatography and incorporate specific probe nucleotides by DNA repair synthesis. Work is underway to develop this methodology. 25 mgs of purified pBR322 plasmid DNA has been isolated. This DNA is 4361 base pairs long and its sequence is known. It will be digested into fragments using the Hae III restriction endonuclease, and the fragments 60 to 500 bps. long will be isolated on neutral RPC-5. These fragments will be used to develop the alkaline RPC-5 chromatography procedure, and for other studies.

3. Specific Objectives for coming year

One objective for next year is to complete the analysis of the Raman spectra of the 95 bp, 157 bp and poly (dG-dC) . poly (dG-dC). This study will provide a quantitative analysis of the effect of a junction between a Z and B type DNA conformations. It should be able to answer the question of how many base pairs of a B type DNA region are influenced by joining it to a Z type region.

A second objective will be to carry out a detailed study of the conformations accessable to the 144 and 64 bp DNAs under different solvent conditions. Both of these fragments contain the catabolite activator protein (CAP) binding site. CAP is know to enhance the binding of RNA polymerase at adjacent recent. We wish to see if the CAP region can assume an unusual DNA conformation suggested by model building studies D.B. McKay and T. A. Steitz, Nature 290, 744, 1981. Additionally we look forward to initiating Raman studies of the complex between RNA polymerase and DNA promoter fragments from the lactose operon and pBR322.

During the next year the Hae III restriction fragments of pBR322 will be isolated using RPC-5. The goal will be to obtain 500 µg-1 mg of fragments about 200 base pairs long. Some of the fragments will be used to develop the alkaline RPC-5, technique to separate strands of a restriction fragment. This technique will be applied to the 64 bp and 144 bp lactose operon fragments. Other fragments will be studied using differential melting curve analysis and Raman spectroscopy.