GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

☐ ORIGINAL ☒ REVISION NO. 1

Project No. G-41-B02 DATE

Project Director: R. M. Wartell School/~~Lab~~ Physics

Sponsor: ~~NIH~~ DHHS/PHS - National Institute of Allergy & Infectious Diseases

Type Agreement: Grant No. 1R01AI16874-02 (02 year)

Award Period: From 9/1/81 To 8/31/82 (Performance) 11/30/82 (if last year) (Reports)

Sponsor Amount: $55,577 Contracted through:

Cost Sharing: $ 2,779 (G-41-313) ~~GTRI~~/GIT

Title: Interaction of RNA Polymerase with DNA

ADMINISTRATIVE DATA OCA Contact William A. Brown x4820

1) Sponsor Technical Contact:

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Grants Management Officer
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Bethesda, MD 20014
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Defense Priority Rating: N/A Security Classification: N/A

RESTRICTIONS

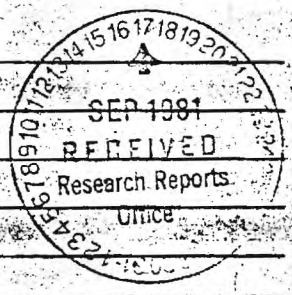
See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with none proposed.

COMMENTS:

Second year follow-on to G-41-B01



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Other

FORM OCA 4:78

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date February 23, 1984

Project No. G-41-B02 School/~~Area~~ Physics

Includes Subproject No.(s) NONE

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

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

Title "Interaction of RNA Polymerase with DNA"

Effective Completion Date: 8/31/82 (Performance) 8/31/82 (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. G-41-B01 Continued by Project No. G-41-B03

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Form OCA 60:1028

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1	GRANT NUMBER G-41-B02/Wartell
SECTION IV—SUMMARY PROGRESS REPORT	AI 16874-03
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT
Wartell, Roger M.	FROM: Sept. 1, 1981 — THROUGH: August 31, 1982
NAME OF ORGANIZATION: Georgia Institute of Technology	

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

Interaction of RNA 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. Publications

J. C. Martin and R. M. Wartell, "Changes in Raman Vibrational Bands of Calf Thymus DNA During the $\overline{B}$ to $\overline{A}$ Transition", Biopolymers (1982) 21, 499-512.

R. M. Wartell, J. Klysik, W. Hillen and R. D. Wells, "Junction Between Z and B Conformations in a DNA Restriction Fragment: Evaluation by Raman Spectroscopy", Proc. Natl. Acad. Sci. (USA) (1982) 79, 2549.

A. S. Benight and R. M. Wartell, "Influence of Base Pair Changes on the Melting Curves of Short DNAs", submitted for publication.

PROGRESS REPORT

1. General Scientific Goals

The overall goals of the project have remained the same as originally proposed. The objective is to determine the conformational properties of DNA restriction fragments containing known or potential transcription initiation or 'promoter' regions and to examine the interactions between these sites and the gene regulation proteins which bind to them. Raman spectroscopy has been the major technique employed. This technique is used to examine the vibrational spectra of DNA in solution.

2. Studies Conducted

Several significant developments occurred during the budget year. A quantitative analysis was carried out on the Raman vibrational spectra of a 157 base pair (bp.) DNA which contained a junction between the left handed $\overline{Z}$ type DNA helix and the more conventional right-handed $\overline{B}$-type DNA helix. This study provides information on the conformational distortion which occurs at such a junction. The 157 bp. DNA was provided by R. D. Wells (University of Alabama, Birmingham, Ala.). It contains a 95 bp. portion of the transcription initiation region of the E. coli lactose operon sandwiched between about 30 bp. (dC-dG) sequences at each end. Analyses of Raman spectra show that in 4.5 M NaCl, B to Z junctions exist between the dC-dG segments and the 95 bp. central region. Intensities of several base ring vibrational bands indicate that the junction is short. Base pair stacking in the 95 bp. segment appears $\overline{B}$ like, whereas the dC-dG regions are in the $\overline{Z}$ conformation. Yet a deoxy ribose-phosphate vibration, characteristic of $\overline{B}$ conformation, has an intensity 85% $\pm$ 15% less than expected if the 95 bp. region was in a $\overline{B}$ conformation. This indicates that $\overline{Z}$ DNA distorts the backbone conformation of neighboring regions although it does not have the same effect on base pair stacking. The transition of the (dC-dG) segments is this DNA from $\overline{B}$ conformation in 0.1 M NaCl to $\overline{Z}$ conformation at 4.5 M NaCl is currently being examined.

Progress was also made in the isolation of milligram amounts of several DNA restriction fragments from the plasmid pBR322. Five DNA fragments of length less than 500 bps. have been obtained. Raman spectroscopy and circular dichroism studies

are underway comparing the normalized intensities of vibrational bands of these and other naturally occurring DNAs with two previously isolated DNA fragments, 144 bps. and 64 bps., from the transcription start region of the E. coli lactose operon. Thermal denaturation studies of these fragments show good agreement with theoretical predictions of the melting behavior for DNA lengths 267 bps. or less. The lack of agreement for a 457 bp. DNA indicates that the current thermodynamic description of internal loop formation is inadequate.

The technique of strand separation of short restriction fragments using alkaline RPC-5 chromatography has met with preliminary success. We have been able to separate and purify small amounts of the two strands of the 144 bp. lactose operon fragment. Initial problems were compounded by the fact that previously published procedures were irreproducable in my laboratory as well as others. The separated strands will be used to incorporate nucleotide analogues (at specific locations by DNA repair synthesis).

Specific Objectives for coming year

The first objective is to complete the detailed comparison of the Raman and circular dichroism spectra of DNA fragments which do or do not contain promoter regions. Much of data has been obtained. Analysis of the conformationally sensitive Raman vibrational bands will be carried out.

A second objective is to employ alkaline RPC-5 chromatography to separate the strands of the lactose operon fragments 144 bp. and 64 bp. long and reconstruct site specific mutations within the binding regions of RNA polymerase. Gel electrophoresis binding assays are currently being developed using RNA polymerase, the catabolite activator protein and the wild type promoter fragments already isolated.

A third objective is to study the conformational transition of the (dC-dG) region of the 157 bp. DNA from its $\overline{B}$ to $\overline{Z}$ conformation. Quantitative analysis of the Raman data will help provide information about the conformational pathway of this transition.