GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: March 18, 1980

Project Title: Organization and Expression of the Genome of Phage T4

Project No: G-32-A04

Project Director: Dr. Dwight H. Hall

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, Maryland 20014

Agreement Period: From March 1, 1980 Until February 28, 1981 (04 year)

Type Agreement: Grant No. 2 RO1 GM24455-04

Amount: $79,416 PHS Funds (G-32-A04)
4,180 GIT Contribution (G-32-330)
$83,596 TOTAL

Reports Required: Annual Progress Reports with Continuation Applications; Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

Technical Matters	Contractual Matters (thru OCA)
Mr. David Beck Program Administrator National Institute of General Medical Sciences Bethesda, Maryland 20014 (301) 496-7175	D. McNish/B. Spinks Grants Management Specialists Office of Associate Director for Program Activities National Institute of General Medical Sciences Bethesda, Maryland 20014 (301) 496-7166

NOTE: CONTINUATION OF G-32-A03 (03 Budget Period)

Defense Priority Rating: None

Assigned to: Biology (School/Laboratory)

COPIES TO:

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GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 3/3/81

Project Title: Organization and Expression of the Genome of Phage T4

Project No: G-32-A04

Project Director: Dr. Dwight H. Hall

Sponsor: DHEW/PHS/NIH - Nat'l Institute of General Medical Sciences; Bethesda, Maryland 20014

Effective Termination Date: 2/28/81 (end of 04 year)

Clearance of Accounting Charges: ---

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
x Other Annual Report of Expenditures due by 5/31/81

NOTE: Follow-on project (05 year) is G-32-A05

Assigned to: Biology (School/~~Laboratory~~)

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CA-4 (1/79)

G-32-1444

SECTION IV Dwight H. Hall

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM 24455-05
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) Hall, Dwight H.	PERIOD COVERED BY THIS REPORT
	FROM 3/1/80 — THROUGH 12/15/80
NAME OF ORGANIZATION Georgia Institute of Technology	

TITLE (Repeat title shown in Item 1 on first page)
Organization and Expression of the Genome of Phage T4

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. (a) Dwight H. Hall, R. Geoffrey Sargent, Kenneth F. Trofatter, and Diane L. Russell. Journal of Virology, 36, 103 (1980). Suppressors of Mutations in the Bacteriophage T4 Gene Coding for Both RNA Ligase and Tail Fiber Attachment Activities.

Paul M. Macdonald and Dwight H. Hall. Abstracts of papers presented at the Bacteriophage Meeting, p. 28 (1980). Mutations in Bacteriophage T4 Genes 41 and 61 Cause Folate Analog Resistance.

(b) Dwight H. Hall and Ronald D. Snyder. Genetics (in press, 1980). Suppressors of Mutations in the rII Gene of Bacteriophage T4 Affect Promoter Utilization.

2. None

3. The long-term goal is to reveal and explain how the genome of phage T4 is organized, why it is organized that way, and how the sequential expression of the genome is regulated. This is being approached by isolating and studying T4 mutants affecting genes that determine related enzymes, namely those involved in the biosynthesis of thymidylic acid in T4-infected Escherichia coli. The aims of the research are:

(a) to study the relation of these genes to each other and the regulation of their expression,
(b) to further characterize the genes in which mutants have been found, and
(c) to isolate and study mutants in more T4 genes controlling enzymes involved in the synthesis of thymidylate.

The goals set for the current year were:

(a) to continue to characterize regulatory far and hus mutants genetically and biochemically, especially those that affect the regB gene,
(b) to further study our temperature-sensitive far mutants, and
(c) to further understand the mechanism of suppression in our false revertants of mutants in gene 63 and its relation to the RNA ligase activity of the gene 63 product.

We have continued to study our folate analog resistant mutant farP13 and other mutants affecting the regB gene. Studies of the growth of these T4 mutants on E. coli mutants with known ribosomal defects indicate that the regB gene product interacts with the ribosomes. Ann Dershowitz has found that these T4-induced ribosomal alterations can selectively change T4 gene expression in the infected cell. For example, on one strain of E. coli there is reduced expression of delayed early genes by both wild type T4 and regB mutants but there is reduced expression of an immediate early gene only by the regB mutant.

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SECTION IV - SUMMARY PROGRESS REPORT (Continued)

We have studied one of our temperature-sensitive far mutants, farP129, and found that it has a mechanism of folate analog resistance quite different from any far mutants we have characterized previously. Some of our results with this mutant are summarized in the following abstract of a talk given at the August, 1980 Bacteriophage Meeting at the Cold Spring Harbor Laboratories in New York.

MUTATIONS IN BACTERIOPHAGE T4 GENES 41 AND 61 CAUSE FOLATE ANALOG RESISTANCE. Paul M. Macdonald and Dwight H. Hall, School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332

Plating techniques that eliminate T4 plaque formation on Escherichia coli by folate analog inhibition of dihydrofolate (FH_2) reductase allow the isolation of folate analog resistant (far) mutants to T4. Many of these far mutants have altered synthesis or structure of the T4-induced FH_2 reductase and some of the other mutants are ribonucleotide reductase deficient. In order to find new types of T4 mutants, we have isolated far mutants at 30°C and screened for those unable to grow at 43°C in the absence of folate analogs. Only one mutant, farP129, out of over 100 tested, has a single mutation causing both folate analog resistance and temperature sensitivity. This mutant induces normal levels of FH_2 reductase (coded by the frd gene) and appears to have normal expression of other T4 genes at 30°C. The results of mapping and complementation studies indicate that the farP129 mutation is in gene 41. Like other mutations in gene 41, farP129 reduces phage-induced DNA synthesis to about 15% that of wild type T4 as measured by thymidine incorporation under restrictive conditions. Surprisingly, four other ts mutants defective in gene 41 (Caltech collection), of four tested, are also far. Three mutants defective in gene 61 have been tested and all are far whereas some other mutants defective in DNA synthesis are not far. The phenotypes of double mutants carrying mutations in genes 41 and 61, 41 and frd, or 61 and frd suggest that the products of these genes interact.

Our studies of the suppressors of mutations in gene 63 indicate that the mechanisms of suppression include delayed lysis and faster addition of tail fibers. The genes that the suppressors do affect do not have functions with any obvious relation to the RNA ligase activity of the gene 63 product. It appears that the major physiological role of the gene 63 product is the attachment of tail fibers.

SECTION IV - SUMMARY PROGRESS REPORT (Continued)

Our studies are providing new types of T4 mutants affecting related functions. Characterization of these mutants, some of which are regulatory, is leading to a better understanding of the organization and expression of the T4 genome.

A knowledge of the mechanisms involved in the control of phage development in infected bacteria should be helpful in the analysis of growth and development of higher organisms. It is conceivable that in some cases very similar mechanisms may be found. For example, it has been suggested that some of the enzymes involved in thymidylate synthesis are under coordinate control in mammalian cells just as they may be in T4-infected E. coli. An understanding of the phage genome may help in studying controlled and uncontrolled growth in higher organisms.

Some mutants are being recognized by their resistance or sensitivity to inhibitors of nucleotide metabolism, such as hydroxyurea and folate analogs, and are providing new insights into mechanisms of drug action and resistance.

The research goals for the coming year are:

(a) to further characterize regulatory far and hus mutants genetically and biochemically, especially those that affect the regB gene,
(b) to further understand the mechanisms of suppression in our false revertants of mutants in gene 63 and its relation to the RNA ligase activity of the gene 63 product and to the pseT gene.
(c) to isolate and characterize more far mutants of different types, particularly temperature-sensitive mutants and mutants derived from E. coli strains containing T4-plasmid hybrids.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

12-18-80

Date

Principal Investigator