**PROJECT ADMINISTRATION DATA SHEET**

**Project No:** G-32-626 (R6117-1A0)

**Project Director:** D. H. Hall

**Sponsor:** DHHS/PHS/NIH/NIGMS

**Type Agreement:** Grant No. 1R01-GM36714-01

**Award Period:** From 4-1-86 To 3-31-87 (Performance) 6-31-87 (Reports)

**Sponsor Amount:**
- **Estimated:** $  
- **Funded:** $ 80,382

**Cost Sharing Amount:** $ 4,653

**Title:** Genetics of the Intron-Containing TD Gene of Phage T4

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**ADMINISTRATIVE DATA**

<table>
<thead>
<tr>
<th>Sponsor Technical Contact</th>
<th>Sponsor Admin/Contractual Matters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Barbara R. Williams</td>
<td>Dona McNish</td>
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<tr>
<td>National Institutes of Health</td>
<td>National Institutes of Health</td>
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<td>National Institute of General Medical Sciences</td>
<td>National Institute of General Medical Sciences</td>
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<tr>
<td>Grant Management Office</td>
<td>Grants Management Office</td>
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<tr>
<td>Bethesda, MD 301/496-7087</td>
<td>Bethesda, MD 301/496-7166</td>
</tr>
</tbody>
</table>

**Defense Priority Rating:** N/A

**Military Security Classification:** N/A

**RESTRICTIONS**

- 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 GIT

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**COMMENTS:**

- No Funds may be expended after 3/31/87

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**COPIES TO:**

- Project Director
- Research Administrative Network
- Research Property Management
- Accounting
- Procurement/EES Supply Services
- Research Security Services
- Reports Coordinator (OCA)
- Research Communications (2)
- GTRI
- Library
- Project File
- Other

*FORM OCA 4:383*
SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 5-22-87

Project No. G-32-626

School/XX Applied Biology

Includes Subproject No.(s) N/A

Project Director(s) D.H. Hall

GTRC / Sponsor

DHHS/FHS/NIH/NIGMS

Title Genetics of the Intron-Containing TD Gene of Phage T4

Effective Completion Date: 3/31/87 (Performance) 6/31/87 (Reports)

Grant/Contract Closeout Actions Remaining:

☐ 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-32-639

continued by Project No. G-32-639

COPIES TO:

Project Director
Research Administrative Network
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Accounting
Procurement/GTRI Supply Services
Research Security Services

Library
GTRC
Project File
Other

Duane H.
Angela DuBose
Russ Embry

FORM OCA 69.285
Publications


Report

1. There has been no change in the general scientific goals of the project.

2. We have been genetically mapping and biochemically characterizing many mutations affecting the expression of the thymidylate synthase and dihydrofolate reductase genes. Our main results are summarized in the following abstract of a presentation given at the 1986 meeting on Molecular Genetics of Bacteria and Phages at the Cold Spring Harbor Laboratory in New York.

TWO SPLICING DOMAINS IN THE INTRON OF THE PHAGE T4 td GENE ESTABLISHED BY NON-DIRECTED MUTAGENESIS. Dwight H. Hall*, Christine M. Povinelli*, Karen Ehrenman**, Joan Pedersen-Lane** and Marlene Belfort**. *School of Applied Biology, Georgia Tech, Atlanta, GA 30332; **Wadsworth Laboratories, N.Y. State Dept. of Health, Albany, NY 12201; "Dept. of Microbiology and Immunology, Albany Medical College, Albany, NY 12208.

The T4 gene (td) coding for thymidylate synthase contains a group I intron similar to the intron in the *Tetrahymena* self-splicing rRNA. We are using the T4 td
gene for genetic analysis of a group I intron. A random saturation mutagenesis approach directed against the intact phage genome was facilitated by rapid phenotypic screening methods for T4 td phage. These non-directed mutations are being localized by recombinational mapping using marker rescue in cells containing defined td subfragments. Among 97 nitrous acid or hydroxylamine induced td mutations that have been localized, 27 map to the intron. None of the mutations are in the middle of the 1017 nucleotide (nt) intron, as defined by a 635 nt deletion. Both this deleted td gene, which is splicing proficient, and the point mutations indicate that the functional domains for splicing in the intron are located within 200 nt of the two intron-exon boundaries.

The base change has been determined for one 5' (T4 tdN57) and one 3' mutant (T4 tdN47). N57 is a C to T transition 10 nt from the 5' splice site in the putative internal guide sequence, while N47 is a G to A transition 49 nt from the 3' splice site in a region of potential secondary structure. Whereas N57 results in greatly reduced levels of cleavage at the 5' splice site, N47 causes both diminished and inaccurate cleavage at this site. A molecular consequence of the 3' N47 mutation therefore appears to be manifested almost 1,000 nt upstream, at the 5' splice site, probably reflecting disruption of the secondary structure of the intron. Further analysis of these and other mutants in the collection will, without any a priori structure-function assumptions, delineate those sequences important in the group I splicing pathway.

Our studies are providing new types of T4 mutants affecting RNA splicing. Characterization of these mutants will lead to a better understanding of the mechanism and the role of RNA splicing in T4-infected E. coli. A knowledge of the mechanisms involved in RNA splicing in phage-infected bacteria should be helpful in the analysis of RNA splicing in eukaryotes. It is likely that in some cases very similar mechanisms will be found.

3. The specific objectives for the coming year are:

   a. to further characterize more td and nrd mutations from our collection genetically and biochemically, especially those that affect RNA splicing, and

   b. to isolate and characterize false revertants of td and nrdB mutants defective in RNA splicing.