GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

CONT. OF G-32-626 [X] ORIGINAL [ ] REVISION NO. _____

Project No./(Center No.) G-32-639 R6117-2A0 GTRC/~~GTX~~ DATE 4 / 20 / 87

Project Director: Dr. Dwight H. Hall School/~~Lab~~ Biology

Sponsor: DHHS/PHS/NIH/National Institute of General Medical Sciences

Agreement No.: Grant No. 5 R01 GM36714-02

Award Period: From 4/1/87 To 3/31/88 (Performance) 6/30/88 Reports

Sponsor Amount:	New With This Change	Total to Date
Contract Value:	$	$
Funded:	$	$ 89,564

Cost Sharing No./(Center No.) F-32-343/(F6117-1A0) Cost Sharing: $ 4,862

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

ADMINISTRATIVE DATA OCA Contact E. Faith Gleason x4-4820

1) Sponsor Technical Contact:

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National Institutes of Health
Westwood Building Rm 939
Bethesda, MD 20892

Military Security Classification: _____ ONR Resident Rep. is ACO: _____ Yes X No

(or) Company/Industrial Proprietary: _____ Defense Priority Rating: _____

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 GIT

COMMENTS:

Continuation of G-32-626

SPONSOR'S I.D. NO. 02.108.001.87.019

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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 4/18/88

Project No. G-32-639 School/~~Lab~~X Biology

Includes Subproject No.(s) N/A

Project Director(s) D. H. Hall GTRC/GIT

Sponsor DHHS/PHS/NIH

Title GENETICS OF THE INTRON-CONTAINING TD GENE OF PHAGE T4

Effective Completion Date: 3/31/88 (Performance) 6/30/88 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [X] Final Invoice or Copy of Last Invoice Serving as Final
- [ ] Release and Assignment
- [ ] Final Report of Inventions and/or Subcontract:
  Patent and Subcontract Questionnaire sent to Project Director [ ]
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. Continued by Project No. G-32-646

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Other

Dwight H. Hall

SECTION IV **PROGRESS REPORT SUMMARY**	GRANT NUMBER GM36714-03	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Dwight H. Hall	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	4/1/87	1/27/88

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Genetics of the Intron-Containing td Gene of Phage T4

(SEE INSTRUCTIONS)

1. There have been no changes 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 ribonucleotide reductase genes. Our main results are summarized in the following abstract of a presentation we will be giving at the UCLA Symposium on Molecular Biology of RNA in Keystone, Colorado, in April 1988.

CLUSTERING OF MUTATIONS AND A SECOND-SITE REVERTANT IN THE GROUP I td INTRON OF BACTERIOPHAGE T4, Dwight H. Hall[1], Michael D. Brown[1], Christine M. Povinelli[1], Deborah Bell-Pedersen[2,3], Karen Ehrenman[2,4], and Marlene Belfort[2]; [1]School of Applied Biology, Georgia Tech, Atlanta, GA 30332; [2]Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201; [3]Dept. of Biological Sciences, SUNY Albany, Albany, NY 12222; [4]Dept. of Microbiology and Immunology, Albany Medical College, Albany, NY 12208.
The isolation and genetic characterization of a large collection of T4 thymidylate synthase-defective (td) mutants implicated the outer boundaries of the 1-kb intron (approximately 220 nucleotides at each end) as the domains functional in splicing (Hall et al., Cell 48, 63, 1987). The sequence changes of 17 mutations that map in or near the intron and have been shown to cause a splicing-defective phenotype have been determined. While the mutations lie scattered throughout the two domains, there is a marked clustering of mutations, each of independent origin, at specific sites. There are two mutations at the same residue in the consensus S element, three at the same site in the phylogenetically conserved P9 stem, and seven mutations in two adjacent residues that may form part of the P6 pairing. Isolation of spontaneous second-site suppressor mutations, using classical phage genetic strategies, is in progress. One false revertant of a mutation in P3[3'], HS9, has the following properties: 1) HS9 grows less well than td^+ in limiting thymidine at 37° and is temperature sensitive; 2) when HS9 is backcrossed to td^+, recombinants with the phenotype of the original td mutant are produced; 3) HS9 contains the original mutation by RNA sequence analysis; 4) HS9 is proficient in RNA splicing by dot blot hybridization analysis; and 5) HS9 has a second-site mutation in P3[5'] that supresses the splicing defect of the original mutant and supports the secondary structure model of the td intron.

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.

SECTION IV - PROGRESS REPORT SUMMARY (Continued)

3. Not Applicable.

4. Not Applicable.

5. Publications.

Belfort, M., J. Pedersen-Lane, K. Ehrenman, D.H. Hall, C.M. Povinelli, J.M. Gott and D.A. Shub, 1987. Processing and genetic characterization of self-splicing RNAs of bacteriophage T4. Molecular Biology of RNA: New Perspectives (Academic Press, Inc.): 45-66.

Brown, M.D., D.H. Hall, C.M. Povinelli, K. Ehrenman, J. Pedersen-Lane, and M. Belfort, 1987. Mutations affecting RNA splicing in the phage T4 thymidylate synthase gene. Abstracts of the Annual Meeting of the American Society for Microbiology:238.

Ehrenman, K., M. Belfort, C.M. Povinelli, and D.H. Hall, 1987. Clustering of mutations in the group I td intron of bacteriophage T4. Abstracts of Papers Presented at the Meeting on RNA Processing (Cold Spring Harbor Laboratory, NY): 48.

Brown, M.D., D.H. Hall, D. Bell-Pedersen, K. Ehrenman, and M. Belfort, 1987. Supressors that relieve the splicing-defective phenotype of intron mutations in the thymidylate synthase gene of phage T4. Abstracts of the Evergreen International T4 Meeting:27.

Ehrenman, K., M. Belfort, C.M. Povinelli, and D.H. Hall, 1987. Clustering of mutations in the group I td intron of bacteriophage T4. Abstracts of the Evergreen International T4 Meeting:26.

Ehrenman, K., M. Belfort, C.M. Povinelli, and D.H. Hall, 1987. Clustering of mutations in the group I td intron of bacteriophage T4. Abstracts of Papers Presented at the Meeting on Molecular Genetics of Bacteria and Phages (Cold Spring Harbor Laboratory, NY):55.

Hall, D.H., M.D. Brown, C.M. Povinelli, D. Bell-Pedersen, K. Ehrenman, and M. Belfort, 1988. Clustering of mutations and a second-site revertant in the group I td intron of bacteriophage T4. Abstracts of the UCLA Symposium on Molecular Biology of RNA. (in press)