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PROJECT ADMINISTRATION DATA SHEET

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Project No. G-32-A06 DATE 3/24/82

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

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

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Award Period: From 3/1/82 To ~~2/28/83~~ 2/29/84 (Performance) 5/31/83 (Reports)

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Cost Sharing: $ 4,532 (G-32-336) GTRI/GIT

Title: Organization and Expression of the Genome of Phage T4

ADMINISTRATIVE DATA OCA Contact Faith G. Costello

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Defense Priority Rating: NA Security Classification: NA

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

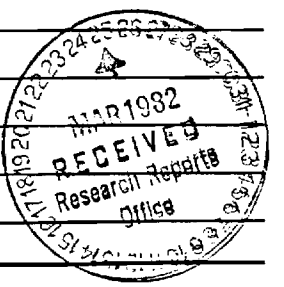
Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT usually; however, we are accountable for all equipment purchased.

COMMENTS:

06 year of continuing grant - continuation of G-32-A05

*includes $1,96e unobligated direct funds balance from 04 year.



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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 6-4-87

Project No. G-32-A06 School/~~Lab~~ Biology

Includes Subproject No.(s) N/A

Project Director(s) Dr. Dwight Hall GTRC / ~~GIT~~

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

Title Organization and Expression of the Genome of Phage T4

Effective Completion Date: 2/29/84 (Performance) 5/29/84 (Reports)

Grant/Contract Closeout Actions Remaining:

- [X] None
- [ ] Final Invoice or Final Fiscal Report
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Transitional control could be achieved by alterations in ribosomes. It has been shown that new T4-induced proteins become associated with ribosomes during infection (59, 60). Studies using ribosomes from T4-infected cells for in vitro protein synthesis have indicated that there are changes in ribosome function during infection (61, 62). Revel et al. (63) have presented evidence that an interference factor is modified early after T4 infection. This interference factor affects the ribosomal initiation factors and therefore the ability of the ribosomes to translate certain mRNAs. These changes in the ribosomes are apparently not essential for T4 growth because late T4 message can be translated in vitro using host ribosomes and factors (64). Translation could also be controlled by the sequence or availability of ribosome binding sites. A ribosome binding site could be buried in secondary structure or covered by a translational repressor as reviewed by Gold et al. (65).

A mutant of T4 which lacks a post-transcriptional regulatory process has been isolated by Wiberg et al. (66). The mutant, SP62, defines a new T4 gene regA and affects the shutoff of early protein synthesis. Karam and Bowles (67) have independently isolated a similar mutant. The extended synthesis of early proteins due to the SP62 mutation is enhanced when a mutation (DO) preventing DNA synthesis is also present in the phage genome. Cells infected with a SP62-DO double mutant overproduce immediate early and delayed early gene products, but at least one example of each class is not overproduced. Since DNA synthesis is known to be involved in the shutoff of early gene expression (29), the SP62 mutation appears to affect a second mechanism for shutoff of early genes which acts in concert with, but independent of the shutoff related to DNA synthesis (66). Karam et al. (68) have identified the regA target site in the T4 rIIB mRNA. The target overlaps the translational initiation region suggesting that the regA protein is a repressor that acts at the level of initiation of translation.

Johnson and Hall (69, 70) have isolated regulatory mutants of T4 by screening for resistance to folate analogs. These mutants, which include farP85 mentioned above, overproduce the phage induced dihydrofolate reductase and several other early enzymes. The overproduction of dihydrofolate reductase is not affected by the absence of DNA synthesis. The mutants show delays in DNA synthesis, phage production, and lysis, and appear to show decreased levels of RNA synthesis. A second type of folate analog resistant (far) mutants that overproduce dihydrofolate reductase have been investigated by Chace and Hall (55). Mutants of this second type appear to have altered expression of fewer genes than the mutants previously described and may affect translation. The gene whose mutation causes overproduction of dihydrofolate reductase in the second type of mutants has been named regB and it is located between the rI and lysozyme genes distant from any of the previously discussed regulatory genes.

C. Progress Report:

Period: June 1, 1979 to May 15, 1982

Professional Personnel:

Dwight H. Hall, Associate Professor, June 1, 1979-present (67%).
Ann J. Dershowitz, Research Scientist I, June 1, 1979-present (50%).
Regine E. Hay, Postdoctoral Fellow, November 15, 1981-February 26, 1982 (80%).

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 in genes that affect the structure or synthesis of related enzymes, namely those involved in the biosynthesis of thymidylic acid in T4-infected Escherichia coli. The specific 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.

We have continued to isolate T4 mutants which produce plaques with a white halo around them when plated on E. coli 0K305, a uracil-requiring strain which is also unable to deaminate cytidine on Petri dishes with synthetic media containing cytidine as the only pyrimidine source. We have used hydroxylamine to produce transition mutants and proflavin

to produce frameshift mutants with this "white halo" phenotype. All of these mutants tested are unable to induce either thymidylate synthetase (td mutants) or dihydrofolate reductase (frd mutants) or both and are very closely linked to each other. Some of the hydroxylamine induced td and frd mutants are suppressible by amber suppressors; that is, when they are plated on a derivative of E. coli OK305 carrying an amber suppressor, their "white halo" phenotype is reduced or eliminated. None of the mutants tested, including the ambers, show any strong polar effects on the other genes in the cluster as judged mostly by their phenotype under various plating conditions.

Some of the amber suppressible td mutants are being mapped carefully in order to be used to study the structure and function of the td gene and its product. The mapping is done by three-factor crosses using one mutation in the frd cistron, which seems to be adjacent to the td cistron. By crossing a frd tdx double mutant with a single mutant tdy selecting for td^+ recombinants, and testing whether these td^+ are frd or frd^+ one can usually determine which of the two td mutations is closer to the frd cistron. The map order of four suppressible td mutants relative to each other and to the frd cistron has been determined.

We have also used hydroxylamine and proflavin to produce false revertants of frd and td mutants. This was done by mutagenizing a frd or td mutant and looking for phage that had lost the "white halo" phenotype. Such phage are almost never true revertants but are instead double mutants containing the original mutation and a new suppressor mutation that somehow prevents the formation of the "white halo." All suppressor mutants tested are unable to induce either ribonucleotide reductase (nrd mutants) or deoxycytidylate deaminase (cd mutants) and are closely linked to each other. This seemed to be a good way to isolate cd mutants but unfortunately (and surprisingly) less than 10% of these mutants are cd. We have therefore developed a new plating technique to recognize cd mutants. This method involves plating T4 on plates containing 5-fluorodeoxyuridine (to make thymidylate limiting in the infected cell) and also 5-methyldeoxycytidine under conditions such that it can be converted to thymidylate only if the phage can induce deoxycytidylate deaminase. We are in the process of isolating more cd mutants, using a combination of the two methods above. These mutants will be used for complementation studies to find out whether there is more than one cd cistron. Mapping studies indicate all these genes are clustered and in the order cd, nrd, td, and frd. None of the cd or nrd mutants tested show any strong polar effects on the other genes in the cluster. Recently, we have found that some alc mutants have deletions that extend into the cd region. These mutants are also being used to study the structure and function of the cd region.

Weil and Terzaghi (71) have reported a general means of selecting for T4 strains which carry deletions of nonessential regions of the genome. Homyk and Weil (72) have found that some deletions produced in this way map in the td region. We have shown that some of these deletions are frd-td-nrd mutants and others are td-nrd mutants and that some of the latter deletions may extend into the nearby denA gene. These deletion mutan do not grow as well as wild type T4 but they do grow, indicating that the absence of th combinations of genes above is not lethal.

Five tk (thymidine kinase) mutants have been isolated which are suppressible by amber suppressors. These nonsense mutants map at three distinct sites within the tk g and the map order of these mutants relative to each other and the rI gene is being det mined for use in studying the structure and function of the tk gene. Two of the suppressible tk mutants have been shown to induce a more heat-labile thymidine kinase than that induced by wild type T4 after infection of an E. coli strain carrying an am suppressor. These results indicate that the tk gene is the structural gene for the T induced thymidine kinase. Five other tk mutants are deletions which extend at least the rI gene and up to fifteen map units beyond the rI gene. The existence of these deletions indicate that there is a large nonessential region of the genome near rI. deletions have been used to map the T4 vs gene, which controls the modification of t host valyl-tRNA synthetase, near the tk gene.

We have started using recombinant DNA techniques and have created and partiall characterized a T4 genome library consisting of E. coli strains containing hybrids and plasmid pBR322. We have found and characterized clones carrying the T4 dihydr

reductase gene (frd). DNAs of phage T4 (containing C in place of HMC) and plasmid vector pBR322 were cut to completion with the restriction endonuclease HindIII, ligated and used to transform the E. coli K strain ED8689 to ampicillin resistance. Those cells that had acquired the plasmid conferred ampicillin resistance but not tetracycline resistance were assumed to contain T4 HindIII fragments inserted into the Tet region. These cells were tested for resistance to trimethoprim, a folate analog that inhibits E. coli dihydrofolate (FH_2) reductase more than it inhibits T4 FH_2 reductase. Two clones out of 250 $Amp^R Tet^S$ cells tested exhibited resistance to trimethoprim. Cell growth curves in the presence of trimethoprim showed that one clone, ED8689/pGS38, was about 1000 fold more resistant to the drug than ED8689/pBR322 cells and that the other clone, ED8689/pGS64, had a lower resistance. Measurement of FH_2 reductase activity of crude extracts from ED8689 cells carrying different plasmids showed a 4-fold higher specific activity for cells carrying pGS38 than for cells carrying pGS64 or pBR322, which had similar activities. However, at a trimethoprim concentration that abolished FH_2 reductase activity in extracts of cells carrying pBR322, activity was detected in extracts of cells carrying either pGS38 or pGS64. Infection of cells carrying pGS38 or pGS64 by a T4 mutant containing a mutation in the structural gene (frd) for FH_2 reductase produces some frd^+ phage by recombination. This marker rescue indicates the presence of a phage sequence in each clone that contains all or part of the T4 frd gene. Isolation of pGS38 DNA and cleavage with HindIII yielded a fragment of about 1000 base pairs, approximately the size of the smallest active fratment thought to code for the T4 FH_2 reductase. Analysis of the fragments produced by restriction endonuclease EcoR1 has been used to demonstrate the orientation of the fragment. Genetically engineered derivatives of pGS38 are being constructed to investigate the expression of the frd gene.

It has been shown by Hänggi and Zachau (23) that the smallest 1000 bp. HindIII fragment will not synthesize an active FH_2 reductase in an in vitro transcription-translation system. EcoR1 cleavage was also shown to inactivate in vitro synthesis of active FH_2 reductase. Mileham et al. (24) as well as our lab have isolated clones carrying the 1000 base pair HindIII fragment, synthesizing active T4 FH_2 reductase. Since the inactivating HindIII site is at the 5' edge of the Hänggi "active" fragment it is possible that ligating this fragment back into another HindIII site reconstitutes an essential part of the structural gene or part of the frd promoter. This is especially interesting since the HindIII site on pBR322 is adjacent to the 5' end of the Tet^R gene Pribnow box. We are preparing to study the structure, function, and expression of the frd gene by generating subclones of pGS38 and using them for physical mapping of different frd mutants. For example, EcoR1 cuts the HindIII fragment into two pieces each of which can marker rescue at least two different frd mutants.

We have found that pGS64 has a second HindIII fragment which contains at least parts of T4 genes 29 and 48, The orientation of the frd gene in pGS64 is the opposite of its orientation in pGS38 (see Figure on Page 1 of Appendix). The expression of the frd gene in pGS64 appears to depend on the presence of the second T4 fragment.

The dihydrofolate (FH_2) reductase specified by the T4 frd gene is apparently non-essential for phage growth on E. coli because the bacterial FH_2 reductase can partially substitute for the phage enzyme. To study the in vivo effect of folate analogs which specifically inhibit the T4 FH_2 reductase, the enzyme was made essential for phage production. Partial inhibition of the E. coli FH_2 reductase with the folate analog trimethoprim strongly inhbiits phage production by a T4 frd mutant and slightly inhibits wild type phage production. Adding an additional T4-specific folate analog, a chlorophenyl triazine, strongly inhibits wild type phage production without preventing the uninfected E. coli cells from multiplying.

We have isolated spontaneous mutants of T4 which are capable of producing progeny in the presence of chlorophenyl triazine and another folate analog pyrimethamine. These mutants are designated folate analog resistant (far) and have been separated into two general classes. Class I mutants induce T4-specific FH_2 reductases which are less sensitive to the action of the folate analogs than the normal FH_2 reductase. Class II mutants induce an unaltered FH_2 reductase but contain mutations in a variety of T4 genes.

Some class II far mutants overproduce the phage induced FH_2 reductase. Deoxycytidylate deaminase, thymidine kinase, and deoxycytidine triphosphatase (dCTPase) and other proteins are also overproduced by 20 minutes after infection at 37°C but some proteins are underproduced. The overproduction of FH_2 reductase by these far mutants is not affected by the absence of DNA synthesis. Other types of mutations which affect the synthesis of early enzymes cause overproduction in the absence of DNA synthesis of some of the above enzymes, but not of FH_2 reductase. Therefore overproducing far mutants, such as farP85, apparently have mutations in a previously undescribed gene controlling the expression of the T4 genome.

In collaboration with Hercules and Sauerbier, it has been shown that farP85 is in the mot gene and affects the utilization of certain promoters. The mot gene function is not required for T4 growth on most hosts, but we have found that it is required for good growth on E. coli CTr5x. Homyk, Rodriguez, and Weil (73) have described T4 mutants, called sip, which partially suppresses the inability of T4rII mutants to grow in λ lysogens. We have found that mutants sip1 and sip2 are resistant to folate analogs, overproduce FH_2 reductase and grow poorly on E. coli CT45x. The results of recombination and complementation studies indicate that sip mutations are in the mot gene. Homyk also described L mutations that reverse the effects of sip mutations. L_2 decreases the folate analog resistance and the inability to grow on CT45x of sip2. L_2 itself is partially resistant to a folate analog. These results suggest that L_2 affects another regulatory gene related to the mot gene. The effects of sip and L on the synthesis of T4-induced proteins are being studies to learn more about the regulation of T4 gene expression.

We have found a second type of far mutants that overproduce FH_2 reductase but not deoxycytidylate deaminase or dCTPase. These mutants are unable to induce thymidine kinase and have an r phenotype and are deletions covering the tk and rI genes. Overproduction of dihydrofolate reductase by the new mutants occurs because enzymatic activity continues to increase for a longer period of time in cells infected by the mutants than in cells infected by wild type phage. This continued increase occurs even in the presence of rifampin, indicating that the overproduction is probably due to a post-transcriptional event. Both these new overproducers and the previously described overproducers have been studied by using polyacrylamide gel electrophoresis. The two types of overproducers appear to be very different. The new overproducers do not show the delay in the synthesis of some proteins and only overproduce a few proteins. The new gene defined by the new overproducers is between the gene coding for thymidine kinase and the gene coding for lysozyme and is called regB. Since our results suggested that the regB gene product might affect the ribosomes of infected E. coli, we have obtained E. coli mutants with known ribosomal defects from Dr. Scott Champney at the University of Georgia. These mutant strains are defective in protein synthesis at high temperature (74). We have found that in some of these E. coli mutants wild type T4 can grow at high temperature if the early part of the infection is at low temperature. This suggests that T4 coded protein synthesized early in infection alters the ribosomes. Regulatory mutants of T4 defective in the regB gene, such as farP13, grow very poorly at high temperature on at lease one of these E. coli mutants (SK1048) under the same conditions (see Table on page 2 of Appendix). These results support the suggestion that the regB gene product may affect the ribosomes of infected E. coli. The availability of different E. coli mutants with known ribosomal defects should make it possible to study this interaction in detail. For example, two E. coli mutants on which regB mutants grow very poorly have alterations in ribosomal protein L22. The regB mutants can grow on a revertant of one of the E. coli mutants which makes a normal L22 protein. Ann Dershowitz has found by enzyme assay and electrophoretic analysis that these T4-induced ribosomal alterations can selectively change T4 gene expression. 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 muzant. We have started using 2-dimensional gels in order to extend and refine this analysis and to identify the regB gene product(s). We have been able to separate many proteins and to detect many differences in farP13-infected cells (see Figure on Page 3 of Appendix). The synthesis of T4-induced proteins on these mutant cells at different temperatures is being

studied to learn more about the regulation of T4 gene expression.

Some of the far mutants do not appear to affect either the structure or the synthesis of the T4 FH_2 reductase. Some of these mutants are unable to induce ribonucleotide reductase. The requirement of stoichiometric amounts of N^5, N^{10}- methylenetetrahydrofolate for the biosynthesis of thymidylate and the by-product FH_2 makes folate analog inhibition of the FH_2 reductase an effective method for depleting all tetrahydrofolate derivatives. Therefore, any mechanism an organism can employ to reduce the amount of thymidylate biosynthesis will conserve the pool of tetrahydrofolate derivatives and perhaps can result in folate analog resistance. Presumably the lack of the phage induced ribonucleotide reductase in these mutants causes their folate analog resistance by limiting thymidylate biosynthesis through reduction of the supply of deoxyuridylate, the substrate for thymidylate synthetase. Mutations in the bacteriophage cd or td genes do not appear to confer folate analog resistance and therefore must not limit synthesis of thymidylate as much as mutations in the nrd genes do. The mechanism of resistance of some far mutants is still not known. Some might affect the permeability of the infected cell as judged by their altered response to acridines.

In order to find new types of folate analog resistant (far) mutants, we have isolated far mutants at low temperature (30°C) and tested them for the ability to grow at high temperature (43°C) in the absence of folate analogs. Mutants that cannot grow at 43°C would define essential genes involved in regulation of gene expression and/or nucleotide metabolism and such mutants should be easier to study than previous far mutants. We have found several different mutants of this type in which the temperature-sensitivity and folate analog resistance seem to be caused by the same mutation. One of these mutations is near or in the mot gene and another is apparently in one of the genes coding for ribonucleotide reductase. A third mutation of this type is in gene 41, one of the genes coding for products required for DNA replication (28). This mutant, farP129, induces normal levels of FH_2 reductase 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 allele-specific suppression of phenotypes shown by double mutants carrying mutations in genes 41 and 61, 41 and frd, or 61 and frd indicates that the products of these genes interact. We suggest that abnormal interactions cause folate analog resistance by altering the structure of the FH_2 reductase. Preliminary results indicate that the cd gene product (dCMP deaminase) also interacts with the product of gene 41. These results suggest that there are important direct interactions in vivo between the nucleotide-synthesizing complex (19, 20) and the DNA replication complex (75). This is not surprising since high localized concentrations of dNTPs are produced by the former complex and are required by the latter complex.

Warner et al. (11) have reported the isolation of T4 mutants unable to degrade the DNA of the host by testing for ability to grow in the presence of hydroxyurea (HU). Since these mutants affect enzymes involved in thymidylate production (from the host DNA) and the mutants of Warner et al. map near the cd, nrd, rd, frd cluster, similar mutants are being isolated and studied in this laboratory. We chose to screen for hydroxyurea-sensitive (hus) mutants among mutagenized phage that make plaques on E. coli OK305 on synthetic plates containing cytidine as the only pyrimidine source and have isolated over fifty mutants. Surprisingly, none of these mutants grow very well under normal conditions (wild type E. coli and rich media) where the mutants of Warner et al. grow as well as wild type T4. Our mutants do not grow well on OK305 on synthetic plates with pyrimidines other than cytidine. We have studied eight of these hus mutants and have shown that they map at eight distinct sites and affect at least six cistrons which are scattered througout the major early region of the T4 genetic map (between gene 38 and rI). Only one of these mutants, husN, is defective in the ability to degrade E. coli DNA and it is probably a leaky mutant in gene 47. The HU sensitivity of three mutants,

hus3 (which is deficient in exonuclease A), hus7 (which is in gene 39) and hus23 (which appears to be in a new gene next to gene 39) is partially due to inefficient utilization of host-derived deoxyribonucleotides as substrates for phage DNA synthesis. This is related to the DNA delay phenotype of these 3 mutants. The mutants hus7 and hus23 also appear to package phage DNA into phage particles inefficiently. The mutants hus1 (in gene 49) and hus13 (which is next to or in gene 45) are apparently HU sensitive due to a defect in packaging DNA into phage particles. The mutant hus13 has other defects, such as delayed expression of the lysozyme gene, that suggest it is a regulatory mutant. Two other mutants (hus19 and hus20) degrade bacterial DNA normally, synthesize phage DNA normally using host-derived deoxyribonucleotides, and package the DNA into phage particles efficiently. Their HU sensitivity is due to defective lysis of the cells and we have identified them as t gene mutants (76). This unexpected finding is even more unusual in that one of the mutants (hus19) only shows defective lysis in the presence of hydroxyurea. If the effect of HU on lysis by these mutants is a consequence of the inhibition of ribonucleoside diphosphate (RDP) reductase by HU, then one might observe an effect on lysis if RDP reductase activity were removed by some other means. We have done this in the case of hus19 by constructing a double mutant with an RDP reductase deficient mutant. Surprisingly, this double mutant shows a lysis defect. In contrast, both single mutants show normal lysis. These results show that the lysis defect of hus19 may simply be a consequence of the inhibition of RDP reductase activity. Recently, we have found that the appearance of lysozyme activity is delayed if the RDP reductase activity is low, suggesting that the decreased lysozyme together with a mutations in the t gene causes the lysis defect.

Our hydroxyurea-sensitive dexA mutant, hus3, is also folate-analog-resistant, apparently due to overproduction of FH_2 reductase. The basis for this regulatory effect is not clear. We have isolated hydroxyurea-resistant derivatives of hus3 and are characterizing them genetically and biochemically to learn more about the function of the dexA gene and other related genes.

Since gene 63 is near the td gene and begins to be expressed early in infection (14), it might have a role in nucleotide metabolism. This possibility is further suggested by our finding that mutants in gene 63 fail to grow on E. coli CK305 when the cells are growing slowly on synthetic medium containing cytidine as the only pyrimidine source. About one in 10^4 phage in a lysate of a gene 63 mutant can grow on the conditions above. These phage that can grow are not wild type but are false revertants. We screened for revertants that could grow at 30°C but not at 43°C on Escherichia coli CK305 when nucleotides are limiting. These false revertants contain the original mutation in gene 63 and new suppressor mutations. Some of these suppressor mutations cause temperature sensitivity by themselves, allowing single mutants carrying the suppressor to be recognized and isolated. The results of mapping and complementation studies indicate that most of these ts suppressors are in the t gene (lysis), one is in gene 5 (baseplate), and one is in gene 18 (sheath). The mutation in gene 18 (tsDH638), suppresses three different amber mutations in gene 63 but does not suppress amber mutations in several other genes. None of the suppressors which have been characterized are in genes with known functions in nucleotide metabolism. However, an intriguing property of these false revertants is that they are very sensitive to hydroxyurea, an inhibitor of nucleotide metabolism. Geoff Sargent in this laboratory has been investigating the mechanism of suppression in these false revertants. We have been unable to detect any normal size gene 63 product in cells infected by these false revertants using SDS-polyacrylamide gel electrophoresis and autoradiography. Other ts mutants in genes 5 and 18 do not suppress amM69 (gene 63) except for one mutant in gene 18, tsA38, which appears to be at or very near the same site as our suppressor tsDH638. These ts suppressors could act by altering the phage particle such that it is infectious with fewer tail fibers than normal. Mutants with these properties have been reported by Crawford (77). We obtained three mutants of this type from Dr. Edward Goldberg at the Tufts University School of Medicine. None of these mutants, which are in genes 5, 8, and 10, suppress amM69 (gene 63). These results indicate that our ts suppressors are fairly unique mutants that suppress by as yet unknown mechanisms.

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. 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.

Publications:

"Growth of Bacteriophage T4 on Mutants of Escherichia coli with Defective Ribosomes," Abstracts of the XI International Congress of Biochemistry (1979):86, with C. E. Smith.

"Gene Expression by Bacteriophage T4 on Mutants of Escherichia coli with Defective Ribosomes," Abstracts of papers presented at the Bacteriophage Meeting (Cold Spring Harbor Laboratory, N.Y.), (1979):107, with C. E. Smith and A. J. Dershowitz.

"Mutations in Bacteriophage T4 Genes 41 and 61 cause Folate Analog Resistance," Abstracts of papers presented at the Bacteriophage Meeting (Cold Spring Harbor Laboratory, N.Y.), (1980):28, with P. M. Macdonald.

"Suppressors of Mutations in the Bacteriophage T4 Gene Coding for both RNA Ligase and Tail Fiber Attachment Activities," Journal of Virology, 36, 103-108, (1980) with R. G. Sargent, K. F. Trofatter and D. L. Russell.

"Evidence for Interactions between Proteins Involved in Initiation of Bacteriophage T4 DNA Replication," Abstract, Journal of Supramolecular Structure and Cellular Biochemistry, (Suppl. 5, 1981):341, with P. M. Macdonald.

"Suppressors of Mutations in the rII Gene of Bacteriophage T4 Affect Promoter Utilization," Genetics, 97, 1-9 (1981), with R. D. Snyder.

"Cloning and Characterization of the Dihydrofolate Reductase Gene of Bacteriophage T4," Abstracts of papers presented at the Bacteriophage Meeting (Cold Spring Harbor Laboratory, N.Y.), (1981):47, with R. G. Sargent.

"Cloning and Characterization of the Dihydrofolate Reductase Gene of Bacteriophage T4," Abstracts of the Annual Meeting of the American Society for Microbiology (1982): 131, with R. G. Sargent and C. M. Povinelli.

D. Methods:

(a) It has been shown that the T4 genes cd, nrd, td, and frd, which control the phage-induced production of deoxycytidylate deaminase, ribonucleotide reductase, thymidylate synthetase, and dihydrofolate reductase, are closely linked and may be part of a genetic unit under common regulatory control. Mutants affecting the expression of these genes are being isolated and tested for effects on translation.

Other methods of analysis that will be employed to study the relation of the cd, nrd, td, frd, and tk genes to each other and the regulation of their expression are the use of double mutants and deletions, as well as other physiological and genetic variations that may lead to an alteration in the time course of the expression of these genes in particular and also of other T4 genes.

We are using T4-plasmid hybrids as a new approach to study the organization are expression of genes in the frd-td region. Presumably it will be easier to study a small section of the T4 genome than it is to study the entire genome. As mentioned in the Progress Report, we have isolated and characterized clones carrying the frd gene. They can be selected by their trimethoprim resistance because this folate analog inhibits the E. coli dihydrofolate (FH_2) reductase but not the T4 enzyme. Derivatives of these clones that overproduce the T4 FH_2 reductase should be resistant to the folate analogs that we have used to select T4 regulatory mutants. If such cells are not found without mutagenesis, we will construct T4-plasmid hybrids using mutagenized T4 DNA. In this way, we should be able to isolate cells that overproduce the T4 FH_2 reductase due to a mutation in the T4 DNA near the frd gene. The mutations causing FH_2 reductase overproduction that we have previously character-

FINAL PROGRESS REPORT

(A) Grant Number: 5R01 GM24455-06

Principal Investigator: Dwight H. Hall

Grantee Institution: Georgia Institute of Technology

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

Period Covered: March 1, 1977 through Feb. 29, 1984.

(B) Summary:

All T4 mutants unable to induce thymidine kinase activity are in a single structural gene located near the rI gene. Folate analog resistant mutants of T4 have been isolated and characterized. Some mutants induce altered dihydrofolate reductases. Some mutants induce a normal amount of an unaltered dihydrofolate reductase but are ribonucleotide reductase deficient. Some mutants overproduce an unaltered dihydrofolate reductase and are new regulatory mutants. Some of these regulatory mutants affect transcription and are in the mot gene and some affect translation and define a new gene regB which is located near the rI gene. The regB gene product appears to affect the ribosomes of infected E. coli (1). Mutations which suppress rII mutants cause overproduction of dihydrofolate reductase and are also in the mot gene (4). Mutations in genes coding for enzymes required for DNA replication can cause folate analog resistance. Studies with mutants suggests that dihydrofolate reductase interacts with these replication enzymes (3,8). The T4 gene controlling the modification of host valyl-tRNA synthetase is also near the rI gene. Two hydroxyurea-sensitive mutants show normal DNA metabolism and their sensitivity to hydroxyurea is due to defective lysis of host cells caused by mutations in the T4 t gene. Gene-specific suppressors of mutations in the T4 gene coding for both RNA ligase and tail fiber attachment activities are in a lysis gene, a baseplate gene, and a sheath gene (2). The denV gene has been physically mapped and sequenced (9).

We have developed new methods to get T4 mutations into plasmids. One method is to cross T4 mutations directly into a plasmid containing the region of T4 DNA where the mutation is located. The plasmid DNA can be isolated after infection by a T4 mutant and used to transform uninfected cells. Recombination between T4 DNA and plasmid DNA is so frequent, as judged by marker rescue, that some plasmid isolated late after infection should have acquired the mutation. We call this procedure "reverse rescue" and we have done it for mutations in the dihydrofolate reductase (frd) gene. The

infecting phage carried mutations in denA and denB to decrease plasmid degradation. Plasmid (pBR322 with the whole frd gene inserted) was isolated 15 minutes after infection in broth at 37°C. In these initial studies, a T4 mutation that could be selected was used and it is present in about one out of 10^4 transformants. A second method is to select mutations in plasmids containing the gene of interest. Starting with cells containing pBR322 with a mutant frd gene inserted, we have been able to select false revertants which have new mutations affecting the structure and/or synthesis of dihydrofolate reductase (5,7).

We have developed a procedure to screen for marker rescue in the T4 thymidine kinase (tk) gene. Positive marker rescue was found in only one of about 300 HindIII clones in pBR322 that were tested. This tk clone has two HindIII fragments, 1.9 kb and 0.8 kb. A subclone containing a 1.3 kb EcoRI subfragment of the 1.9 kb fragment shows positive marker rescue with six different tk mutants. The tk plasmid from this subclone confers fluorodeoxyuridine resistance to thymidine kinaseless E. coli indicating that the plasmid expresses the T4 tk gene. The regB gene is near the tk gene and regB mutants can be recognized by their folate-analog-resistant (FAR) phenotype. Growth of wild type T4 on mutagenized tk clone produces T4 mutants with a FAR phenotype. If these mutations are within the 1.9 kb fragment, then at least part of the regB gene would appear to be on the same HindIII fragment as the tk gene (7).

(C) Publications:

1. Hall, D.H., and C.E. Smith, 1979. Growth of bacteriophage T4 on mutants of Escherichia coli with defective ribosomes. Abstracts of the XIth International Congress of Biochemistry: 86.

2. Hall, D.H., R.G. Sargent, K.F. Trofatter, and D.L. Russell, 1980. Suppressors of mutations in the bacteriophage T4 gene coding for both RNA ligase and tail fiber attachment activities. Journal of Virology 36: 103-108.

3. Hall, D.H., and P.M. Macdonald, 1981. Evidence for interactions between proteins involved in initiation of bacteriophage T4 DNA replication. Abstract, Journal of Supramolecular Structure and Cellular Biochemistry Suppl. 5: 341.

4. Hall, D.H., and R.D. Snyder, 1981. Suppressors of mutations in the rII gene of bacteriophage T4 affect promoter utilization. Genetics 97:1-9.

5. Hall, D.H., R.G. Sargent, and C.M. Povinelli, 1982. Cloning and characterization of the dihydrofolate reductase gene of bacteriophage T4. Abstracts of the Annual Meeting of the American Society for Microbiology: 131.

6. Brody, E., D. Rabussay, and D.H. Hall, 1983. Regulation of transcription of prereplicative genes. Bacteriophage T4 (American Society for Microbiology): 174-183.

7. Hall, D.H., C.M. Povinelli, and K.A. Gutekunst, 1983. Molecular cloning and manipulation of the regions of the T4 genome coding for dihydrofolate reductase and thymidine kinase. Abstracts of the Evergreen International T4 Meeting: 53.

8. Macdonald, P.M., and D.H. Hall, 1984. Genetic evidence for physical interactions between enzymes of nucleotide synthesis and proteins involved in DNA replication in bacteriophage T4. Genetics 107: 343-353.

9. Radany, E.H., L. Naumovski, J.D. Love, K.A. Gutekunst, D.H. Hall, and E.C. Friedberg, 1984. Physical mapping and complete nucleotide sequence of the denV gene of bacteriophage T4. Journal of Virology 52:846-856.