JOURNAL OF VIROLOGY, Apr. 1975, p. 929-945
Vol. 15, No. 4
Printed in U.S.A.
Copyright © 1975 American Society for Microbiology

Characterization of New Regulatory Mutants of Bacteriophage T4

II. New Class of Mutants

KENNETH V. CHACE[1]* AND DWIGHT H. HALL

Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Received for publication 8 November 1974

New mutants of bacteriophage T4 that overproduce the enzyme dihydrofolate reductase were investigated. Unlike previously described overproducers of this enzyme (Johnson and Hall, 1974), these mutants did not overproduce deoxycytidylate deaminase. Overproduction of dihydrofolate reductase by the new mutants occurred because enzymatic activity continued 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 occurred 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 were studied by using polyacrylamide gel electrophoresis. The two types of overproducers appeared to be very different. The previously described overproducers showed a delay and/or reduction in the synthesis of several proteins that normally started to be made 4 to 6 min after infection. Several proteins could be seen to be overproduced on the gels. The new overproducers did not show the delay in the synthesis of some proteins and only overproduced 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.

After infection of *Escherichia coli*, bacteriophage T4 induces several enzymes that are involved in pyrimidine metabolism. The genes coding for four of these, deoxycytidylate (dCMP) deaminase, dihydrofolate (FH_2) reductase, thymidylate synthetase, and ribonucleotide reductase (11, 13, 26, 39) map in the same general area of the genome. However, two other enzymes, deoxycytidine triphosphatase (dCTPase) (23) and thymidine kinase (3), are not coded for by genes in this area although they are also involved in pyrimidine metabolism. To see whether the genes that are linked and have a similar function are expressed, using a common control mechanism, Johnson and Hall (17, 18) isolated mutants of T4 that overproduce FH_2 reductase. These mutants, called *far* mutants, were isolated by virtue of their ability to grow in the presence of folate analogues that inhibit T4-induced FH_2 reductase. In addition to overproducing FH_2 reductase, they also overproduce dCMP deaminase, dCTPase, and thymidine kinase but underproduce thymidylate synthetase. This seems to indicate that there is no direct relationship between the control of expression of these genes and their location on the genetic map. These mutants were thought to map at two different sites (18). However, evidence to be presented in this paper shows that two of these mutants, *farP14* and *farP85*, map very near each other, although they were originally thought to map at far distant sites.

Several other *far* mutants have been isolated, and it seemed desirable to see whether any of these also overproduce FH_2 reductase. If another type of overproducer could be isolated, more information could obtained about the regulation of these enzymes.

Polyacrylamide gel electrophoresis has been used by Hosoda and Levinthal (16) and O'Farrell et al. (22) to identify many T4-induced proteins as bands on the gels. This technique could be used to study the synthesis of many proteins by mutants of T4 that overproduce FH_2 reductase. This might give more information about what causes the overproduction.

MATERIALS AND METHODS

Bacteriophage strains. T4D*o*, an osmotic shock-resistant derivative of T4D(11), was the standard strain of bacteriophage used. Amber mutants used to identify bands on the polyacrylamide gels plus *amE51*

[1] Present address: Department of Biology, University of Virginia, Charlottesville, Va. 22903.

(gene 56), *amH17* (gene 52), and *amE727* (gene 49) used in mapping studies were isolated by Epstein et al. (7) and by Edgar and Wood (6) and were obtained from C. Levinthal, W. B. Wood, D. P. Snustad, R. B. Luftig, and J. S. Wiberg. The mutant in gene 42, *amN55X5*, used to construct a double mutant with *farP13*, was backcrossed to wild-type T4D five times by J. S. Wiberg and was obtained from him. The amber mutant in lysozyme, *am8-82*, was isolated by R. L. Russell and was obtained from W. H. McClain. The deletion *eG298*, a lysozyme mutant of T4 isolated in the laboratory of G. Streisinger, was obtained from J. Owen. The *rI* mutant, *r48*, was isolated by Doermann and Hill (4) and was obtained from W. B. Wood. The *rII* deletion mutants *r638* and *r1272* were isolated by S. Benzer. The mustant *r638* deletes the entire *rIIB* cistron (1) and was used to identify the product of *rIIB* on the gels. The mutant *r1272* deletes both the *rIIA* and the *rIIB* cistrons (1) as well as the adjacent D1 and D2A regions (2) and was used to identify the *rIIA* band on the gels. The band missing in gels prepared using cells infected by this mutant corresponds to that identified as the *rIIA* band by O'Farrell et al. (22). The *rII* mutants were obtained from I. Tessman. The *nrdC19* mutant was isolated from an *nrdC19-frd1* double mutant obtained from I. Tessman. Its isolation was described by Johnson and Hall (17). The *far* mutants *farP12*, *farP13*, *farP14*, *farP23*, and *farP85* were isolated by Johnson and Hall (17) and were obtained from them. The *tk* deletions *tk2* and *tk3* have been previously described (3).

Bacterial strains. Most bacteriophage stocks were obtained by making liquid lysates on *E. coli* S/6. Stocks of the *tk* deletions, *far* deletions, and amber mutants were made on *E. coli* CR63, a K strain of *E. coli* permissive for the growth of amber mutants. Enzyme studies were done with *E. coli* B. *E. coli* OK305 was used for some mapping. It is a derivative of *E. coli* B that requires pyrimidine for growth and is deficient in cytidine deaminase activity (13). It was obtained from O. Karlström.

Media. Broth medium, synthetic medium, and agar plates containing about 30 ml of broth or synthetic medium were prepared as described by Goscin and Hall (10). 3XD, the glycerol-Casamino Acids medium of Fraser and Jerrel (8), prepared without gelatin, was used to prepare phage stocks and enzyme extracts. *E. coli* OK305 was grown on synthetic medium supplemented with uracil (20 μg/ml). M9 medium contained NH_4Cl (1.0 g), KH_2PO_4 (3.0 g), $Na_2HPO_4 \cdot 7H_2O$ (11 g), NaCl (0.5 g), $FeCl_3 \cdot 6H_2O$ (2.7 mg), $MgSO_4$ (0.12 g), glucose (5 g), and water to make a total volume of 1 liter.

Chemicals. Cytidine, uridine, uracil, bromodeoxyuridine, dCMP, dCTP, dUMP, dTMP, dTDP, dTTP, and rifampin were purchased from Calbiochem. ATP and sodium nicotinamide adenine dinucleotide phosphate were purchased from P-L Biochemicals. Glycine and folic acid were puchased from Sigma Chemical Co. Lauryl sodium sulfate (SDS) was purchased from Schwarz/Mann. Acrylamide (electrophoresis grade) and *N,N'*-methylenebisacrylamide were purchased from Eastman Kodak Co. Pyrimethamine was supplied by James J. Burchall of Burroughs Wellcome and Co. [*methyl*-^{3}H]dTMP (39 Ci/mmol) was purchased from Schwarz/Mann. ^{14}C-labeled L-amino acid mixture was purchased from New England Nuclear. FH_2 was prepared as described by Futterman (9).

Bacteriophage crosses. Phage crosses were performed as described by Hall et al. (13) with a few modifications. All crosses involving amber mutants or *tk* or *far* deletion mutants were done with *E. coli* CR63 instead of *E. coli* B. The crosses involving *eG298* were done in 3XD medium instead of broth medium and were incubated for 2 h at 37 C after infection.

Preparation of extracts for enzyme assays. Extracts were prepared as described by Hall (11). Cells were grown in 3XD medium at 37 C to a concentration of 2×10^8 cells/ml. L-Tryptophan (20 μg/ml) was added to the cells, and immediately afterward phage were added. The infection was stopped by rapidly chilling the cells on ice, and the cells were concentrated 20-fold by centrifuging for 5 min at $6{,}000 \times g$ and resuspending in a solution containing 0.024 M Tris-hydrochloride buffer (pH 7.5), 0.038 M mercaptoethanol, and 0.01 M NaN_3. After sonic treatment, the extracts were centrifuged at $20{,}000 \times g$ for 20 min.

Enzyme assays. FH_2 reductase and dCMP deaminase assays were performed as described by Warner and Lewis (34) as modified by Hall (11). The product of the dCMP deaminase, dUMP, was isolated by column chromatography as described by Price and Warner (23). The dCTPase assays were performed as described by Price and Warner (23). The deoxynucleotide kinase assays were performed as described by Duckworth and Bessman (5). The substrate used for the assay was dTMP, and both dTDP and dTTP were measured as products. The products were separated from the substrate by using thin-layer polyethyleniminie chromatography as described previously (3).

Protein assays. Protein assays were performed by the method of Lowry et al. (19) using bovine serum albumin as a standard.

SDS-polyacrylamide gels. SDS-polyacrylamide gels were made as described by Studier (31). A slab gel apparatus purchased from Hoeffer Scientific Instruments was used. *E. coli* B cells were grown to a concentration of 2×10^8 in M9 medium. The cells were then subjected to UV radiation for 22 min at a distance of 12 cm from a UVSL-25 lamp purchased from Ultraviolet Products, Inc. The shortwave setting of the lamp was used. After irradiation, the cells were incubated at 37 C for 15 min while being aerated vigorously. The irradiation and aeration prevent protein synthesis in uninfected cells but allow T4 protein synthesis after infection. All phage stocks used to infect the cells were prepared in M9 medium so that no amino acids would be present to dilute the label.

One milliliter of cells was then infected at 37 C and labeled with ^{14}C-labeled amino acid mixture. The label was chased by adding 1 ml of M9 medium containing 15 mg of Casamino Acids. After 1 min of further incubation, the tubes containing infected cells were placed on ice. They were then concentrated by centrifugation at $4{,}500 \times g$ for 10 min and resuspended in 0.1 ml of cracking buffer containing 0.05 M Tris-hydrochloride (pH 6.8), 1% SDS, 1% mercapto-

ethanol, 0.002 M EDTA, and 10% glycerol. The tubes containing the samples were then placed in boiling water for 5 min and stored at 4 C.

The gels had an acrylamide *N,N'*-methylenebisacrylamide ratio of 30:0.8. The resolving or separating gel contained 0.19 M Tris-hydrochloride (pH 8.8), 0.1% SDS, 0.002 M EDTA, and between 10 and 18% acrylamide. After the separating gel was poured and polymerized, using small amounts of ammonium persulfate and *N,N,N',N'*-tetraethylmethylenediamine, a stacking gel was poured on top. This contained 0.063 M Tris-hydrochloride (pH 6.8), 0.1% SDS, 0.002 M EDTA, and 5% acrylamide. This was polymerized in the same manner as the separating gel. The separating gel was 9.5 cm high and 20 cm wide. Both 0.75- and 1.5-mm-thick gels were used. The stacking gel was 1.0 cm high. Either one or two gels can be electrophoresed at the same time. When two 12.5% acrylamide gels were run, they were run at 40 V for 8 h. One 12.5% gel was run at 30 V for 8 h. Linear gradient gels were also made by using a Glenco gradient maker. When two gradient gels with the gradient running from 10 to 18% acrylamide were electrophoresed, they were run for 13 h at 40 V. One 10 to 18% gradient gel was run for 13 h at 35 V.

RESULTS

Characterization of three folate analogue resistant mutants. Two mutants of T4, called *tk2* and *tk3*, which appear to have deletions, have previously been described (3). They have two phenotypes, the inability to induce thymidine kinase and a large clear plaque similar to that formed by rapid lysis (*r*) mutants, that cannot be separated by crossing to wild-type phage. Three other similar deletion mutants have been isolated in the same way and have been called *tk19*, *tk21*, and *tk25*. In the course of studying the *tk* mutants, the investigation of several folate analogue resistant (*far*) mutants isolated by Johnson and Hall (17) was undertaken. These three mutants, *farP12*, *farP13*, and *farP23*, all were able to form plaques in the presence of bromodeoxyuridine and light and so seemed to be unable to induce thymidine kinase activity. They also made large plaques like those made by *r* mutants. The *r*, *far*, and *tk* phenotypes of these mutants could not be separated by crossing to wild-type phage. Therefore, it appeared that these were mutants with deletions of the same area of the genome as the *tk* deletion mutants already described. One mechanism of folate analogue resistance is overproduction of the enzyme FH_2 reductase (17, 18). Therefore, the activity of FH_2 reductase was assayed in *E. coli* B cells after infection by *farP13* and the *tk* deletions (Table 1). As can be seen, the *far* deletion overproduced this enzyme, whereas none of the *tk* deletions did. The FH_2 reductase overproducers described by Johnson and Hall (18) overproduced dCMP deaminase, dCTPase, and thymidine kinase. Therefore, extracts were made from cells infected by *far*⁺, *farP12*, *farP13*, and *farP23*, and the activities of several T4-induced enzymes were compared (Table 2). Only FH_2 reductase was overproduced by all three *far* mutants. Deoxynucleotide kinase was overproduced by two of the mutants, *farP12* and *farP13*, but it was not overproduced to anywhere near the same extent that FH_2 reductase was. One enzyme, dCTPase, was produced in significantly

TABLE 1. *FH_2 reductase activity induced by several mutants of T4*[a]

Phage	FH_2 reductase relative sp act[b]
far⁺-tk⁺ (T4Do)	100
tk2	118
tk3	103
tk19	115
tk21	114
tk25	113
farP13	249

[a] *E. coli* B was infected at a multiplicity of 4 to 6 phage per cell for 20 min at 37 C except the *tk19* sample, which was infected for 15 min.

[b] Expressed as percentage of the specific activity present in cells infected by wild-type phage. The activity present in uninfected cells was subtracted from each sample.

TABLE 2. *Activities of several early enzymes induced by far mutants*[a]

Phage	Sp act			
	FH_2 reductase[b]	dTMP kinase[c]	dCTPase[d]	dCMP deaminase[e]
far⁺(T4Do)	22 ± 2	122 ± 13	90 ± 14	145 ± 29
farP12	47 ± 7[f]	140 ± 9[f]	81 ± 23	130 ± 20
farP13	50 ± 3[f]	167 ± 13[f]	99 ± 21	130 ± 21
farP23	46 ± 7[f]	118 ± 9	66 ± 12[f]	121 ± 15

[a] *E. coli* was infected at a multiplicity of 4 to 6 phage per cell for 15 min at 37 C. Each value gives the mean ± standard deviation for eight extracts, four extracts from each of two separate experiments.

[b] Expressed as nanomoles of tetrahydrofolate formed per minute per milligram of protein.

[c] Expressed as nanomoles of dTDP and dTTP formed per minute per milligram of protein.

[d] Expressed as nanomoles of dCMP formed per minute per milligram of protein.

[e] Expressed as nanomoles of dUMP formed per minute per milligram of protein.

[f] $P < 0.01$ as tested by Student's *t* test when compared with value for *far*⁺.

lower amounts by *farP23*. *far23* induced significantly less deoxynucleotide kinase than *farP12* and *farP13* and significantly less dCTPase activity than *farP13*. These results are in marked contrast to those obtained with previously described *far* overproducers. Those mutants overproduce dCMP deaminase two- to fourfold (18), whereas the mutants just described produced normal amounts of dCMP deaminase (Table 2). The overproducers described by Johnson and Hall (18), which include *farP14* and *farP85*, will be called class I overproducers, and the overproducers just described will be called class II overproducers.

The synthesis of FH_2 reductase after infection by *farP13* was compared with its synthesis after infection by wild-type T4 (Fig. 1). Whereas wild-type T4 only synthesized FH_2 reductase for the first 5 min of infection, *farP13* continued to synthesize it for the first 10 to 12 min of infection. The other two class II overproducers, *farP12* and *farP23*, also continued to synthesize FH_2 reductase between 5 and 10 min after infection (results not shown).

A double mutant was constructed by crossing *farP13* to *amN55X5*, a mutant which cannot synthesize DNA, defective in gene 42. A recombinant *farP13-amN55X5* double mutant was selected from the progeny of this cross that was unable to grow on strains of *E. coli* nonpermissive for the growth of amber mutants and that made the large clear plaque characteristic of *farP13* on permissive strains of *E. coli*. Many early enzymes that normally stop being made 10 min after infection continue to be made for an additional 10 to 20 min if *E. coli* is infected by a mutant of T4 unable to synthesize DNA (36). One mutant of T4, SP62, overproduces early enzymes to a much greater extent when it is coupled with a second mutant that is unable to synthesize DNA (37). Table 3 compares the amounts of activity of three early enzymes in far^+ and *farP13* extracts made 10 and 20 min after infection and in *amN55X5* and *amN55X5-farP13* extracts made 10 and 30 min after infection. Neither the *far* nor the amber mutation caused FH_2 reductase to be synthesized later than 10 min after infection. In some cases, FH_2 reductase continued to be synthesized for as late as 15 min after infection by *farP13* (Fig. 1), but synthesis usually stopped by 10 min after infection. Both the *far* mutation and the amber mutation caused dTMP kinase to be synthesized later than 10 min after infection. In the *far*-amber double mutant, overproduction of dTMP kinase was greater than in either the *far* or amber single mutant. The amber but not the *far* mutation caused dCMP deaminase to continue to be synthesized later than 10 min after infection. There was no indication that class II overproducers acted like SP62 in having a greater effect when coupled with a mutation that prevented DNA synthesis.

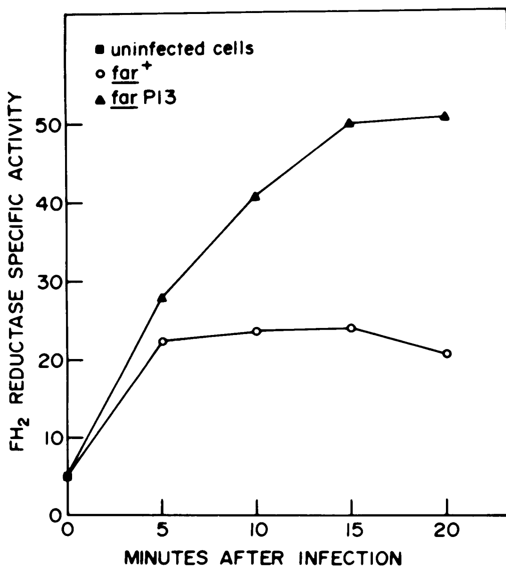

FIG. 1. *Induction of dihydrofolate reductase activity after infection of E. coli B at 37 C by farP13 and far^+. Specific activity is expressed as nanomoles of tetrahydrofolate formed per minute per milligram of protein.*

Mapping of far and tk deletions. To find where the locus causing overproduction in the class II mutants is on the T4 genetic map, it was desirable to find the map position of the *tk* and *far* deletions. The *r* phenotype was used to identify the deletions in these crosses. Wild-type plaques are surrounded by a white halo of rapidly growing cells when plated on *E. coli* OK305 growing on synthetic medium agar containing cytidine and the folate analogue pyrimethamine (17). However, *nrd* mutants make no halo and *r* mutants make a thin halo. The *tk* and *far* deletions were crossed to *nrdC19* and wild-type recombinants were detected by their white halo. The map distances obtained in these crosses are given in Table 4. Since *farP13* did not recombine with *nrdC19* and did not make a white halo when plated on *E. coli* OK305 in the presence of pyrimethamine, it probably extends through the *nrdC* locus. Therefore, it crossed to *amE727*, an amber mutant defective in gene 49. Since amber mutants cannot form plaques on *E. coli* OK305, wild-type recombinants could again be identi-

TABLE 3. *Enzyme synthesis after infection by farP13, amN55X5, and farP13-amN55X5*[a]

Phage	Relative sp act[b]								
	FH_2 reductase			dCMP deaminase			dTMP kinase		
	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
far$^+$-*am*$^+$(T4Do)	100	83		100	87		100	98	
farP13	178	209		112	117		81	155	
amN55X5	100		99	100		141	100		267
amN55X5-farP13	167		183	112		179	92		311

[a] *E. coli* B was infected at a multiplicity of 4 to 6 phage per cell at 37 C.
[b] Expressed as percentage of specific activity present in *far*$^+$-*am*$^+$-infected cells at 10 min after infection (for *far*$^+$-*am*$^+$- and *farP13*-infected cells) or as percentage of specific activity present in *amN55X5*-infected cells at 10 min after infection (for *amN55X5*- and *amN55X5-farP13*-infected cells).

TABLE 4. *Genetic map distances between far and tk deletions and several genetic markers*[a]

Phage	*nrdC19*	*amE727* (gene 49)	*eG298*	*am8-82* (gene *e*)
tk2	1.5 ± 0.2		7 ± 1	[c]
tk3	12 ± 1		[b]	[c]
tk19	8 ± 1		7 ± 1	[c]
tk21	0.6 ± 0.2		6 ± 1	[c]
tk25	8 ± 1		[b]	[c]
farP12	10 ± 1		[b]	7 ± 1
farP13	[b]	2.7 ± 0.2	[b]	5 ± 1
farP23	14 ± 1		[b]	8 ± 1
nrdC19		5.0 ± 0.2		
r48	18 ± 2		11 ± 1	[c]

[a] Entries are recombination frequencies, given in map units, for the appropriate mutant pairs.
[b] No recombinants were found.
[c] Mutants showed maximum recombination.

fied by their large white halo when plated on OK305 growing on synthetic medium agar plates containing cytidine and pyrimethamine. At the same time, *nrdC19* was crossed to *amE727* and recombinants were scored in the same way (Table 4). To determine the map position of the right end of the deletions, they were crossed to *am8-82*, which contains an amber mutation in the lysozyme gene. Recombinants were identified in the same way as they were in the cross to *amE727*. Only the *far* deletions lay close enough to *am8-82* for meaningful map distances to be obtained (Table 4). The *tk* and *far* deletions were then crossed to *eG298*, a lysozyme mutant carrying a deletion that extends from the gene coding for lysozyme towards *rI* (J. Owen, personal communication). Like the two amber mutants, *eG298* would not form plaques on *E. coli* OK305, so wild-type recombinants could again be identified by their white halos. The map distances are also shown in Table 4. A deletion map of all the *tk* and *far* deletions is shown in Fig. 2. The mutation causing overproduction of FH_2 reductase must lie to the right of *rI*, since some of the *tk* deletions that did not overproduce extended farther to the left than some *far* deletions that did. Since *tk3* and *tk25* overlapped *eG298* and did not overproduce, the mutation causing overproduction must lie farther to the right of *r48* than the left end of *eG298* does. Since *farP23* did overproduce, the mutation causing overproduction must lie at least as far to the left of *am8-82* as *farP23* does. Therefore, the gene whose mutation causes overproduction of FH_2 reductase in class II overproducers, which has been named *regB*, must be more than 11 map units to the right of *r48* (*rI* gene) and at least 8 map units to the left of *am8-82* (*e* gene). A second gene, deleted in *farP12* and *farP13* but present in the shorter *farP23* deletion, may cause increased synthesis of dCTPase and deoxynucleotide kinase.

Overproduction of FH_2 reductase by eG298. The results of mapping *far* and *tk* mutants suggest that the gene whose mutation causes overproduction in class II overproducers lies within deletion *eG298*. Therefore, extracts were prepared from *eG298*-infected cells and assayed for FH_2 reductase and dCMP deaminase (Table 5). It is clear that *eG298* overproduces FH_2 reductase but not dCMP deaminase and so shows the same phenotype as the class II overproducers do.

Complementation studies of far overproducers. Complementation studies were performed by infecting *E. coli* B with two different FH_2 reductase overproducers or with wild-type T4 and an FH_2 reductase overproducer. Two overproducers of the class I type, *farP85* and *farP14*, and three of the class II type, *farP12*, *farP13*, and *farP23*, were studied. Enzyme extracts were assayed for FH_2 reductase and, in some cases, dCMP deaminase. These studies (Table 6) revealed several interesting facts. The mutants, particularly *farP23*, seemed to

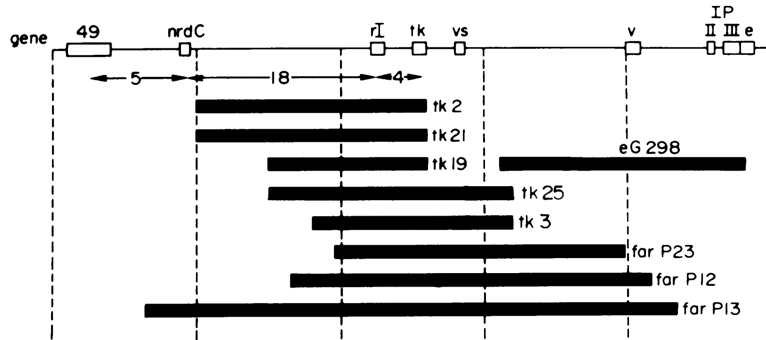

FIG. 2. *Partial genetic map of T4, modified from Wood (38), showing the locations of the tk and far deletions. The distance between the dashed lines is approximately 5,000 nucleotide pairs. The average frequencies of recombinants obtained in two-factor crosses are shown.*

TABLE 5. *FH_2 reductase and dCMP deaminase activities induced by eG298*[a]

Phage	Sp act	
	FH_2 reductase[b]	dCMP deaminase[c]
e^+(T4Do)	27	128
eG298	71	125
Uninfected cells	8	<10

[a] *E. coli* B was infected at a multiplicity of 4 to 5 phage per cell for 15 min at 37 C.

[b] Expressed as nanomoles of tetrahydrofolate formed per minute per milligram of protein.

[c] Expressed as nanomoles of dUMP formed per minute per milligram protein.

be partially dominant to wild-type phage for the overproduction of FH_2 reductase. This may be an indication that the product of the gene whose mutation causes overproduction is needed in stoichiometric amounts for wild-type function (28). Although *farP23*, *farP14*, and *farP85* all mapped in different places (18), they failed to complement each other as judged by overproduction of reductase. This is not surprising in the case of complementation studies involving *farP23*, which is partially dominant to wild type, but is surprising in the case of *farP14* and *farP85*, which seem to be recessive to wild type. The two class I mutants, *farP14* and *farP85*, also failed to complement each other to give normal production of dCMP deaminase, although they were clearly recessive to wild type for production of this enzyme. Although the partial dominance of the mutant makes the results difficult to interpret, the class II mutants did not seem to complement each other. This was expected since they appeared to delete the same gene.

Mapping of farP14 and farP85. Since complementation studies indicate that *farP14* and *farP85* fail to complement each other, it seemed possible that they were in the same gene. Therefore, it was necessary to reexamine the map positions of these two mutations. The original studies (18) indicated that *farP14* mapped between genes 56 and 39, and *farP85* mapped between genes 52 and *t*. Therefore, both *farP14* and *farP85* were crossed to *amH17*,

TABLE 6. *Complementation studies in vivo of far overproducers*[a]

Phage	Sp act[b]	
	FH_2 reductase[c]	dCMP deaminase[d]
far^+(T4Do)	$25 \pm 4, n = 14$	$112 \pm 25, n = 10$
farP85	$41 \pm 5, n = 8^e$	$437 \pm 83, n = 8^e$
farP14	$42 \pm 3, n = 8^e$	$262 \pm 66, n = 8^e$
farP12	$42 \pm 2, n = 5^e$	
farP13	$43 \pm 4, n = 5^e$	
farP23	$45 \pm 13, n = 8^e$	
farP85 + far^+	$27 \pm 5, n = 7$	$97 \pm 33, n = 7$
farP14 + far^+	$29 \pm 4, n = 7$	$110 \pm 17, n = 7$
farP12 + far^+	$28 \pm 3, n = 5$	
farP13 + far^+	$26 \pm 3, n = 5$	
farP23 + far^+	$33 \pm 3, n = 6^e$	
farP14 + *farP85*	$39 \pm 6, n = 7^e$	$282 \pm 73, n = 7^e$
farP23 + *farP14*	$38 \pm 12, n = 6^f$	
farP23 + *farP85*	$37 \pm 3, n = 6^e$	
farP12 + *farP23*	$44 \pm 3, n = 3^e$	
farP12 + *farP13*	$47 \pm 3, n = 3^e$	
farP13 + *farP23*	$39 \pm 8, n = 3^e$	

[a] *E. coli* B cells were singly infected at a multiplicity of 4 or mixedly infected at a multiplicity of 4 for each phage. Cells were infected for 15 min at 37 C.

[b] Value given is mean ± standard deviation. *n* is the number of samples.

[c] Expressed as nanomoles of tetrahydrofolate formed per minute per milligram of protein.

[d] Expressed as nanomoles of dUMP formed per minute per milligram of protein.

[e] $P < 0.01$ as tested by Student's *t* test when compared with extracts made after far^+ infection.

[f] $P < 0.02$ as tested by Student's *t* test when compared with extracts made after far^+ infection.

an amber mutant defective in gene 52, and *amE51*, an amber mutant defective in gene 56, as well as to each other. The progeny from these crosses were plated on *E. coli* OK305 growing on synthetic medium agar plates containing uracil (20 μg/ml). Under these conditions, both *farP14* and *farP85* formed plaques much smaller than the plaques made by wild-type phage and amber mutants could not form plaques. These crosses indicate that *farP85* has a recombination frequency of 4 $\pm$ 1% with *amH17* and *farP14* has a recombination frequency of 3$\pm$1% with *amH17*. Both *farP14* and *farP85* showed maximum recombination with *amE51*. It was also determined that *farP14* and *farP85* have a recombination frequency of 1$\pm$1% with each other. It therefore appears that *farP14* and *farP85* lie very close to each other between genes 52 and *t*. The mapping and complementation studies indicate that they are in the same gene. The map position of this gene is shown in Fig. 3. Another *far* overproducer, *farP3*, was also originally described as mapping between gene 56 and *dex A* (18). The map position of this mutant has not been reexamined.

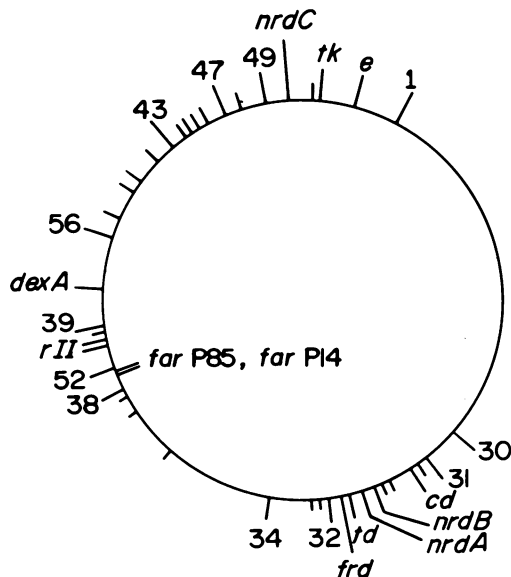

FIG. 3. *Genetic map of T4, modified from Edgar and Wood (6), showing the position of farP14 and farP85.*

SDS-polyacrylamide gel electrophoresis of the far overproducers. To test the effect of the *far* FH_2 reductase overproducers on all proteins synthesized after T4 infection, extracts prepared from *E. coli* B infected by *far* FH_2 reductase overproducers and wild-type phage were prepared and electrophoresed on SDS-polyacrylamide gels. T4-infected cells were pulse labeled with radioactive amino acids for a short interval and then the label was chased with an excess of nonradioactive amino acids. The amount of the different T4-induced proteins made during each pulse could be observed after electrophoresis of the extracts and autoradiography of the gels. *E. coli* protein synthesis was prevented by irradiating the cells with UV light before infection. The protein bands were identified by running gels of extracts of cells infected by amber or deletion mutants and looking for missing bands. This method was originally used by Hosoda and Levinthal (16) and was perfected by O'Farrell et al. (22).

The first study examined cells infected by wild-type T4, *farP14*, *farP85*, and *far23* labeled in 3-min pulses from 0 to 21 min after infection at 37 C. The synthesis of the product of gene 43 was delayed in cells infected by the class I overproducers, *farP14* and *farP85* (Fig. 4). This protein appeared during the 3- to 6-min pulse in *far*$^+$-infected and the class II overproducer, *farP23*-infected cells, but did not appear until the 6- to 9-min pulse in *farP14*- and *farP85*-infected cells. The product of gene 32 is synthesized at a lower rate in *farP14*- and *farP85*-infected cells than in *far*$^+$- or *farP23*-infected cells during the 3- to 6-min pulse and the 6- to 9-min pulse. The product of the *rIIA* cistron was synthesized at a higher rate during the 6- to 9-minute pulse in cells infected by *farP14* and *farP85* than in cells infected by *far*$^+$ and *farP23*. Extracts prepared from *farP23*-infected cells seemed to resemble those prepared from *far*$^+$-infected cells quite closely. A number of bands that normally first appeared during the 9- to 12-min pulse in *far*$^+$- and *farP23*-infected cells were delayed in appearance in *farP14*- and *farP85*-infected cells (Fig. 5). Also, the product of the *rIIA* cistron, which was no longer made in wild type- and *farP23*-infected cells in the 9- to 12-min pulse, continued to be made during this pulse in cells infected by *farP14* and *farP85*. In general, the band pattern seen when extracts prepared from cells infected by *farP14* and *farP85* are electrophoresed resembled the band pattern seen with *far*$^+$- and *farP23*-infected cell extracts from the preceding 3-min pulse. The gels support the complementation and enzyme data in indicating that *farP14* and *farP85* are very similar mutants, whereas *farP23* is a very different mutant.

To look more closely at protein synthesis in the mutants, cells infected by *far*$^+$, the class I overproducer *farP14*, and the class II overproducer *farP23* were labeled with 1-min pulses of

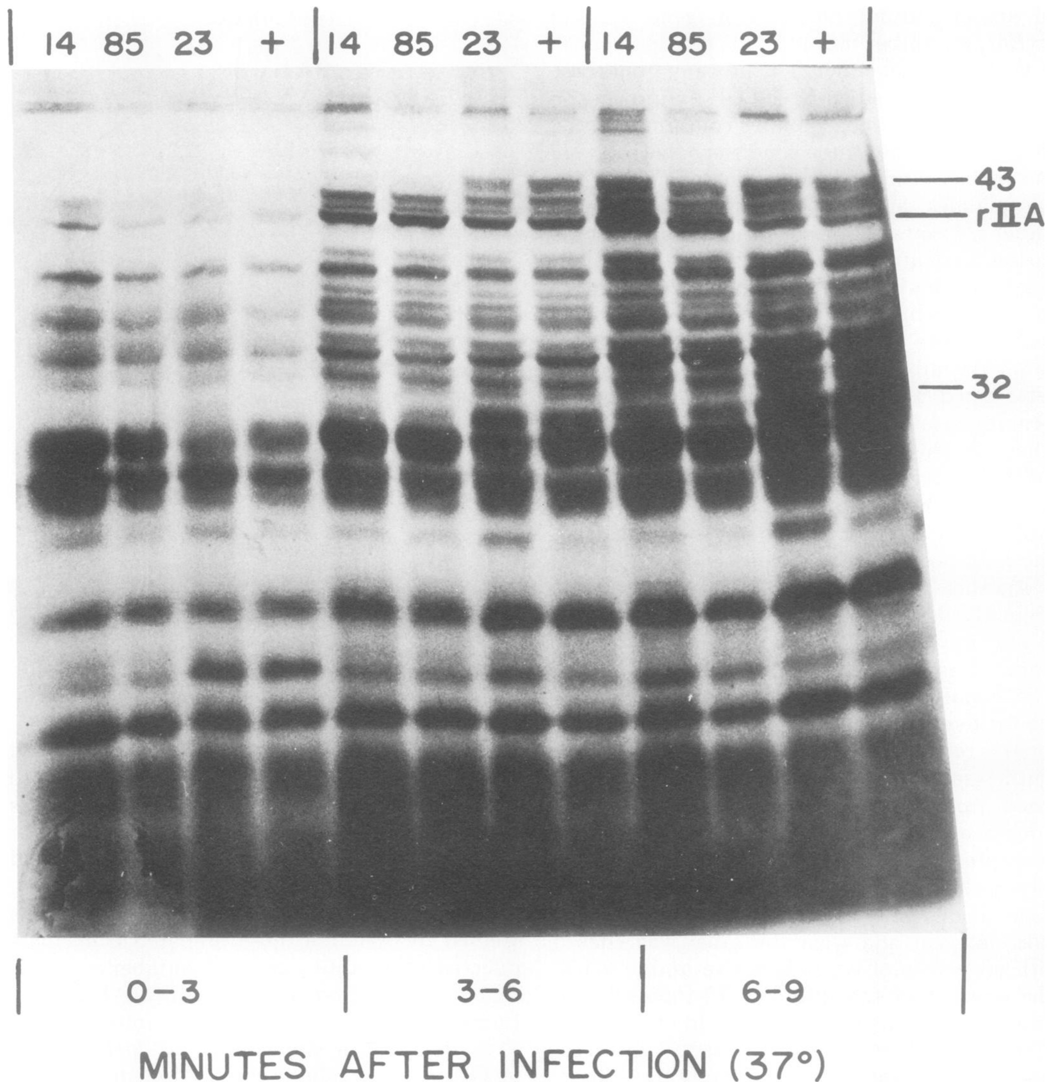

FIG. 4. Autoradiogram of an SDS-polyacrylamide slab gel showing patterns of protein synthesis after infection of E. coli B by farP14 (14), farP85 (85), farP23 (23), and far$^+$ (+). Proteins were pulse labeled with a mixture of ^{14}C-labeled amino acids for the times indicated. The gels contained 12.5% acrylamide. The numbers on the right edge of the figure refer to the genes coding for the proteins indicated.

radioactive amino acids from 0 to 13 min after infection. Extracts from these infected cells were electrophoresed on 10 to 18% acrylamide gradient gels. These gels gave better resolution of protein bands than the 12.5% acrylamide gels used in the preceding study.

Figures 6 and 7 show photographs of autoradiograms of these gels. The synthesis of gene 43 and gene 45 products was delayed in *farP14*-infected cells. The synthesis of gene 32 product was also delayed, and its synthesis continued at a lower rate in cells infected by this mutant. As seen in the 12.5% acrylamide gel, the product of the *rIIA* cistron was made at a higher rate from 4 to 11 min after infection in *farP14*-infected cells than in *far*$^+$- or *farP23*-infected cells. Some effects not obvious in the 12.5% acrylamide gels can be seen in the gradient gels. The product of gene 39 was synthesized for a longer period of time in cells infected by both *farP14* and *farP23* than in cells infected by *far*$^+$. This is the only protein visible on the gels that acts like FH_2 reductase by being overproduced in cells infected by *farP14* and *farP23*. An unidentified band seen above the product of gene 45 in Fig. 6 and 7 was present in cells infected by *farP23*.

This band has not been seen in cells infected by *farP12* and *farP13*, the other two class II overproducers, and its presence in *farP23*-infected cells appears to be due to a fragment of a protein produced by the *farP23* deletion ending in the middle of a gene. A band designated as X appeared to be made 1 to 3 min after infection in cells infected by far^+ and *farP23* but 6 to 13 min after infection in cells infected by *farP14*.

Sippel and Hartmann (27) showed that the antibiotic rifampin interfered with the initiation of RNA synthesis. O'Farrell and Gold (21) found that adding rifampin shortly after infection by T4 decreased the amount of the products of genes 43, 45, 46, 32, and *rIIB* made while increasing the amounts of the products of genes *rIIA*, 39, and 52 made after infection of *E. coli*. The proteins made in reduced amounts were called quasi-late proteins. They believe that the overproduction of some gene products occurs because the mRNA coding for these products has greater access to the ribosomes when rifampin is added shortly after infection. This occurs because the rifampin prevents the initiation of synthesis of message coding for quasi-late proteins, and consequently the mRNA coding for early proteins has less competition for binding to the ribosomes. If this is true, the major defect in the class I overproducer *farP14* could be a failure or delay in initiation of transcription from the quasi-late promoters that are used for transcription of mRNA coding for the quasi-late proteins whose appearance is delayed in *farP14*. Adding rifampin shortly after infection should prevent initiation at quasi-late promoters in both wild type- and *farP14*-infected cells, and the amount of overproduction of enzymes should be the same in both cases.

When rifampin was added 2 min after infection, both FH_2 reductase and dCMP deaminase activities were higher 15 min after infection than when no rifampin was added (Table 7). However, both enzyme activities were higher in

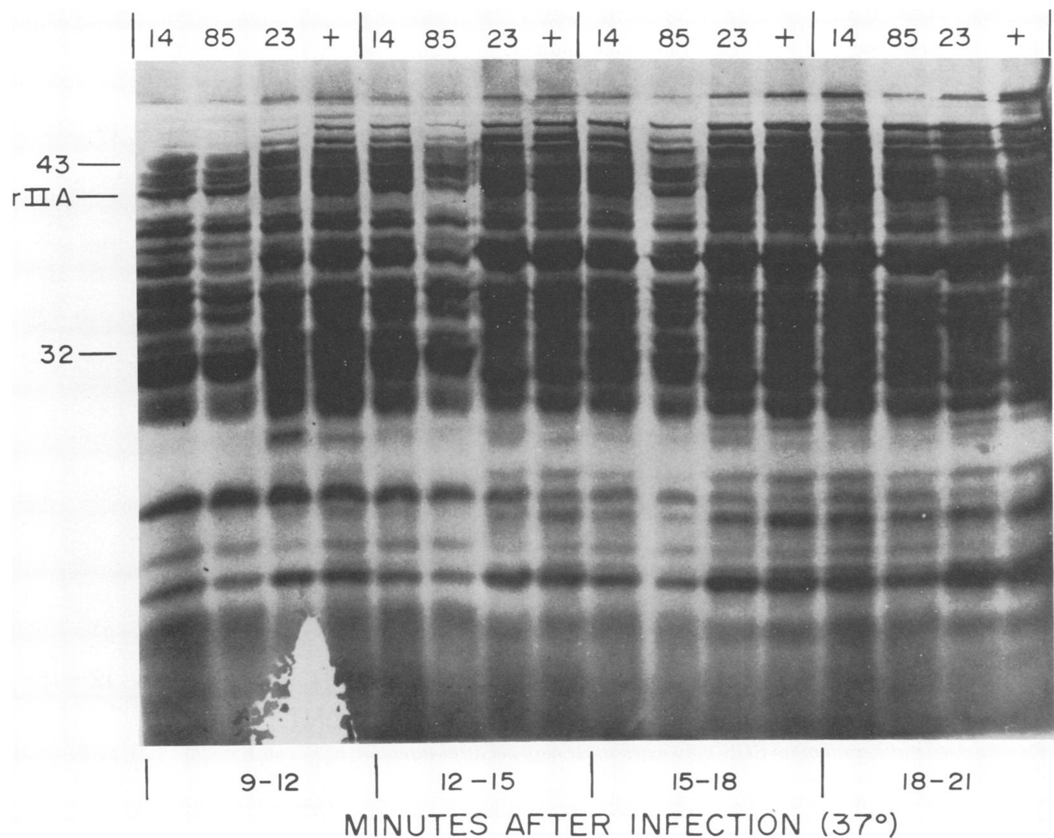

FIG. 5. *Autoradiogram of an SDS-polyacrylamide slab gel after infection of E. coli B by farP14 (14), farP85 (85), farP23 (23), and far⁺ (+). Proteins were pulse labeled with a mixture of ^{14}C-labeled amino acids for the times indicated. The gels contained 12.5% acrylamide. The numbers on the left edge of the figure refer to the genes coding for the proteins indicated.*

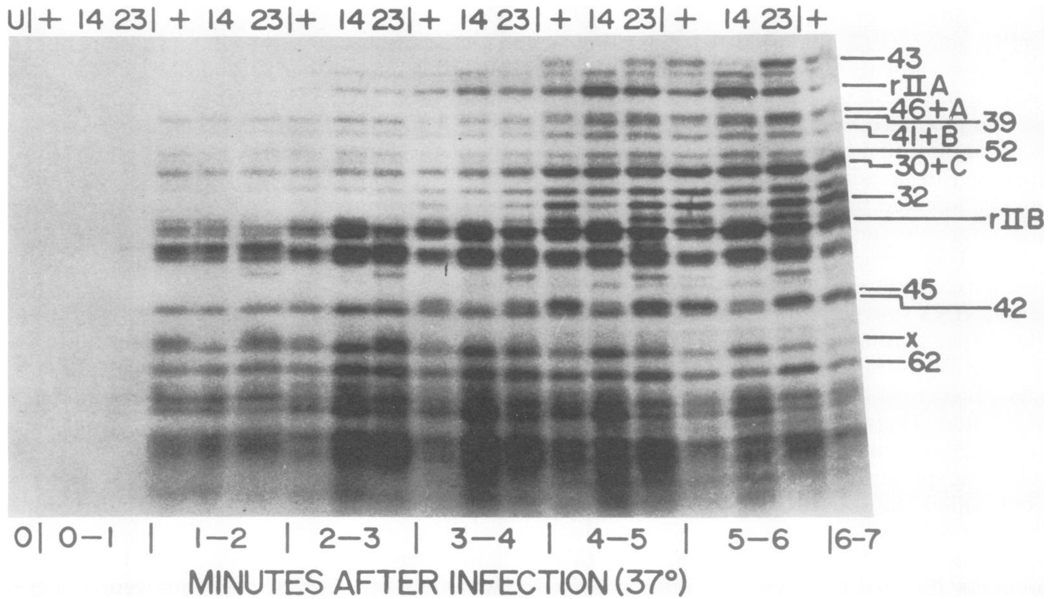

FIG. 6. *Autoradiogram of an SDS-polyacrylamide slab gel after infection of E. coli B by far⁺ (+), farP14 (14), and farP23 (23). U designates uninfected cells. Proteins were pulse labeled with a mixture of ^{14}C-labeled amino acids for the times indicated. The gels contain a gradient of 10 to 18% acrylamide. The numbers on the right edge of the figure refer to the genes coding for the proteins indicated.*

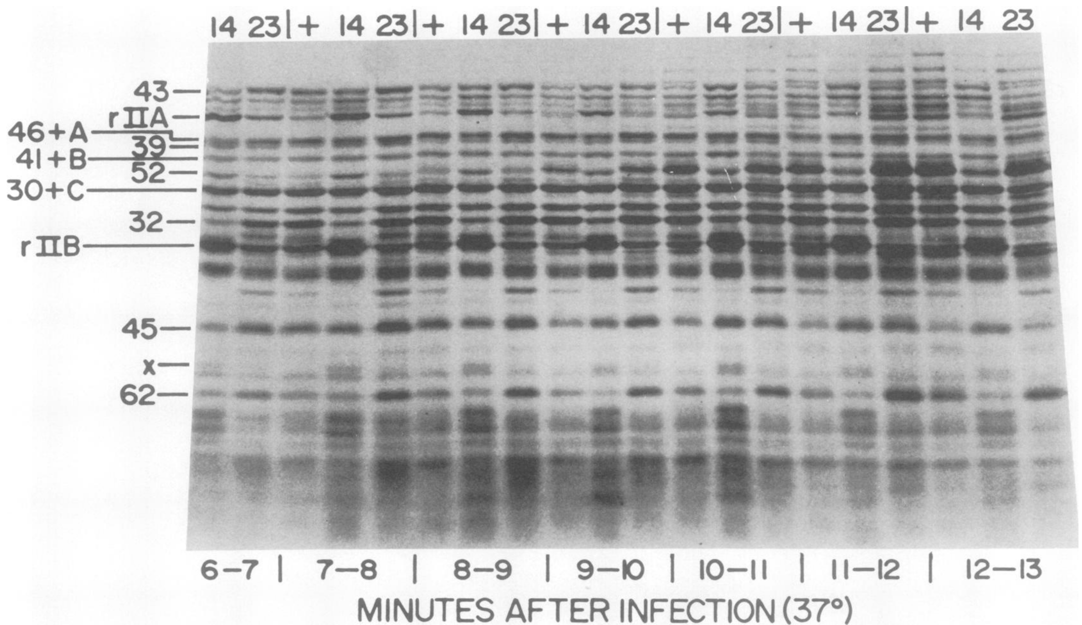

FIG. 7. *Autoradiogram of an SDS-polyacrylamide slab gel after infection of E. coli B by far⁺ (+), farP14 (14), and farP23 (23). Proteins were pulse labeled with a mixture of ^{14}C-labeled amino acids for the times indicated. The gels contain a gradient of 10 to 18% acrylamide. The numbers on the left edge of the figure refer to the genes coding for the proteins involved.*

cells infected by *farP14* than by *far⁺*-infected cells, even when rifampin was added 2 min after infection. Either quasi-late protein synthesis was reduced more in cells infected by *farP14* than in cells infected by *far⁺* when rifampin was added 2 min after infection, or some additional factor caused overproduction by *farP14*.

It seemed desirable to see what effect rifam-

pin has on the expression of what is most likely the primary phenotype of the class I overproducers, the delay in synthesis of quasi-late proteins such as the products of gene 32 and gene 43. *E. coli* B was infected with *far*$^+$ or *farP14* phage, and rifampin (200 μg/ml) was added either 1 or 2 min after infection. The infected cells were labeled with 2-min pulses of ^{14}C-labeled amino acids. The extracts made from these infected cells were then electrophoresed. The autoradiogram made after electrophoresis is shown in Fig. 8. A slight reduction in the synthesis of gene 43 and gene 32 products could be seen in *farP14*- as compared with *far*$^+$-infected cells even when rifampin was added shortly after infection. This could be seen most clearly during the 4- to 6-min pulses in the case of gene 43 and during the 6- to 8-min pulses in the case of gene 32. No apparent overproduction by *farP14* of the products of genes 39, 52, and *rIIA* could be seen when extracts of cells infected with this mutant were compared with extracts of cells infected with *far*$^+$ when rifampin was added to both cultures shortly after infection. Gene 39 product was not well resolved in these gels, particularly in the samples where rifampin was added 2 min after infection so that its overproduction would be difficult to detect. The overproduction of FH_2 reductase by *farP14* in the presence of rifampin could be accounted for by the reduction in the synthesis of quasi-late proteins under these conditions. If the overproduction of other early enzymes when rifampin was added shortly after infection was only as great as the overproduction of FH_2 reductase, it would probably not be possible to see this on the gels.

Rifampin was also used to see whether the synthesis of FH_2 reductase between 5 and 10 min after infection in the class II overproducers was due to an additional 5 min of transcription. Even more FH_2 reductase activity was present in cells infected with *farP23* if rifampin was added 4 min after infection than when no rifampin was added (Table 8). It seems very likely that the mutation present in *farP23* affects a post-transcriptional step in FH_2 reductase formation.

Since the phenotype seen in class II overproducers can be explained by a stabilization in the translation of some early species of T4 mRNA, it seems possible that some host mRNA might also be stabilized and that host protein synthesis might continue for a longer time after infection by the overproducer than after infection by wild-type phage. Figure 9 shows autoradiograms made after electrophoresis of extracts made from cells infected by *far*$^+$, *farP14*, and *farP23* as well as extracts made from uninfected cells. The cells in this case were not treated with UV light before infection. The infected cells were pulse labeled with ^{14}C-labeled amino acids from 3 to 5 min after infection and the uninfected cells were also pulse labeled for 2 min. Three bands, indicated by the arrows, indicate host proteins made in much greater quantity in cells infected by *farP23* than in cells infected by *far*$^+$ or *farP14*.

TABLE 7. *Effect of rifampin on enzyme induction by farP14*[a]

Phage	Time of rifampin addition (min after infection)	Sp act	
		FH_2 reductase[b]	dCMP deaminase[c]
far$^+$		28 ± 1	51 ± 13
farP14		37 ± 3[d]	74 ± 14
far$^+$	2	44 ± 2	107 ± 21
farP14	2	55 ± 3[d]	125 ± 7

[a] Cells were infected at a multiplicity of 4 to 6 phage per cell for 15 min at 37 C. Rifampin (200 μg/ml) was added at the time shown. Each value shown is the mean ± standard deviation for three samples.

[b] Expressed as nanomoles of tetrahydrofolate formed per minute per milligram of protein.

[c] Expressed as nanomoles of dUMP formed per minute per milligram of protein.

[d] $P < 0.01$ when compared with the value for *far*$^+$ using Student's *t* test.

DISCUSSION

The isolation of mutants of T4 that overproduce the enzyme FH_2 reductase demonstrates that there is some regulation of T4 protein synthesis. These mutants fall into two classes. Class I overproducers, which include *farP14* and *farP85*, are characterized by a delay and/or reduction in the synthesis of several quasi-late proteins after infection and the increased synthesis of several early proteins. Class II overproducers, which include *farP12*, *farP13*, and *farP23*, are characterized by the overproduction of a few proteins. Only two proteins, FH_2 reductase and gene 39 product, have been shown to be overproduced by both classes of overproducers.

The class II overproducers have an effect on far fewer proteins than the class I overproducers do. Three proteins, FH_2 reductase, gene 39 product, and gene 62 product, are synthesized at a higher rate between 5 and 12 min after infection by the class II overproducers than after infection by wild-type phage. Both FH_2 reductase and gene 39 product are not made

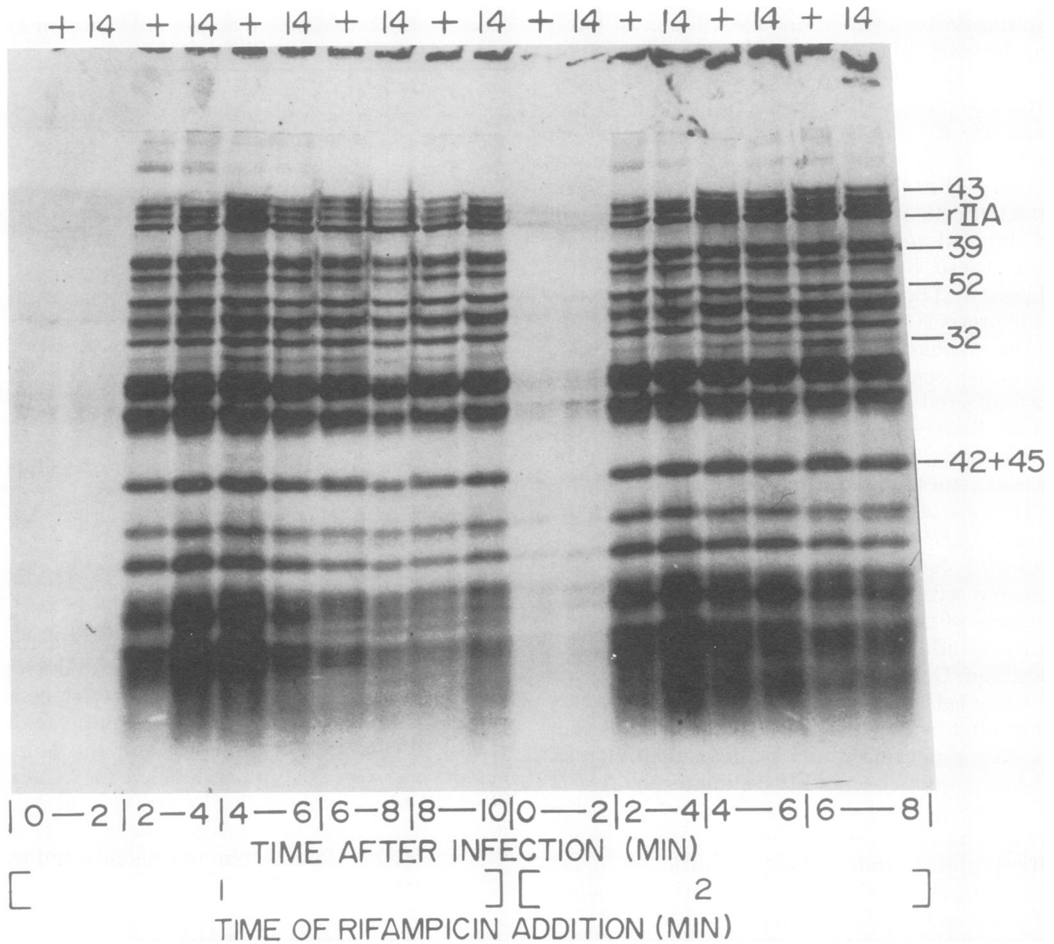

FIG. 8. *Autoradiogram of an SDS-polyacrylamide slab gel after infection of E. coli B by far⁺ (+) and farP14 (14). Proteins were pulse labeled with a mixture of ^{14}C-labeled amino acids for the times indicated. Rifampin (200 μg/ml) was added at 1 or 2 min after infection as indicated. The gels contain a gradient of 10 to 18% acrylamide. The numbers on the right edge of the figure refer to the genes coding for the proteins indicated.*

TABLE 8. *Effect of rifampin on FH_2 reductase induction by farP23*[a]

Phage	Time of rifampin addition (min after infection)	FH_2 reductase sp act[b]
far$^+$		22 ± 1
farP23		39 ± 3
far$^+$	4	23 ± 2
farP23	4	50 ± 7

[a] Cells were infected at a multiplicity of 4 to 6 phage per cell for 15 min at 37 C. Rifampin (200 μg/ml) was added at the time shown. Each value shown is the mean ± standard deviation for three samples.

[b] Expressed as nanomoles of tetrahydrofolate formed per minute per milligram of protein.

later than 5 to 6 min after infection by wild-type phage. It seems that the class II overproducers cause extended synthesis of some proteins that normally stop being made 5 to 6 min after infection. Synthesis of gene 62 product is different from that of most proteins made after infection by wild-type phage since it starts to be made 1 min after infection and continues to be made at least until 12 to 13 min after infection. This presents the possibility that gene 62 product is made first from RNA synthesized from an early promoter and later, when synthesis from this RNA stops, synthesis from message initiated at a quasi-late promoter begins. Overproduction of gene 62 product might be caused by continued synthesis of protein from the message made from the early promoter while it is synthe-

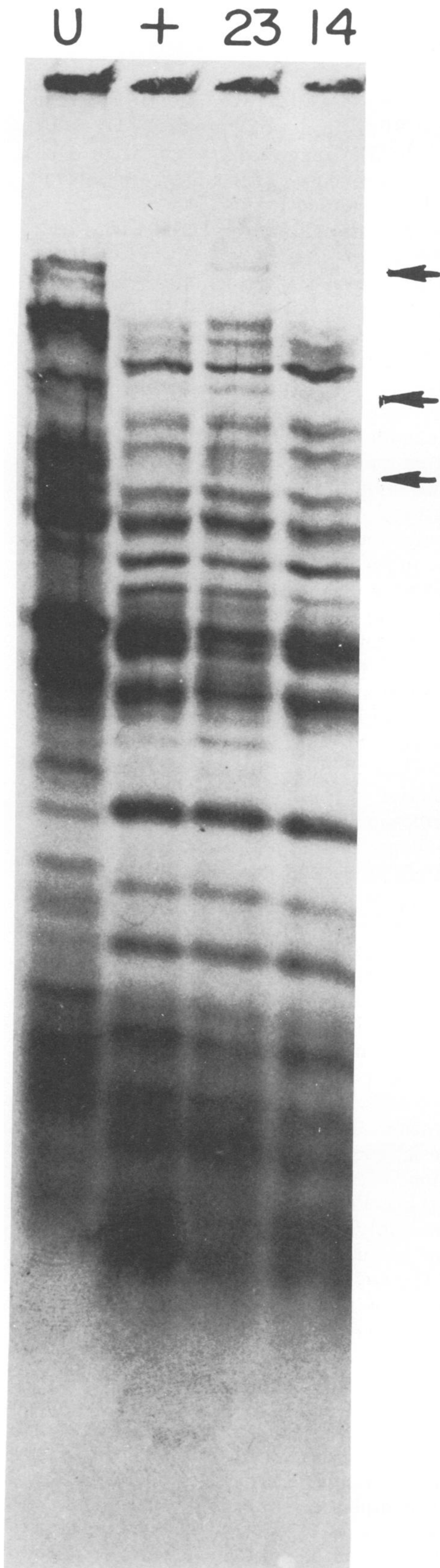

FIG. 9. *Autoradiogram of an SDS-polyacrylamide slab gel after infection of E. coli B by far⁺ (+), farP23 (23), and farP14 (14). U designates uninfected cells. Proteins were pulse labeled with a mixture of* ^{14}C-*labeled amino acids 3 to 5 min after infection. The gels contain 10 to 18% acrylamide.*

sized as normal by using a quasi-late promoter. Hercules and Sauerbier (14) have shown that late in infection gene 62 product is made from the end of a multicistronic message whose promoter is near gene 45, which codes for a quasi-late protein. Since gene 62 product is also made early in infection, it must also be made by using an early promoter.

The experiment with rifampin shows that the overproduction of FH_2 reductase by class II overproducers occurs when no RNA initiation can take place. Experiments by Trimble et al. (33) indicate that rifampin acts within 30 s after its addition to *E. coli* B infected with T-even phage. Experiments in our laboratory (results not shown) show that when rifampin is added with T4 phage, no phage protein synthesis occurs. Therefore, it is plain that no new FH_2 reductase message could have been initiated later than 5 min after infection when rifampin was added 4 min after infection. The increased synthesis is likely due to increased translation of message made during the first 5 min.

Trimble et al. (32) showed that the amount of mRNA coding for FH_2 reductase increases sharply from 1 to 4 min after infection but then decreases. The mRNA coding for other early enzymes continues to increase at least until 6 min after infection. It appears likely that the mRNA coding for FH_2 reductase is stabilized in some way between 5 and 10 min after infection by class II overproducers. It is possible that wild-type phage modifies the *E. coli* translational system shortly after infection so that mRNA coding for FH_2 reductase can no longer be translated. There are indications that some sort of modification in the translational machinery does occur after T4 infection (24). The continued translation of the mRNA coding for FH_2 reductase by the class II overproducers could stabilize it. Another possibility is that a nuclease specific for a small number of species of mRNA is made in wild-type T4-infected cells but not in cells infected by class II overproducers. The increased stability of the mRNA may lead to its increased translation just as its increased translation may lead to its increased stability. Some host proteins are also made for a longer period of time than normal after infection by class II overproducers. It is likely that the mRNA coding for these proteins is also stabilized. Another mutant of T4, called SP62, may also affect the stability of early message (25). The class II overproducers are different from SP62 in the following ways: (i) the affected genes are not close to each other (Results; 37); (ii) preventing DNA synthesis causes additional overproduction by SP62 but not by class II overproducers (Results; 37); and (iii) cells infected by SP62 do not overproduce FH_2 reductase or dCMP deaminase, even when DNA synthesis is prevented (J. Johnson and D. Hall, personal communication).

When *E. coli* cells are infected with *farP23* and wild-type phage, the amount of FH_2 reductase synthesized is intermediate between the amount made in cells infected by *farP23* and the amount made by wild type-infected cells. When *E. coli* is infected with a T4 mutant defective in the synthesis of some enzyme together with wild-type phage, the amount of enzyme made is related to the ratio of wild-type to mutant phage used in the infection (35). In the experiments described, cells were infected at a ratio of wild type to *farP23* of 1:1. Therefore, only about half as much of the product missing in *farP23* should be made as is made in cells infected only with wild-type phage. If this product is an enzyme, such as nuclease, it can be used repeatedly and its reduction by one-half would have little effect. However, if the product is bound in some way to the ribosomes, its reduction could leave some ribosomes unaltered and cause continued translation of FH_2 reductase message. Thus, class II overproducers may be defective in the modification or synthesis of some ribosomal protein or factor.

The experiments performed so far do not absolutely prove that the class II overproducers affect translation. It is possible that the mRNA coding for FH_2 reductase is synthesized more rapidly during the first 4 min of infection by the class II overproducers than during the same period after infection by wild-type T4. However, there is no evidence that FH_2 reductase is made at a higher rate during the first 4 min in class II overproducer-infected cells than by wild-type infected cells. Also, it is difficult to imagine how a mutation affecting the control of messenger synthesis could have its effect so early in infection.

The class I overproducers are very similar to, and probably defective in the same gene as, a mutant isolated by Mattson et al. (20) called *tsG1*. The *tsG1* mutation shows a recombination frequency of 4.6% with *amH17*, an amber mutant defective in gene 52, and 8.4% with *a3* (*t* gene). Johnson and Hall (18) found that *farP85* shows a recombination frequency of 3.4% with *amH17* and 11% with *amB5*, another mutant defective in the *t* gene. As shown in Results, *farP14* maps very close to this position and appears to be in the same gene as *farP85* based on the complementation studies. Like *farP14*-infected cells, *tsG1*-infected cells are delayed in the synthesis of the products of genes 43, 45, and

32 and overproduce the product of the *rIIA* cistron. Unlike *tsG1*, *farP14* and *farP85* are not temperature sensitive. Their efficiency of plating is the same at 32, 37, and 42 C (J. Johnson and D. Hall, personal communication). It is interesting that the proteins found to be produced in very low amounts when rifampin is added 1 min after infection, the products of genes 43, 45, 46, 32, and *rIIB* (21), include all the proteins whose synthesis is delayed after infection by *farP14*. The proteins overproduced when rifampin is added 1 min after infection, the products of genes 39, 52, and *rIIA* (21), are also overproduced by *farP14*. As has been shown, FH_2 reductase and dCMP deaminase are overproduced when rifampin is added shortly after infection by wild-type phage as well as in *farP14*-infected cells when no rifampin is added. The mutant *farP14* overproduces *rIIB* products, whereas this protein is made in lower than normal amounts when rifampin is added 1 min after infection by wild-type phage. However, the overproduction of *rIIB* product is probably due to translation of the multicistronic message coding for both *rIIA* and *rIIB* products. O'Farrell and Gold (21) showed that *rIIB* product is made at twice the rate of *rIIA* product even when rifampin is added very early after infection, so increased synthesis of the multicistronic message should lead to increased synthesis of *rIIB* product even when no *rIIB* product is made from mRNA initiated at the *rIIB* quasi-late promoter. Also, the synthesis of the product of gene 46 does not appear to be delayed in *farP14*-infected cells. However, a second protein, called A (Fig. 6), co-migrates with gene 46 product and makes it difficult to see when gene 46 product is first made.

Hercules and Sauerbier (15) have presented evidence that *tsG1* affects recognition of quasi-late promoters. They irradiated T4 phage with increasing doses of UV light and then infected the cells with these phage. They reasoned that the further away a gene was from its promoter, the more chance there would be that the DNA between the gene and the promoter would be damaged by UV light and the more sensitive to UV light the synthesis or protein from that gene would be. A few minutes after infection, synthesis of the products of gene 43 and 45 shows less sensitivity to UV irradiation of the phage. This implies that a new promoter is recognized at this time that is closer to genes 43 and 45. This new promoter does not seem to be recognized in the case of *tsG1*, the mutant that acts like the class I overproducers, because the synthesis of the products of gene 43 and 45 does not become less sensitive to the amount of UV light the phage was irradiated with at the same time it does in cells infected by wild-type phage. It seems most likely that *tsG1* (and class I overproducers by implication) directly affects recognition of quasi-late promoters. These mutations could alter promoter recognition by affecting the alterations of RNA polymerase that have been observed (29, 30). However, the possibility is not excluded that *tsG1* prevents translation of message initiated at these promoters.

Although all proteins that are overproduced by *farP14* (a class I overproducer) start to be made during the first 3 min after infection, not all proteins made during the first 3 min after infection are overproduced by *farP14*. This may occur because the mRNA coding for some of the proteins not overproduced is no longer present in the cells between 6 and 10 min after infection when most of the overproduction occurs.

The FH_2 reductase overproducers supply enough information to allow a model of T4 control of protein synthesis to be constructed. The message coding for many early enzymes immediately after infection is made by using the *E. coli* RNA polymerase and is translated by using the *E. coli* translation machinery. A few minutes after infection, new promoters begin to be recognized on the T4 DNA, perhaps due to changes in RNA polymerase. In class I overproducers, these promoters are not recognized or their recognition is delayed. At the same time these new promoters are recognized, the promoters for some of the early message may stop being recognized. The protein missing in class II overproducers could prevent translation of some early message, either by causing its degradation or by directly preventing its translation. It also prevents the translation of some host message. Many of the quasi-late proteins are needed for DNA synthesis to occur. Shortly after DNA synthesis starts, message for early proteins stops being made and translated, and message for late proteins starts to be made and translated.

This rather complicated pattern insures that sufficient amounts of all proteins are made. Since delay in the synthesis of quasi-late proteins causes overproduction of early proteins, it seems likely that if the quasi-late proteins were made immediately after infection, the amount of some early proteins would be greatly diminished. Delay in the synthesis of quasi-late and late proteins allows sufficient early protein to be made.

The overproducers isolated so far present no evidence that the expressions of genes closely linked to the gene coding for FH_2 reductase are controlled together. The two types of mutants

that cause the overproduction of FH_2 reductase do so indirectly, either by decreasing the synthesis of other proteins or by stabilizing the mRNA coding for FH_2 reductase. The genes affected by these mutants are unlinked to each other or to the mutation causing the overproduction. There is no evidence for a classic operon as is found in bacteria.

ACKNOWLEDGMENTS

We thank Marcia Rementer and Jerry Collins for their excellent technical assistance and James Johnson for helpful discussions and advice.

This investigation was supported by Public Health Service research career development award GM-33299 to D.H.H. and Public Health Service research grant GM-16306, both from the National Institute of General Medical Sciences. K.V.C. performed the work while a predoctoral trainee supported by Public Health Service training grant GM-00233 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Benzer, S. 1961. On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S.A. **47**:403-415.
2. Bruner, R., A. Souther and S. Suggs. 1972. Stability of cytosine-containing deoxyribonucleic acid after infection by certain T4 *r*II-D deletion mutants. J. Virol. **10**:88-92.
3. Chace, K. V., and D. H. Hall. 1973. Isolation of mutants of bacteriophage T4 unable to induce thymidine kinase activity. J. Virol. **12**:343-348.
4. Doermann, A. H., and M. B. Hill. 1953. Genetic structure of bacteriophage T4 as described by recombination studies of factors influencing plaque morphology. Genetics **38**:79-90.
5. Duckworth, D. H., and M. J. Bessman. 1967. The enzymology of virus-infected bacteria. X. A biochemical-genetic study of the deoxynucleotide kinase induced by wild type and amber mutants of phage T4. J. Biol. Chem. **242**:2877-2885.
6. Edgar, R. S., and W. B. Wood, 1966. Morphogenesis of bacteriophage T4 in extracts of mutant infected cells. Proc. Natl. Acad. Sci. U.S.A. **55**:498-505.
7. Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. B. de la Tour, R. Chevally, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis. 1963. Physiological studies of conditional mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**:375-394.
8. Fraser, D., and E. A. Jerrel. 1953. The amino acid composition of T3 bacteriophage. J. Biol. Chem. **205**:291-295.
9. Futterman, S. 1957. Enzymatic reduction of folic acid and dihydrofolic acid to tetrahydrofolic acid. J. Biol. Chem. **228**:1031-1038.
10. Goscin, L. A., and D. H. Hall. 1972. Hydroxyurea-sensitive mutants of bacteriophage T4. Virology **50**:84-94.
11. Hall, D. H. 1967. Mutants of bacteriophage T4 unable to induce dihydrofolate reductase activity. Proc. Natl. Acad. Sci. U.S.A. **58**:584-591.
12. Hall, D. H., and I. Tessman. 1966. T4 mutants unable to induce deoxycytidylate deaminase activity. Virology **29**:339-345.
13. Hall, D. H., I. Tessman, and O. Karlström. 1967. Linkage of T4 genes controlling a series of steps in pyrimidine biosynthesis. Virology **31**:442-448.
14. Hercules, K., and W. Sauerbier. 1973. Transcription units in bacteriophage T4. J. Virol. **12**:872-881.
15. Hercules, K., and W. Sauerbier. 1974. Two modes of in vivo transcription for genes 43 and 45 of phage T4. J. Virol. **14**:341-348.
16. Hosoda, J., and C. Levinthal. 1968. Protein synthesis by *Escherichia coli* infected with bacteriophage T4D. Virology **34**:709-727.
17. Johnson, J. R., and D. H. Hall. 1973. Isolation and characterization of mutants of bacteriophage T4 resistant to folate analogs. Virology **53**:413-426.
18. Johnson, J. R., and D. H. Hall. 1974. Characterization of new regulatory mutants of bacteriophage T4. J. Virol. **13**:666-676.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem **193**:265-275.
20. Mattson, T., J. Richardson, and D. Goodin. 1974. Mutant of bacteriophage T4D affecting expression of many early genes. Nature (London) **250**:48-50.
21. O'Farrell, P. Z., and L. M. Gold. 1973. Bacteriophage T4 gene expression. Evidence for two classes of prereplicative cistrons. J. Biol. Chem. **248**:5502-5511.
22. O'Farrell, P. Z., L. M. Gold, and W. M. Huang. 1973. The identification of prereplicative bacteriophage T4 proteins. J. Biol. Chem. **248**:5499-5501.
23. Price, A. R., and H. R. Warner. 1968. A structural gene for bacteriophage T4-induced deoxycytidine triphosphate-deoxyuridine triphosphate nucleotidohydrolase. Virology **36**:523-526.
24. Revel, M., Y. Pollack, Y. Groner, R. Scheps, H. Inouye, H. Berissi, and H. Zeller. 1973. IF3-interference factors: protein factors in *Escherichia coli* controlling initiation of mRNA translation. Biochimie **55**:41-51.
25. Sauerbier, W., and K. Hercules. 1973. Control of gene function in bacteriophage T4. J. Virol. **12**:538-547.
26. Simon, E. H., and I. Tessman. 1963. Thymidine-requiring mutants of phage T4. Proc. Natl. Acad. Sci. U.S.A. **50**:526-532.
27. Sippel, A., and G. Hartmann. 1968. Mode of action of rifampin on the RNA polymerase reaction. Biochim. Biophys. Acta **157**:218-219.
28. Snustad, D. P. 1968. Dominance interactions in *Escherichia coli* cells mixedly infected with bacteriophage T4D wild type and *amber* mutants and their possible implication as to type of gene product function: catalytic vs. stoichiometric. Virology **35**:550-563.
29. Stevens, A. 1972. New small polypeptides associated with DNA dependent RNA polymerase of *Escherichia coli* after infection with bacteriophage T4. Proc. Natl. Acad. Sci. U.S.A. **69**:603-607.
30. Stevens, A. 1974. Deoxyribonucleic acid dependent polymerases from two T4 phage-infected systems. Biochemistry. **13**:493-503.
31. Studier, F. W. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol **79**:237-248.
32. Trimble, R. B., J. Galivan, and F. Maley. 1972. The temporal expression of T2r$^+$ bacteriophage genes *in vivo* and *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **69**:1659-1663.
33. Trimble, R. B., G. F. Maley, and F. Maley. 1972. Relationship between *Escherichia coli* B titer and the level of deoxycytidylate deaminase activity induced on bacteriophage T2r$^+$ infection. J. Virol. **9**:454-464.
34. Warner, H. R., and N. Lewis. 1966. The synthesis of deoxycytidylate deaminase and dihydrofolate reductase and its control in *Escherichia coli* infected with bacteriophage T4 and T4 *amber* mutants. Virology **29**:172-175.
35. Wiberg, J. S. 1967. *Amber* mutants of bacteriophage T4 defective in deoxycytidine di- and triphosphatase: on the role of 5-hydroxymethylcytosine in bacteriophage deoxyribonucleic acid. J. Biol. Chem. **242**:5824-5829.

36. Wiberg, J. S., M. L. Dirkson, R. H. Epstein, S. E. Luria, and J. M. Buchanan. 1962. Early enzyme synthesis and its control in *E. coli* infected with some *amber* mutants of bacteriophage T4. Proc. Natl. Acad. Sci. U.S.A. **48**:293-302.
37. Wiberg, J. S., S. Mendelsohn, V. Warner, K. Hercules, C. Aldrich, and J. L. Munro. 1973. SP62, a viable mutant of bacteriophage T4D defective in regulation of phage enzyme synthesis. J. Virol. **12**:775-792.
38. Wood, W. B. 1974. Genetic map of bacteriophage T4. *In* R. C. King (ed.), Handbook of genetics. Plenum Publishing Corp., New York.
39. Yeh, Y. C., E. J. Dubovi, and I. Tessman. 1969. Control of pyrimidine biosynthesis by phage T4: mutants unable to catalyze the reduction of cytidine diphosphate. Virology **37**:615-623.