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Utilization of Early Promotors in Mutant *far*P85 of Bacteriophage T4

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We show that *far*P85 is a recessive mutant of T4 incapable of activating the delayed early promotors for genes 43 and 45 and that the *far*P85 mutation is in the same complementation group as the *ts*G1 mutation, which is located in the "modifier of transcription" (*mot*) gene.

Maximal expression of some T4 delayed early genes is achieved by activation of a set of "delayed early promotors" (16) shortly after infection (3, 4, 10, 12, 13). The T4 mutant *ts*G1 (*mot*$^-$; 10) is defective in activating some delayed early promotors" (16) shortly after infectransition from immediate early to delayed early promotor utilization (6), and it has reduced and delayed expression of some early genes (10). Another T4 mutant, *far*P85, isolated on the basis of resistance to folate analogues (8), was shown to overproduce dihydrofolate reductase and several other early enzymes (9). Recently, Chace and Hall (1) showed that the *far*P85 was also delayed in the expression of certain other early genes. Moreover, the *ts*G1 and *far*P85 mutations were found to map close to each other, between genes 52 and *t* (9, 10), and it is conceivable that the *far*P85 mutant is also defective in utilizing delayed early promotors and that both mutations affect the same gene.

To elucidate the mechanism by which the *far*P85 mutation alters the expression of T4 early genes, we have tested the ability of this mutant to make the shift from immediate early to delayed early promotors. The delayed early promotors for genes 43 and 45 are located much closer to these genes than the immediate early promotors from which they are transcribed immediately after infection (4). Therefore, the transition to delayed early promotor utilization manifests itself in a shortening of the transcriptional distance. Transcriptional distances were measured by the "UV mapping technique," which we used previously to define transcription units in the early region of bacteriophage T4 and to determine the effect of the *ts*G1 mutation on promotor recognition (5, 6). The technique is based on the fact that UV-induced photoproducts in DNA cause premature termination of transcription (11, 15). Therefore, the UV sensitivity of the expression of a particular gene becomes a function of its distance from the promotor.

We show here, by UV mapping, that the *far*P85 mutation prevents the utilization of the delayed early promotors for the expression of genes 43 and 45. In addition, by measuring promotor recognition in complementation experiments, we show that the *far*P85 mutation is recessive to the wild-type allele and that the *far*P85 mutation and the *ts*G1 mutation are in the same complementation group.

MATERIALS AND METHODS

Bacteriophage. Bacteriophage T4D was from the collection of R. S. Edgar. The T4 mutant *ts*G1 (10) was isolated by and obtained from T. Mattson. The T4 mutant *far*P85 was isolated by Johnson and Hall (8).

Bacteria. Experiments were carried out in host *Escherichia coli* B_{s-1} (7). All phage lysates were prepared in *E. coli* B_{s-1} at 30°C.

Chemicals and radiochemicals. *N,N'*-methylenebisacrylamide and acrylamide were purchased from Bio-Rad Laboratories, Richmond, Calif. Rifampin, sodium dodecyl sulfate (SDS), Tris, and ^{14}C-labeled amino acids, algal profile (approximate average specific activity of 300 mCi/mmol), were purchased from Schwarz/Mann, Orangeburg, N.Y.

Media. M9 (KH_2PO_4, 3.0 g; $Na_2HPO_4 \cdot 12H_2O$, 15 g; NH_4Cl, 1.0 g; water, 1,000 ml; glucose, 0.4% [wt/wt], and $MgSO_4$, 10^{-3} M, pH 6.9) was used for growth of bacteria; 2×10^{-5} M tryptophan was added for adsorption of T4.

Growth and infection of bacteria. Cultures of bacteria (10 to 20 ml), grown in M9 medium to a density of 5×10^8 cells/ml, were chilled and UV irradiated at 254 nm (incident dose, 2,600 ergs/mm^2) on a watch glass (14 cm in diameter) with rapid stirring. Bacteria were then incubated at 37 or 42°C for 5 min before infection. Bacteriophage were UV irradiated in 5-ml batches at a concentration of 5×10^{10}/ml in M9 medium in the same manner as the bacteria; the dose rate was 14 ergs/mm^2 per s. Bacteria were infected with irradiated or unirradiated

phage at a multiplicity of infection of 10. Aliquots (1 ml) of infected cultures were labeled with ^{14}C-amino acids (2 to 4 μCi/ml) beginning at 6 min after infection. At 9 min after infection, a 10^3-fold excess of nonradioactive amino acids was added, and incubation was continued for an additional 3 min. The infective centers were then chilled on ice and sedimented. The pellets were suspended in 0.1 ml of "sample buffer" (0.05 M Tris-hydrochloride, pH 6.8; 1% SDS; 1% mercaptoethanol; and 15% glycerol) and boiled for 2 to 3 min.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was used as described by Studier (18). The dried gels were autoradiographed with Dupont Cronex DC2 film, and the developed film was traced with a Beckman Analytrol densitometer.

RESULTS

UV sensitivity of the expression of early genes of *far*P85. To determine whether the *far*P85 mutation affects utilization of delayed early promotors, the UV sensitivities of the expression of some of the T4 early genes were evaluated in this mutant. *E. coli* B_{s-1} was infected at 37°C with *far*P85, which had been UV irradiated for the times indicated in Fig. 1. Early T4 proteins were labeled with ^{14}C-amino acids from 6 to 9 min after infection and resolved on SDS-polyacrylamide gels (Fig. 1A). The loss of expression of several of the T4 early genes, as determined by densitometry of autoradiograms of the gels, is shown in Fig. 2A. The

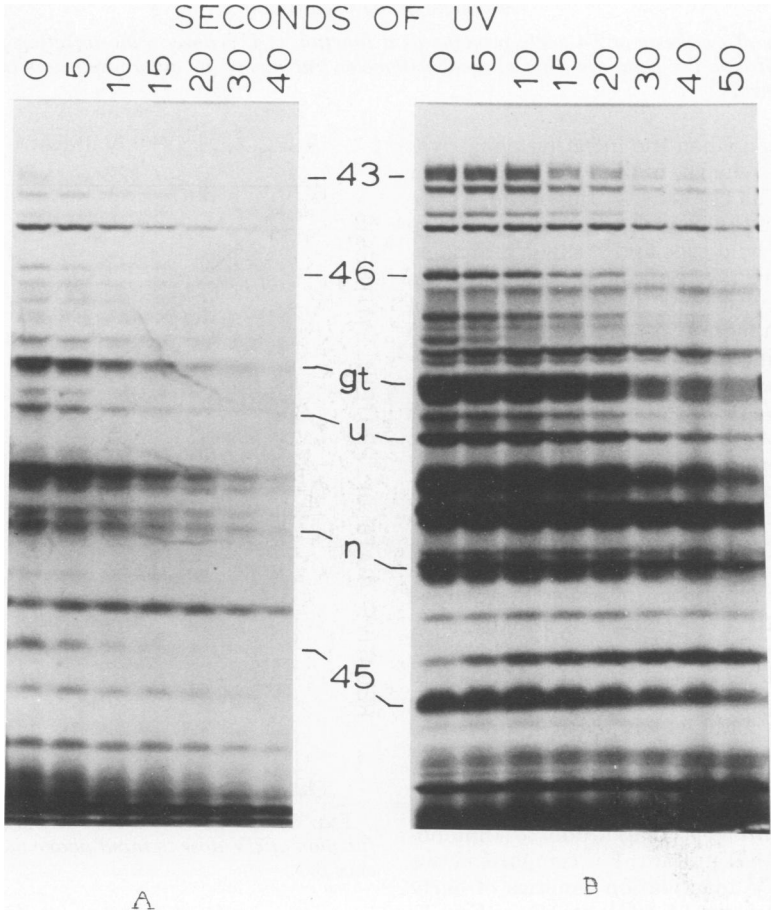

FIG. 1. *Autoradiograms of proteins synthesized in T4-infected E. coli B_{s-1} and resolved on 10% SDS-polyacrylamide gels. (A) T4 farP85; (B) wild-type T4. Samples of 1 ml of bacteria (5 × 10^8 cells/ml, UV irradiated with 2,600 ergs/mm^2) were infected with UV-irradiated T4 farP85 or wild-type T4 at a multiplicity of infection of 10 at 37°C. ^{14}C-labeled amino acids were supplied from 6 to 9 min after infection, and the cells were harvested and prepared for SDS-gel electrophoresis of proteins. Protein bands are identified by comparison with extracts from T4 amber-infected cells and their position is indicated by gene number. Protein band gt is missing in T4 αgtam8, βgtam10 (2); u and n are unidentified proteins which we evaluated for reference purposes.*

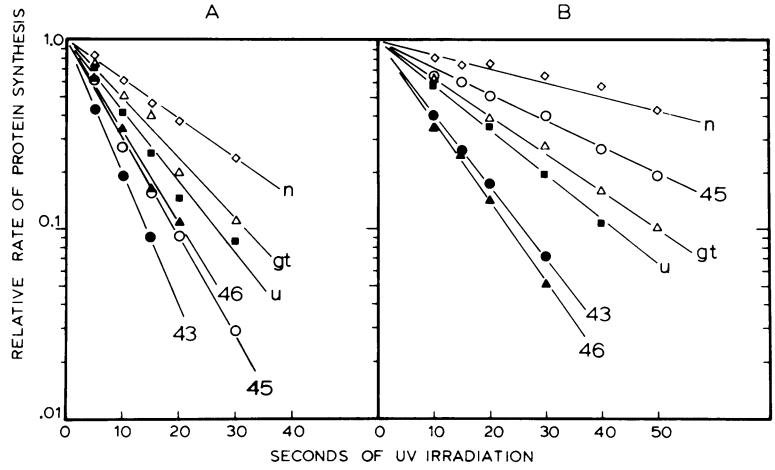

FIG. 2. *Rates of synthesis of T4 early proteins as a function of UV dose to the infecting phage. (A) T4 farP85; (B) wild-type T4. Data are from microdensitometer tracings of the autoradiograms in Fig. 1 and of parallel experiments.*

results obtained when the infecting phage was wild type are shown in Fig. 1B and 2B for comparison. The UV sensitivities of the expression of some of the genes (*n*, *gt*, *u*, and 46) are similar in the wild-type and the mutant phage. However, there is a striking difference in the UV sensitivity of gene 45, the expression of which is much more UV sensitive in the *far*P85 mutant than in wild-type T4. The UV sensitivity of gene 43 expression is also increased in the mutant. Thus, it appears that, in the *far*P85 mutant, genes 43 and 45 are transcribed from promotors more distant than the ones utilized in T4 wild type.

To be assured that the apparent failure to utilize the nearby promotors for the transcription of these genes was not simply due to a delay in their recognition, we also determined the UV sensitivities of expression of the early genes in the *far*P85 mutant with pulse times for ^{14}C-labeled amino acid incorporation from 8 to 11 min after infection. The results were the same as those obtained with 6- to 9-min pulses (data not shown).

The increase in UV sensitivity of the expression of genes 43 and 45 observed in the *far*P85 mutant is similar in magnitude to that observed in the *ts*G1 mutant. For comparison, we include the UV inactivation kinetics of early gene expression in T4 *ts*G1 at 42°C (Fig. 3, published previously, see reference 6). The effect on promotor recognition by the *far*P85 and the *ts*G1 mutations appears to be very similar.

Complementation of *far*P85. To determine whether the mutation *far*P85 and *ts*G1 are in the same gene, we have carried out complementation tests, measuring the UV sensitivities of

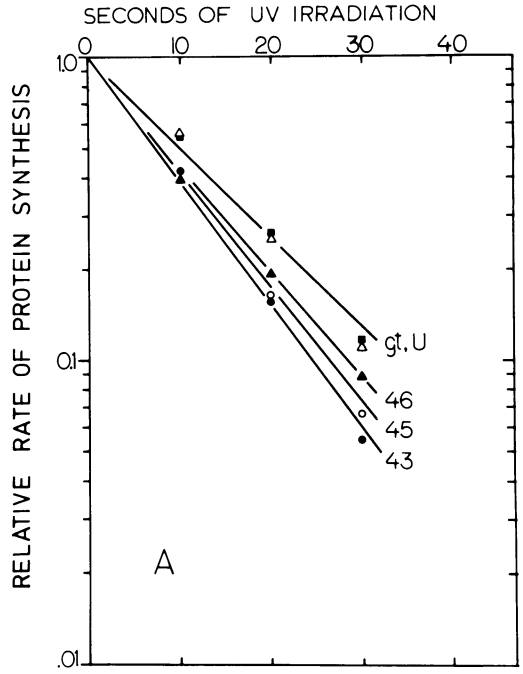

FIG. 3. *Rate of synthesis of T4 early proteins as a function of UV dose to input phage tsG1 (from reference 6).*

the expression of the early genes in cells co-infected with both mutants.

First, it was necessary to determine whether the *far*P85 mutation was recessive and could be complemented by wild-type T4. To accomplish this, *E. coli* B_{s-1} was co-infected at 37 and 42°C with *far*P85 and a mutant of T4 carrying amber

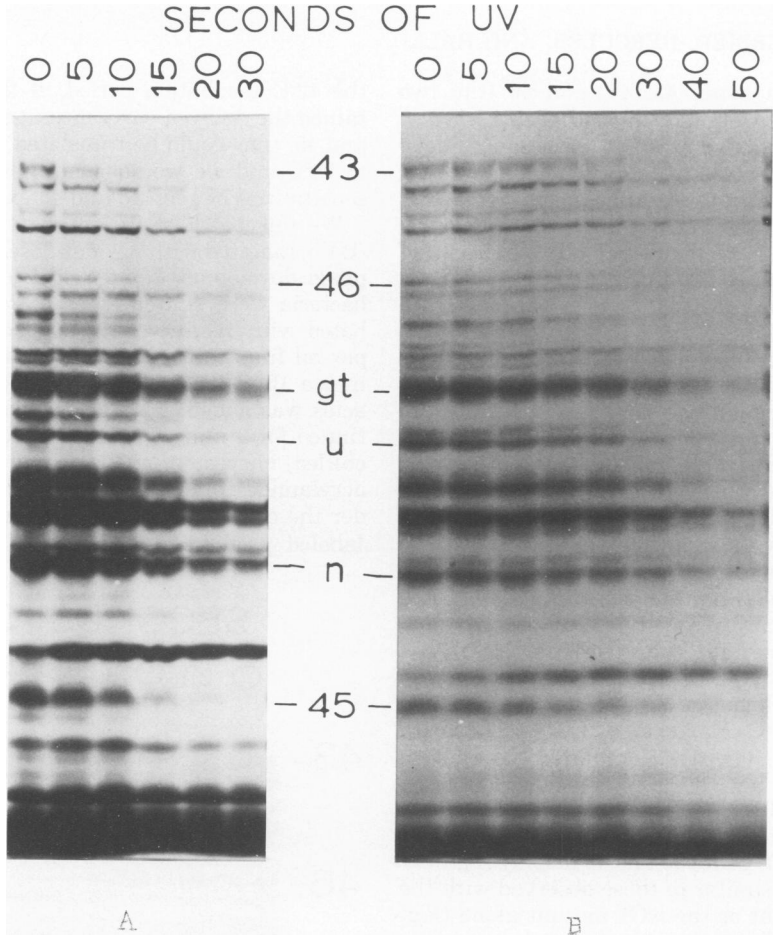

FIG. 4. *Autoradiograms of proteins synthesized in cells infected with a mixture of T4 farP85 and T4 carrying amber mutations in genes 43, 45, and 46. (A) Experiment carried out at 37°C; (B) at 42°C. Other experimental conditions were the same as in Fig. 1.*

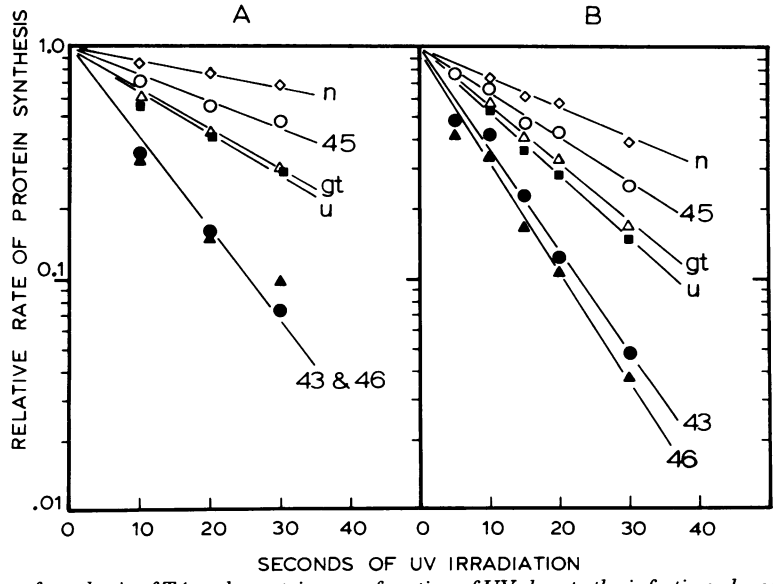

FIG. 5. *Rate of synthesis of T4 early proteins as a function of UV dose to the infecting phage. (A) T4 farP85 plus T4 amE4322, amE10, amB14 at 37°C; (B) same at 42°C. Densitometric evaluation of Fig. 4.*

mutations in genes 43, 45, and 46 (the two phage strains were mixed together in a 1:1 ratio and UV irradiated prior to infection). Phage proteins were labeled from 6 to 9 min after infection and resolved on polyacrylamide gels (Fig. 4). Gels were evaluated by autoradiography and densitometry (Fig. 5). The presence of the amber mutations in the *far*P85$^+$ phage assures that the products of genes 43, 45, and 46 that we monitor on the gels are expressed from the *far*P85 mutant genome. The UV sensitivities of the expression of the T4 early genes measured under these conditions (Fig. 5) are identical to those measured in wild-type T4 (Fig. 2B). Thus, the *far*P85 mutation must be recessive, and the *far*P85$^+$ gene product must be trans-active both at 37 and 42°C. Similar conclusions were drawn previously about the *ts*G1 mutation at 42°C (6).

Since the *ts*G1 mutant was originally isolated as a temperature-sensitive mutant (10) and most of its mutant characteristics have been studied only at 42°C, it was necessary to carry out the complementation test between *ts*G1 and *far*P85 at 42°C. *E. coli* B_{s-1} was infected with *far*P85 and *ts*G1 (UV irradiated together in a 1:1 ratio), and the UV sensitivities of the expression of the early genes were determined as described above. Under these conditions (Fig. 6 and 7), genes 43 and 45 display high UV sensitivities similar to those observed with the *far*P85 mutant or the *ts*G1 mutant alone (Fig. 2A and 3). Thus, the mutants fail to complement for the recognition of delayed early promotors at 42°C.

Is the *mot* gene controlling transcription or translation of delayed early messages? The preceding experiments show that T4 *mot*$^-$ does not express genes 43 and 45 from the nearby delayed early promotors, and we have assumed that this is due to a failure to activate these promotors. It is conceivable, however, that the *mot* gene controls a translational function and that in the *mot*$^-$ state the delayed early messages for genes 43 and 45 are not translated. This latter possibility is made unlikely by the following reasoning and experimentation.

If we infect bacteria with T4 *ts*G1 at 42°C, the synthesized RNA will be characteristic of the *mot*$^-$ state. If we, then, shift the infected cells to 25°C, RNA synthesis will convert to the *mot*$^+$ pattern. If we measure the UV sensitivities of gene expression after the shift-down, we should observe the low sensitivities characteristic of genes 43 and 45 in the *mot*$^+$ state. However, if we terminate transcription initiation upon shifting down to 25°C by rifampin addition (17), proteins will be translated exclusively from RNA initiated in the *mot*$^-$ state, i.e., during the 42°C incubation period. If this RNA contained the delayed early messages for genes 43 and 45, they could be translated after the shift to 25°C and we would again observe low UV sensitivities of gene 43 and 45 expression.

We infected host B_{s-1} at 42°C with T4 *ts*G1 (UV irradiated with various doses) and allowed phage development for 6 min. Then the infected bacteria were shifted down to 25°C and incubated with 10 μCi of ^{14}C-labeled amino acids per ml from 6 to 14 min after infection. At 14 min a 10^3-fold excess of nonradioactive amino acids was added, and the incubation was continued for 3 min. The infected cells were then chilled, harvested, and prepared for SDS-polyacrylamide gel electrophoresis of proteins. Under the conditions of this experiment, the ^{14}C-labeled proteins are translated from mRNA

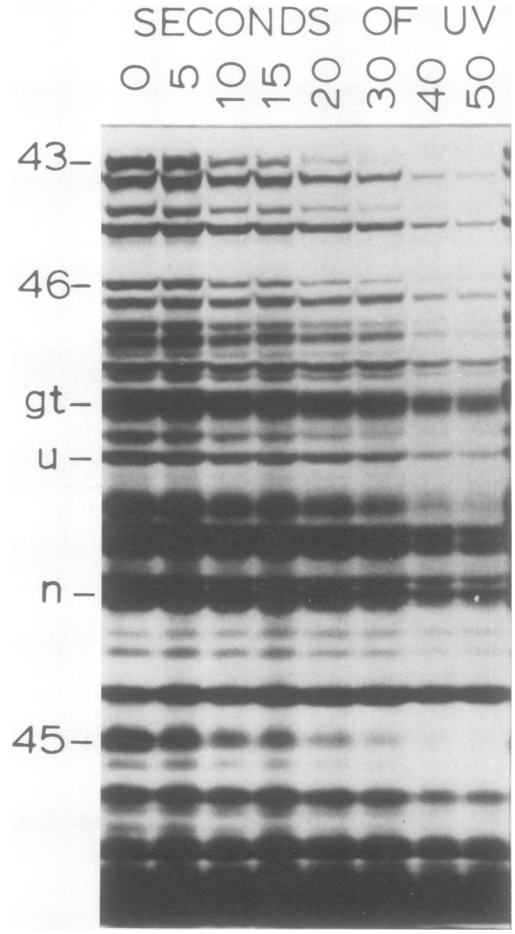

FIG. 6. *Autoradiograms of proteins synthesized at 42°C in cells infected by a mixture of T4 farP85 and T4 tsG1. Experimental conditions were the same as in Fig. 1.*

synthesized at 42°C and from mRNA synthesized after the shiftdown to 25°C. The expression of genes 43 and 45 shows low sensitivity to UV (Fig. 8A), meaning that the delayed early promotors for these genes were utilized and the delayed early transcripts were translated.

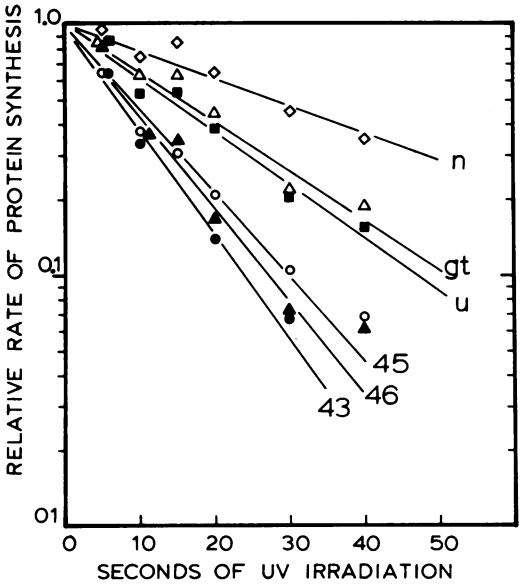

FIG. 7. *Rates of synthesis of T4 early proteins as a function of UV dose to a mixture of infecting T4 farP85 plus T4 tsG1. Densitometric evaluation of Fig. 6.*

When the same experiment was performed but rifampin (200 μg/ml) was added to the bacteria at the time of shift-down, we observed high UV sensitivities of expression for genes 43 and 45. In this case, proteins must be translated from mRNA that had been initiated during the 42°C incubation period (0 to 6 min). If this RNA contained delayed early messenger for genes 43 and 45, the UV sensitivity of expression of these genes would be the same as in the experiments without rifampin. However, we observe high UV sensitivities for the expression of the two genes (Fig. 8B). It is, therefore, obvious that no delayed early mRNA for genes 43 and 45 was available for translation after the shift-down to 25°C. We cannot rule out that delayed early transcripts had been produced and degraded immediately. We consider this unlikely since T4 mRNA half-lives are usually on the order of 5 to 12 min (14) and since we have no evidence that phage mRNA's are subject to accelerated breakdown in the absence of translation (4, 14).

DISCUSSION

Our results indicate that *far*P85 fails to make the transition from immediate early to delayed early promotor utilization in the expression of genes 43 and 45. We also showed that the *far*P85 mutation is recessive and located in the same complementation group as the *ts*G1 mutation.

T4 *far*P85 expresses the *mot*⁻ phenotype at

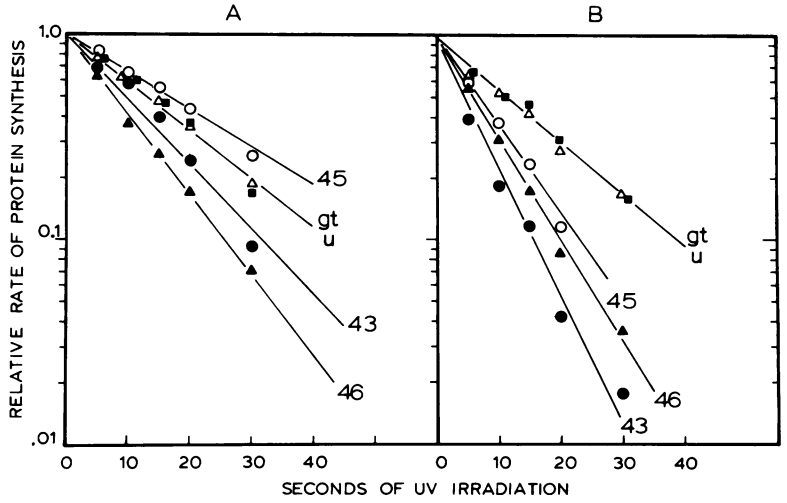

FIG. 8. *Rates of synthesis of T4 early proteins as a function of UV dose to the input T4 tsG1. Infection was carried out at 42°C, and phage development proceeded at this temperature for 6 min; then we shifted the infected cells to 25°C and allowed for ^{14}C-labeled amino acid uptake from 6 to 14 min postinfection at 25°C. (A) Densitometric evaluation of an autoradiogram of labeled proteins from this experiment; (B) from a similar experiment in which 200 μg of rifampin per ml was added at the time of the down-shift to 25°C, i.e., at 6 min postinfection.*

37°C, as indicated by the lack of activation of the delayed early promotors for genes 43 and 45. This mutant is viable at 37°C, although the onset of DNA synthesis is somewhat delayed and the burst size is reduced to about one-half of normal (9). Similarly, T4 *ts*G1 can be propagated at 30°C (10) while it expresses the *mot*$^-$ phenotype (Hercules, unpublished data). At 25°C, however, T4 *ts*G1 is phenotypically *mot*$^+$ (Sauerbier, unpublished data; Fig. 8). Thus, it appears that the *mot* gene function is not required for, but may enhance, growth of T4.

We have not rigorously ruled out the possibility that the *mot*$^-$ state prevents the translation of the delayed early messages for genes 43 and 45 instead of preventing their transcription from the delayed early promotors. This possibility is rendered unlikely, however, by the results shown in Fig. 8.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Chace, K. M., and D. H. Hall.** 1975. Characterization of new regulatory mutants of bacteriophage T4. II. New class of mutants. J. Virol. 15:929-945.
2. **Georgopoulos, G. P., and H. Revel.** 1971. Studies with glucosyl transferase mutants of the T-even bacteriophages. Virology 44:271-285.
3. **Grasso, R. J., and J. M. Buchanan.** 1969. Synthesis of early RNA in bacteriophage T4-infected *Escherichia coli* B. Nature (London) 224:882-885.
4. **Hercules, K., S. Jovanovich, and W. Sauerbier.** 1976. Early gene expression in bacteriophage T7. I. In vivo synthesis, inactivation, and translational utilization of early messenger RNAs. J. Virol. 17:642-658.
5. **Hercules, K., and W. Sauerbier.** 1973. Transcription units in bacteriophage T4. J. Virol. 12:872-881.
6. **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.
7. **Hill, R. F.** 1960. A radiation sensitive mutant of *Escherichia coli*. Biochim. Biophys. Acta 30:636-637.
8. **Johnson, J. R., and D. H. Hall.** 1973. Isolation and characterization of mutants of bacteriophage T4 resistant to folate analogs. Virology 53:413-426.
9. **Johnson, J. R., and D. H. Hall.** 1974. Characterization of new regulatory mutants of bacteriophage T4. J. Virol. 13:666-676.
10. **Mattson, T., J. Richardson, and D. Goodin.** 1974. Mutant of bacteriophge T4D affecting expression of many early genes. Nature (London) 250:48-50.
11. **Michalke, H., and H. Bremer.** 1969. RNA synthesis in *Escherichia coli* after irradiation with ultraviolet light. J. Mol. Biol. 41:1-23.
12. **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.
13. **Salser, W., A. Bolle, and R. Epstein.** 1970. Transcription during bacteriophage T4 development: a demonstration that distinct subclasses of the "early" RNA appear at different times and that some are "turned off" at late times. J. Mol. Biol. 49:271-295.
14. **Sauerbier, W., and K. Hercules.** 1973. Control of gene function in bacteriophage T4. IV. Post-transcriptional shutoff of expression of early genes. J. Virol. 12:538-547.
15. **Sauerbier, W., R. L. Millette, and P. B. Hackett, Jr.** 1970. The effects of ultraviolet irradiation on the transcription of T4 DNA. Biochim. Biophys. Acta 209:368-386.
16. **Schmidt, D. A., A. J. Mazaitis, T. Kasai, and E. K. F. Bautz.** 1970. Involvement of a phage T4 σ factor and an anti-terminator protein in the transcription of early T4 genes. Nature (London) 225:1012-1016.
17. **Sippel, A., and G. Hartmann.** 1968. Mode of action of rifampicin on the RNA polymerase reaction. Biochim. Biophys. Acta 157:218-219.
18. **Studier, F. W.** 1972. Bacteriophage T7. Science 176:367-376.