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Transcription Units in Bacteriophage T4

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We have investigated the possibility of assigning genes of T4 bacteriophage to their units of transcription (scriptons) by studying gene expression from UV-irradiated DNA templates. Since RNA chains are prematurely terminated on UV-irradiated DNA templates and since the promotor distal part of the RNA chain is deleted, the expression of any gene is inversely proportional to the distance between the promotor and the promotor distal end of the gene. We find that the early genes, 43, 45 and rIIB, are promotor proximal. Since at least genes 43 and rIIB are classified as delayed early genes, these results suggest that their synthesis may require the recognition of new promotors. Additional early genes (44, 62, 42, 46, 47, 55, and rIIA) and some late genes (34, 37, and 38) have also been assigned positions relative to their promotors.

The timing of synthesis of T4-specific proteins is complex (11). Some early proteins appear immediately after infection, others are delayed in their appearance by a few minutes (11, 31, and Gold, personal communication). Later, the synthesis of many early proteins decreases, whereas others are synthesized throughout the latent period (11, and our own observations). Late T4 proteins appear shortly after DNA replication begins and continue to be synthesized until lysis (5, 7, 11). Some of these regulatory steps appear to occur on the transcriptional level. Messenger RNA for late proteins cannot be detected until shortly before the late proteins appear (4, 9). Some species of mRNA for early T4 proteins cannot be detected in cells infected in the presence of chloramphenicol (15, 21). These species may correspond to the mRNA for delayed early proteins. Such data have been interpreted to mean that a T4-specific protein is required either to activate new promotors controlling delayed early genes (23, 28, 29) or to prevent termination and allow read-through into the delayed early genes (18, 23).

Shutoff of synthesis of some of the early proteins probably occurs on the post-transcriptional level since mRNA for early T4 genes continues to be synthesized throughout the latent period (4, 9), whereas the synthesis of most early proteins ceases at about 10 min (11). Regulation of the relative amounts of the late proteins may occur either on the transcriptional or the translational level.

To provide a basis for distinguishing between the possible modes of regulation of T4 early proteins and to provide evidence for or against translational regulation of T4 late proteins, we have developed a method for determining the location of promotors in T4 and for identifying co-transcribed genes. The method is based on the facts that (i) UV-induced damage to DNA causes premature termination of transcription into RNA at the site of the UV damage and (ii) that the RNA polymerase cannot reinitiate transcription between the UV-induced damage and the end of the transcription unit (22). Therefore, in UV-irradiated phage, the functional survival of promotor proximal genes should be less UV-sensitive than that of promotor distal genes.

We have used two methods to assay for survival of genes after UV irradiation. These are (i) to measure the ability of UV-irradiated wild-type T4 to complement T4 carrying conditional-lethal mutations in various T4 genes; UV-irradiated T4 will complement mutants in promotor proximal genes well and will complement mutants in promotor-distal genes poorly; (ii) to measure the amount of specific T4 proteins, resolved on SDS polyacrylamide gels, which can be synthesized in vivo from increasingly UV-irradiated phage; synthesis of more promotor-distal proteins should be more affected by UV-irradiation.

MATERIALS AND METHODS

Phage and bacterial strains. *Escherichia coli* B_{s-1} (10), B_{s-1}, str^R, and $B_{s-1}(\lambda)$ (all nonpermissive for T4 *amber* mutants) and *E. coli* CR63 (permissive for T4 *amber* mutants) were used.

Wild-type T4 (T4D$^+$) and all mutants of T4D were originally obtained from the collection of R. S. Edgar, unless otherwise specified. T4B was obtained from S. Benzer. The T4B deletion mutants r1589 (6) and r1695 (19) were kindly provided by S. P. Champe. The mutants used are listed in Table 1. Mutants having the designation "X5" have been backcrossed with T4D$^+$ five times (30) in the laboratory of J. S. Wiberg. Mutants will usually be referred to by gene number only.

Complementation conditions. *E. coli* B_{s-1}, B_{s-1},-str^R, or $B_{s-1}(\lambda)$ were grown in tryptone broth (10 g of Difco tryptone and 5 g of NaCl in 1,000 ml of water) to a concentration of 5×10^8 to 6×10^8 cells per ml. (The str^R strain was used to reduce transmission of phage amber mutants [Karam, personal communication and 14].) Chloramphenicol (50 μg/ml) was added to prevent temporal exclusion of the phage and 3 min later the cells were infected at an MOI of 2 to 4 with UV-irradiated T4D$^+$ and the unirradiated T4 amber mutant at an MOI of 0.04. The infected cells were incubated at 37 C for 10 min, then T4 antiserum was added to inactivate unadsorbed phage. Two minutes later the infected cells were chilled, diluted, and plated with *E. coli* CR63 to determine the number of infected cells which release phage, and with *E. coli* B_{s-1} to determine the number of infected cells which release wild-type recombinants. All manipulations are carried out in yellow light to prevent photoreactivation.

Transmission coefficients. Transmission (fraction of infected nonpermissive cells which release phage) of the T4 mutants was measured under the same conditions as complementation except that UV-irradiated T4D$^+$ was omitted.

UV-irradiation. Phages were irradiated by UV light of 254 mμ.

SDS slab-gel electrophoresis. *E. coli* B_{s-1} was grown in M9 medium (3.0 g of KH_2PO_4, 6.6 g of Na_2HPO_4, and 1.0 g of NH_4Cl per liter with 0.4% glucose and 10^{-3} M $MgSO_4$) to a concentration of 5×10^8 to 6×10^8 cells per ml, tryptophane (2×10^{-5} M) was added, and cells were infected at an MOI of 10 at 37 C. (When early T4 proteins were to be labeled, cells were UV-irradiated at 2,610 ergs/mm^2 before infection to prevent labeling of bacterial proteins.) One-milliliter samples were labeled with 2 μCi of ^{14}C-amino acids (reconstituted protein hydrolysate, algal profile, 57 mCi per atom carbon, Schwarz/Mann) for 3 min. During this time there is little depletion of radioactive amino acids. Completion of nascent, radioactive proteins was allowed through a 3-min incubation period with a 10^3-fold excess of nonradioactive amino acids. The cells were then chilled on ice and 5 ml of ice-cold 0.1 M Tris-hydrochloride (pH 6.8) was added. The cells were sedimented and suspended in 0.1 ml of sample buffer (0.05 M Tris-hydrochloride, pH 6.8; 1% SDS, Bio-Rad; 1% mercaptoethanol; 15% glycerol; and 0.1% bromophenol blue) and heated for 2 min in a boiling water bath. Samples of 0.01 ml were loaded on the gels. The method used for vertical slab-gel electrophoresis is that described by Studier (27). We have found that resolution and banding patterns on the gels is significantly influenced by the source of the chemicals used. We have used Eastman Kodak acrylamide, Bio-Rad *N-N'*-methylene-bis-acrylamide, Bio-Rad sodium dodecyl sulfate (SDS) and Schwarz/Mann Tris-base (ultra pure).

Densitometer tracings of autoradiograms were made by using a Beckman Model RB Analytrol with the film densitometer attachment and a slit width of 0.1 mm.

RESULTS

(i) **Kinetics of gene inactivation by UV-irradiation.** The UV sensitivity of a given gene is mainly determined by the distance between the promotor and the promotor-distal end of the particular gene (22). The kinetics of inactivation of complementing ability ought to be the same for all genes, i.e., an initial shoulder followed by a first order loss in complementing ability. The shoulder should be largely determined by the multiplicity of infection, and the final slope by the distance between the promotor and the promotor distal end of the gene. Dose-effect curves for the complementation of amber mutants in genes 45 and 44 are shown in Fig. 1. They are in reasonable agreement with the expectations outlined above. Furthermore, these data indicate that gene 44 is more promotor distal than gene 45.

TABLE 1. *Properties of T4 mutants used*

Mutants of	Gene	Mutation	Transmission coefficients		
			B_{s-1}	B_{s-1}, str^R	B_{s-1} (λ)
T4D	30	amH39X	0.075	0.039	
	30	amH39x5			
	32	amE315x1	<0.001	<0.001	
	34	amN58			
	37	amB280			
	38	amB262			
	39	amN116			
	42	amN55x5	<0.001	<0.001	
	43	amB22x5	0.050	0.001	
	43	amE4322[a]		0.002	
	44	amNG485	0.003	0.003	
	44	amN82	<0.001	<0.001	
	45	amE10	<0.001	<0.001	
	46	amB14x5	0.21	0.10	
	47	amA456x5	0.21	0.17	
	55	amBL292	0.10	<0.001	
	56	amE51x5	0.002	<0.001	
	62	amE1140		0.004	
	rIIA	r219[b]			0.002
	rIIB	rb50[b]			0.002
T4B	rIIA	r1589[c]			
	rIIA	r1695[c]			

[a] From J. W. Drake.

[b] From A. H. Doermann; point mutation.

[c] From S. P. Champe; deletion mutation (see Table 2).

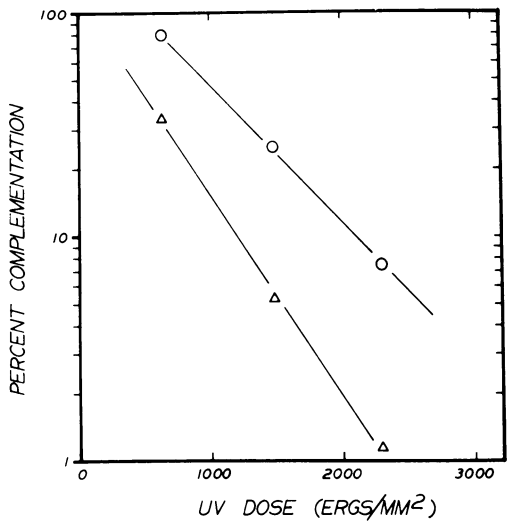

FIG. 1. *Efficiency of complementation as a function of UV dose. Complementation of amN82 (gene 44) and amE10 (gene 45) by wild-type T4, UV-irradiated at the doses indicated in the figure, was carried out in E. coli B_{s-1} as described in Materials and Methods, the MOI of the UV-irradiated phage was 2. The ordinate (percent complementation) is the percent of amber mutant infected cells which release phage. The complementation values have been corrected for exclusion of the amber mutant by the UV-irradiated phage (the loss of infective centers due to co-infection with the UV-irradiated phage was determined in parallel experiments in which the amber mutant had been replaced by T4D$^+$) and the transmission of the amber mutants in the absence of UV-irradiated phage. Symbols: ○, amE10; △, amN82. Polarity of amber mutations in gene 45 on gene 44 could not be detected under the conditions of our experiments.*

(ii) **Complementation by UV-irradiated T4D$^-$ of amber mutants in early genes of T4.** Relative UV-sensitivities can also be obtained from the complementation efficiencies of the various genes at a single UV dose (provided that the UV dose is high enough so that the comparison is made well beyond the shoulder of the dose-effect curves). If these complementation efficiencies are plotted as a function of distance along the DNA (approximated by genetic map distances and known molecular weights of the protein products) one can deduce the positions of the promotors. This type of evaluation is illustrated for genes 42 through 45 in Fig. 2. It can be seen that the complementation values decrease from gene 45 towards gene 62 indicating that these genes are on the same transcription unit (scripton). Theoretically, the slope of the curve defined by the complementation values of these genes should extrapolate to the position of the promotor at 100% complementation (provided that the UV sensitivity is a function of distance only). However, one can see that if this slope is applied to the scripton containing gene 43 where the position of the promotor would be limited to the region be-

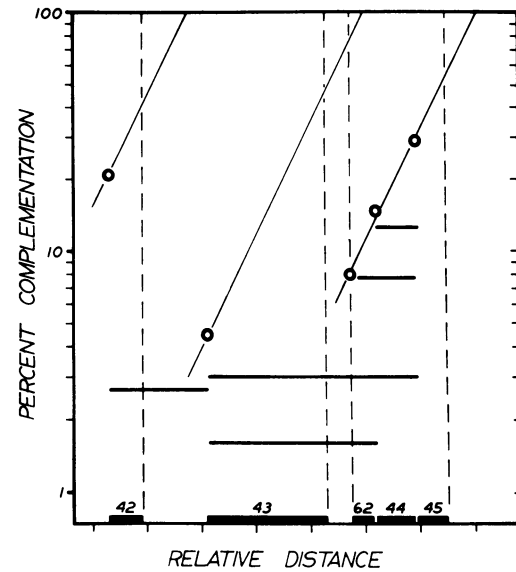

FIG. 2. *Complementation of T4 amber mutants in genes 42, 43, 62, 44, and 45 by UV-irradiated T4D. Complementation was carried out in E. coli $B_{s-1}str^R$ as described in Materials and Methods. The UV dose was 1,680 ergs/mm^2 and the MOI of the UV-irradiated phage was 4. The complementation values have been corrected for exclusion of the amber mutant by the UV-irradiated phage and the transmission of the amber mutants in the absence of UV-irradiated phage. Adsorption rates of all of the mutants used here were similar. The abcissa is proportional to an approximation of the distance along the DNA based on the molecular weights of genes 45, 44 and 62 (27,000, 35,000, and 20,000, respectively, ref. 3 and Alberts, personal communication), gene 43 (110,000, ref. 8) and gene 42 (30,000, our own observations); the assumption that genes 45, 44, and 62 are adjacent to each other (Wood, personal communication), separated by 100 nucleotide pairs; and mapping data of gene 43 mutants relative to genes 42 and 44 (2, 19, Wood, personal communication). The complementation values of single mutants are indicated by the open circles, complementation values for the double mutants are indicated by the level of the horizontal bars and the genes mutated in the double mutant are indicated by each end of the bar. Complementation values are the average of several experiments. Mutants used were 45 (amE10), 44 (amNG485 and amN82), 43 (amB22x5 and amE4322), 62 (amE1140), 42 (amN55x5).*

tween the end of gene 62 and the beginning of gene 43, the position of the promotor must correspond to a complementation value between 47 and 74%. (This overall reduction in complementation may reflect a nonspecific effect of the UV irradiation which results in a general reduction of the ability to complement, or it may mean that the promotor region is especially UV sensitive). Using this information, one would predict that the promotor for genes 45, 44, and 62 is close to the beginning of gene 45. The promotor for gene 42 would be within about 900 base pairs of gene 42. These results are in agreement with those of Stahl et al. (24), who found that an amber mutation in gene 45 was polar on gene 44 but not on gene 43. Gene 62 was not tested.

That we are actually measuring complementation rather than simply the production of wild-type genomes by recombination between the amber mutant and the UV-irradiated wild-type phage is evidenced by the fact that the number of cells releasing wild-type phage was a constant fraction of the total number of phage-releasing cells (about 30% for the UV doses used). In addition, most of the genes tested here control functions required for DNA replication and it is unlikely that these functions could be expressed sufficiently from recombinant genomes. Support for this conclusion comes from experiments performed by Stahl (cited in 24) and in this laboratory where the nonpermissive host was co-infected by mutants at two different sites in the same early gene and the number of cells releasing phage was measured. In some cases this number was slightly above the level of wild-type revertants in the amber mutant stock but this number is about 1,000-fold below what we observe in our experiments. Therefore, production of wild-type phage cannot be a prerequisite for complementation.

(iii) **Complementation by UV-irradiated T4D$^+$ of double mutants in early genes of T4.** Another prediction of our method for mapping scriptons concerns the efficiency of complementation of double mutants. When the genes mutated in the double mutant are in the same scripton, the double mutant will be complemented by the UV-irradiated T4 with the same efficiency as that of the more promotor distal of the two genes. When the genes are in separate scriptons, the double mutant will be complemented with an efficiency equal to the product of the efficiencies with which the individual mutants were complemented.

The levels of complementation for double mutants involving genes 45, 44, 43, and 42 are indicated by the horizontal bars in Fig. 2. The double mutants in the same scripton (i.e., 45, 44, and 45,62) are complemented with about the same efficiency as the more promotor distal of the two genes; the double mutants in separate scriptons (i.e., 43,45, 43,44, and 43,42) are complemented with an efficiency which is considerably lower than that of either of the two single mutants. It is clear that the complementation of the mutants in separate scriptons is not as low as the product of the efficiencies of the two single mutants. This would be explained if the overall lowering of complementation efficiencies discussed above is due to a nonspecific effect of the UV-irradiation. In this case the predicted values for complementation would be the product of the efficiencies with which the individual mutants would be complemented divided by the factor by which all of the complementation values have been reduced. The predicted values (assuming that the position of the promotor corresponds to 55% complementation) for the double mutants 43,45, 43,44, and 43,42 would then be 2.9, 1.5, and 2.1%, respectively, which compares reasonably well with the observed complementation efficiencies (see Fig. 2).

(iv) **Complementation by a promotor deletion mutant.** Further verification of the validity of our method comes from results obtained with a promotor deletion mutant in the rII region of T4. There is evidence for the existence of a promotor between the rIIA and rIIB cistrons in T4 (23) as indicated in Table 2. Our data (Table 2, line 1) support this notion in that mutants in the rIIB cistron are complemented by UV-irradiated T4D$^+$ much more efficiently than mutants in the rIIA cistron. (This result has

TABLE 2. *Complementation of rII mutants by UV-irradiated wild-type T4B and T4B rII deletion mutants*[a]

UV-irradiated phage		Complementing phage	
		rIIA r219	rIIB rb50
rIIA rIIB	Wild-type T4B	14.0	44.6
rIIA rIIB	T4B r1589		6.5
rIIA rIIB	T4B r1695		40.1

[a] Complementation was carried out in $B_{s-1}(\lambda)$ as described in Materials and Methods except that chloramphenicol was omitted, the MOI of the UV-irradiated phage was 2, and the UV dose was 840 ergs/mm^2. The location and extent of the rII deletion mutations is indicated by the heavy bar in the diagram of the rII region.

been observed previously and was interpreted in terms of the relative lengths of the rIIA and rIIB cistrons, ref. 17.) Since transcription of rII is known to proceed from left to right (see Table 2) (23), i.e., the B cistron following the A cistron, the more efficient complementation by the B cistron reveals the existence of a promotor between rIIA and rIIB.

The mutant r1589 in the rII region of T4 is deleted in both the end of the rIIA cistron and the beginning of the rIIB cistron (6); therefore, a promotor lying between rIIA and rIIB would also be deleted in this mutant. This mutant retains rIIB function. If UV-irradiated T4 r1589 is used in place of UV-irradiated T4D to test complementation of a mutant in the rIIB cistron, our model predicts that the complementation of the rIIB mutant by UV-irradiated r1589 should be lower than that of the rIIA mutant by the UV-irradiated wild-type T4. This would be so since the expression of rIIB now requires read-through from the promotor which precedes rIIA. The data shown in Table 2, lines 2 and 3, bear out this prediction. Complementation of an rIIB mutant by UV-irradiated r1589 is almost 10-fold lower than the complementation by UV-irradiated r1695 (a deletion mutant in the rIIA cistron [19]) and it is twofold lower than the complementation of rIIA by UV-irradiated wild-type T4B.

(v) **Complementation of amber mutants in additional early genes.** Complementation by UV-irradiated wild-type T4 of amber mutants in genes 46, 47, and 55 is shown in Fig. 3. Here it was necessary to use a lower UV dose (840 ergs/mm^2) since mutants in genes 46 and 47 have high transmission even on the str^R strain of *E. coli*. The complementation values for these genes can be fit onto a single line, which would indicate that they are on the same transcription unit. The slope of this line compares well with the slope of the line (dashed line, Fig. 3) defined by the complementation values of genes 43 and 45 under these conditions. The position of the promotors (assuming that genes 43 and 45 are both promotor proximal) would correspond to a complementation value of about 68%. From these data, it appears that the promotor for genes 46 and 47 is ahead of gene 55. The complementation of the double mutant 46, 55 probably does not help in the assignment of these genes to scriptons since it is known that mutation of gene 55 allows gene 46 mutants to make normal amounts of phage DNA (5). The high complementation value obtained with the 46,55 mutant is probably indicative of this interaction.

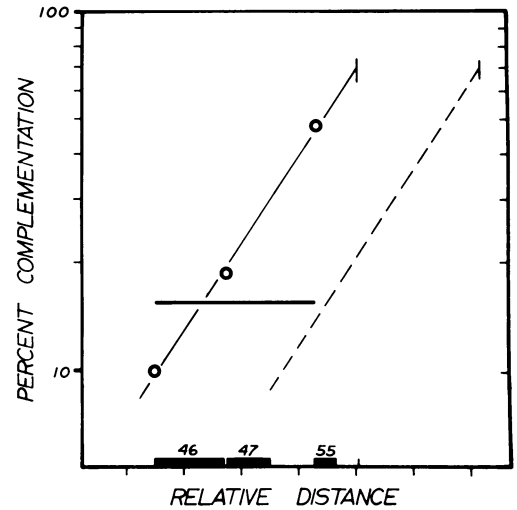

FIG. 3. *Complementation of T4 amber mutants in genes 46, 47, and 55 by UV-irradiated wild-type T4. Complementation was carried out in E. coli* B_{s-1},*str*^{R} *as described in Materials and Methods. The UV dose was 840 ergs/mm*^{2} *and the MOI of the UV-irradiated phage was 4. The complementation values have been corrected for exclusion of the amber mutant by the UV-irradiated phage and the transmission of the amber mutants in the absence of UV-irradiated phage. The abcissa is proportional to an approximation of the distance along the DNA based on the molecular weights of genes 46 and 47 (about 60,000 and 40,000, respectively, our own observations) and gene 55 (about 20,000, ref. 26); the assumption that genes 46 and 47 are adjacent to each other (separated by 100 nucleotide pairs); and mapping data on the distance between gene 47 and 55 (Wood, personal communication). Mutants were 46 (amB14x5), 47 (amA456x5), 55 (amBL292). Symbols are as in Fig. 2. The slope indicated by the dashed line is based on the assumption that the promotors for genes 45 and 43 are immediately ahead of each of these genes and on an extrapolation of their complementation efficiencies at UV doses of 1,135 ergs/mm*^{2} *and 1,680 ergs/mm*^{2} *(from Fig. 2).*

Genes 56, 30, and 32 (molecular weights about 20,000, 58,000, and 35,000, respectively, ref. 31 and 1) have also been tested and their complementation values under the conditions of Fig. 1 were found to be 31, 11, and 18%, respectively, indicating that they all may be relatively promotor proximal. We were unable to link these genes to others on the same scripton due to lack of nonleaky, adjacent genes.

Genes 41, 61, 39, 60, 52, 57, 59, and 63 could not be tested by the complementation assay because of high transmission coefficients (32, our own observations).

Most late genes could not be tested by this method, probably a result of inefficient expression of these genes from UV-irradiated DNA templates (which presumably do not replicate) (5).

(vi) **Inactivation of T4 genes by UV-irradiation as determined by a decrease in the rate of synthesis of specific T4 proteins resolved on SDS polyacrylamide gels.** To extend scripton mapping to early genes which could not be tested by complementation due to high transmission and to late genes, we have determined UV-sensitivities of T4 genes from the reduction in the rate of synthesis of the proteins as a function of UV dose.

For the early genes, cells were infected with UV-irradiated wild-type T4, pulse labeled with ^{14}C-amino acids, then lysed and the proteins electrophoresed on SDS polyacrylamide slab gels. Figure 4 shows the protein patterns obtained when cells are infected with increasingly UV-irradiated phage. It can be seen that different proteins disappear at different rates and that these rates are not strictly a function of the molecular weight of the protein as it would be if the UV target size were determined solely by the size of the individual gene. The protein bands are identified by gene as the band missing in extracts of cells infected by amber mutants. The decrease in rate of synthesis of particular proteins was quantitated by densitometer tracings of autoradiograms of the gels and plotted as a function of UV dose. Figure 5 shows UV inactivation curves for early genes. (Some of the early genes could not be evaluated by this method because their protein products could not be identified on the gels, or could not be resolved from other proteins.)

Since the UV sensitivity, or the slope of the inactivation curve, for a particular gene is a measure of the distance between the promotor and the end of that gene, the slopes of these curves ought to give the distance directly. However, we have observed that in addition to the direct effects of UV-irradiation on transcription units, there is an "indirect effect" which reduces the amount of protein which can be synthesized from genes which are undamaged at the time of infection. The existence of this "indirect effect" was shown by the following experiment: cells were mixedly infected with UV-irradiated phage carrying amber mutations in genes 43 and 45 and unirradiated wild-type phage. It is seen in Fig. 6 that the rate of synthesis of the products of genes 43 and 45 from the unirradiated wild-type phage decreases as a function of the UV dose to the irradiated mutant phage and that the decrease is the same for both proteins. The magnitude of this "indirect effect" under the conditions of the UV-inactivation experiment shown in Fig. 5 can be calculated from the relative UV sensitivities of genes 43 and 45 measured under these conditions, compared to their predicted relative sensitivities (based on the assumption that these genes are promotor proximal). The rate of inactivation due to the "indirect effect" (indicated by the dashed line in Fig. 6) can be used to correct the slopes obtained in Fig. 5 so that the relative distances of these genes from their promotors can be determined. Figure 7 shows a plot of the corrected UV sensitivities of the early

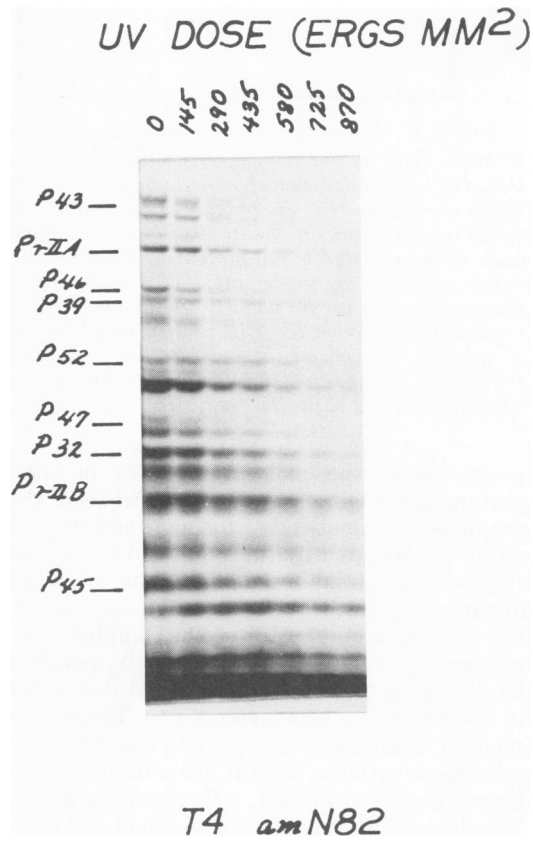

FIG. 4. *SDS polyacrylamide gel electrophoresis of T4 proteins synthesized early after infection by UV-irradiated phage. E. coli B_{s-1} (UV-irradiated at 2,610 ergs/mm^2) was infected at a MOI of 10 by a mutant in gene 44 (amN82), UV-irradiated at the doses indicated in the figure. After 6 min at 37 C, cells were pulse labeled for 3 min with ^{14}C amino acids and samples were prepared as described in Materials and Methods. Electrophoresis was carried out on a 12.5% gel for 17.5 h at 50 V.*

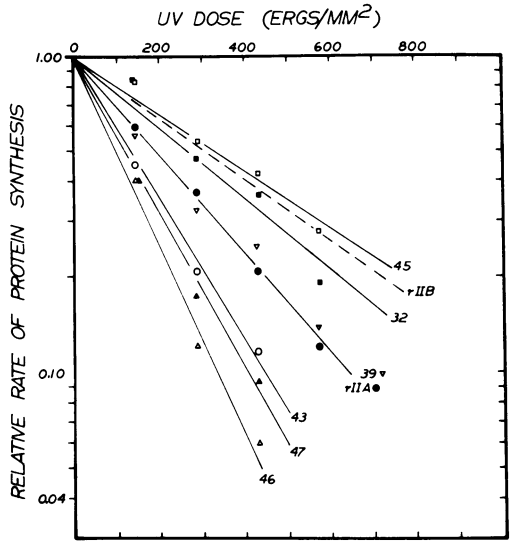

FIG. 5. *UV inactivation of synthesis of early T4 proteins. The autoradiogram shown in Fig. 4 was traced by using a densitometer and individual protein bands were evaluated. The amount of material in the bands (which is proportional to the rate of ^{14}C-amino acids incorporated into that protein during the 3-min pulse) is plotted as a percentage of the amount of material present in the band in the absence of UV irradiation. Although the rIIB protein could not be completely resolved on the gels, its UV sensitivity could still be measured by comparing the protein patterns obtained with rIIB$^-$ and rIIB$^+$ phage.*

genes versus the molecular weight of their protein products. The distance between the promotor proximal end of the gene and its promotor is indicated by the vertical distance between the point and the solid diagonal line. The distances obtained by this method are consistent with those obtained from the complementation assay. Genes rIIA and rIIB both appear to lie very close to their promotors and they differ in UV-sensitivity by a factor of two. The assignment of a promotor between rIIA and rIIB is in agreement with the work of Schmidt et al. (23). Genes 46 and 47 appear to be promotor-distal. The dashed diagonal line is the predicted position of promotor proximal genes based on the previous observation (22) of one chain terminating UV lesion formed in DNA per 1,000 nucleotide pairs per 1,000 ergs/mm^2 (see Discussion).

Late genes. Experimental conditions for measuring the UV sensitivity of late genes of T4 have to satisfy the following criteria: (i) the requirements for efficient expression of the late genes, such as DNA, replication should be met before the phage are UV-irradiated; (ii) late mRNA synthesis should not begin before UV-irradiation. These criteria were reasonably well met by the following conditions: cells were infected by unirradiated wild-type T4, at 7 min after infection (37 C), infective centers were chilled and UV-irradiated, then returned to 37 C, and 3 min later they were pulse-labeled with ^{14}C-amino acids. (That late mRNA is not present at the time of UV-irradiation [7 min] was indicated by the fact that in parallel experiments, when rifampin was added at 7 min, late proteins did not appear.)

Figure 8 shows the gel patterns obtained for samples prepared under the above conditions. The rate of synthesis of late proteins as a function of UV dose to the infective center can then be evaluated as for the early proteins.

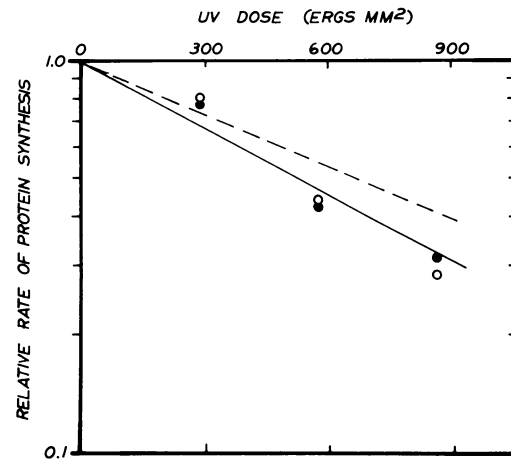

FIG. 6. *Reduction in gene specific protein synthesis as a result of co-infection with UV-irradiated phage. $B_{s\text{-}1}$ (UV-irradiated at 2,610 ergs/mm^2) was infected at 37 C by unirradiated wild-type T4 (MOI = 2) and UV-irradiated 43,45 (amE4322,amE10) (MOI = 10). Samples were pulse labeled with ^{14}C amino acids from 3 to 6 min after infection and samples were prepared and electrophoresed on a 10% gel for 16 h at 28 V as described in Materials and Methods. The amount of gene 43 (●) and 45 (○) protein synthesized was evaluated from densitometer tracings of autoradiograms of the gel and these values are plotted as a percentage of the amount synthesized when cells were co-infected with unirradiated mutant phage. The dashed line indicates the "indirect effect" of UV-irradiation calculated by determining the change in slope of the UV inactivation of genes 43 and 45 shown in Fig. 5 required to give their UV sensitivities a ratio of 4:1. (The 4:1 ratio is based on an assumption that these genes are promotor proximal and the fact that the gene 43 protein (about 110,000 daltons, ref. 8) is about four times the size of the gene 45 protein (about 27,000 daltons; Alberts, personal communication).*

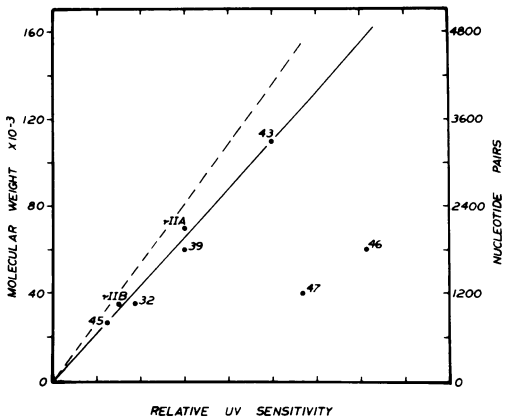

FIG. 7. *Relative UV sensitivities of the early genes as a function of protein size. The relative slopes of the inactivation curves of the early genes (obtained from a correction of the slopes shown in Fig. 5) are plotted as a function of the molecular weights of the protein products (determined by their mobilities on the gels). The diagonal line is determined by the positions of genes 45 and 43. Assuming that these genes are promotor proximal, the distance in nucleotide pairs (right ordinate) between a given gene and its promotor is the vertical distance between the point plotted for that gene and the diagonal line. The dashed diagonal line is the predicted position of promotor proximal genes based on the previous observation (22) of one chain terminating UV lesion formed in DNA per 1,000 nucleotide pairs per 1,000 ergs/mm².*

Inactivation curves for the tail fiber genes 34, 37, and 38, are shown in Fig. 9. Since genes 34 through 38 are located next to each other, the relative sensitivities of these genes would indicate that genes 34 and 37 (protein molecular weights, 150,000 and 120,000, respectively, ref. 16) are on separate scriptons and that gene 38 (protein molecular weight, 26,000, ref. 16) is co-transcribed with gene 37. (Genes 35 and 36 were not included in this evaluation because their proteins were not well resolved on our gels.) These observations agree with those of Stahl et al. (25), and King and Laemmli (16) who studied the polar effect of amber mutations in these genes.

The assignment to scriptons of additional late genes will be the subject of a future publication.

DISCUSSION

We have investigated the possibility of assigning genes to scriptons by studying gene expression from UV-irradiated DNA templates. Since RNA chains are prematurely terminated on UV-irradiated DNA templates and the promotor distal parts of the RNA chain is deleted (22), the expression of any gene is inversely proportional to the distance between promotor and the promotor distal end of the gene. We have assigned to scriptons several early genes of T4 and the tail fiber genes 34, 37, and 38. Our assignments agree with those of Stahl et al. (25), and King and Laemmli (16) who studied the polar effect of amber mutations on neighboring genes. Additional early genes could be assigned to scriptons once their protein products have been identified on gels. The assignment of additional late genes to scriptons will be the subject of a subsequent paper.

We find that the early genes 43 and rIIB are

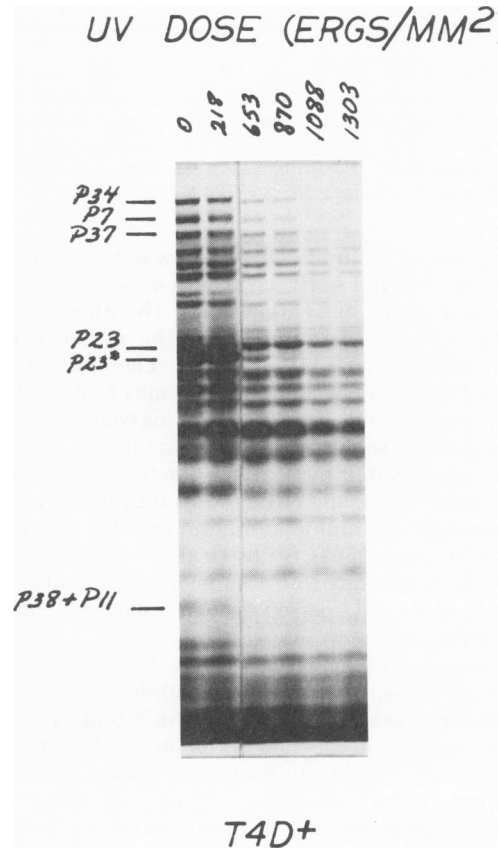

FIG. 8. *SDS polyacrylamide gel electrophoresis of T4 proteins synthesized following UV-irradiation of infective centers. E. coli B_{s-1} was infected by wild-type T4 (MOI = 10). After 7 min at 37 C, infective centers were chilled and UV-irradiated and then returned to 37 C. Three min later, infective centers were pulse labeled for 3 min with ^{14}C-amino acids and samples were prepared as described in Materials and Methods. Electrophoresis was carried out on a 10% gel for 13.5 h at 28 V.*

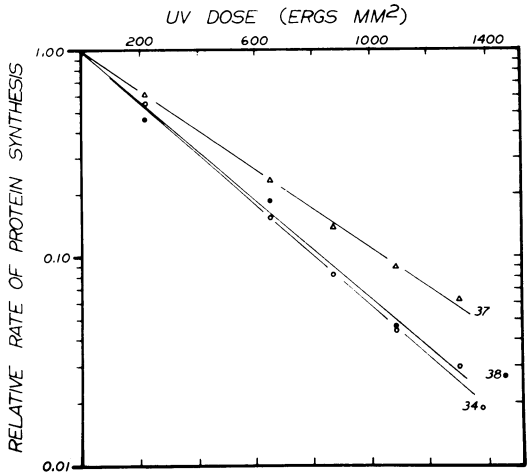

FIG. 9. *UV inactivation of synthesis of the T4 protein products of the late genes 34, 37, and 38. Autoradiograms of gels prepared by using the samples shown in Fig. 8 were evaluated in the same way as those for Fig. 5. P38 from a 20% gel (50 h at 50 V) and P34 and P37 from a 10% gel (24 h at 28 V).*

promotor proximal. These genes were assigned to the delayed early class of T4 genes due to their kinetics of synthesis and the absence of gene 43 and rIIB mRNA in the presence of chloramphenicol (12, 13, 23). Therefore, the synthesis of these gene products may require the recognition of new promotors. This conclusion is consistent with the facts that in the tsG1 mutant isolated by Mattson the full expression of genes 43, 45, and rIIB is substantially delayed (personal communication) and that the expression of these genes is drastically reduced when rifampin is added at 1 min after infection at 30 C (L. Gold, personal communication, own observations).

The complementation assay we have used should reveal the maximum number of promotors since genes whose expression is required for the activation of new promotors should be provided by the unirradiated amber mutant phage. (Also, this assay would not reveal a requirement for an anti-terminator for transcription of promotor distal genes, nor would it reveal secondary promotors whose activation requires transcription up to that promotor from a preceding promotor.) However, when the gel assay is used to establish gene inactivation curves, a gene which is transcribed from two independent promotors, *a* and *b* (where *b* must be activated by the function of an activator gene), might display a biphased survival curve. The amount of gene product produced would be the sum of the amount synthesized due to initiation at promotor *a* (and having a sensitivity determined by the distance between *a* and the gene), and the amount synthesized due to initiation at promotor *b* (and having a sensitivity determined by the distance between *b* and the gene). (Since we have used a high MOI the sensitivity of the activator gene can be disregarded.) Whenever the sensitivity of the transcription from promotor *a* differs from the sensitivity of the transcription from promotor *b*, a biphased survival curve will be observed. However, when the contribution to the total amount of protein originating from the more sensitive transcription unit is small (less than 20%) it becomes difficult to experimentally establish the biphased nature of the survival curve.

We have not observed biphased survival curves for genes 43 and rIIB, which suggests either a minor contribution to the total protein by the more sensitive mode of transcription, or a coincidentally equal sensitivity for both modes of transcription.

When the effects of UV-irradiation on RNA transcription were examined directly, it was found that an average of one chain terminating UV-lesion was formed in DNA per 1,000 nucleotide pairs by 1,000 ergs/mm² (22). Assuming that UV-inactivation of transcription is reflected directly in the reduction in the rate of synthesis of proteins, the rate of inactivation of genes measured by our gel assay would be described by the dashed line in Fig. 7. The observed rate of inactivation (indicated by the solid line in Fig. 7) would be equivalent to one chain terminating UV-lesion per 800 nucleotide pairs per 1,000 ergs/mm². Considering that these measurements have been made by very different methods, the two values are in good agreement. This agreement also indicates that repair is not extensive between the time of infection and the pulse label in our experiments.

The purpose of this investigation was to establish the validity of scripton mapping by utilizing the RNA chain terminating effect of UV irradiation. We have assayed for the UV sensitivity of gene expression by two techniques: (i) by complementation of amber mutants by UV-irradiated wild-type phage and (ii) by the rate of production of proteins from UV irradiated DNA. Each assay makes several, but different, assumptions as to its applicability. When applied to the same set of genes, 45, 44, 62, and 55, 47, 46, both approaches show these genes to be on separate scriptons, 45 to 62 and 55 to 46. Furthermore, both assays make the same scripton assignments as the amber polarity effect utilized by Stahl et al. (24), and by King and Laemmli (16). We, therefore, con-

clude that our method and the assumptions implicit in the complementation assay (limited to the genes tested in this paper) and the protein labeling assay for gene survival are correct. We think the method will be applicable in many systems in which the gene order is known and where the gene products can be quantitated.

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