Two Modes of In Vivo Transcription for Genes 43 and 45 of Phage T4

KATHLEEN HERCULES AND WALTER SAUERBIER

Department of Biophysics and Genetics, University of Colorado Medical School, Denver, Colorado 80220

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Sensitivities of the expression of early genes of phage T4 to UV light were determined at various stages of intracellular development of T4 wild type, a DNA-negative mutant (T4 DO), and T4 tsG1, (a mutant that exhibits delayed expression of some T4 early genes). Whereas the sensitivities of some genes in the T4 wild type and T4 DO remain constant, genes 43 and 45 exhibit greatly reduced sensitivities several minutes after the onset of phage development. Since UV sensitivities are a measure of the distance of a gene from its promoter, these observations indicate a switch from distal, "immediate early" promoters to proximal, "delayed early" promoters for genes 43 and 45. In the tsG1 mutant this decrease in UV sensitivities of genes 43 and 45 does not occur at 42 C, suggesting that at high temperature this mutant does not utilize the delayed early promoters.

We have recently assigned several genes of phage T4 to their units of transcription. The assignment was made through preferentially inhibiting transcription of promoter distal genes by UV irradiation of the template DNA (3). In these experiments, gene expression was determined by quantitating the synthesis of gene products through radioactive labeling and autoradiography, or by complementation of conditionally lethal mutants by UV-irradiated T4 wild type. In the first case, the promoter proximity of genes was determined only for one period after infection (6 to 9 min); in the second case, conditions were such that the closest promoter to a given gene was able to function. The present experiments, in which the timing of promoter accessibility is investigated, reveal that the expression of certain genes is first controlled by a more distal promoter.

By comparing the timing of early gene expression in T4 and the mutant tsG1, Mattson (personal communication) concluded that some early T4 genes are transcribed from more than one promoter. It was speculated that bacterial RNA polymerase initiates at one type of promoter, and after alteration by T4 (11, 17, 18) it begins transcription at another type of promoter. In some cases the new promoter appears to be closer to the gene of interest (10). This concept was also used by O'Farrell and Gold (7) to account for the different patterns of synthesis of early proteins in T4-infected cells and in cells in which RNA synthesis was inhibited by rifampin shortly after phage infection.

Since determinations of UV sensitivities of gene expression provide a direct measure of the distance between a gene and its promoter, we investigated the promoter proximity of genes 43 and 45 by their expression from UV-irradiated T4 DNA. We studied the expression of these genes at early and late times after infection, utilizing a DNA-negative mutant (T4 DO), T4 wild type, and the tsG1 mutant isolated by Mattson. We observed that immediately after infection genes 43 and 45 are transcribed from promoters that are at different sites on the DNA than the ones used for transcription of these genes several minutes after the onset of normal phage development. In contrast, T4 tsG1 utilizes only one distal set of promoters for transcribing genes 43 and 45 when phage development proceeds at 42 C.

MATERIALS AND METHODS

Bacteriophage. T4D, T4 *amN122* (gene 42), and T4 *amE10* (gene 45) are from the collection of R. S. Edgar. T4 *am4322* (gene 43) was obtained from J. Drake, T4 *amB14x5* (gene 46) from J. S. Wiberg and *agtm* 8, *βgtam* 10 from H. Revel. The T4 mutant tsG1 was generously provided by T. Mattson. (The *amN122* mutant used here does not contain the secondary mutation *m* in gene 43.)

Bacteria. Experiments were carried out with host *Escherichia coli* B₈₋₁ (4). Lysates of T4 amber mutants were prepared and assayed with host *E. coli* CR63. Stocks of tsG1 were prepared in strain B₈₋₁ at 30 C.

Chemicals and radiochemicals. Sodium dodecyl sulfate (SDS) and *N,N'*-methylene-bis-acrylamide were purchased from Bio-Rad Laboratories, Rich-

mond, Calif.; acrylamide was purchased from Eastman Kodak Co., Rochester, N.Y.; the following items were obtained from Schwarz/Mann, Orangeburg, N.Y.: rifampin, tris-(hydroxymethyl)aminomethane, ^{14}C -labeled amino acids, algal profile (approximate average specific activity of 300 mCi/mmol).

Media. M9 (KH_2PO_4 , 3.0 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 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; see reference 1) was used for growth of bacteria and infective centers. T4 adsorption was carried out in the presence of 2×10^{-5} M tryptophan.

Growth of bacteria and infections with T4. Growth temperature for bacteria and infective centers was 30, 37, or 42 C. Bacteria were infected at an absorbancy of 0.5 measured at 600 nm (A_{600}) which equals about 5×10^8 cells per ml. Multiplicity of infection was 10 phages per cell.

UV irradiation at 254 nm. Portions (10 to 20 ml) of bacteria in M9 medium, grown to a density of $A_{600} = 0.5$, were chilled and irradiated (incident dose 2,600 ergs/mm 2) on a watch glass (10-cm diameter) with rapid stirring. UV irradiation of the bacterial cells was used to prevent synthesis of bacterial proteins. Bacteriophage were irradiated in 5-ml batches at a concentration of 5×10^{10} /ml in M9 medium in the same manner as the bacteria. Under these conditions there is no measureable attenuation of the light due to the phage or the medium. Bacteriophage were irradiated for the times indicated in Fig. 1-7. The dose rate was 14.4 ergs per mm 2 per s, which corresponds to about 0.6 lethal hits per s. All experiments were performed in dim, yellow light.

Labeling of proteins. Samples (1 ml) of infected cultures were incubated with ^{14}C -labeled amino acids (2 $\mu\text{Ci}/\text{ml}$) for periods varying from 3 to 5 min. During these pulses there was little depletion of radioactive amino acids. Completion of nascent, radioactive proteins was allowed through a 3-min incubation with a 10^3 -fold excess of nonradioactive amino acids. The infective centers were then chilled by addition of 5 ml of cold Tris buffer (0.1 M Tris-hydrochloride, pH 6.8) and sedimented. The supernatant was removed, and the pellets were suspended in 0.1 ml of the 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. The SDS polyacrylamide gel electrophoresis was used as described by Studier (16). The dried gels were autoradiographed with Dupont Cronex DC2 film, and the developed film was traced with either a Beckman Analytrol or a Joyce-Loebl densitometer.

RESULTS

Expression of genes 43 and 45 from immediate early promoters in wild-type T4 and a DNA-negative mutant. In the following experiments we allowed RNA chain initiation at 30 C for a period of only 1.5 min after infection by UV-irradiated T4 wild type or T4 *amN122* (gene 42). Further initiation was inhibited by adding rifampin (12) to a concentration of 200 $\mu\text{g}/\text{ml}$.

Thereafter, early T4 proteins were labeled with ^{14}C -labeled amino acids from 6 to 9 min after infection and resolved on SDS polyacrylamide gels (Fig. 1). The reduction in the expression of the individual early T4 genes as a function of UV dose can be quantitated by densitometry of the autoradiograms of Fig. 1. This is shown for T4 *amN122* (Fig. 2A) and for wild-type T4 (Fig. 2B). Gene 43 is the most sensitive in both cases, followed by gene 46, then 45, and then U and gt, which display similar sensitivities.

Expression of genes 43 and 45 from UV-irradiated wild-type T4 and T4 DO from 6 to 9 min after infection. In these experiments *E. coli* B $_{8-1}$ was infected at 37 C with UV-irradiated wild-type T4 or T4 *amN122*, and early T4 proteins were labeled with ^{14}C -labeled amino acids from 6 to 9 min after infection. The proteins were separated on SDS polyacrylamide gels (Fig. 3) and quantitated by densitometry of autoradiograms. The loss of expression of several early T4 genes as a function of UV dose is shown in Fig. 4. The relative UV sensitivities of the genes shown are similar for T4 DO (Fig. 4A) and wild-type T4 (Fig. 4B). However, in comparison with Fig. 2 where rifampin was added at 1.5 min after infection, the UV sensitivities of genes 45 and 43 are much less in relation to the other genes. (In addition to the relative change in UV sensitivities of genes 43 and 45, there is an overall decrease in the UV sensitivities measured later after infection. This point and our rationale for determining which genes shift in UV sensitivities and which remain constant will be discussed below.)

Expression of genes 43 and 45 from UV-irradiated tsG1 at 42 C. Recently a temperature-sensitive mutant of T4, tsG1, has been isolated by Mattson (personal communication) in which the expression of some of the early T4 genes is delayed. It was proposed that this delay was due to a failure to activate certain promoters and that the genes whose expression was delayed (e.g., genes 43, 45, rIIB, and 32) could be transcribed only by readthrough from distant promoters. To test this proposal and to determine whether the promoters used for the transcription of genes 43 and 45 in this mutant are at the same positions as those used at early times in the normal infection, we have measured the UV sensitivities of the expression of these genes in the tsG1 mutant. The experimental approach was the same as that described above, except that the infections were carried out at 42 C. Figure 5 shows autoradiograms of gels containing proteins labeled from 6 to 9 min after infection by tsG1 (Fig. 5, left frame) or by

FIG. 1. Autoradiograms of proteins synthesized in T4-infected cells in the presence of rifampin and resolved on 10% SDS polyacrylamide gels. Samples of 1 ml of *E. coli* B₈, (5×10^8 cells per ml, UV-irradiated with 2,600 ergs/mm²) were infected with T4 amN122 (left) or wild-type T4 (right) at multiplicity of infection of 10 at 30 C. At 1.5 min after phage addition, 200 μ g of rifampin was added, and ¹⁴C-labeled amino acids were supplied from 6 to 9 min. From left to right in each frame: increasing UV dose to the input phage. Protein bands were identified by gene number on the basis of comparisons with protein banding patterns of extracts prepared after infection with amber mutants in various genes. The identified protein bands are indicated by gene number; 43 is the gene 43 protein, etc., gt is a band missing in T4 α gtam8, β gtam10 (2), and U is an unknown protein band. A protein coelectrophoreses with the gene 46 protein. Since it contributes little to the label at this position, it does not significantly interfere with evaluations of the UV sensitivity of gene 46. The relative locations of some of the protein bands (see gene 45 protein) were affected by slight variations in the conditions of electrophoresis (e.g., different lots of Tris base); protein band identifications were made for each set of conditions.

wild-type T4 (Fig. 5, right frame) at 42 C. The loss of expression of genes as a function of UV dose is shown in Fig. 6A for tsG1 and in Fig. 6B for wild-type T4. Clearly, genes 43 and 45 are much more UV sensitive in the tsG1 mutant than in wild-type T4, whereas many of the other genes have similar UV sensitivities.

Expression of genes 43 and 45 from UV-irradiated tsG1 under permissive conditions. To determine whether the increased UV sensi-

tivity of genes 43 and 45 in the tsG1 mutant is an inherent property of the DNA of that phage or whether it is due to mutational loss of activity of a diffusible product, it is necessary to measure the UV sensitivity of the genes in this mutant under permissive conditions, i.e., when the diffusible product is active. We find that even at 30 C genes 43 and 45 have increased UV sensitivity, although the tsG1 mutant was originally isolated as a temperature-sensitive mu-

FIG. 2. Rates of synthesis of early T4 proteins as a function of UV dose to input phage T4 amN122 (A) or wild-type T4 (B) in the presence of rifampin. Data are evaluations of microdensitometer tracings of the autoradiograms in Fig. 1.

FIG. 4. Rates of synthesis of early T4 proteins as a function of UV dose to input phage T4 amN122 (A) or wild-type T4 (B). Data are evaluations of microdensitometer tracings of the autoradiograms in Fig. 3.

FIG. 3. Autoradiograms of proteins synthesized in T4-infected cells and resolved on SDS polyacrylamide gels. Experimental conditions were the same as those given in the legend to Fig. 1, except that experiments were carried out at 37°C and no rifampin was used. Left, Cells infected by T4 amN122, proteins resolved on 12.5% gel. Right, Cells infected by wild-type T4, proteins resolved on 10% gel.

FIG. 5. Autoradiograms of proteins synthesized in cells infected by *tsG1* or wild-type *T4* at 42 C and resolved on 10% SDS polyacrylamide gels. Experimental conditions were the same as those given in the legend to Fig. 1 except that the experiments were carried out at 42 C and no rifampin was used. Left, Cells infected by *tsG1*. Right, Cells infected by wild-type *T4*.

FIG. 6. Rate of synthesis of early *T4* proteins as a function of UV dose to input phage *tsG1* (A) or wild-type *T4* (B). Data are evaluations of microdensitometer tracings of the autoradiograms in Fig. 5.

tant. Therefore, we tested the UV sensitivity of genes 43 and 45 in mixed infections of UV-irradiated *tsG1* and an unirradiated mutant of *T4* containing amber mutations in genes 43, 45, and 46. Here, the triple-amber mutant will provide the product which is mutated in *tsG1*, and genes 43, 45, and 46 will be expressed only from UV-irradiated *tsG1*. The loss of expression of genes 43, 45, and 46 as a function of UV dose measured under these conditions is shown in Fig. 7. Clearly, the genes 43 and 45 of *tsG1* have UV sensitivities relative to gene 46, which are characteristic of *T4* wild type several minutes after infection (Fig. 4). Thus, the mutation in *tsG1* appears to affect promoter selection through loss of a diffusible, trans-active product.

Comparison of UV sensitivities of genes

FIG. 7. Rates of synthesis of early T4 proteins as a function of UV dose to input phage tsG1 in a co-infection with a T4 phage carrying amber mutations in genes 43, 45, and 46. Experimental conditions were the same as those given in the legend to Fig. 1 except that the cells were infected with tsG1 (multiplicity of infection of 5) and with T4 amE4322, amE10, amB14x5 (multiplicity of infection of 5) at 42 C, and no rifampin was used. Only the tsG1 mutant was UV-irradiated. Data are evaluations of microdensitometer tracings of autoradiograms of proteins resolved on 10% SDS polyacrylamide gels. Since, in this experiment, the protein band which coelectrophoreses with the gene 46 protein is expressed from the unirradiated triple amber mutant phage and therefore contributes more heavily (especially at the higher UV doses) to the total label at this position on the gel (see the legend to Fig. 1), a correction has been made to eliminate the contribution of this band to the measurement of the UV sensitivity of the gene 46 protein.

under various experimental conditions. To facilitate comparison of the UV sensitivities of the genes under the various experimental conditions outlined above, we have normalized all of the UV sensitivities observed to the sensitivity of one protein band, U, under one set of experimental conditions (Table 1). From the values in Table 1 we conclude that gt and 46, in addition to U, maintain a constant UV sensitivity under all experimental conditions used. Therefore, each of these genes is transcribed from only one promoter location. We call these genes "invariant" as opposed to genes 43 and 45 which display varying sensitivities.

DISCUSSION

The UV sensitivity of expression of genes from UV-irradiated DNA is a direct function of the distance between promoter and the end of the gene (6, 9, 15). Measuring UV sensitivities of gene expression, therefore, provides a simple means of assigning genes to descriptions (3). However, when such assignment is to be made at the various stages of intracellular T4 develop-

ment, a complication arises from repair of UV lesions by phage-specific repair mechanisms. Absolute UV sensitivities (survival versus UV dose) decrease as more time becomes available for repair. Relative UV sensitivities (sensitivities of genes relative to each other), however, remain unaffected by repair. We have demonstrated this by comparing the relative UV sensitivities of gene expression in T4D and in mutants deficient in the repair genes *v* and *x* (unpublished data). Since the absolute UV sensitivities of all genes decrease when measured at progressively later times in phage development and since, in addition, relative UV sensitivities change at later times, it is not

TABLE 1. Relative UV sensitivities^a of the expression of several early genes of T4 under various experimental conditions

Protein	UV sensitivities of expression					
	Fig. 2 (+ rifampin, 30 C)		Fig. 4 (37 C)		Fig. 6 (42 C)	
	T4 D0	T4	T4 D0	T4	tsG1	T4
43	2.53	2.61	1.69	1.40	2.48	1.25
45	1.47	1.53	.42	.35	2.10	.30
46	2.01	2.04	2.08	1.60	1.85	1.80
gt	.92	.88	.87	.81	1.00	.83
U	1.00	1.00	1.00	1.00	1.00	1.00

^a The relative UV sensitivities have been derived from the observed UV sensitivities (Fig. 2, 4, and 6) by the following arithmetic manipulations. First, the sensitivity of each gene was expressed relative to the sensitivity of the protein band U for each labeling condition. Then, a contribution by the "nonspecific" reduction of protein synthesis (3) was subtracted from these relative sensitivities. The numerical value for the nonspecific reduction was obtained from the observed ratio of sensitivities of genes 45 versus 43 at developmental conditions when both genes are promoter proximal (6 to 9 min after infection at 37 C), and the sensitivity was predicted from gene size (a 1:4 ratio). (We have previously shown the applicability of this operation, ref. 3.) Thereafter, the corrected sensitivities were again normalized against the sensitivity of protein band U. The factor obtained from the data from T4 D0, 6 to 9 min after infection at 37 C was applied to the data from T4 D0 plus rifampin; the factor obtained from the data from T4, 6 to 9 min after infection at 37 C, was applied to the data from T4 plus rifampin. A separate calculation was made for the data from T4 at 42 C assuming that genes 43 and 45 are also promoter proximal under these conditions. Since the data from tsG1 at 42 C could not be standardized in the same manner as the others, they were standardized by averaging the relative sensitivities of gene 46 protein under all of the other conditions and using this value as a standard for normalizing the sensitivities of the other proteins in the tsG1 at 42 C.

self-evident whether a particular gene has increased its sensitivity relative to another one, or whether the other gene has decreased its sensitivity. However, this distinction can be made with a reasonable degree of confidence because several genes of T4 do not alter their relative UV sensitivities during phage development, and we therefore assume that these genes are transcribed from the same promoter location throughout early T4 development. Whenever we say that the UV sensitivity for the expression of a particular gene increases or decreases we mean a change relative to a set of genes with invariant relative UV sensitivities. The following "invariant" genes were used to calibrate the UV sensitivities of genes 43 and 45: gene products or protein bands *gt*, *U*, and 46 (Fig. 1).

When initiation of transcription is limited to 1.5 min after infection at 30 C, genes 43 and 45 display high UV sensitivities. We estimate (Table 1 and reference 3) that under this condition genes 43 and 45 are transcribed from promoters that precede these genes by about 1,600 nucleotide pairs. When transcription is not inhibited by rifampin and proteins are labeled between 6 and 9 min after infection at 37 C, genes 43 and 45 show greatly reduced UV sensitivities. Transcription now starts at promoters which are adjacent to or very close to genes 43 and 45. (We have performed control experiments which show that it is not the presence of rifampin *per se* which causes genes 43 and 45 to have high UV sensitivities but that the high UV sensitivities are due to limiting initiation of transcription to a period of 1.5 min postinfection).

Since, in the *tsG1* mutant, genes 43 and 45 display high UV sensitivities even late in the early phase of development (6 to 9 min), we conclude that these genes are transcribed only from distant promoters.

The approximate positions of "immediate early" and "delayed early" promoters for the transcription of genes 43 and 45 are shown in Fig. 8. It is not known what mechanisms activate delayed early promoters. It is conceivable that they become available only after alteration (11) or modification (18) by T4 of bacterial RNA polymerase.

The terms immediate early and delayed early are used to refer to the times when promoters are utilized for transcription. This is in analogy to the usage of these terms in describing the times when mRNA or protein species are synthesized after T4 infection. However, genes that have been classified as delayed early are not necessarily transcribed from delayed early promoters; e.g., Jayaraman (5) discusses the possi-

FIG. 8. Transcriptional map of the 25 to 38 segment (W. B. Wood, *Handbook of Genetics*, 1974, in press) of the T4 genome. The beginning and direction of transcription units are indicated by arrows. "Delayed early" scriptons were identified previously (3, 13, 14). "Immediate early" scriptons are identified by our present experiments. The terminator site of a scripton is not defined.

bility that expression of gene 43 is delayed early because an anti-*rho* factor is required for its transcription *in vivo*.

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