SEQUENCE AND TRANSCRIPTS OF THE BACTERIOPHAGE T4 DNA REPAIR GENE *uvsY*

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ABSTRACT

We have cloned, sequenced and analyzed transcription of the phage T4 *uvsY* gene. This gene is transcribed from a single gp MotA-dependent middle promoter to give a major transcript of approximately 930 nucleotides and a minor transcript of approximately 620 nucleotides. All *in vivo* and *in vitro uvsY* transcripts show anomalous migration in agarose gels. The *uvsY* transcript contains an open reading frame coding for an 137 amino acid [15.8 kilodaltons (kD)] UvsY protein and two unidentified open reading frames, ORF UvsY.-1 (9.0 kD) and ORF UvsY.-2 (6.0 kD). Our DNA sequence differs in only three places from that published by TAKAHASHI *et al.* However, one of these changes alters the predicted carboxy terminus of the UvsY protein. Marker rescue experiments map gene *25* to the region upstream of *uvsY*. Gene *25* is likely, although not certain, to correspond to an ORF that is found upstream from *uvsY* and is translated in the same direction.

THE *uvsY* gene of bacteriophage T4, first recognized as a DNA repair gene (BOYLE and SYMONDS 1969), is specifically required for error-prone repair. This function may be related to the role of *uvsY* in homologous recombination (HAMLETT and BERGER 1975; CUNNINGHAM and BERGER 1977, 1978) and DNA replication (MELAMEDE and WALLACE 1977). This gene is largely dispensable for T4's life cycle; unirradiated *uvsY* mutants produce sufficient progeny to form plaques (for reviews, see BERNSTEIN and WALLACE 1983; CONKLING and DRAKE 1984a,b). Either alternative pathways can compensate for defective *uvsY* functions or the mutations found until now are leaky.

The DNA arrest phenotype of *uvsY* mutants (MELAMEDE and WALLACE 1977) is less severe than that of mutants of other recombination-deficient genes, *e.g.*, genes *46* or *47*. It could be a consequence of recombination deficiency since, under normal conditions, recombinational intermediates are required to initate all but the first few rounds of T4 DNA replication (LUDER and MOSIG 1982; DANNENBERG and MOSIG 1981, 1983). On the other hand, the position of *uvsY* near an origin of DNA replication between position 107 and 121 kb on the standard T4 map (for reviews see KOZINSKI 1983; MOSIG

Abbreviations: bp = base pair, cpm = counts per minute, dC-DNA = T4 DNA containing cytosine instead of glucosylated hydroxy-methyl-cytosine, gp = gene product, kb = kilobase pair, kD = kilodalton, ORF = open reading frame, pfu = plaque forming units, SDS = sodium dodecyl sulfate.

1983; YEE and MARSH 1985; KREUZER and ALBERTS 1986; RUEGER and KUTTER 1984) could suggest that its product is required at that origin.

When we began the work reported here, HALPERN, MATTSON and KOZINSKI (1979) had mapped this origin in a clone containing genes *uvsW* through *29*; our results, confirming and refining this location, suggested that it lies in *Xba*I fragment 12 (MACDONALD *et al.* 1983). Since at that time the *Hin*dIII sites were not well mapped (KUTTER and RUGER 1983) and did not correspond to the *Hin*dIII fragments that we isolated from this region (MACDONALD and MOSIG 1984), we cloned various *Hin*dIII and *Pst*I restriction fragments from total T4 DNA by first enriching for those fragments that hybridize to *Xba*I fragments 12 and 7. We located the *uvsY* gene on a 2.4-kb *Pst*I fragment and an overlapping *Hin*dIII fragment (Figure 1), in agreement with other laboratories (TAKAHASHI and SAITO 1982; DEVRIES and WALLACE 1983; TAKAHASHI *et al.* 1985).

Here, we describe the precise location and sequence of the *uvsY* gene on this *Hin*dIII fragment.[1] Downstream from *uvsY* on this *Hin*dIII fragment there are two unidentified open reading frames which could code for proteins of molecular mass 8952 daltons (ORF UvsY.-1) and 6056 daltons (ORF UvsY.-2). Upstream of *uvsY* there are two incomplete open reading frames, of which one could correspond to the late gene *25*.

While this manuscript was in preparation, TAKAHASHI *et al.* (1985) published a sequence of the *uvsY* gene, and we also learned that T. C. LIN and W. KONIGSBERG (personal communication) had independently sequenced the *uvsY* gene. Our sequence agrees perfectly with that of LIN and KONIGSBERG and, for the most part, with that of TAKAHASHI *et al.* A minor difference with the sequence of TAKAHASHI *et al.*, however, alters the predicted carboxy terminus of the deduced protein.

We show, in addition, that *uvsY* is transcribed from a single gp MotA-dependent middle promoter and that most *uvsY* transcripts terminate downstream from all three open reading frames.

MATERIALS AND METHODS

Bacteria: *E. coli* JM103 (*Δlac-pro, thi, strA, supE, endA, sbcB15, hsdR14/F' traD36, proAB, lacI*^{q}, *lacZ ΔM15*) and TB-1 (*Δlac-pro, r*$^-$, *strA, sup*°, *ara, thi, φ80dlacZ ΔM15, hsdR*) were obtained from BRL, and UT481 (*met, thy, Δ(prolac), r*$^-$*m*$^-$, *supD, tn10/F' traD36, proAB, lacI*^{q}, *lacZ ΔM15*) was a gift from C. LARK. *E. coli* B and CR63 have been maintained in this laboratory.

Bacteriophage: T4D wild type, the *uvsY* amber mutant y_{10} (from BOYLE and SYMONDS 1969), the dC-DNA producing strain GT7 (*56*$^-$*42*$^-$*denB*$^-$*alc*$^-$ from WILSON, TANYASHIN and MURRAY 1977; WILSON *et al.* 1979), the *motA*$^-$ strain *sip1* (HOMYK, RODRIGUEZ and WEIL 1976; HALL and SNYDER 1981), and the amber mutants *S-52* (*25*$^-$) and *S-29* (*51*$^-$) from A. H. DOERMANN have all been maintained in this laboratory.

Vectors: All initial cloning was done in pUC plasmids or in mp derivatives of the M13 phage vectors (MESSING 1983), and all were obtained from Bethesda Research Laboratories. For generation of strand-specific probes, appropriate T4 DNA fragments were inserted into the pGEM-3 vector purchased from Promega Biotec and were transcribed according to their protocol. Preparation of vector and T4 DNA as well as

[1] A preliminary account of this work was presented at the Evergreen International T4 Meeting, August 1985.

hybridization (SOUTHERN 1975) and enrichment for cloning DNA fragments was as described (MACDONALD and MOSIG 1984).

Chemicals and reagents: Restriction enzymes were purchased from Bethesda Research Laboratories, International Biotechnologies, Boehringer-Mannheim Biochemicals, or New England Biolabs. Nuclease S1 and ribonuclease A (type IIIA) were purchased from Sigma. Avian reverse transcriptase was purchased from Life Sciences. T7 polymerase was purchased from United States Biochemical. SP6 polymerase and T1 ribonuclease were purchased from Bethesda Research Laboratories. T4 DNA ligase and T4 DNA polymerase were purified by L. ROWEN in this laboratory. Deoxynucleotide and dideoxynucleotide triphosphates used for DNA sequencing and for synthesis of hybridization probes were purchased from Pharmacia. ^{35}S-[α-thio]dCTP (>1000 Ci/mmol) and ^{32}P-UTP (>600 Ci/mmol) were purchased from New England Nuclear. The ^{32}P-dCTP (800 Ci/mmol) was purchased from Amersham.

Analysis of RNA synthesized *in vivo* by Northern blot analysis: RNA was isolated from infected and uninfected *E. coli* B as described (MACDONALD, KUTTER and MOSIG 1984). RNA was denatured by glyoxalation and separated by electrophoresis (MCMASTER and CARMICHAEL 1977; MANIATIS, FRITSCH and SAMBROOK 1982). Size standards were generated from restriction digests of pBR322 (see legend of Figure 4), and after phenol extraction, the DNA fragments were glyoxalated and size fractionated in parallel with the RNA samples. The nucleic acids were blotted to Biodyne nylon membrane (Pall Ultrafine Filtration Corp.; THOMAS 1980), and the membrane was baked at 80° for 1–2 hr. The blots were probed with strand-specific RNA probes described below. Hybridization conditions and autoradiography were as previously described (MACDONALD and MOSIG 1984), except that the posthybridization washes were done at 65° in 15 mM NaCl, 1.5 mM Na_3 citrate and 0.1% SDS.

Conditions for synthesizing unlabeled RNA *in vitro* were identical to those for making labeled RNA except for omitting the label and increasing the UTP concentration to 0.5 mM. The RNA was ethanol-precipitated two times and was resuspended in TE buffer (10 mM Tris-HCl, 1 mM Na_2 EDTA, pH 8.0) containing 2 mM vanadyl ribonucleosides (Bethesda Research Laboratories).

Nuclease mapping of the *uvsY* transcript ends: Hybridizations and subsequent ribonuclease digestions were done as described by MELTON *et al.* (1984). The temperatures for both hybridization and nuclease digestion are indicated in the figure legends. Nuclease S1 digestions were done by diluting the hybridization mix with 0.3 ml of digestion buffer (0.28 M NaCl, 50 mM sodium acetate, pH 4.6, 4.5 mM $ZnSO_4$) and incubating for 30 min. The nuclease S1 digestions were stopped by adding 50 μl of stop buffer (4.0 M ammonium acetate, 0.1 M Na_2 EDTA). All samples were phenol/chloroform extracted once, and the RNA was ethanol-precipitated and dried. All samples were resuspended in 5 μl of TBE loading buffer (90% formamide, 0.05 M Tris-borate, 0.001 M Na_2 EDTA, pH 8.3, 0.1% xylene cyanol and 0.1% Bromophenol blue), denatured at 90° for 3 min and stored briefly on ice before loading onto either 8% or 4% acrylamide-urea sequencing gel (MANIATIS, FRITSCH and SAMBROOK 1982). The 4% gels were covered with plastic wrap and autoradiographed directly at −70°. The 8% gels were fixed in 5% methanol:5% acetic acid for 20 min, dried onto Whatman 3MM paper and autoradiographed at room temperature or at −70°.

Radioactive probes: DNA probes were labeled by replacement synthesis (O'FARRELL 1981; MACDONALD and MOSIG 1984).

RNA probes were made by transcribing appropriate DNA fragments subcloned into pGEM-3 with either SP6 or T7 RNA polymerase. Template DNA was prepared by the alkaline lysis method (MANIATIS, FRITSCH and SAMBROOK 1982), except that the DNA was resuspended in TE without ribonuclease. Template DNA (1–3 μg) was restricted, extracted first with phenol/chloroform and then with ether, precipitated with ethanol and dried. The DNA was resuspended and the probes were prepared as described by the supplier of each polymerase, except for the omission of bovine serum albumin in the reaction buffers. The activity of all probes was determined by precipitating an

aliquot with trichloroacetic acid followed by scintillation counting (LUDER and MOSIG 1982). The probe used for the blot in Figure 3b was prepared from the 0.65-kb *Bgl*II/*Cla*I DNA fragment by nick translation using a kit purchased from Amersham (catalog no. 5000).

DNA sequencing and analysis: All sequencing was done by the chain termination method (SANGER, NICKLEN and COULSON 1977; SMITH 1980) using ^{35}S-[α-thio]dCTP as the label. Sequences were analyzed on an Apple IIe by the University of Minnesota sequence analysis program of R. LARSON and J. MESSING (version 2.1), and a protein analysis program was kindly provided by W. MCALLISTER, adapted to the Apple IIe (MOSIG and MACDONALD 1986), or on a MacIntosh with DNA Inspector II (GROSS 1986).

Generation of deletion subclones for sequencing: For sequencing larger fragments, a nested set of deletions was generated from one end of the insert DNA (HENIKOFF 1984). This procedure relies on *E. coli* exonuclease III's preference for 5′ overhangs as a substrate. Briefly, a double restriction digest provides both a 5′ and a 3′ overhang between the cloning site and the annealing site of the universal primer. After terminating the Exo III reaction, the single-stranded regions are removed with nuclease S1 and the ends of the resulting DNA molecules are made blunt with T4 polymerase. The ligation and transfection steps were done as previously described (MACDONALD and MOSIG 1984).

Genetic complementation: Stocks of wild-type T4 and *uvsY* amber mutant y_{10} were diluted to a titer of 10^9 pfu/ml in diluting fluid, and 5-ml samples were irradiated in 100 × 15 mm dishes with one 15-watt General Electric germicidal lamp at a distance of 130 cm (MOSIG 1985). TB-1 cells (*sup*°), transformed with either pUC19 or various T4 clones in that vector, were used as plating bacteria. Media and plating conditions for T4 growth were as described (MOSIG 1985).

Marker rescue was done by the "spot test" protocol described by MATTSON *et al.* (1977).

RESULTS

Cloning T4 DNA fragments: The T4 DNA fragments of interest were identified by Southern blot analysis of total *Hin*dIII or *Pst*I restriction digests of T4 dC-DNA using as probes the labeled *Xba*I fragments 7 or 12 from a total T4 dC-DNA digest. Only the probe from the *Xba*I fragment 12 hybridized to a 1.4-kb *Hin*dIII fragment. This 1.4-kb fragment was cloned into pUC8 to give pMG1400, and transformants were screened by colony blot hybridization (GRUNSTEIN and HOGNESS 1975; MANIATIS, FRITSCH and SAMBROOK 1982) with probes from *Xba*I fragments 7 and 12. The identity of pMG1400 was confirmed by probing a Southern blot of a T4 dC-DNA *Xba*I digest with the labeled 1.4-kb insert (see Figure 1a, data not shown).

Two T4 *Pst*I fragments (1.9 and 2.4 kb, shown in Figure 1a) hybridized to the 1.4-kb T4 insert of pMG1400. These *Pst*I fragments were also cloned into pUC8. The 2.4-kb fragment (pMG824) hybridized only to *Xba*I fragment 12, whereas the 1.9-kb fragment (pMG819) hybridized to both *Xba*I fragments 7 and 12. These results, together with marker rescue data, are summarized in Figure 1a.

To determine the precise position of *uvsY* and to facilitate sequencing, the 1.4-kb *Hin*dIII fragment was digested with *Cla*I to generate three fragments (Figure 1c) which were subcloned into one of the M13mp vectors.

DNA sequence: The sequence of the 1.4-kb *Hin*dIII fragment, shown in Figure 2, has a *Pst*I and a *Bgl*II site as well as two *Cla*I sites predicted from

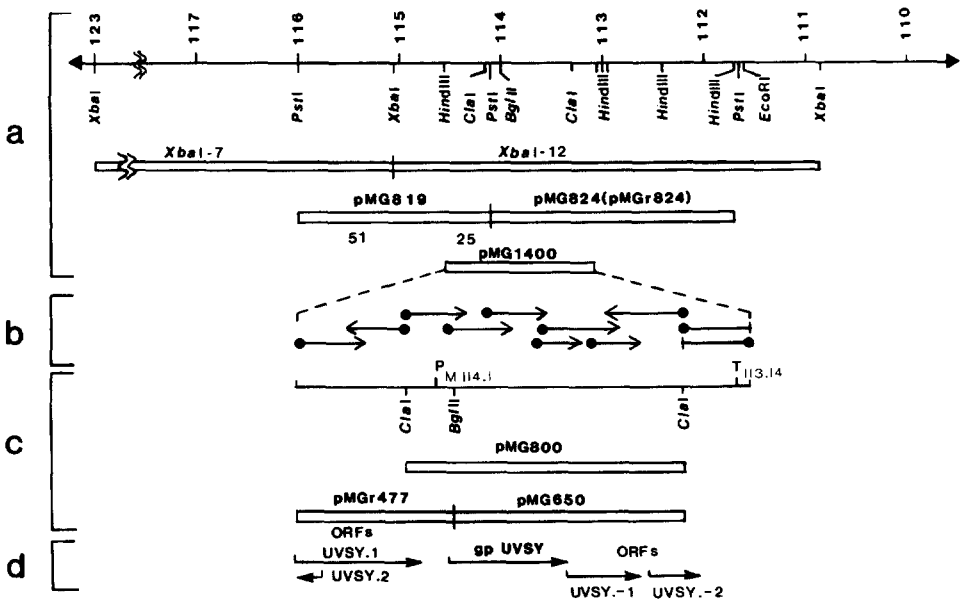

FIGURE 1.—Partial restriction map of the cloned region. a, The map units are distances (in kilobases) of DNA from the *rIIa/rIIB* junction (RUEGER and KUTTER 1984); the indicated DNA fragments were used as probes (*Xba*I 7 and 12) or were cloned (pMG819, pMG824, pMGr824 and pMG1400). The pMG819 clone rescued *S-29*$^+$ (gene *51*) and *S-52*$^+$ (gene *25*), whereas the pMG814 clone rescued only *S-52*$^+$. b, Sequencing strategy. Each filled circle indicates the start of the sequence for each clone. An arrowhead or vertical bar indicates the end of the sequence for each clone. An arrow means that the cloned fragment is unsequenced past the arrow. A vertical bar means that the entire cloned fragment is sequenced. c, Expanded partial restriction map of the pMG1400 clone and subclones of the 1.4-kb fragment. $P_M114.1$ is the *uvsY* middle promoter, and T113.14 is a major transcription terminator that is probably *rho*-independent. d, The gp UvsY and unidentified ORFs predicted from the sequence.

the restriction enzyme analysis. The G+C content from nucleotides 407 to 610 (28%) is considerably lower than that of surrounding segments of the same size (37–38%). Beginning at nucleotide 422, a pair of overlapping 13 nucleotide direct repeats (containing only one mismatch) were found, just five or six nucleotides downstream of the start of the *uvsY* transcript (see overlapping arrows, Figure 2). This repeated sequence contains six thymine pairs which could form dimers after UV irradiation (see DISCUSSION).

The sequence in Figure 2 from position 1–354 has not been published previously. The sequence from 355–1357 differs in three places (indicated with asterisks) from the published sequence (TAKAHASHI *et al.* 1985). We find an additional G at nucleotide 795 (changing the predicted reading frame of *uvsY*) and at position 1336 (with no consequence for translation). A TG instead of GT at positions 906/907 changes two stop codons to a *TyrGlu* in ORF UvsY.-1. These positions correspond to nucleotides 440, 980 and 552, respectively, in the sequence of TAKAHASHI *et al.* (1985). Amino acid composition of the purified gp UvsY supports the DNA sequence presented here (T. C. LIN and W. KONIGSBERG, personal communication).

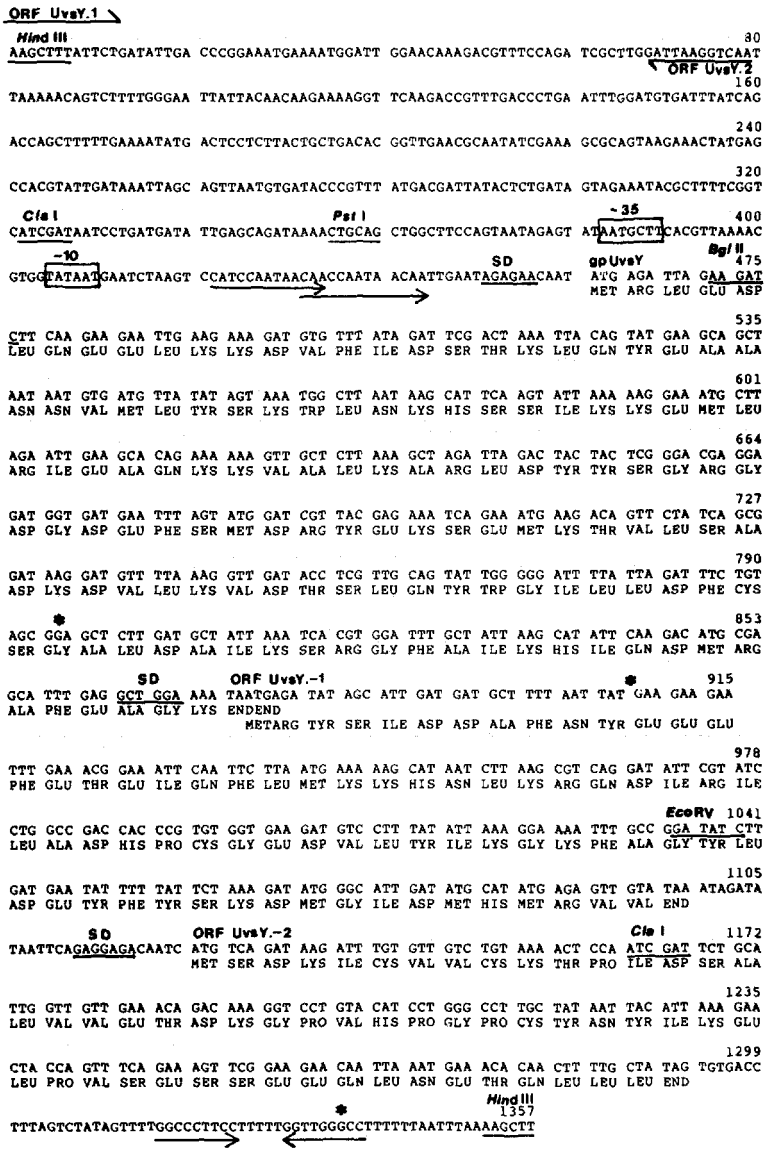

FIGURE 2.—DNA sequence of the 1.4-kb insert of pMG1400. The P_M114.1 promoter recognition sequences are boxed, and the terminator sequence of T113.14 is highlighted by opposing horizontal arrows. Shine-Dalgarno sequences are underlined, and their ORFs are named over the start codon. The incomplete ORFs (UvsY.1 and UvsY.2) are labeled at the first codon, and an arrow for each indicates direction. (See Figure 6 for the predicted amino acid sequence of the peptides.) ORF UvsY.1 terminates at nucleotide 381, which overlaps the −35 region of the *uvsY* promoter. The direct repeats are indicated by horizontal arrows.

Transcription: The DNA sequence predicts a gp Mot-dependent middle promoter (boxed in Figure 2; BRODY, RABUSSAY and HALL 1983; HALL and SNYDER 1981). Since PULITZER, COLOMBO and CIARAMELLA (1985) have shown the existence of two additional T4 coded genes (*motB* and *motC*) that can

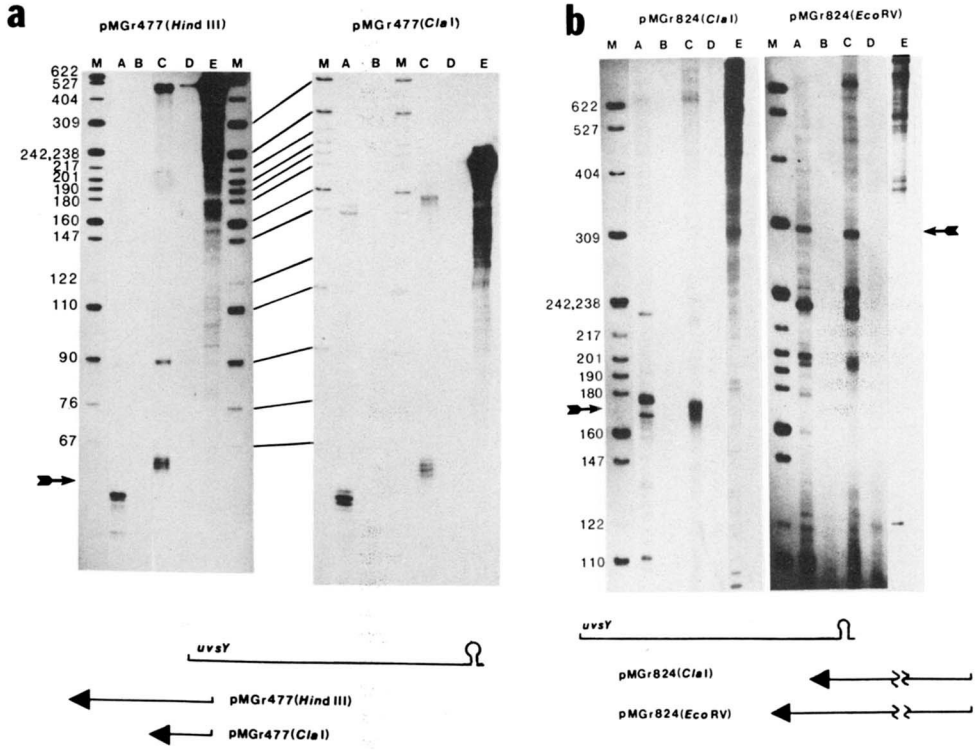

FIGURE 3.—Nuclease mapping of ends of *in vivo uvsY* transcripts. Both hybridization and nuclease digestion were done at 30° in 3a and 37° in 3b. The positions of protected fragments are indicated by arrows. The size standards (lane M) were generated by replacement synthesis (O'FARRELL 1981) of an *Hpa*II digest of pBR322. The RNase A digestions (lanes A and B) and the nuclease S1 digestions (lanes C and D) are shown for each probe in Figure 3a and b. Nuclease digestions were done after hybridization with (lanes A and C) or without (lanes B and D) RNA isolated early from wild-type T4 infected cells. The probe alone is shown in lane E. The insert indicates the extent of homology that each probe has for the *uvsY* transcript. Each lane, except the size standards, represents a reaction that initially had 5×10^5 cpm of probe. A 90-nucleotide band seen with both probes (Figure 3a) when total SP6 transcripts were used disappeared when the pMGr477(ClaI) probe was gel purified before hybridization (MANIATIS, FRITSCH and SAMBROOK 1982). The true protected bands remained unchanged.

mediate transcription of T4 middle promoters, we call here the original *mot* gene "*motA*." A putative Rho-independent terminator is seen at nucleotides 1316–1338.

To confirm these predictions, the *in vivo uvsY* transcript ends were mapped by protection from ribonuclease A (RNase) or nuclease S1 digestion (Figures 3a and b), after hybridization with various overlapping complementary RNAs. To make the necessary probes, two fragments were subcloned into the transcription vector pGEM-3. For mapping the 5′ end, a 477-bp *Bgl*II/*Hin*dIII fragment of pMG1400 was subcloned to give pMGr477 (Figures 1c and 4a). For mapping the 3′ end, the 2.4-kb fragment of pMG824 was subcloned to give pMGr824 (Figures 1a and 4b). Positive clones were identified by colony

blot hybridization, and the orientation of each fragment was determined by asymmetric restriction digest analysis.

To map the 5′ end, pMGr477 (Figure 3a) was transcribed *in vitro* from the T7 promoter to either the *Hin*dIII or *Cla*I site (Figure 4a). If there were a single *uvsY* promoter, the two probes should protect transcript segments of the same size. Protection of the transcripts from nuclease S1 with both probes confirms that the transcript starts at or near nucleotides 414–418, *i.e.*, at an appropriate distance from the consensus −10 region of the promoter. RNase A gives slightly shorter protected segments, probably because it more readily detects fraying at the ends. If hybridizations and digestions are done at 37°, the protected fragments in the nuclease S1 digestion decrease in size by 3–4 nucleotides and approach the size of the RNase digestion products which do not change in size (data not shown).

For mapping the 3′ end, pMGr824 was transcribed *in vitro* from the SP6 promoter after digestion with *Eco*RV or *Cla*I. If transcripts terminated after the palindrome (positions 1317–1338 in Figure 2), the probes were expected to protect *uvsY* transcript segments of approximately 300 or 175 nucleotides, respectively (Figures 2 and 3b), and these predicted fragments were found (Figure 3b, arrows). The transcripts terminated at different positions in the stretch of uridines downstream from the palindrome (Figure 2). The three other protected fragments in Figure 3b (see the A lanes for a band positioned at 234 nucleotides and the C lanes for bands positioned at 227 and 243 nucleotides) probably originate downstream of the *uvsY* transcript, since they all have the same size regardless of the size of the protecting probe.

Northern blots (Figure 4a) of RNA isolated from cells infected with wild-type (lane C) or *motA*$^-$ T4 phage (lane B) or from uninfected bacteria (lane A) show directly that the major *uvsY* transcript (detected with the 694 nucleotides *Bgl*II/*Cla*I strand-specific probe made from pMGr650, see Figure 1c) is gp MotA-dependent. The DNA sequence and the nuclease protection studies predict the *uvsY* transcript to be approximately 930 nucleotides long. A size of 940 nucleotides is estimated when the *uvsY* transcript is compared with three differently sized transcripts synthesized *in vitro* from *uvsY*-containing clones in the pGEM-3 vector (Figure 4b). DNA size standards derived from pBR322 cannot be used to estimate the size of the *uvsY* transcripts. The *uvsY* transcript as well as each of the transcripts synthesized from *uvsY* templates *in vitro* migrate slower than expected from a comparison with DNA size standards derived from pBR322 restriction fragments (Figure 4a and b). We do not know the reason for the anomalous migration of the *uvsY* transcripts. It may result from the formation of A·U-rich hairpins which would be unaffected by glyoxalation. Such a structure (nucleotides 893–951) was pointed out by TAKAHASHI *et al.* (1985). Note that all *in vitro* transcripts (Figure 4b) could form that structure. The base composition of the 5′ region of the *uvsY* transcript (approximately the first 200 nucleotides) is relatively G + C-poor (28%) when compared to the rest of the transcript (36%). This uneven distribution may also affect the migration of the glyoxalated transcript. Since no *in vivo* transcripts longer than 930 nucleotides are detected, it is unlikely that many *uvsY*

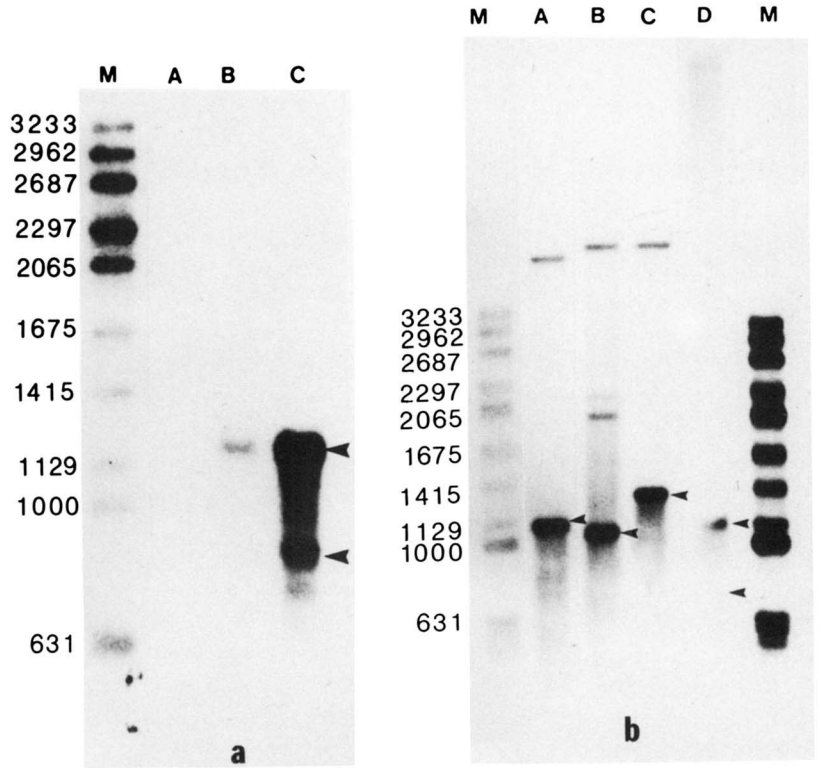

FIGURE 4.—Northern blots of RNA synthesized *in vivo*. a, Northern blots were done with 10 μg of RNA isolated from uninfected cells (lane A), *motA*$^-$ infected cells (lane B) and wild-type infected cells (lane C). RNA was isolated from infected and uninfected cultures grown at 30° for 10 min and prepared as described in the MATERIALS AND METHODS section. The probe is described in the text. b, RNA generated *in vitro* with T7 RNA polymerase from T4 fragments cloned into pGEM-3. The length of each defined RNA is 885 nucleotides (*Bgl*II to *Hin*dIII, lane A), 800 nucleotides (*Pst*I to *Cla*I, lane B) and 1000 nucleotides (*Pst*I to *Hin*dIII, lane C) (see Figure 2). For comparison, *in vivo* wild-type RNA (lane D) was isolated and prepared as described for samples in Figure 4a. The hybridization probes contained 1–5 × 10^5 cpm. The size standards (lane M) for both Figure 4a and b are prepared from five different restriction digests of pBR322. The restriction enzymes used and the fragment sizes, in nucleotides, produced are as follows: *Pvu*II and *Sal*I to make 2962 and 1415; *Pvu*II and *Bam*HI to make 2687 and 1675; *Pst*I and *Bam*HI to make 3233 and 1129; *Pvu*II and *Eco*RI to make 2297 and 2065; *Eco*RI and *Hin*fI to make 1000, 631, 517, 506, 396, 344, 298, 221, 220, 154 and 75. The smaller fragments were not efficiently transferred during blotting.

transcripts originate upstream of the middle promoter shown in Figure 2. In addition, no transcripts were found when Northern blots of wild-type early RNA were probed with the insert from pMG819 (data not shown).

Figure 4a (lane C) also shows a smaller minor band (made prominent by overexposing the autoradiogram). The minor band has been seen in all different RNA preparations. On the average, it represents about 15% of the hybridization signal. If not a degradation product (*i.e.*, by site-specific RNase processing), then the minor band could represent a *uvsY* transcript terminated after a potential hairpin at positions 1000–1041 in Figure 2, as proposed by

TAKAHASHI *et al.* (1985). If the formation of this hairpin is regulated by coupled transcription/translation of ORF UvsY.-1 it might regulate expression of ORF UvsY.-2.

Figure 4a (lane B) also shows low levels of the *uvsY* transcript in the *motA*$^-$ infection. Other *motA*-dependent genes show similarly low levels in the absence of gp MotA, both *in vivo* and *in vitro* (BRODY, RABUSSAY and HALL 1983; MACDONALD and MOSIG 1984). The UV inactivation curves of *motA*$^-$ phage are only slightly steeper than those of wild-type phage (data not shown). This and the result in Figure 4a (lane B) suggest that the low level of *uvsY* transcripts in *motA*$^-$ infections may produce sufficient UvsY protein for DNA repair.

Open reading frames: The DNA sequence predicts three ORFs in the transcript initiated from P_M114.1 (Figure 2). Only one is of sufficient size (15,837 daltons) to code for gp UvsY (apparent molecular mass 16 kD, YONESAKI *et al.* 1985). Complementation tests of the *uvsY* mutant y_{10} with three *Cla*I-derived subclones of pMG1400 confirm that this sequence is *uvsY*. Only one subclone (pMG800) containing an 0.8-kb fragment complemented y_{10} (Figure 5). Complementation of the *uvsY* gene was lost when P_M114.1 and the start codon were deleted from this clone (pMG650, Figures 1 and 5).

ORF UvsY.-1 and ORF UvsY.-2 could potentially code for 8952 dalton and 6056 dalton proteins, respectively, but such proteins have not yet been identified. Genes for many small T4-coded proteins are not yet mapped on the T4 genome (for review, see BURKE *et al.* 1983). Intriguingly, however, the deduced amino acid sequence of ORF UvsY.-2 predicts a possible metal binding domain that might bind to nucleic acids (BERG 1986; MILLER, MCLACHLAN and KLUG 1985). Starting at the sixth amino acid, the sequence Cys-X_2-Cys-X_{17}-His-X_3-Cys (where X may be any amino acid) fits the proposed structure, except for the larger size and the hydrophobic potential of the middle spacer (X_{17}).

Marker rescue experiments assign the wild-type allele of the gene *25* amber mutation *S-52* to the region upstream of *uvsY*. Therefore, we scanned the upstream sequence for open reading frames that might correspond to gp 25. This 15-kD protein is a structural component of the outer wedges (1/6 arm) of the phage base plate (BERGET and KING 1983). Gp 25 also has lysozyme activity (SZEWCZYK, BIENKOWSKA-SZEWCZYK and KOZLOFF 1986). In contrast to the basic lysozymes coded by genes *e* (TSUGITA *et al.* 1968) and *5* (KAO and MCLAIN 1980), the gene-*25* lysozyme is an acidic protein (NAKAGAWA, ARISAKA and ISHII 1985). There is a large open reading frame upstream from, and in the same direction as, *uvsY* (UvsY.1 in Figures 1, 2 and 6). A methionine codon at nucleotide 28 (Figures 1 and 6) would start a 13.5-kD peptide with a net charge of −4 at pH 7. A larger protein could be initiated upstream of the sequenced region, since the reading frame is open from the beginning of the clone. The sequenced portion of the ORF UvsY.1 could code for a peptide of 14.5 kD with a net charge of −6, which is closer to the reported size and isoelectric point of gp 25. We cannot, however, unambiguously assign ORF UvsY.1 to gene *25*, since there is another possible ORF (ORF UvsY.2) on the opposite strand that could start and extend beyond the sequenced region (toward gene *51*). The incomplete ORF UvsY.2 could start with UUG at position

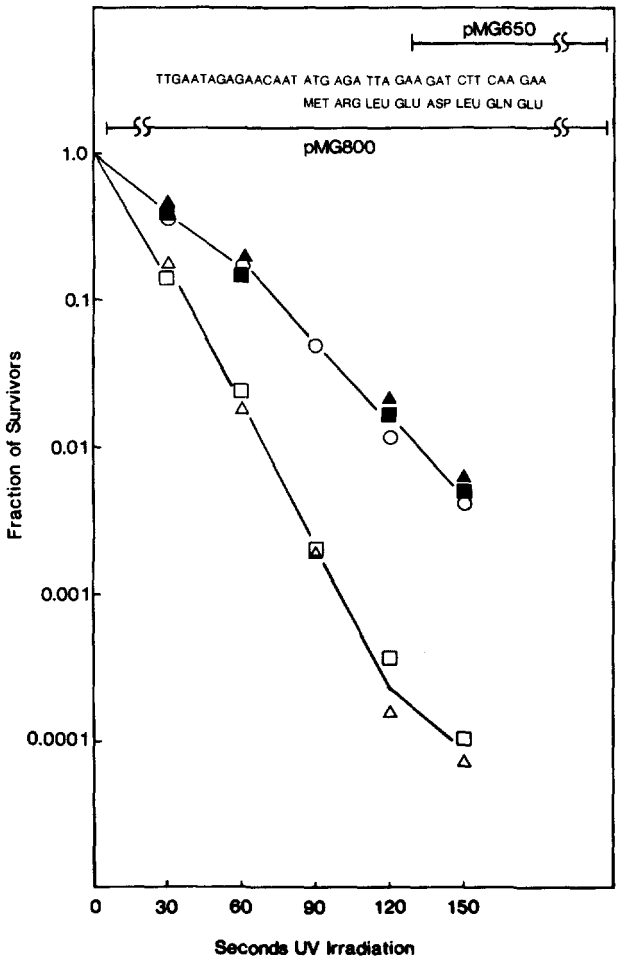

FIGURE 5.—Complementation of *uvsY*. y_{10} (open symbols) and wild type (closed symbols) phage particles were UV-irradiated as described in MATERIALS AND METHODS and were plated on exponential-phase TB-1 cells carrying different T4 clones (○, pMG800; or △ and ▲, pMG650) or vector alone (pUC19) (□ and ■).

79 in the opposite direction of *uvsY*. The putative initiation codon for ORF UvsY.2 is not preceded by a good Shine-Dalgarno sequence. A possible late promoter (TATAAACAC) for ORF UvsY.2, with less than perfect match to the consensus sequence (CHRISTENSEN and YOUNG 1983), is found from nucleotides 508–500. These considerations, taken together, make it likely, although not certain, that ORF UvsY.1 corresponds to gene *25*.

DISCUSSION

An 0.8-kb *Cla*I restriction fragment spanning position 114 kb on the standard T4 map (RUEGER and KUTTER 1984) contains the entire *uvsY* gene. The DNA sequence (Figure 2) predicts a molecular mass for gp UvsY of 15.8 kD, in excellent agreement with the apparent size of the purified protein on SDS

ORF UvsY.1 Start nucleotide: 1, Stop nucleotide: 381

lys-leu-tyr-ser-asp-ile-asp-pro-glu-met-lys-met-asp-trp-asn-lys-asp-
val-ser-arg-ser-leu-gly-leu-arg-ser-ile-lys-asn-ser-leu-leu-gly-ile-ile-
thr-thr-arg-lys-gly-ser-arg-pro-phe-asp-pro-glu-phe-gly-cys-asp-leu-
ser-asp-gln-leu-phe-glu-asn-met-thr-pro-leu-thr-ala-asp-thr-val-glu-
arg-asn-ile-glu-ser-ala-val-arg-asn-tyr-glu-pro-arg-ile-asp-lys-leu-ala-
val-asn-val-ile-pro-val-tyr-asp-asp-tyr-thr-leu-ile-val-glu-ile-arg-phe-
ser-val-ile-asp-asn-pro-asp-asp-ile-glu-gln-ile-lys-leu-gln-leu-ala-ser-
ser-asn-arg-val-STOP

ORF UvsY.2 Start nucleotide: 79, Stop nucleotide: 1

leu-thr-leu-ile-gln-ala-ile-trp-lys-arg-leu-cys-ser-asn-pro-phe-ser-phe-
pro-gly- gln-tyr-gln-asn-lys-ala-

FIGURE 6.—ORFs predicted for gp 25. Refer to Figure 2 to position the indicated start and stop sites of each ORF with the DNA sequence. See text for discussion of each ORF.

polyacrylamide gels (16 kD, YONESAKI *et al.*, 1985), but slightly smaller than that predicted by TAKAHASHI *et al.* (1985). Because of an additional base that we found, the predicted amino acid sequence of the carboxy terminal portion differs from that of TAKAHASHI *et al.* (1985).

uvsY is a "delayed early" gene. *In vivo* transcription of *uvsY* depends on gp MotA, an activator of T4 middle promoters (Figure 4). The upstream sequence (Figure 2) contains the consensus sequence for T4 middle promoters (BRODY, RABUSSAY and HALL 1983). Many T4 delayed early genes are transcribed both from early and middle promoters (BRODY, RABUSSAY and HALL 1983; PULITZER, COLOMBO and CIARAMELLA 1985), but *uvsY* does not have an upstream early promoter (Figures 2 and 4).

The amino acid composition deduced from the DNA sequence and the direct amino acid analysis of purified gp UvsY (T. C. LIN and W. KONIGSBERG, personal communication) indicate that the protein is slightly acidic, with the charges evenly distributed. The sequence also predicts a hydrophilic protein with a strong hydrophobic domain in the C-terminal segment of the protein (Figure 7), but this domain is not long enough to span a membrane. There is no obvious similarity to the helix-turn-helix motif (PABO and SAUER 1984) or to metal-binding domains of other DNA binding proteins (BERG 1986; MILLER, MCLACHLAN and KLUG 1985). Perhaps gp UvsY functions in DNA recombination, replication and repair mainly by interacting with and modulating other DNA binding proteins in a manner analogous to UmuDC modulation of RecA (LU, SCHEUERMANN and ECHOLS 1986), or perhaps with a product of ORF UvsY.-2 that might help the function of gp UvsY. The hydrophobic C-terminal domain of gp UvsY may be important in these interactions and, if not occupied by other proteins, could lead to self-aggregation. The gp UvsY binds specifically to gp UvsX (FORMOSA and ALBERTS 1984), a Rec A-like DNA binding protein that is required for synapsis of homologous DNA (GRIFFITH and FORMOSA 1985; YONESAKI *et al.* 1985), as well as to gp 32 (FORMOSA, BURKE and ALBERTS 1983), a single-stranded-DNA binding protein (ALBERTS and FREY

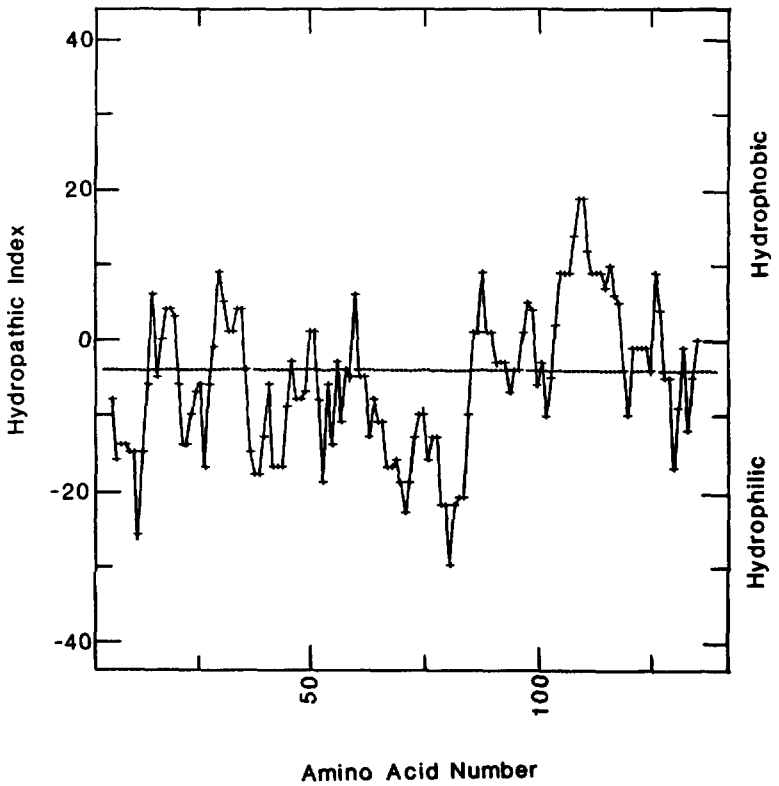

FIGURE 7.—Hydropathy plot based on KYTE and DOOLITTLE (1982) of the deduced amino acid sequence of gp UvsY. The mean hydropathicity value is −0.5204.

1970). It may also interact with the dda helicase and the dCMP-hydroxymethylase (gp 42) in a recombination/replication machine (ALBERTS 1984; FORMOSA and ALBERTS 1984).

A search for homologies with other proteins in the Protein Identification Resource (PIR) databank revealed several patches of gp UvsY which resemble patches of other proteins. Most interesting is the similarity with a C-terminal domain of the replication initiation protein of plasmid R6K (GERMINO and BASTIA 1982) (Figure 8). This similarity extends, in an optimizing search, beyond the segments that were aligned in the initial search. (In contrast, the 14 amino acid similarity with an immunoglobulin protein, found in the initial search immediately following that for the R6K initiation protein, does not increase after an optimizing search.) This C-terminal domain does not contain the DNA binding site of the plasmid's initiator protein and is, in fact, not essential for initiation of plasmid replication (CROSA, LUTTROPP and FALKOW 1978). Nevertheless, it is intriguing to relate this observation to the proposal that phage evolution may include the exchange of DNA patches with other phages and plasmids that reside in the same hosts (CAMPBELL and BOTSTEIN 1983). Note that the *uvsY* region of the T4 genome, which is expressed before replication, appears sandwiched between two large clusters of late genes. Per-

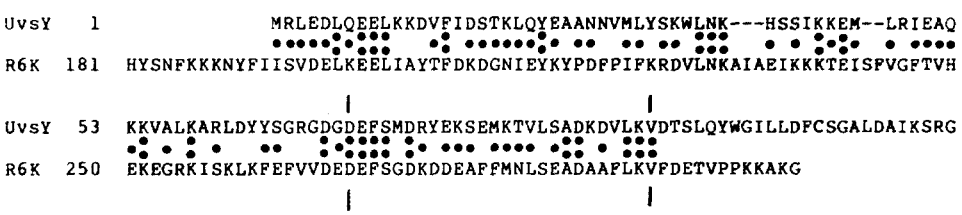

FIGURE 8.—Homology of gp UvsY with R6K initiator protein, found with the program of LIPMAN and PEARSON (1985) in the PIR databank of Georgetown University. For simplicity, the single-letter amino acid code is shown. The upper line is gp UvsY, and the lower line is the sequence for R6K initiator protein. Two dots indicate exact homology, and a single dot shows functional homology.

haps this gene and the nearby origin of replication (see Introduction) were acquired from a relative of R6K. The two overlapping direct repeats in the 5′ region of *uvsY* (Figure 2) may be remnants of such an origin.

In contrast, no homologies were found with the *umuDC* genes of *E. coli* or the corresponding *mucAB* genes of plasmid pKM101 (KITAGAWA *et al.* 1985; PERRY *et al.* 1985). We had considered these possibilities, since, like *uvsY* in T4, *umuDC* or *mucAB* are essential for UV mutagenesis in *E. coli* (WALKER 1984). The lack of homology is consistent with the finding that *umuC* does not compensate for a T4 *uvsY* mutation in T4 DNA repair (DRAKE 1985). Since clones containing T4 genes *uvsY* and *uvsW* might partially substitute for defective *recA* mutants of *E. coli* (DEVRIES and WALLACE 1983), we also looked for homology with the *E. coli recA* gene, but we found none.

As discussed in the Introduction, the role of gp *uvsY* in DNA replication and in DNA repair can be attributed, at least in part, to its role in homologous recombination. If it participates in an early step of recombination, mutational defects must affect initiation of secondary replication forks (LUDER and MOSIG 1982), as well as repair of radiation damages via recombination (BERNSTEIN and WALLACE 1983; CONKLING and DRAKE 1984a,b; MOSIG 1985). In this respect, it is interesting to note that WOMACK (1965) reported unusually high frequencies of marker rescue in the region surrounding *uvsY* (as well as in three other regions of the T4 genome), and ROTTLANDER, HERMANN and HERTEL (1967) found increased heteroduplex frequencies in the same regions. Whereas at least one of the other regions showing high marker rescue (genes *34* and *35*) coincides with a hot spot of recombination that distorts the map over a large distance and probably affects both patch and splice recombinants (MOSIG 1966, 1968), the hot spot near *uvsY* affects localized recombination with little distortion of the overall map (MOSIG 1966, 1968). These results could be explained if short single-stranded T4 DNA, generated and displaced from a nearby origin (see below), initiate specifically patch-type recombination that can initiate secondary replication forks in this region. Thymine dimers, possibly those generated in the direct repeats (Figure 2) and subsequent attempts at repair, might further stimulate both recombination and initiation of secondary replication forks. Any possible additional role of *uvsY* in initiation

from the nearby origin remains to be shown. It is, however, necessary to characterize this origin region in more detail. At this time there are different criteria by which it is defined: (1) it incorporates radioactive label preferentially after infection (HALPERN, MATTSON and KOZINSKI 1979); (2) it forms replication loops visible in electronmicrographs (YEE and MARSH 1985); (3) it incorporates more label after ^{32}P-induced radiation damage than without such damage (MACDONALD *et al.* 1983); (4) it can drive plasmid DNA replication (KREUZER and ALBERTS 1985; 1986); and (5) it out-replicates other T4 regions from certain defective particles, particularly in the presence of rifampicin (KREUZER and ALBERTS 1985). Before any possible function of *uvsY* in direct origin initiation can be assessed, it remains to be shown whether all of the above criteria define the same origin, whether initiation occurs by the same mechanism and at the same site, and whether all domains of this origin region are shared by several different initiation mechanisms or if some domains are specific for only one specific mechanism.

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