

## CLONING AND PHYSICAL MAPPING OF AN EARLY REGION OF THE BACTERIOPHAGE T4 GENOME

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### ABSTRACT

We have cloned DNA restriction fragments from the largely nonessential region of bacteriophage T4 located between genes 39 and 56. The cloned DNA fragments were used to construct a precise map of the sites in this region recognized by eight restriction endonucleases. This restriction map allowed us to compare the cytosine-containing T4 DNA used for cloning with the hydroxymethylcytosine-containing DNA of wild-type T4; there were no detectable rearrangements in the region tested. We were also able to determine the physical locations of several deletion end points and of several genes.

**W**E have found a strong origin of bacteriophage T4 DNA replication in a region encompassing the two adjacent *Xba*I fragments 15 and 17, between genes 56 and 39 (MOSIG *et al.* 1981; MACDONALD *et al.* 1983; MOSIG 1983). This region of the T4 genome is poorly understood; in fact, it appears largely nonessential, since large segments of the T4 genome between genes 56 and 39 can be deleted. HOMYK and WEIL (1974), WILSON and ABELSON (1972) and LITTLE (1973) isolated and characterized viable deletion mutants (called *del(39-56)1-12*, *mb6*, and *sud1*, respectively) in which different segments of this region are missing. Some of these deletion mutants do not use this origin (G. MOSIG, unpublished results) and, therefore, must lack DNA sequences and/or proteins required for initiation at this origin.

To facilitate analysis of the gene 39–56 region, particularly the replication origin at 15.8 kb from the *rIIA/rIIB* junction (coordinate 15.8), we have cloned *Hind*III and *Eco*RI restriction endonuclease fragments from the region between genes 39 and 56 into M13 and plasmid-cloning vectors.

Glucosylated hydroxymethylcytosine, which replaces cytosine in wild-type T4 DNA, prevents digestion by most restriction endonucleases. Large segments of the bacteriophage T4 genome have, however, been cloned since T4 strains were constructed that incorporate cytosine into their DNA (SNYDER, GOLD and KUTTER 1976; WILSON, TANYASHIN and MURRAY 1977), the regions identified to date are summarized by KUTTER and RUGER (1983).

Most T4 DNA clones have been identified on the basis of a screenable

Abbreviations used: dC-DNA = cytosine-containing DNA; HmDC-DNA = hydroxymethylcytosine-containing DNA; XGal = 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside; IPTG = Isopropyl  $\beta$ -D-thiogalactopyranoside; bp = base pairs.

phenotype (MATTSON *et al.* 1977; WILSON, TANYASHIN and MURRAY 1977; VELTEN and ABELSON 1980; REVEL 1981; PUROHIT *et al.* 1981). Some other clones have been identified by hybridization with purified T4 tRNAs (FUKADA, GOSSENS and ABELSON 1980). (See KUTTER and RUGER 1983 for the map positions of clones identified to date.).

We have used DNA hybridization probes to identify clones from the gene 39-56 region, taking advantage of the *Xba*I and *Kpn*I restriction maps of the T4 genome (O'FARRELL, KUTTER and NAKANISHI 1980; MARSH and HEPBURN 1981; NIGGEMANN *et al.* 1981). The use of restriction fragments as specific probes (MILEHAM, REVEL and MURRAY 1980) allowed us to identify clones of overlapping restriction fragments without relying on selectable phenotypes. The cloned fragments were then used to confirm and extend the restriction map of the region and to locate the gene 56-proximal end points of the deletions in three mutants, *del(39-56)7*, *del(39-56)11* and *del(39-56)12* (HOMYK and WEIL 1974). This allowed the mapping of genes *dda*, *mod* and *soc* to certain restriction fragments.

#### MATERIALS AND METHODS

*Phage:* T4GT7 (WILSON *et al.* 1979), a quadruple mutant containing *amE51* (gene 56), *amC87* (gene 42), *NB5060* (a deletion spanning *rII*B, *denB* and *ac*) and a mutation in *alc*, was from G. WILSON. When this mutant is grown on the amber-suppressing host K803 (WOOD 1966), it produces glucosylated HMdC-DNA, which is resistant to most restriction enzymes. When the mutant is grown on B834galU56(sup<sup>0</sup>), it produces dC-DNA which is sensitive to restriction enzymes. *del(39-56)7*, *del(39-56)11* and *del(39-56)12* (HOMYK and WEIL 1974) were from J. WEIL. T4D *os-ac* was from our collection.

*Bacteria:* *E. coli* CR63, used for propagation of the *del(39-56)7*, *del(39-56)11* and *del(39-56)12* phages was from our collection. *E. coli* B834galU56 (RUNNELS and SNYDER 1978), used for the growth of T4GT7 in order to prepare T4 C-DNA, was from L. SNYDER. *E. coli* JM103, which allows selection of recombinants on XGal plates (MESSING and VIEIRA 1982; FELTON 1983), was the host for M13 phage and pUC9 plasmid and was obtained from Bethesda Research Laboratories.

*Cloning vectors:* M13mp7, M13mp9 and pUC9 (MESSING and VIEIRA 1982; VIEIRA and MESSING 1982) were obtained from Bethesda Research Laboratories.

*Media:* YT medium was 5 g of NaCl, 8 g of Bactotryptone and 5 g of yeast extract in 1 liter of water. 2×YT was 5 g of NaCl, 16 g of Bactotryptone and 10 g of yeast extract in 1 liter of water. YT agar was YT medium plus 15 g of agar/liter. YT top agar was YT medium plus 8 g of agar/liter.

*Preparation of DNA:* Plasmid and M13 RF (replicative form) DNAs were extracted from cells by the cleared lysate method (CLEWELL and HELINSKI 1972) and purified by cesium chloride-ethidium bromide gradient centrifugation. T4 DNA was extracted from cesium chloride gradient-purified phage by extraction with SDS and phenol. Unless otherwise noted, T4 dC-DNA was used in most experiments.

*Gels:* Agarose, alkaline agarose and urea-acrylamide gels were prepared and run as described by MANIATIS, FRITSCH and SAMBROOK (1982). Alkaline agarose gels were dried prior to autoradiography. Acrylamide gels were covered with Saran-wrap and autoradiographed directly.

*Elution of DNA from agarose gels:* DNA from agarose gels was eluted either by direct electrophoresis onto DEAE membrane (Schleicher and Schuell Inc.) or by electroelution from a gel slice into gel running buffer in a dialysis membrane. DNA was eluted from the DEAE membrane by washing in 1 M NaCl, 10 mM Tris-Cl (pH 8.0), 1 mM EDTA at 55°. DNA was concentrated by ethanol precipitation.

*Hybridization and autoradiography:* DNA was transferred to nitrocellulose (Schleicher and Schuell Inc.) by the method of SOUTHERN (1975). The blots, which were baked (80°) for 2 hr at reduced pressure, were prehybridized for 1 hr at 65° in 2 × SSPE [1 × SSPE is 0.18 M NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 1 mM EDTA (pH 7.4)] and 1 × Denhardt's (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin). Hybridization, 12 hr at 43°, was in 45% formamide, 7 × SSPE and 2 × Denhardt's containing 25,000–100,000 cpm of the denatured probe. After hybridization the filters were washed extensively at 45° in 2 × SSPE and 0.2% SDS, blotted dry and used to expose Kodak XAR5 film at –70° using Dupont Cronex Lightning Plus intensifying screens.

*Preparation of hybridization probes:* The replacement synthesis method of O'FARRELL (1981) was used. Briefly, 3 µg of T4 dC-DNA or 1.0 µg of a M13-clone RF DNA or plasmid DNA was digested to completion at 37° with one or more restriction endonucleases, in 1 × TA buffer (33 mM Tris-acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 100 µg/ml of bovine serum albumin, 0.5 mM dithiothreitol). T4 DNA polymerase was added directly and, after an appropriate length of time for digestion by the polymerase 3' exonuclease, dATP, dGTP and dTTP were added to 125 µM each, along with 10–30 µCi α<sup>32</sup>P-dCTP (Amersham Corporation, 800 Ci/mmol). The proper duration of the exonuclease step was determined empirically. The <sup>32</sup>P-dCTP was chased by the addition of cold dCTP to 125 µM. After inactivation of the T4 DNA polymerase (15', 70°), the mixture was freed of nucleotides by spin dialysis (NEAL and FLORINI 1973) through Sephadex G-50 medium. Fragments were immediately separated by electrophoresis through an agarose gel. DNA was eluted as described before.

*Restriction digests:* Restriction endonuclease digests were done either as suggested by the vendor or in 1 × TA buffer.

*Purification of T4 restriction fragments for cloning:* Fifty micrograms of T4 dC-DNA were digested to completion with either *EcoRI* or *HindIII*, and the fragments were separated in preparative 1.0% agarose gels containing 0.5 µg of ethidium bromide/ml. The DNA was viewed by illumination with long wave UV light, the desired bands were cut out and the DNA was electroeluted. Rarely, a particular fragment was well resolved and could be excised individually. More often, two or three fragments were purified together. The eluted DNA was phenol extracted twice, ether extracted once, ethanol precipitated and resuspended in a small volume of water. Recovery was usually greater than 50%. Twenty percent of each DNA sample was electrophoresed parallel to 1.5 µg of T4 dC-DNA cut with *HindIII* or *EcoRI* in a 1.0% agarose gel and blotted onto nitrocellulose filters. Southern hybridization revealed which of the different sets of excised fragments contained those of interest, as well as the degree of cross-contamination.

#### *Recombinant DNA procedures*

*Ligations:* Ligations (10 µl) were done in 70 mM Tris-Cl (pH 7.5), 7 mM MgCl<sub>2</sub> and 70 µM ATP containing 20 ng of linearized vector, a one- to three-fold molar excess of the gel-purified T4 DNA restriction fragment and 0.1 unit of T4 DNA ligase. After 12 hr at 8°, 46 µl of water and 4 µl of 0.2 M EDTA were added. This mixture was used directly for transformation.

*Transformations:* *E. coli* JM103 was grown to midlog phase in YT medium, pelleted and resuspended in ½ volume of ice-cold 50 mM CaCl<sub>2</sub> (adjusted to pH 7.5 with Tris). After 20 min on ice the cells were pelleted again and resuspended in 1/10 of the original volume in ice-cold 50 mM CaCl<sub>2</sub> (pH 7.5). These competent cells (0.3 ml) were mixed with an aliquot (6–10 µl) of the ligation mixture and held on ice for 40 min, then at 42° for 2 min, and finally on ice for 10 min. For M13 hybrids, IPTG (10 µl of 100 mM in water), XGal (50 µl of 20 mg/ml in dimethyl formamide), log phase JM103 (0.3 ml) and YT top agar (3 ml) were added, and the mixture was spread on a YT agar plate and incubated overnight at 37°. For *E. coli* JM103 transformed with plasmid hybrids, the log phase JM103 and top agar were omitted, and the transformation mixture was spread on YT agar plates containing ampicillin, 50 µg/ml.

*Identification of cloned DNA:* for M13, colorless plaques from the XGal containing plates (*i.e.*, recombinant phage) were picked into 2 ml of 2 × YT and incubated with aeration for 5 hr at 37°. After pelleting the cells, 17 µl of supernatant was mixed with 3 µl of loading buffer (50% glycerol, 0.1% SDS, 0.25% bromophenol blue) and electrophoresed into a 0.7% agarose gel. The

clones of interest were identified by hybridization with appropriate probes after SOUTHERN (1975) transfer of the DNA to nitrocellulose.

For the plasmids, colorless colonies from the XGal plates were picked onto duplicate YT agar plates (ampicillin, 50  $\mu\text{g}/\text{ml}$ ) and incubated at 37° overnight; clones of interest were detected by colony filter hybridization (GRUNSTEIN and HOGNESS 1975).

*S1 mapping of deletion end points:* Probes labeled at the 3' ends were prepared as described for hybridization probes, except that the exonuclease step was omitted, and only 5  $\mu\text{Ci}$  of  $^{32}\text{P}$ -dCTP was used. *TaqI*-digested DNA of the deletion mutant to be mapped (5  $\mu\text{g}$ ) was ethanol precipitated together with the labeled probe DNA (50 ng). This DNA was resuspended in 30  $\mu\text{l}$  of hybridization buffer [40 mM PIPES (pH 6.4), 1 mM EDTA (pH 8.0), 0.4 M NaCl, 80% formamide], heated at 75° for 15 min, gradually cooled to 30° and incubated at 30°. After 14 hr, 90 units of nuclease S1 (Sigma Chemical Company) in 0.3 ml of buffer [0.28 M NaCl, 50 mM sodium acetate (pH 4.6), 4.5 mM ZnSO<sub>4</sub>, 20  $\mu\text{g}/\text{ml}$  of denatured calf thymus carrier DNA] was added, and the reaction mixture was incubated at 37° for 30 min. The reaction was terminated by adding 50  $\mu\text{l}$  of stop buffer (4.0 M ammonium acetate, 0.1 M EDTA). The sample was phenol/chloroform extracted and the DNA precipitated with 1 volume of isopropanol. The DNA was resuspended in 50 mM NaOH for analysis in an alkaline agarose gel or in TBE loading buffer [45 mM Tris-borate (pH 8.3), 1 mM EDTA, 0.1% bromophenol blue 0.1% xylene cyanol, 80% formamide] for analysis in a urea-acrylamide gel.

*Chemicals and enzymes:* XGal was from Bethesda Research Laboratories, Inc. IPTG was from Sigma. Restriction enzymes were from Bethesda Research Laboratories, Inc., New England Biolabs or Boehringer. T4 DNA polymerase and T4 DNA ligase were gifts from M. BESSMAN and L. ROWEN, respectively.

## RESULTS

*Cloning:* To reduce the number of clones to be screened, we first enriched for the *HindIII* and *EcoRI* restriction fragments that hybridize to specific *XbaI* and *KpnI* restriction fragments which have been mapped to the gene 39–56 region. *HindIII* or *EcoRI* digests of T4 DNA were separated by agarose gel electrophoresis, transferred to nitrocellulose and hybridized with  $^{32}\text{P}$ -labeled *XbaI* 15, *XbaI* 17 or *KpnI* 7 (see Figures 1 and 3A). All of the five *HindIII* and six *EcoRI* restriction fragments detected by this screening were excised from preparative agarose gels. The screening was repeated with aliquots of the partially purified restriction fragments run parallel to an appropriate digest of whole T4 DNA to ensure that the correct fragments had been isolated. *EcoRI* fragments were cloned in M13mp7 or pUC9, and *HindIII* fragments were cloned in M13mp9 (VIEIRA and MESSING 1982; MESSING and VIEIRA 1982). Recombinants were detected by their white colony or white plaque phenotype on XGal plates (MILLER 1972). Plasmid recombinants were tested for the presence of the desired insert by colony filter hybridization (GRUNSTEIN and HOGNESS 1975). M13 recombinants were tested by SOUTHERN (1975) hybridization of M13 phage DNA.

Clones containing four of the five *HindIII* fragments and five of the six *EcoRI* fragments were isolated (Figure 3). These clones were screened as positive by the hybridization test using *XbaI* 15, *XbaI* 17 or *KpnI* 7 as probes.

We also cloned a 4.5-kb *HindIII* fragment because one had been reported in the region adjacent to gene 39 (O'FARRELL, KUTTER and NAKANISHI 1980). However, hybridization of the  $^{32}\text{P}$ -labeled cloned fragment to *BglII*, *XbaI* and *SalI* restriction fragments of T4 DNA (data not shown) revealed that the 4.5-

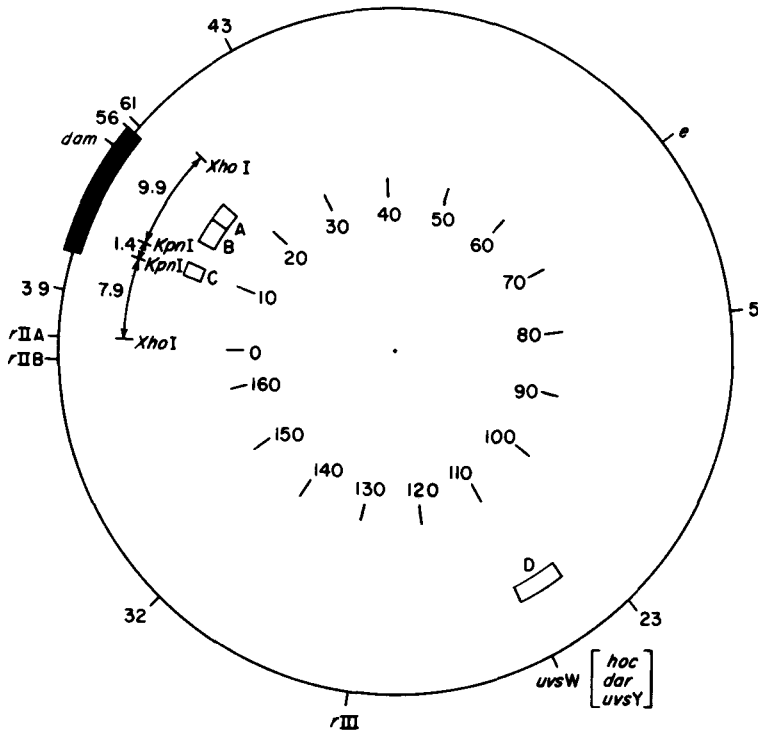


FIGURE 1.—The T4 genome. Only a few genes are marked. The region described in this paper is indicated by a filled bar. The *Xba*I 17, *Xba*I 15 and *Kpn*I 7 restriction fragments used as hybridization probes are labeled A, B and C, respectively. Restriction fragments generated by digestion with *Xho*I and *Kpn*I that were used in orienting the T4 insert DNAs in Figure 2B are shown inside the map. The position of the cloned *Hind*III restriction fragment that is not from the gene 39-56 region (see text) is labeled D. The numbering refers to coordinates on the T4 map and gives the distance in kilobases from the *rIIA/rIIB* junction (KUTTER and RUGER 1983).

kb *Hind*III fragment is actually located near map coordinate 110, where genes *dar*, *hoc* and *uvsY* have been mapped (Figure 1).

*Location of the cloned T4 DNA segments on the T4 map:* The positions of the cloned DNAs from the *Xba*I 15 and 17 region (Figure 3C) agree with the published *Eco*RI and *Hind*III restriction maps (O'FARRELL, KUTTER and NAKANISHI 1980). For confirmation, each T4 insert was labeled and hybridized to Southern blots of *Xba*I digested total T4 DNA. These T4 inserts hybridized to the expected *Xba*I fragment or fragments and only to these fragments (data not shown).

On the other hand, the relative positions of the T4 inserts in Figure 3B could not be deduced from the published restriction maps of *Hind*III and *Eco*RI (O'FARRELL, KUTTER and NAKANISHI 1980). Therefore, we mapped them relative to fragments produced by a *Kpn*I-*Xho*I double digest. This digest produces the 1.4-kb *Kpn*I fragment (shown in Figure 3) as well as a 7.9-kb *Xho*I-*Kpn*I fragment that includes gene 39 and a 9.9 kb *Xho*I-*Kpn*I fragment that includes gene 56 (Figure 1). M13r51 hybridizes to the 9.9-kb-*Xho*I-*Kpn*I

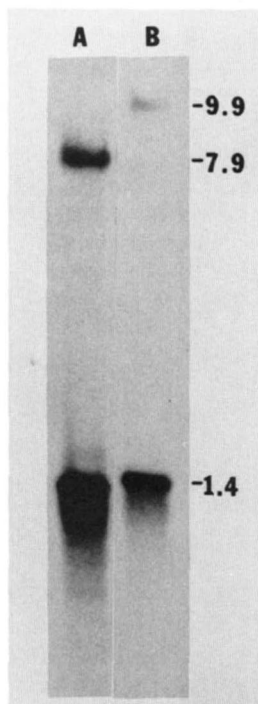


FIGURE 2.—Hybridization of the T4 DNA inserts of M13r51 and M13h91 to a *XhoI-KpnI* digest of total T4 dC-DNA. Lane A, the M13h91 insert was used as probe, hybridizing to the 1.4- and 7.9-kb fragments. Lane B, the M13r51 insert was used as probe, hybridizing to the 1.4- and 9.9-kb fragments. See Figure 1 for the locations of these restriction fragments.

fragment (Figure 2) and so must overlap *KpnI* 7 on the gene 56 proximal side. Hybridization of the insert of M13h91 to the 7.9-kb-*XhoI-KpnI* fragment (Figure 2) demonstrates that this fragment overlaps the *KpnI* 7 fragment on the gene 39 proximal side. These data establish the arrangement shown in Figure 3.

*The restriction map of the gene 39-56 region:* The DNA used for cloning was from a T4 mutant phage that incorporates cytosine into its DNA (dC-DNA) instead of the usual hydroxymethylcytosine (HMdC-DNA). One potential consequence of this substitution is instability of the T4 genome. Because we wish to examine structural and physiological features of the cloned DNA near the replication origin (located at approximately coordinate 15.8) it was important to determine whether the dC-DNA used for cloning was rearranged, deleted or duplicated, as compared with HMdC-DNA. This comparison required a fine structure restriction site map of the cloned region and, most importantly, the position of *TaqI* sites on that map. *TaqI*, unlike most other restriction endonucleases, cuts HMdC-DNA and, therefore, allows direct comparison of T4 HMdC- and dC-DNA.

Restriction sites within cloned fragments were determined by standard techniques (see MANIATIS, FRITSCH and SAMBROOK 1982). To locate restriction sites not covered by our clones, Southern blots of T4 dC-DNA digested with

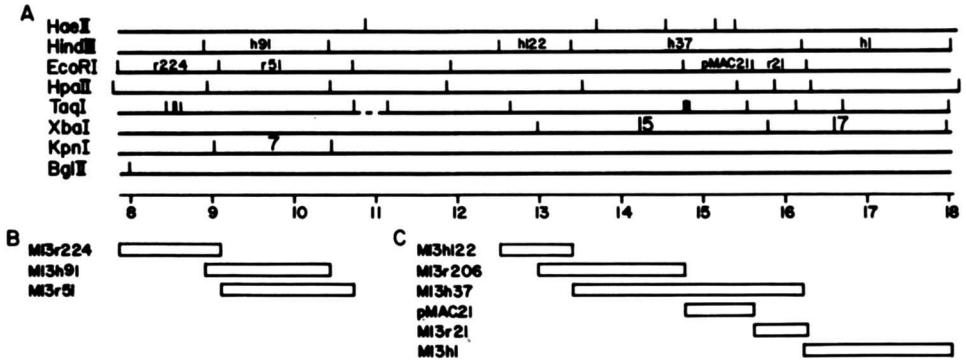


FIGURE 3.—A, Restriction map of the cloned region of the T4 genome discussed in this paper. The *Xba*I 15-*Xba*I 17 junction at 15.8 was used as a reference point since our clones do not extend into the *rII* region. The restriction fragments used for probes (*Kpn*I 7, *Xba*I 15 and *Xba*I 17) are numbered according to MARSH and HEPBURN (1981). B, Cloned segments that hybridize to the *Kpn*I 7 probe. C, Cloned segments that hybridize to the *Xba*I 15 and/or *Xba*I 17 probes. The numbering of the T4 genome coordinates is the same as in Figure 1.

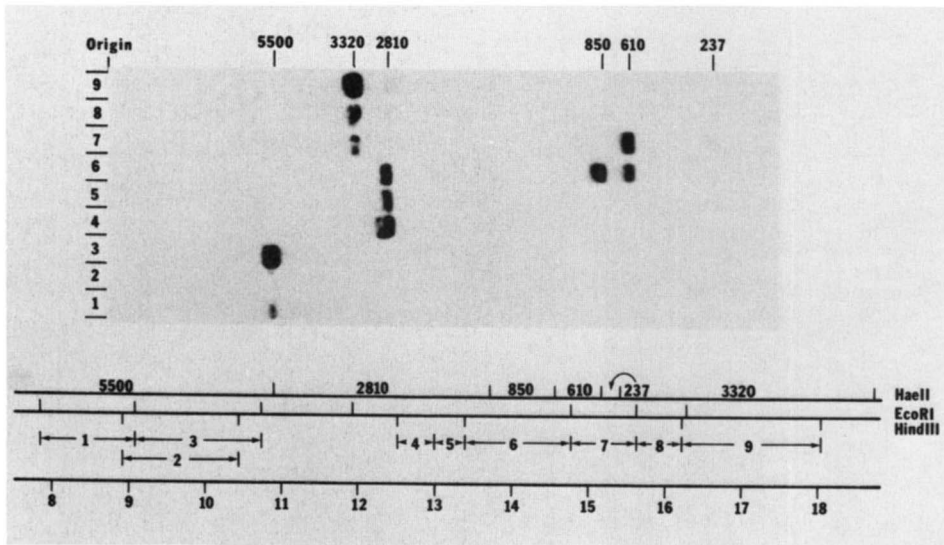


FIGURE 4.—Southern hybridization of cloned T4 DNA to *Hae*II-digested T4 dC-DNA, and the deduced *Hae*II restriction map. A, Identical strips numbered 1-9 were hybridized with the probes shown in B. Standards used for size determination were restriction fragments of pBR322. B, The deduced *Hae*II restriction map and the probes used for part A. Precise mapping of the three *Hae*II sites within the M13h37 T4 insert (Figure 2) allowed alignment of the *Hae*II map with the *Eco*RI and *Hind*III maps. The numbering of the T4 genome coordinates is the same as in Figure 1.

various restriction enzymes were cut into strips, and each individual strip was hybridized to a different probe. As an example, the autoradiogram of the probed *Hae*II digests is shown in Figure 4. The banding pattern observed by autoradiography, when taken in conjunction with the location of restriction sites within the cloned fragments, allowed us to position other restriction sites.

The map we deduce for eight restriction endonucleases is presented in Figure 3. The only uncertainty in this map concerns the presence of possible *TaqI* sites in the dotted segment at coordinates 10.8–11.1. An additional *TaqI* site (not marked in any figure), which is at least partially resistant to digestion, is located within the coordinates 16.7–18.0 *TaqI* fragment (see legend to Figure 5).

*Integrity of the cloned DNA fragments relative to T4 dC-DNA:* We tested each clone to determine whether (1) the entire T4 dC-DNA restriction fragment had been cloned and (2) the fragment had experienced gross rearrangements during cloning. Phage T4 dC-DNA and cloned DNAs were digested with the restriction endonuclease used in cloning, either alone or with a second restriction endonuclease that cleaved within the insert DNA. The fragments pro-

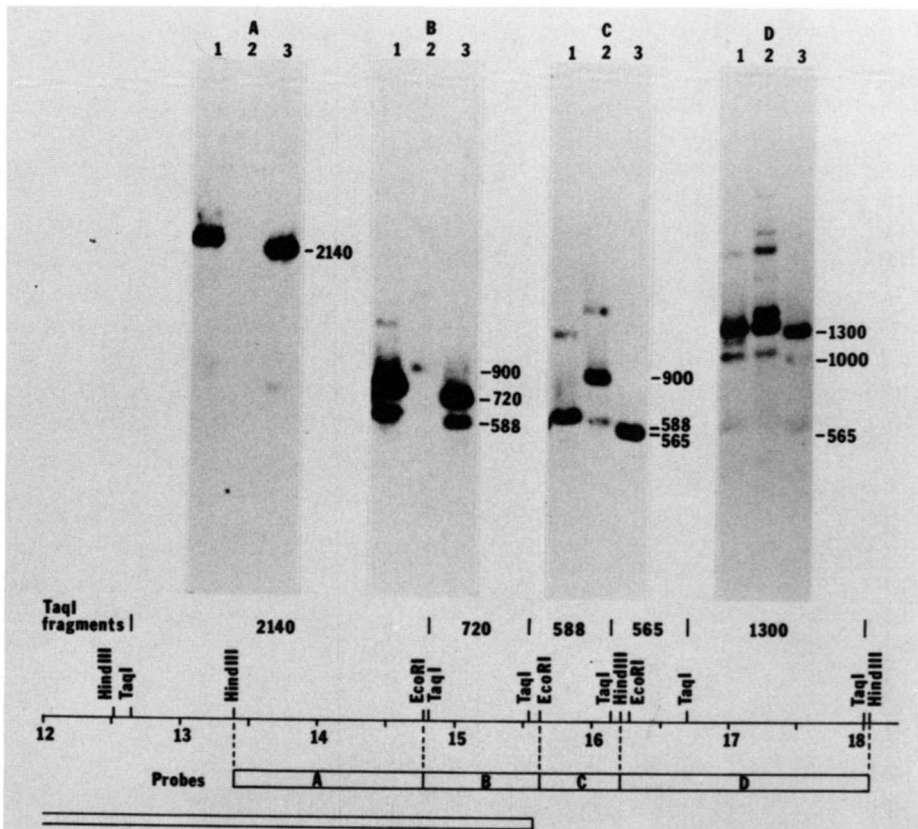


FIGURE 5.—Southern hybridization of *TaqI*-digested T4 *os-ac* (1), T4 *del(39-56)12* (2) and T4 GT7 (3) DNAs using the cloned probes shown below. The *TaqI* restriction fragments that hybridize to the various probes are presented in a linear order beneath the autoradiograms, and their lengths (in base pairs) are shown next to the appropriate bands in the autoradiograms. The marked positions correspond to the *TaqI* fragments of T4 dC-DNA. The same *TaqI* fragments from T4 HMdC-DNA migrate slightly slower in agarose gels. The 1000-bp *TaqI* fragment seen in panel D appears to result from cleavage at a partially resistant *TaqI* site within the 1300-bp *TaqI* fragment. The numbering of the T4 genome coordinates is the same as in Figure 1.



duced were separated by agarose gel electrophoresis, transferred to nitrocellulose and hybridized with  $^{32}\text{P}$ -labeled insert DNA. By this test, all but one of the restriction fragments were judged to have been cloned intact (data not shown).

The one exception is an altered and incomplete restriction fragment in clone M13r206. This clone contains, in addition to the T4 insert shown in Figure 3, 182 bp at the left end. As determined by DNA sequencing, these 182 bp are identical with the 182 bp from the *EcoRI* site at coordinate 15.6 to the *XbaI* site at coordinate 15.8 (data not shown). The explanation for this rearrangement is unknown.

*Integrity of T4 dC-DNA relative to T4 HMdC-DNA:* We compared, by Southern blots, hybridization of labeled restriction fragments (probes A through D in Figure 5) to *TaqI* digests of T4 dC-DNA and T4 HMdC-DNA. As seen in Figure 5, the five detectable *TaqI* fragments between coordinate 12.65 and 18.0 are of the same length for both T4 dC-DNA and T4 HMdC-DNA. (Note that the substitution of HMdC for dC slightly retards the mobilities of the DNA fragments in the agarose gels.) For all probes, the hybridization patterns in lanes 1 (HMdC-DNA) and 3 (dC-DNA) are identical. This shows that there are no major differences between wild-type T4 and the dC-DNA-containing phage in this region.

*Mapping deletion end points:* HOMYK and WEIL (1974) isolated a series of mutants that have deletions extending various distances into the region that we have cloned. Some of these mutants do not initiate replication at the origin near coordinate 15.8 and, therefore, must lack DNA sequences and/or proteins required for initiation at this origin. As a first step in identifying the missing component(s) we have more precisely mapped the gene 56 proximal end points of three deletions. The approximate end point of *del(39-56)12* was determined by Southern blotting of *TaqI* restriction fragments and probing with the DNA fragments A through D as shown in Figure 5. As mentioned before, five different *TaqI* fragments were detected in wild-type T4 DNA by probing with these fragments. In contrast, only the 1300- and 565-bp *TaqI* fragments were present in *del(39-56)12* (lane 2, panels C and D), therefore, the deletion end point must be within the adjacent 588-bp *TaqI* fragment. In *del(39-56)12*, this 588-bp fragment is replaced by a 900-bp fusion fragment (lane 2, panels B and C). Since probe B hybridized weakly to the 900-bp fragment, the *del(39-56)12* end point must be between the *TaqI* and *EcoRI* sites near coordinate 15.5.

The position of this end point was refined and confirmed by nuclease S1 mapping. As described in METHODS AND MATERIALS, *TaqI* digested *del(39-56)12* HMdC-DNA was hybridized in liquid to the 588-bp 3'-end labeled *TaqI* fragment from clone M13h37 (probe E in Figure 7) and treated with nuclease S1. The products were fractionated by electrophoresis in a 50% urea (w/v) 4% acrylamide gel (Figure 6). Protection from S1 of an approximately 550 nucleotide fragment (lane 2, marked with a ■) locates the *del(39-56)12* end point at approximately 30 bp to the left of the *EcoRI* site at coordinate 15.6 (bands that migrate slower than the probe are artifacts). The same probe, when hy-

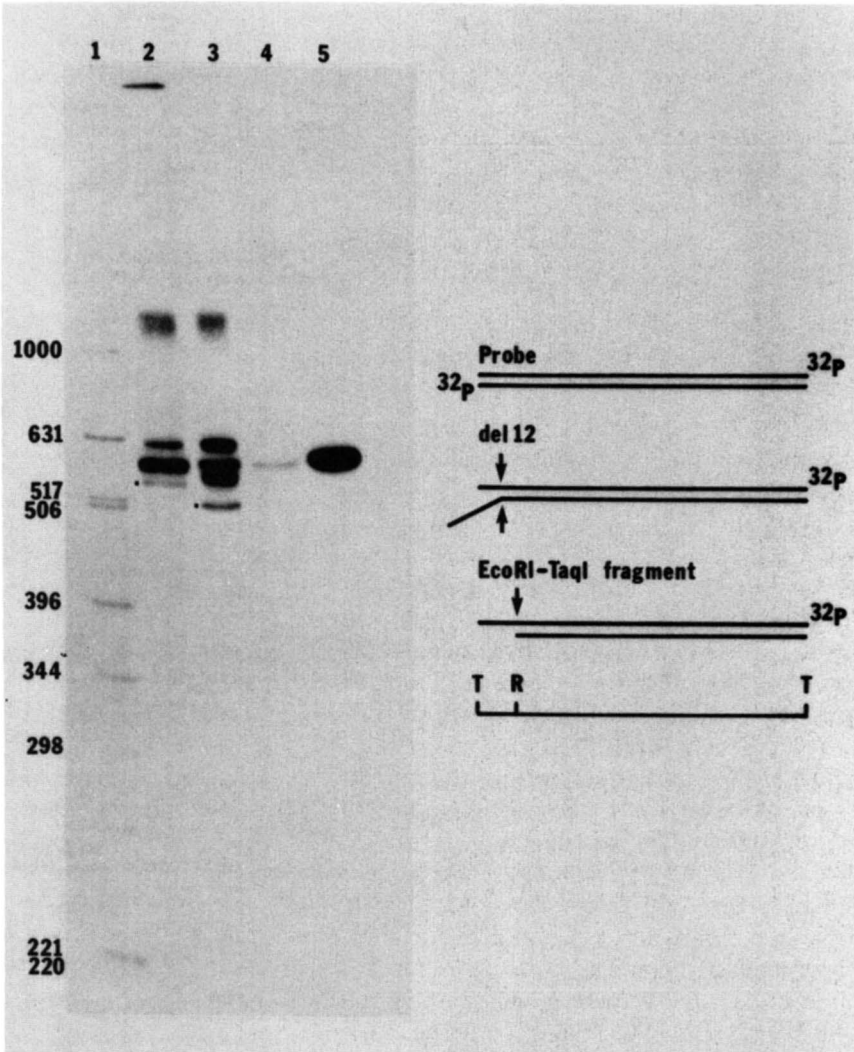


FIGURE 6.—Autoradiogram of a *del(39-56)12* S1 mapping gel, with a schematic representation shown at the right. Lane 1, pBR322 digested with *Hinf*I and *Eco*RI. Lane 2, the  $^{32}$ P-labeled 588-bp *Taq*I fragment (probe E in Figure 5) hybridized to *Taq*I-digested *del(39-56)12* DNA and digested with S1. The protected strand is marked (■). Lane 3, probe E hybridized to the 517-bp *Eco*RI-*Taq*I fragment and digested with S1. The protected strand is marked (■). Lane 4, probe E digested with S1. Lane 5, probe E, no S1 digestion.

bridized to the cloned 517-bp *Eco*RI-*Taq*I fragment, protects only 517 bases of the probe from S1 digestion, as expected (lane 3, marked with a ■). This experiment also shows that the base sequence of the T4 HMdC-DNA and cloned dC-DNA in the region spanning coordinates 15.6 and 16.1 is identical or nearly identical, since no mismatches in heteroduplexes were cleaved by S1 nuclease to yield fragments smaller than 550 nucleotides in lane 2 of Figure 6.

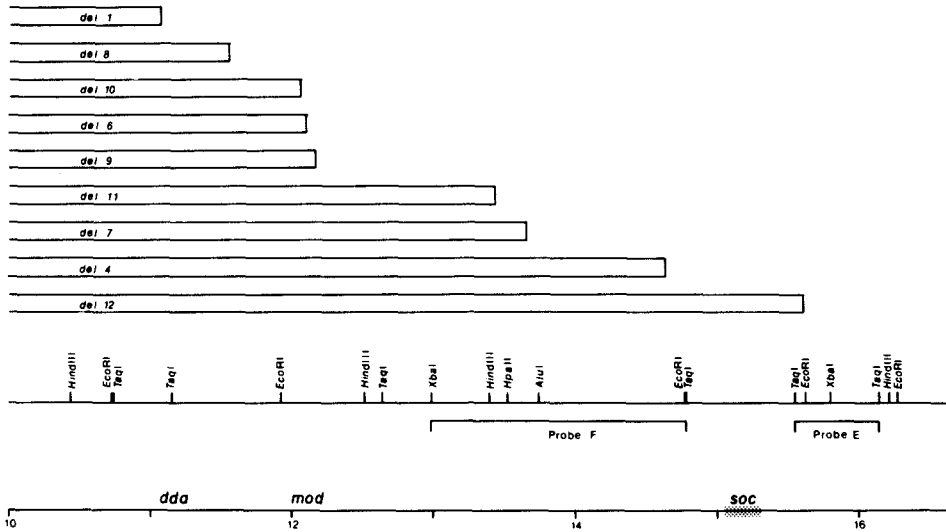


FIGURE 7.—A map of the end points of nine of the deletions present in some of the mutants isolated by HOMYK and WEIL (1974). The (39-56) designations are omitted in the figure. The *del(39-56)12* end point was mapped as shown in Figure 6, using probe E. Probe F, which extends from the *XbaI* site at 13 to the leftmost of the three *TaqI* sites at 14.75, was used to map *del(39-56)7* and *del(39-56)11*. As controls, three restriction fragments from M13h37 (Figure 3) were also hybridized to probe F, treated with nuclease S1 and run on the same alkaline agarose gel as the S1 resistant products of the hybrids formed between *del(39-56)7* or *del(39-56)11* and probe F. These three restriction fragments shared the same *TaqI* site at the right end of probe F and extended to the *AluI* site, the *HpaII* site or the *HindIII* site within probe F. The only *AluI* and *HpaII* site shown in the restriction map are the relevant ones within probe F. The locations of the mapped genes are shown. The numbering of the T4 genome coordinates is the same as in Figure 1.

The end points of *del(39-56)7* and *del(39-56)11* were determined in the same manner, using probe F in Figure 7, except that the S1-resistant DNA fragments were fractionated in a 1% alkaline agarose gel (data not shown). Using electron microscopy of heteroduplexes, HOMYK (1974) measured the distances from the *del(39-56)7* end point to the end points of the deletions indicated by slashed bars in Figure 7. We correlated his data to our *del(39-56)7* end point to more precisely locate the end points of these deletions on the physical map.

#### DISCUSSION

We have cloned restriction fragments covering most of the region between coordinates 8 and 18 on the T4 genome, including a replication origin at approximately coordinate 15.8. One *HindIII* restriction fragment that is missing among our clones (Figure 3) is partially present in another clone. D. HINTON and N. NOSSAL (personal communication) have cloned an *EcoRI* fragment whose hybridization and restriction patterns are consistent with its being the 2.9-kb fragment from coordinates 11.85-14.7. In addition, we have constructed a fine structure restriction map of this region.

Using the cloned fragments and this restriction map, we have shown that no

major rearrangements are present in the phage dC-DNA (relative to HMdC-DNA), at least between coordinates 12.65 and 18.0, and that the base sequences of T4 HMdC- and dC-DNA are identical or nearly identical, at least in the region between coordinates 15.6 and 16.1 on the T4 map. In addition, we have precisely mapped the end points of three deletions that eliminate some nonessential genes of T4.

These results, together with different kinds of information obtained by other investigators and by us, which will be described, position an origin region of DNA replication and several known T4 genes more precisely on the T4 map. They also suggest that additional, as yet unidentified genes may exist in that region.

We now summarize what is known about genes in this region, starting at the gene 56 end. The 4.8-kb *EcoRI* fragment (coordinates 16.3–21.1) could not be cloned intact in our vectors, possibly because this *EcoRI* fragment contains genes 56 and 61. Gene 56 codes for a dCTPase and could be detrimental to *E. coli* DNA synthesis (although the T4 DNA used for cloning has an amber mutation in gene 56, *E. coli* JM103 carries the suppressor *sup E*). The product of gene 61 is part of the T4 primase (LIU *et al.* 1979; NOSSAL 1980). We do not know whether this gene would be detrimental to *E. coli*. The *dam* gene, which codes for a DNA-adenine methylase, is present on the cloned 1.8-kb *HindIII* fragment (coordinates 16.2–18.0) (SCHLAGMAN and HATTMAN 1983). Other genes may be present in the region of the 4.8-kb *EcoRI* fragment, as the three known genes do not exhaust the coding capacity of 4.8 kb. Three possible candidates are genes *uvsZ* (CUPIDO, SCHEIJ-VISSER and VAN DER REE 1982), *uvsX* (CHILDS 1980; WAKEM and EBISUZAKI 1981; CUPIDO, GRIMBERGEN and DEGROOT 1981) and *sp* (EMRICH 1968). Perhaps the strong promoter located at coordinate 19.2 (NIGGEMANN *et al.* 1981) is responsible for our inability to clone this restriction fragment; a strong promoter could have an adverse effect on the cloning vector.

A replication origin region (MOSIG *et al.* 1981; MACDONALD *et al.* 1983; MOSIG 1983) is located at coordinate 15.8 in the *EcoRI* fragment overlapping the *XbaI* 15-*XbaI* 17 junction.

DNA sequence analysis of the region between coordinates 14.9 and 16.3 reveals two open reading frames. They would code for a protein of 224–274 amino acids and a protein of 80 amino acids (P. M. MACDONALD and G. MOSIG, unpublished results). The 80-residue protein is gp Soc (MACDONALD, KUTTER and MOSIG 1984), the late, small outer capsid protein (ISHII and YANAGIDA 1975). We are presently trying to determine the identity of the protein corresponding to the larger open reading frame.

Two genes, *dda* and *mod*, which could not be cloned intact, have been mapped to the region of the 2.1-kb *HindIII* fragment (coordinates 10.4–12.5) by their presence or absence in the various deletion mutants of HOMOYK and WEIL (1974). The *mod* gene product modifies the *E. coli* RNA polymerase (HORVITZ 1974). It has been proposed to alter the transcription specificity from host to phage DNA (MAILHAMMER *et al.* 1975) and, therefore, would be expected to seriously interfere with *E. coli* RNA synthesis. Gp Dda, which was

first identified as a DNA-dependent ATPase (BEHME and EBISUZAKI 1975), is also a DNA helicase (KUHN *et al.* 1979; ALBERTS *et al.* 1983). Either or both of these gene products could be important in initiation of replication at the coordinate 15.8 origin. The DNA-dependent ATPase activity of *dda* is present in *del(39-56)1* (HOMYK and WEIL 1974). However, the 47-kilodalton Dda gene product is missing in two-dimensional polyacrylamide gels of *del(39-56)1* extracts (T. FORMOSA and B. ALBERTS, personal communication). Therefore, it seems likely that a truncated version of the Dda protein that is made by *del(39-56)1* retains the ATPase activity. Since only *del(39-56)1* and *del(39-56)8* retain Mod activity, it is probable that *mod* is present intact on the 2.1-kb *Hind*III fragment (see Figure 7). Thus, we believe that our failure to clone this fragment intact is due to the presence of the complete *mod* and/or *dda* genes. As mentioned before, this region has been partially cloned as an *Eco*RI fragment, possibly because this clone does not contain either of the intact genes.

*dexA*, which codes for exonuclease A, and *pseF*, which codes for a 5'-phosphatase (DEPEW and COZZARELLI 1974), are missing in at least some of the HOMYK and WEIL (1974) deletions. Certain suppressors of some gene 32 mutations, *sud* mutations (LITTLE 1973), probably map in the same region. Gene *mb*, which is necessary for the processing of the T4 ser, pro and ile tRNAs and band 2 RNA (WILSON and ABELSON 1972; GUTHRIE and MCCLAIN 1973; MCCLAIN, GUTHRIE and BARRELL 1973), is also located in this region. We do not know whether *pseF* and *mb* are present in the cloned region.

Our results facilitate more precise studies on the requirements for specific proteins and DNA sequences to initiate at the replication origin that is contained in this region. In addition, they help in understanding the role of nonessential genes in phage T4 in general. Knowledge of the physical location of the end points of the deletions and of the cloned segments and restriction sites is extremely valuable for studies on the organization and expression of this region of the T4 genome. As an example, the following paper (MACDONALD, KUTTER and MOSIG 1984) describes the identification of the exact location of the late gene *soc* and the control of its expression.

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