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Genetic Analysis of Gene 1.2 of Bacteriophage T7: Isolation of a Mutant of *Escherichia coli* Unable to Support the Growth of T7 Gene 1.2 Mutants

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The product of gene 1.2 of bacteriophage T7 is not required for the growth of T7 in wild-type *Escherichia coli* since deletion mutants lacking the entire gene 1.2 grow normally (Studier et al., J. Mol. Biol. **135**:917-937, 1979). By using a T7 strain lacking gene 1.2, we have isolated a mutant of *E. coli* that was unable to support the growth of both point and deletion mutants defective in gene 1.2. The mutation, *optA1*, was located at approximately 3.6 min on the *E. coli* linkage map in the interval between *dapD* and *tonA*; *optA1* was 92% cotransducible with *dapD*. By using the *optA1* mutant, we have isolated six gene 1.2 point mutants of T7, all of which mapped between positions 15 and 16 on the T7 genetic map. These mutations have also been characterized by DNA sequence analysis. *E. coli optA1* cells infected with T7 gene 1.2 mutants were defective in T7 DNA replication; early RNA and protein synthesis proceeded normally. The defect in T7 DNA replication is manifested by a premature cessation of DNA synthesis and degradation of the newly synthesized DNA. The defect was not observed in *E. coli opt*$^+$ cells infected with T7 gene 1.2 mutants or in *E. coli optA1* cells infected with wild-type T7 phage.

In recent years, the combination of genetic and biochemical studies has provided detailed information on the replication of bacteriophage T7 DNA (for reviews, see references 15 and 21). The products of at least six genes of phage T7 are essential for T7 DNA replication: gene 1 (RNA polymerase), gene 2 (inhibitor of *Escherichia coli* RNA polymerase), gene 3 (endonuclease), gene 4 (primase and DNA-dependent nucleoside triphosphatase), gene 5 (subunit of DNA polymerase), and gene 6 (exonuclease). T7 encodes a DNA ligase, the product of gene 1.3, although the host ligase is sufficient for phage growth. T7 also induces a single-stranded DNA binding protein, the product of gene 2.5, but its role in DNA replication is unknown since no alterations in T7 DNA metabolism have been observed in cells infected with gene 2.5 mutants (F. W. Studier, personal communication). At least one host protein is essential for T7 DNA replication; thioredoxin, the product of the *trxA* gene of *E. coli*, is a subunit of T7 DNA polymerase. Other host proteins, such as DNA polymerase I, DNA ligase, DNA gyrase, DNA binding protein, and RNA polymerase may also play important, although not essential, roles in T7 DNA replication.

Although these known enzymes can carry out most of the events occurring at the replication fork (15), it seems likely that additional proteins will be required for initiation of DNA replication and for the formation and processing of concatemers. If additional proteins of replication are the products of T7 genes, it is reasonable to assume that they are among the proteins synthesized early in the infection cycle. Although the early genes of T7 have been analyzed extensively (19, 22, 23, 25), there remain a few genes whose functions are unknown; among these are genes 1.1 and 1.2.

Genes 1.1 and 1.2 are transcribed in vivo by *E. coli* RNA polymerase starting from promoters located at the extreme left end of the molecule (25); these genes are also transcribed in vitro by T7 RNA polymerase, using the T7 RNA polymerase promoters located at positions 14.5 and 14.7 (11, 12). The primary transcript made by *E. coli* RNA polymerase is processed by *E. coli* RNase III to yield five mature mRNAs. One of these mRNAs, the 1.1 mRNA, codes for two small proteins. Studier et al. (25) tentatively designated these two proteins as the gene 1.1 and gene 1.2 proteins, although the relative positions of the two genes were not known.

We recently reported the 682-base-pair nucleotide sequence of the T7 DNA molecule between positions 14.45 and 16.15 (16). Within this

region, shown schematically in Fig. 1, we have identified the sequence of the primary origin of T7 DNA replication (26), the termination of gene 1, all of genes 1.1 and 1.2, the start of gene 1.3, and a number of regulatory sequences, including three T7 RNA polymerase promoters and two RNase III cleavage sites. From the nucleotide sequence it can be deduced that genes 1.1 and 1.2 code for proteins of 42 and 85 amino acids, respectively, in agreement with the molecular weights determined for the two proteins coded by the 1.1 mRNA (25). The proximity of genes 1.1 and 1.2 to the primary origin of T7 DNA replication provided additional incentive to investigate the role of their protein products in T7 DNA replication.

The gene 1.1 and 1.2 proteins are clearly not required for the growth of T7 since deletions that lack these genes grow normally in wild-type *E. coli* (23, 25). Genetic analysis of such nonessential genes is difficult, as is the biochemical analysis of their products. However, if the nonessential nature of the gene arises from the ability of a host protein to substitute for the phage-specified protein, then a feasible approach is to identify host mutants in which the T7 function is essential for growth. Such a situation occurs for gene 1.3, whose product is T7 DNA ligase (10, 22). T7 gene 1.3 mutants do not grow in ligase-deficient strains of *E. coli*, although they grow normally in wild-type *E. coli* (7, 10). Studier (22) has used this phenotype of T7 gene 1.3 mutants to isolate both *E. coli* and T7 ligase mutants.

In the study described in this paper, we used a similar approach to isolate a mutant of *E. coli* that was unable to support the growth of T7 deletion mutants lacking genes 1.1 and 1.2. Genetic and DNA sequence analyses of the phage mutations responsible for this phenotype showed that they reside in gene 1.2. We also mapped the host gene, *optA*, responsible for the nonessential nature of gene 1.2 of phage T7. Our preliminary characterization of T7 gene 1.2 mutant-infected *E. coli optA1* indicates that the failure of T7 growth resulted from a defect in T7 DNA replication.

MATERIALS AND METHODS

Bacterial and phage strains. All bacterial strains except *E. coli* 011′ were derived from *E. coli* K-12. A list of the strains used and their properties is shown in Table 1. The following T7 phage strains were obtained from F. W. Studier: wild-type T7; T7 deletion mutants LG3, LG37, LG13, D2, D303, LG12, LG30, LG16, D24, LG26, and D53 (25); T7 amber mutants 1*am*342a, 3*am*29, 4*am*20, 5*am*28, and 6*am*147 (20); and a point mutant of gene 1.3, A22 (22). T7 mutants are designated hereafter by subscript notation indicating the deletion or mutated gene. P1*vir* phage was obtained from M. Syvanen.

Media. T broth contained 10 g of tryptone (Difco), 5 g of NaCl, and 20 mg of thymine per liter. TY broth contained 10 g of tryptone, 1 g of yeast extract (Difco), 1 g of glucose, 8 g of NaCl, and 0.22 g of $CaCl_2$ per liter. TY broth plates were prepared from the same medium containing 1.5% agar. M9 buffer contained 1 g of NH_4Cl, 3 g of KH_2PO_4, 6 g of Na_2HPO_4 and 1 ml of 1 M $MgSO_4$ per liter. M9 media was M9 buffer containing the indicated amino acids (60 μg/ml) and 5 g of glucose per liter. M9CAA media contained 2 g of vitamin-free Casamino Acids (Difco), 5 g of glucose, and 10 mg of adenine per liter of M9 buffer. L broth was prepared by the method of Lennox (8). L broth plates were prepared from L broth containing 1.5% agar. EMBO plates were prepared as previously described (17).

Chemicals. *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine was purchased from Aldrich Chemical Co. Chloramphenicol was obtained from Calbiochem. Unlabeled amino acids were obtained from Sigma Chemical Co. [^{3}H]thymidine, [^{3}H]uridine, and a ^{14}C-labeled L-amino acid mixture were obtained from New England Nuclear Corp.

Mating and transduction procedures. All bacterial matings and transductions, using P1*vir*, were performed as previously described (17). Genetic crosses of T7 phage were carried out as described by Studier (20), except that chloramphenicol (100 μg/ml) was used instead of KCN (3).

Isolation of *optA1* mutant. Mutagenized *E. coli* was used to isolate a host mutant that could not support the growth of T7 phages lacking genes 1.1 and 1.2. *E. coli* PA610 was mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, 1 mg/ml, by the method of Adelberg et al. (1). For mutant segregation, portions were diluted 20-fold into L broth and incubated at 30°C for 16 h. After segregation, approximately 1,000 cells were spread on each TY broth plate and incu-

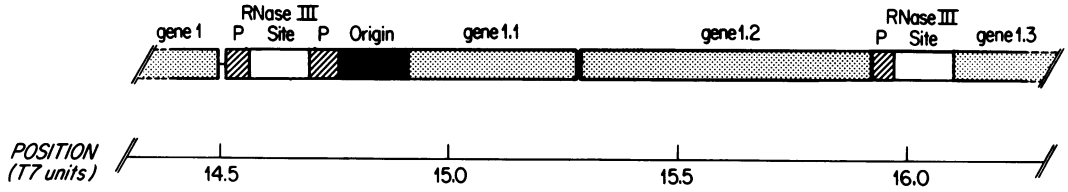


FIG. 1. *Schematic representation of the genetic elements between positions 14.5 and 16.3 (16). P represents T7 RNA polymerase promoter.*

TABLE 1. *E. coli* strains

Strain	Genotype[a]	Source or reference
PA610	*thr leu purE his lys argH thi ara lacY gal malA mtl xyl str^r tonA supE*	4
HR40	*thr leu purE his lys argH thi ara lacY gal malA mtl xyl str^r tonA supE optA1*	PA610
HR44	*thr leu his lys argH thi ara lacY gal malA mtl xyl str^r tonA su$^-$ optA1*	HR40 × KL208
KL208	Hfr	9
AT982	Hfr *dapD4 thi*	18
HMS96-1	F$^-$ *dapD4 thi*	AT982
011′	*thy su$^+$*	20
011′L1	*thy su$^+$ lig*	22
BL2	*su$^-$ lig*	22
W3110	*su$^-$*	5

[a] The genetic symbols used are those of Bachmann and Low (2).

bated at 37°C for 18 h. The colonies were replica plated onto EMBO plates seeded with 3×10^7 $T7_{LG37}$ phage and incubated at 37°C for 18 h. Pink colonies that had not been lysed by the phage were purified by streaking on TY broth plates. These isolates were then scored by spot tests for their ability to support the growth of, or to be killed by, T7 wild-type, $T7_{1.3}A22$, and $T7_{LG37}$ phages. One *E. coli* mutant, designated HR40 *optA1*, was killed by, but failed to produce a burst of $T7_{LG37}$ phage while supporting the normal growth of T7 wild-type and $T7_{1.3}A22$ phages.

Isolation of $T7_{1.2}$ mutants. Wild-type T7 phage were mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, and $T7_{1.2}$ mutants were isolated by the agar overlay method (20). The mutagenized lysate was plated on *E. coli opt$^+$* at a concentration that gave approximately 300 plaques per plate. After the top agar hardened, the plates were overlayered with soft agar containing *E. coli optA1* and incubated at 25°C for 24 h. Turbid plaques were stabbed with a toothpick which was then stabbed into two plates containing lawns of *E. coli optA1* and *opt$^+$*, respectively. Phage that could grow only on the *opt$^+$* hosts were saved as candidates for $T7_{1.2}$ mutants. Two combinations of *E. coli* strains were used for the initial screening by the agar overlay method. *E. coli* PA610 *opt$^+$ su$^+$* and *E. coli* HR40 *optA1 su$^+$* should yield all classes of mutants except amber mutants; T7 HS1, HS3, HS4, and HS7 were isolated by using these *su$^+$* strains. *E. coli* W3110 *opt$^+$ su$^-$* and *E. coli* HR44 *optA1 su$^-$* should yield all classes of mutants; T7 HS9 and HS10 were isolated by using these *su$^-$* strains.

Kinetics of DNA, RNA, and protein synthesis. *E. coli* HR44 *optA1* was grown in a gyratory shaker at 30°C in M9CAA medium to carry out radioactive labeling with thymidine or uridine. Labeling with radioactive amino acids was carried out in M9 medium supplemented with threonine, leucine, histidine, arginine, and lysine. At a density of 3×10^8 cells per ml, the bacteria were infected with various T7 phages at a multiplicity of 7. To measure the rate of DNA, RNA, or protein synthesis, 0.2-ml samples were removed from the culture and placed in tubes containing 10 μl of [^{3}H]thymidine (50 μCi/ml), [^{3}H]uridine (50 μCi/ml), or a mixture of ^{14}C-labeled amino acids (50 μCi/ml). After 90 s at 30°C, synthesis was terminated by the addition of 3 ml of cold 0.3 N trichloroacetic acid. The acid-insoluble material was collected on Whatman GF/C filters and washed five times with 3 ml of cold 1 N HCl followed by 3 ml of ethanol. The radioactivity was determined in a toluene-based solvent in a liquid scintillation counter.

Preparation of radioactively labeled T7 phages. An overnight culture of *E. coli* 011′ grown in M9CAA medium supplemented with 2 μg of thymine per ml was diluted 25-fold into M9CAA medium containing 2 μg of thymine and [^{3}H]thymidine per ml (4 μCi/ml). The cells were grown in a gyratory shaker at 30°C to a density of 5×10^8 cells per ml. The bacteria were infected at a multiplicity of 0.08 with T7 phage, and shaking was continued until complete lysis occurred. The radioactively labeled phages were purified by differential centrifugation and CsCl density-gradient centrifugation (14). The specific activity of the purified phages was about 10^5 cpm per 10^{10} phage.

T7 map. In this paper, we use the conventions of Studier et al. (25) for designating lengths of T7 DNA and positions in the T7 DNA molecule. DNA lengths are given in base pairs or T7 units, a T7 unit being equal to 1% of the total length of wild-type T7 DNA (approximately 400 base pairs). Positions in the T7 DNA molecule are given in T7 units beginning at the genetic left end.

RESULTS

Isolation of *E. coli optA1* mutant. Approximately 150 colonies that were not lysed by $T7_{LG37}$ (see above) were classified according to their sensitivities to wild-type T7 and a T7 ligase mutant, $T7_{1.3}A22$. About two-thirds of the *E. coli* mutants were able to support the growth of T7 wild-type phage but not that of $T7_{1.3}A22$ and, thus, most likely represented *E. coli* mutants having reduced levels of DNA ligase (7, 10). Most of the remaining mutants were resistant to both T7 phages. One isolate, however, designated HR40 *optA1*, was sensitive to both wild-type T7 and $T7_{1.3}A22$ phages. $T7_{LG37}$ failed to produce progeny phage in this mutant strain.

T7 gene 1.2 mutants cannot grow on the *optA1* mutant. The LG37 deletion removes the DNA between positions 14.55 and 19.3 on the T7 DNA molecule (25). This region contains the primary origin of T7 DNA replication (16, 26), the termination site for early RNA transcription (23), and genes 1.1, 1.2, and 1.3 (16, 25). We

initially identified which of these genes or regulatory sites in the deleted region was responsible for the ability of T7 phage to grow on the *optA1* mutant.

The availability of T7 mutants carrying deletions of various sizes in this region (25) provided a way to carry out preliminary mapping studies. Among the deletion mutants tested, LG12, LG30, LG16, D24, LG26, and D53 grew as well as wild-type T7 phage on *E. coli optA1*. However, LG3, LG13, D2, and D303, like LG37, could not grow on the *optA1* strain. The common region that was present in the former group of deletion mutants but lacking in the latter group was the nonoverlapping portion of the two deletions D303 and LG12 between positions 15.0 and 16.0 (Fig. 2). This segment contains genes 1.1 and 1.2 (16, 25).

To identify whether gene 1.1 or 1.2 was responsible for the phenotype, we isolated several T7 point mutants that could not grow on *optA1* mutants. The plating properties of six such T7 mutants, isolated from a stock of T7 phage mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (see above), are shown in Table 2. All six T7 mutants grew well on *E. coli opt*$^+$ strains and on *E. coli lig* mutants. The ability to plate on the *lig* mutants of *E. coli* indicated the presence of an active gene 1.3 protein (T7 ligase). Four of the T7 mutants (HS1, HS3, HS4, and HS7) did not plate on either *su*$^+$ or *su*$^-$ strains of *E. coli optA1*. The other two mutants (HS9 and HS10) grew normally at 25°C on *E. coli su*$^+$ *optA1*, but failed to plate on *E. coli su*$^-$ *optA1*. Furthermore, growth of these two mutants was temperature sensitive in the *su*$^+$ strains. As will be described below, however, these two mutations were not amber mutations.

A series of genetic crosses established that the six point mutations lay between positions 15.0 and 16.0 (Fig. 2). They represented at least four different loci within this narrow region, none of which could recombine with the deletion mutant D303. No detectable recombination was observed between HS3 and HS4 or between HS9 and HS10.

Concurrently with these studies, we determined the nucleotide sequence of the T7 DNA molecule between positions 14.45 and 16.15 (16). Within this region we identified gene 1.1 (position 14.95 to 15.26) and gene 1.2 (position 15.27 to 15.91). The precise locations of five of the point mutations were determined by sequencing the gene 1.1–1.2 region of the mutant DNAs (16). Three mutations (HS1, HS3, and HS7) lay within the coding sequence of gene 1.2 and affect the length of the gene 1.2 protein. In HS1, the initiation codon (ATG) was changed to the noninitiating ATA isoleucine codon. In HS3 and HS7, two TGG tryptophan codons were changed to chain-terminating TGA codons. The other two mutants, HS9 and HS10, whose DNA was sequenced, had an identical nucleotide change in the intercistronic region between gene 1.2 and gene 1.3 within the RNase III processing site (Fig. 1). These base changes were not the result of a secondary mutation since certain spontaneous revertants of HS9, able to grow on *E. coli optA1*, were found by DNA sequence analysis to be true revertants; other revertants have a secondary mutation within the same RNase III processing site. The mechanism by which the

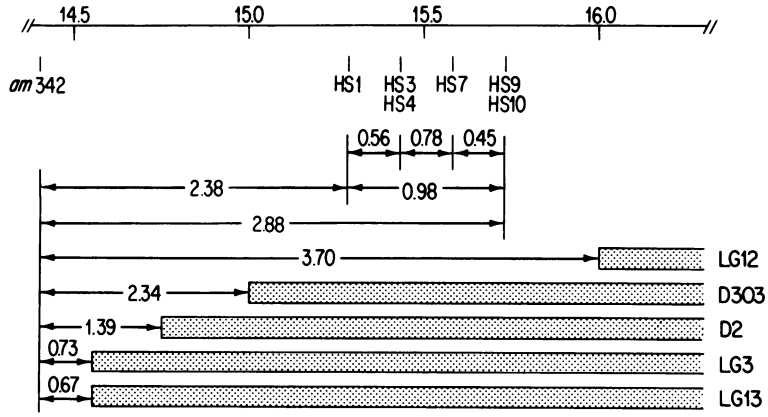


FIG. 2. *Mapping of gene 1.2 mutations. The top line indicates the physical position of the region of T7 DNA in T7 units. The positions of point mutations in gene 1.2 (HS1 through HS10) are shown below the line; am342 is the farthest-right mutation known in gene 1 (25). The boxes indicate the left ends of several deletion mutations in this region. Approximate recombination frequencies between several pairs of mutations are also shown. To order the six point mutations in gene 1.2, many genetic crosses were carried out, several of which are shown here.*

TABLE 2. *Plating properties of mutant T7 phages on E. coli optA1*

E. coli strain	Genotype	Temp (°C)	Plating efficiency of following phage[a]:								
			HS1	HS3	HS4	HS7	HS9	HS10	LG13	D303	LG12
011′	*su*$^+$ *opt*$^+$	25, 37	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PA610	*su*$^+$ *opt*$^+$	25, 37	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
W3110	*su*$^-$ *opt*$^+$	25	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.4
		37	0.6	0.6	0.6	0.9	0.6	0.6	1.0	0.4	0.4
HR40	*su*$^+$ *optA1*	25	10^{-6}	10^{-6}	10^{-5}	10^{-5}	0.4	0.4	10^{-7}	10^{-7}	1.0
		37	10^{-6}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-4}	10^{-7}	10^{-7}	0.9
HR44	*su*$^-$ *optA1*	25, 37	10^{-6}	10^{-6}	10^{-6}	10^{-5}	10^{-5}	10^{-5}	10^{-7}	10^{-7}	0.5
BL2	*su*$^-$ *lig*	25	0.6	0.7	0.6	0.5	0.7	0.6	10^{-5}	10^{-5}	10^{-5}

[a] The plating efficiencies on all strains are expressed relative to that obtained on *E. coli* 011′ at 37°C. HS1 through HS10 are point mutants in gene 1.2. LG13 and D303 are deletion mutants lacking genes 1.1, 1.2, and 1.3. LG12 is a deletion mutant lacking gene 1.3. Since the high concentration of phage in a plate caused partial clearing even under restrictive conditions, the values indicate the upper estimates of reversion frequencies.

HS9 and HS10 mutations affect the expression of gene 1.2 is not clear, but a likely possibility is that they alter the RNase III processing of the 1.1 mRNA, thus affecting the translation of gene 1.2

The relative genetic positions of the mutations HS1, HS3, HS7, HS9, and HS10 determined by phage crosses were identical to the base changes found from the sequencing studies. Therefore, we conclude that the gene 1.2 protein is essential for the growth of T7 in the *optA1* mutant.

Genetic mapping of *optA1*. The *optA1* mutation has been mapped by Hfr mating and by P1 transduction. Preliminary Hfr mating experiments fixed the position of the *optA1* mutation between 0 and 10 min on the *E. coli* genetic map (2). Then, *E. coli* HR40 *optA1 thr leu tonA lac purE str*^{r} was mated with *E. coli* Hfr KL208; KL208 transfers its DNA counterclockwise from 30 min (9). After 55 min at 37°C, mating was interrupted, and *purE*$^+$ *str*^{r} recombinants were selected and tested for each unselected genetic marker. As shown in Fig. 3, the frequency of transfer of each unselected marker decreased exponentially as the distance between the unselected marker and the selected marker (*purE*) increased (27). This experiment placed the location of *optA* very near *tonA* (Fig. 3).

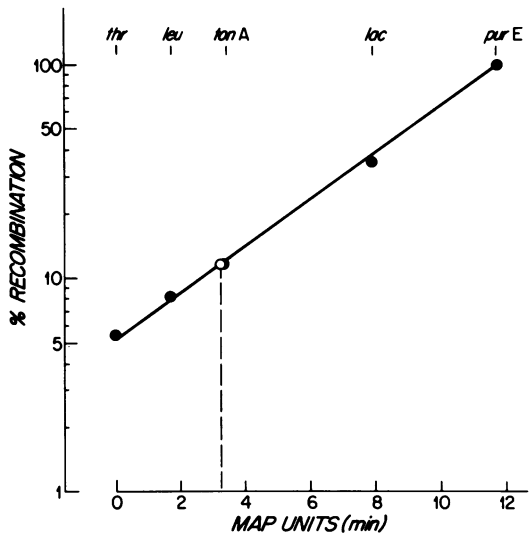


FIG. 3. *Hfr mapping of optA1 mutation. An Hfr strain, KL208, and F*$^-$ *strain, HR40, were mated, and purE*$^+$ *str*^{r} *recombinants were selected. After purification, they were tested for the unselected markers thr, leu, tonA, lac and optA. The frequencies of donor alleles (●) were plotted against the locations of the genes on E. coli genetic map on a semilogarithmic scale (27). The location of the optA1 mutation is indicated by an open circle. (○).*

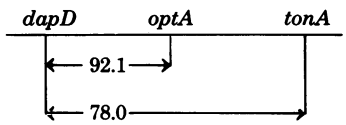
Since the lesion in strain HR40 appeared to lie near *tonA*, P1 transduction was carried out with *dapD*, a gene close to *tonA*, as a selective marker. Transduction was mediated by phage P1*vir*. The donor was HR40 *dapD*$^+$ *optA1 tonA*$^-$, and the recipient was HMS96-1 *dapD*$^-$ *optA*$^+$ *tonA*$^+$. Selection was for *dapD*$^+$, and transductants were scored for the unselected *optA* and *tonA* genotypes. As shown in Table 3, the *optA1* mutation lies close to *dapD* between the *dapD* and *tonA* genes. From the cotransduction frequency of 92.1%, the distance between *dapD* and *optA* is 0.05 min or approximately 2,000 base pairs (28).

DNA, RNA, and protein synthesis in T7-

TABLE 3. *Mapping of optA1 mutation by P1 transduction*[a]

dapD	*optA*	*tonA*	No. of recombinants (%)
1	1	1	184 (76.7)
1	1	0	37 (15.4)
1	0	1	3 (1.3)
1	0	0	16 (6.7)

[a] P1*vir* phage grown on the donor strain *E. coli* HR40 was used to infect *E. coli* HMS96-1. *dapD*$^+$ transductants were selected, purified, and scored for the *optA* and *tonA* genotypes by cross-streaking against T7 wild-type, $T7_{LG37}$, and T5 phages. The donor and recipient alleles are represented by 1 and 0, respectively, and their relative positions are as follows:



infected *optA1* mutant cells. In a wild-type T7 infection of *E. coli opt*$^+$ or *E. coli optA1* in M9 medium at 30°C, lysis began approximately 28 min after infection and was completed by 40 min; identical results were obtained when $T7_{1.2}$ mutants infected *E. coli opt*$^+$ cells. However, $T7_{1.2}$-infected *E. coli optA1* cells began lysis at approximately 33 min and did not complete lysis until about 60 min. Although lysis was complete at 60 min, the $T7_{1.2}$-infected *E. coli optA1* cells produced less than one progeny phage per infected cell.

In an attempt to define the biochemical lesion responsible for this inability of $T7_{1.2}$ mutants to grow in *E. coli optA1* cells, we examined DNA, RNA, and protein synthesis under a variety of conditions. In the experiments described here, *E. coli opt*$^+$ cells infected with either T7 wild-type phage or $T7_{1.2}$ mutants were quantitatively identical to *optA1* cells infected with wild-type T7 phage. Therefore, we will present only the results obtained from the infection of *E. coli optA1* with T7 wild-type or $T7_{1.2}$ phage. Since the gene 1.2 point mutants were somewhat leaky, we used deletion mutants LG12 and LG13 as gene 1.2$^+$ and 1.2$^-$, respectively. These two deletion mutants have almost identical right-hand endpoints within a T7 RNA polymerase promoter at positin 19.35 (16, 25). However, since these studies were carried out with T7 deletion mutants lacking both genes 1.1 and 1.2, it is possible that the observed defects reflect the combined absence of the products of genes 1.1 and 1.2.

The kinetics of DNA, RNA, and protein synthesis after infection of *E. coli optA1* cells with 1.2$^-$ phage were compared (Fig. 4) with those obtained with 1.2$^+$ phage under identical conditions. In both instances, the rate of DNA synthess increased rapidly at about 10 min after infection, presumably due to synthesis of T7 DNA (Fig. 4A). However, DNA synthesis in 1.2$^-$-infected *optA1* cells shut off prematurely at approximately 16 min after infection, a time at which the rate of DNA synthesis was maximal in 1.2$^+$-infected cells. The transient DNA synthesis in 1.2$^-$-infected *optA1* cells was dependent on both T7 gene 4 and gene 5 proteins since mutations in either gene abolished this phase of DNA synthesis (Fig. 4A).

Although less striking, RNA synthesis in 1.2$^-$-infected *optA1* cells also shut off earlier than in 1.2$^+$-infected cells (Fig. 4B). The kinetics of protein synthesis, on the other hand, appeared identical in 1.2$^-$-and 1.2$^+$-infected *optA1* cells.

Fate of newly synthesized DNA in $T7_{1.2}$-infected *optA1* cells. Newly synthesized DNA in *optA1*cells infected with 1.2$^+$ phage was stable over the entire period of infection as measured by the acid insolubility of DNA synthesized early in infection (Fig. 5); such was also the case for DNA synthesized in wild-type T7-infected *E. coli opt*$^+$ cells. In contrast, newly synthesized DNA in *optA1* cells infected with 1.2$^-$ phage was rapidly degraded to acid-soluble material; 80% of the DNA synthesized between 12 and 14 min after infection was rendered acid soluble by the time lysis took place. The degradation of newly synthesized DNA was partially inhibited by a mutation in gene 3 (endonuclease) and was completely abolished by a gene 6 mutation (exonuclease) (Fig. 5).

We also examined the fate of intracellular tritium-labeled parental T7 DNA injected by the infecting phage. As shown in Fig. 6B, the parental DNA in $T7_{1.2}$-infected *E. coli optA1* cells was stable until lysis. The introduction of gene 3 and gene 6 mutations was not required for stability.

The early cessation of DNA synthesis in $T7_{1.2}$-infected *E. coli optA1* cells shown in Fig. 4A could be suppressed if both the T7 endonuclease and exonuclease were eliminated. As shown in Fig. 7, the introduction of gene 3 and gene 6 mutations into the $T7_{1.2}$ phage effectively inhibited the early shutoff of DNA synthesis in *optA1*-infected cells. the introduction of a gene 3 mutation alone into $T7_{1.2}$ phage partially inhibited the early shutoff (data not shown). The evaluation of the effect of a gene 6 mutation alone is difficult to interpret since gene 6 mutations themselves lead to early shutoff of T7 DNA synthesis (20).

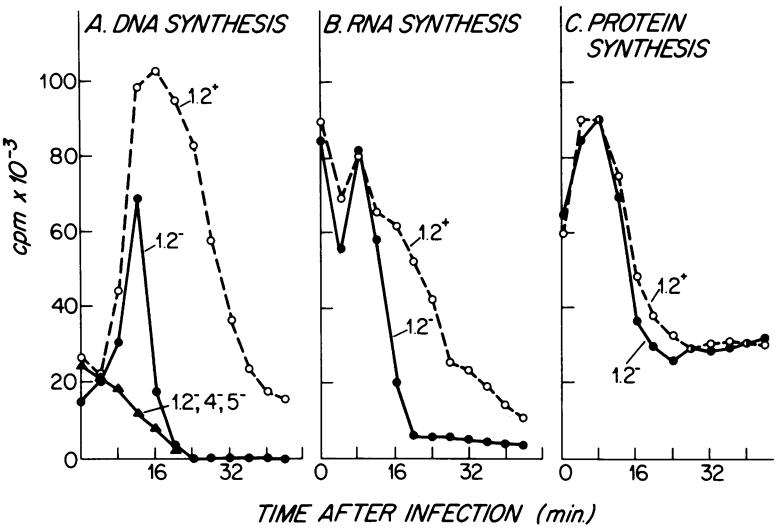


FIG. 4. *Time course of the rates of DNA, RNA, and protein synthesis in T7-infected optA1 cells. The rates of DNA (A), RNA (B), and protein (C) synthesis were measured (see the text) at intervals after infection of E. coli HR44 optA1 with* $T7_{LG(1.2^+)}$ (○) *or* $T7_{LG13(1.2^-)}$ (●). *Also shown in (A) are the results obtained after infection of E. coli HR44 with* $T7_{LG13(1.2^-),4}$ *and* $T7_{LG13(1.2^-),5}$ *both of which gave identical results* (▲).

DISCUSSION

Recent genetic studies by Studier et al. (25) have shown that the early region of the T7 genome contains at least nine genes: 0.3, 0.4, 0.5, 0.6, 0.7, 1, 1.1, 1.2, and 1.3. Of these early genes, only the product of gene 1, the T7 RNA polymerase, is essential for the growth of T7 phage. In addition to gene 1.2 described in this paper, the functions of three other nonessential early genes (genes 0.3, 0.7, and 1.3) have been characterized genetically and biochemically (7, 10, 13, 24). An important step toward analyzing these nonessential genes was the realization that their products might be essential in some mutant strains of *E. coli*. Thus, T7 DNA ligase, the product of gene 1.3, is essential in ligase-deficient *E. coli* strains (7, 10); protein kinase, the product of gene 0.7, is essential in some *E. coli* strains having an altered RNA polymerase (6, 22); the product of gene 0.3, an inhibitor of the host restriction system, is required for the growth of unmodified T7 on *E. coli* strains having a restriction system (24).

By analogy, the studies described here were based on the premise that the products of genes 1.1 and 1.2 might be essential for the growth of phage T7 in some mutants of *E. coli*. The *E. coli optA1* mutant was isolated by its inability to support the growth of a deletion mutant of T7 lacking genes 1.1 and 1.2. Since the available deletion mutants in this region all lacked both genes 1.1 and 1.2, it was necessary to isolate point mutants of T7 that could not grow on

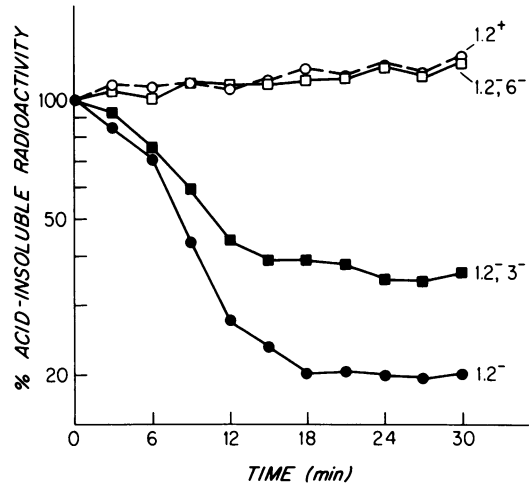


FIG. 5. *Stability of the newly synthesized DNA in optA1 cells infected with T7 phage. E. coli HR44 su⁻ optA1 was grown in M9CAA medium at 30°C. At a density of 3 × 10⁸ cells per ml, cells were separately infected with* $T7_{LG12(1.2^+)}$ (○), $T7_{LG13(1.2^-)}$ (●), $T7_{LG13(1.2^-),3}$ (■), *or* $T7_{LG13(1.2^-),6}$ (□) *at a multiplicity of 7. At 12 min after infection, 2.5 μCi of [³H]thymidine per ml was added; at 14 min after infection, 0.7 mg of thymidine per ml was added to stop the further incorporation of radioactive thymidine. At the indicated times after the addition of nonradioactive thymidine, 0.2-ml samples were withdrawn, and the acid-insoluble radioactivity was measured. The acid-insoluble radioactivities at 0 min were taken to be 100%. The actual values at 0 min were as follows:* $T7_{LG12}$, *135,000 cpm;* $T7_{LG13}$, *95,600 cpm;* $T7_{LG13.3}$, *92,800 cpm;* $T7_{LG13.6}$, *85,600 cpm.*

optA1 strains to identify the essential gene. Genetic mapping of the phage mutations, combined with nucleotide sequencing of the mutant phage genomes in this region (16), identified the mutated gene as gene 1.2. The *E. coli* mutation is designated *opt* for one point two. Since no 1.1^- 1.2^+ phage mutants are available, we do not know if the gene 1.1 protein is also essential for growth in the *optA1* strain. Furthermore, in interpreting the physiological studies it should be recalled that the T7 mutants used lacked both genes 1.1 and 1.2. Therefore, it is possible that the observed defects reflect the combined absence of the gene 1.1 and 1.2 gene products.

The *optA1* gene of *E. coli* was mapped on the basis of the inability of $T7_{1.2}$ mutants to grow on *E. coli optA1*. Both conjugational and transductional mappings established the location of the *optA* gene between the *dapD* and *tonA* genes.

What is the relationship between the T7 gene 1.2 product and the *optA1* gene product? One obvious possibility is that the products of the two genes are proteins that have similar or identical activities, either of which is sufficient for phage growth. Alternatively, the *optA1* protein could be activated either by a host protein or by the phage gene 1.2 protein. An *optA1* mutation altering the *optA* protein in such a way that it could no longer be activated by the host system would render the 1.2 protein essential.

If the gene 1.2 and *optA1* proteins play similar roles in T7 DNA replication, by what mechanism do they prevent the early discontinuation of DNA synthesis and the concomitant degradation of the newly synthesized DNA? They could, by an unknown mechanism, protect the newly synthesized DNA from degradation by phage and host nucleases. On the other hand, they could be directly involved in the replication process; the accumulation of aberrant replication structures in their absence might provide substrates for the nucleases. Only the purification and characterization of these two gene products will delineate their roles in T7 DNA metabolism and provide insight into the role of the *optA* protein in *E. coli*.

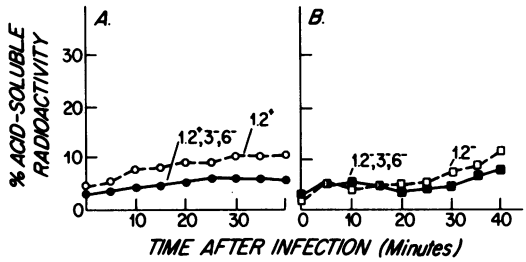


FIG. 6. *Stability of the infecting phage DNA in optA1 cells. E. coli HR44 su⁻ optA1 was grown in M9CAA medium at 30°C. At a density of 3 × 10^8 cells per ml, cells were infected separately with [^{3}H]thymidine-labeled $T7_{LG12(1.2^+)}$ (○) or $T7_{LG12(1.2^+),3,6}$ (●) (A), and $T7_{LG13(1.2^-)}$ (□) or $T7_{LG13(1.2^-),3,6}$ (■) (B) at a multiplicity of 7 and shaken at 30°C. At the indicated times, 0.5-ml samples were withdrawn, mixed with 0.5 ml of cold 0.6 N trichloroacetic acid, and kept on ice for 30 min. After centrifugation at 8,000 rpm for 10 min, the radioactivity in 0.5 ml of supernatant fluid was measured in a liquid scintillation counter. The acid-insoluble radioactivity at 0 min was taken to be 100%.*

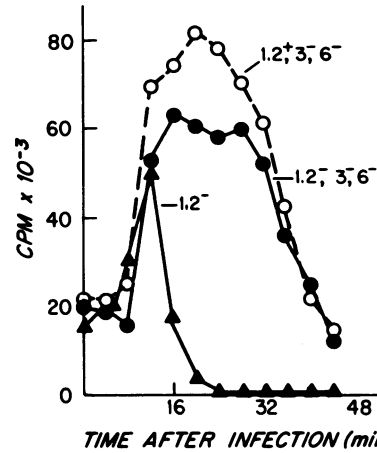


FIG. 7. *Effect of mutations in genes 3 and 6 on the time course of DNA synthesis in optA1 mutant cells infected with $T7_{1.2}$ mutants. E. coli HR44 su⁻ optA1 was infected with $T7_{LG12(1.2^+),3,6}$ (○), $T7_{LG13(1.2^-),3,6}$ (●), or $T7_{LG13(1.2^-)}$ (▲), and the rates of DNA synthesis were measured as described in the text.*

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