J. (1951), *J. Biol. Chem. 193,* 265.
Minkley, E., and Pribnow, D. (1973), *J. Mol. Biol. 77,* 255.
Siegel, R. B., and Summers, W. C. (1970), *J. Mol. Biol. 49,* 115.
Skare, J., Niles, E. G., and Summers, W. C. (1974), *Biochemistry 13,* 3912.
Staynov, D. Z., Pinder, J. C., and Gratzer, W. B. (1972), *Nature (London), New Biol. 235,* 108.
Studier, F. W. (1973), *J. Mol. Biol. 79,* 237.
Studier, F. W., and Maizel, J. V. (1969), *Virology 39,* 575.
Summers, W. C. (1969), *Virology 39,* 75.
Summers, W. C., Brunovskis, I., and Hyman, R. W. (1973), *J. Mol. Biol. 74,* 291.
Summers, W. C., and Siegel, R. B. (1970), *Nature (London) 228,* 1160.
Summers, W. C., and Szybalski, W. (1968), *Virology 34,* 9.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.
Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L., and Treiber, G. (1970), *Virology 40,* 734.
Yphantis, D. (1964), *Biochemistry 3,* 297.

Localization of the Leftmost Initiation Site for T7 Late Transcription, *in Vivo* and *in Vitro*†

James Skare, Edward G. Niles,* and William C. Summers

ABSTRACT: T7 RNA polymerase transcribes the late region of the T7 genome *in vitro* and *in vivo*. The strongest leftmost initiation site on the T7 DNA for late transcription has been localized by a multistep hybridization enrichment technique utilizing both *in vivo* and *in vitro* late T7 mRNA and T7 DNA with deletions from 2.9 to 7.0% and from 15.2 to 23.5% from the left end of the T7 DNA. These hybridization results are compared to electron microscopic heteroduplex analysis of hybrids constructed from T7 right-strand DNA and total late T7 *in vitro* RNA. Both methods yield the conclusion that the strongest leftmost initiation site for T7 RNA polymerase lies to the left of gene 1.3, at about 15% from the left end of the T7 DNA. In addition, from the heteroduplex analysis of the *in vitro* T7 late RNA and the T7 right-strand DNA, it is apparent that the total late region is transcribed *in vitro* by T7 RNA polymerase.

In vivo and *in vitro,* transcription of bacteriophage T7 can be divided into two classes. The early region, from 1 to 20% from the left end of the T7 DNA, is transcribed by host RNA polymerase (Siegel and Summers, 1970; Hyman, 1971). The transcript is processed by ribonuclease III into discrete monocistronic mRNAs (Dunn and Studier, 1973a,b; Nikolaev *et al.,* 1973). The late region of the T7 DNA is transcribed by T7 RNA polymerase, the product of T7 gene *1* (Summers and Siegel, 1970; Chamberlin *et al.,* 1970). *In vitro,* seven major T7 late RNA species are synthesized which vary in molecular weight from 0.27×10^6 to $3\text{-}5 \times 10^6$ daltons (Golomb and Chamberlin, 1974a; Niles *et al.,* 1974). Evidence exists that the largest T7 late RNAs may also be cleaved by ribonuclease III (Dunn and Studier, 1973a).

Analysis of *in vivo* T7 RNA and protein synthesis (Summers *et al.,* 1973; Studier, 1973) shows that genes 0.3, 0.7, 1, 1.1, and 1.3 (Figure 1) are early functions whose genes are transcribed by the *Escherichia coli* RNA polymerase. The remainder of the T7 genes are transcribed by the T7 RNA polymerase. However, gene 1.3 (DNA ligase) is unusual in that its protein continues to be made at times when the other early proteins are no longer made. Studier (1972) has suggested that gene 1.3 might be transcribed by both the *E. coli* and the T7 RNA polymerase. If this is true, the leftmost initiation site for late transcription is to the left of gene 1.3.

In order to identify the strongest leftmost initiation point for late transcription, multistep hybridization experiments have been carried out using T7 late RNA synthesized both *in vivo* and *in vitro,* and T7 DNAs which are deleted from 2.9 to 7.0% and from 15.2 to 23.5% from the left end of the T7 DNA. To corroborate these experiments, and to determine if the total late region of the T7 DNA is transcribed *in vitro* by T7 RNA polymerase, electron microscopic measurements of heteroduplex molecules between *in vitro* T7 late RNA and T7 right-strand DNA heteroduplex molecules have been made.

The results of the two techniques demonstrate that late transcription, catalyzed by T7 RNA polymerase, begins to the left of gene 1.3 at about 15% from the left end of the T7 DNA. Furthermore, the whole late region of the DNA is transcribed *in vitro.*

Materials and Methods

Strains. Growth and purification of T7 have been previously described (Yamamoto *et al.,* 1970). $T7^+$, T7 deletions H1, LG3, LG26, and LG37, a generous gift from F. W. Studier, have also been described (Summers *et al.,* 1973; Simon and Studier, 1973).

T7 RNA Polymerase. The isolation of T7 RNA polymerase has been described in detail by Niles *et al.* (1974). The procedure yields electrophoretically pure enzyme free from contaminating nuclease activity. During the purification, chromatography of the enzyme on phosphocellulose yields three activity peaks. The activity peak which elutes at about 0.38 M KCl, ac-

† From the Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received May 16, 1974.* E. G. N. is a recipient of a National Cancer Institute Postdoctoral Fellowship (CA 53115). W. C. S. is a Faculty Research Awardee of the American Cancer Society (PRA-97). J. S. is supported by a training grant from the U. S. Public Health Service (GM-711). The work was supported by a grant from the American Cancer Society (VC-60C).

counting for about 80% of the T7 RNA polymerase activity, was purified to homogeneity and used in these experiments.

The RNA synthesis mixture contained in 100 μl: 40 mM Tris-Cl (pH 8), 20 mM $MgCl_2$, 10 mM β-mercaptoethanol, 50 μg/ml of T7 DNA, 0.4 mM of each nucleoside triphosphate, and an appropriate amount of enzyme. [^{3}H]UTP (10 μCi/μmol) was employed in standard syntheses, and to assay T7 RNA polymerase activity, a 50-μl aliquot of the mixture was adsorbed to a Whatman No. 3MM filter disk, followed by four washes in 5% cold trichloroacetic acid. The dry filter disks were counted by liquid scintillation methods.

For large scale RNA synthesis, a 1-2-ml reaction mixture containing 10 μg/ml of rifampicin was used, and the product labeled either with [^{14}C]ATP (1 μCi/μmol) or [^{3}H]UTP (630 μCi/μmol). Enough enzyme was added to incorporate 50% of the triphosphate in 15 min at 37°C.

RNA Purification. In vivo late T7 RNA was made as follows. *E. coli* SY106 (20 ml), growing at 30° in K broth, was infected with T7 (m.o.i. = 10). Four minutes after infection, 4 mg of rifampicin was added. At 9 min after infection, 400 μCi of [^{3}H]uridine was added, and at 14 min after infection, the culture was poured onto crushed ice. RNA was extracted as previously described by Summers (1970).

RNA-DNA Hybridization. The methods of hybridization using denatured DNA bound to membrane fibers (S&S, B6) followed those of Bϕvre and Szybalski (1971). All routine hybridization employed filters to which 20 μg of heat-denatured DNA had been fixed. Under these hybridization conditions, the DNA was in excess.

Electron Microscopy; RNA Purification. For the heteroduplex analysis of the RNA-DNA hybrid, it was necessary to remove any double-stranded DNA from the *in vitro* T7 late RNA. The RNA was precipitated from the reaction mixture by the addition of three volumes of 95% ethanol and left overnight at −20°. The precipitate was collected by centrifugation and the pellet was dissolved in 2 × SSC (0.30 M NaCl-0.03 M sodium citrate). Cs_2SO_4 was added to the RNA solution to a concentration of 0.89 g/ml. ^{32}P-Labeled T7 DNA (a gift from R. A. Ludwig) was added to mark the position of double-stranded DNA, and the solution was centrifuged for 24 hr to equilibrium at 32,000 rpm, 5° in a Beckman angle 40 rotor. Fractions (0.1 ml) were collected and the position of the [^{14}C]ATP-labeled RNA and ^{32}P-labeled DNA was determined by counting 5-μl aliquots of each fraction. The RNA banded in Cs_2SO_4 at a density of 1.65 g/cm^3 and the DNA at 1.43 g/cm^3. The RNA fraction, free from contaminating double-stranded DNA, was dialyzed against 0.1 M Tris-Cl (pH 8)-0.15 M NaCl-1 mM EDTA, to remove the Cs_2SO_4 and then frozen in separate aliquots at −20°. The homogeneity of the RNA was tested by gel electrophoresis according to Niles *et al.* (1974). This purified T7 late RNA exhibited the expected six major bands with no evidence of degradation.

T7 DNA Strand Separation and Purification. The complementary strands of T7 DNA were separated by equilibrium density gradient sedimentation in the presence of poly(UG) as described by Summers and Szybalski (1968). The right-strand DNA region was pooled and judged to be about 10% contaminated with left-strand DNA. The pool was dialyzed against 10 mM Tris, 0.1 M NaOH, 0.15 mM NaCl, and 1 mM EDTA and sedimented in a 5-20% sucrose density gradient in the same solvent for 2 hr, 5° at 60,000 rpm in a SW 65.1 rotor. Fractions (0.1) were collected and the T7 right-strand DNA was localized by reading the absorption at 260 nm. This step serves to remove the poly(UG) and to select for intact right-strand DNA.

In order to eliminate left-strand DNA contamination of the T7 right-strand DNA, the DNA pool was dialyzed against 0.1 M Tris (pH 8)-0.15 M NaCl-1 mM EDTA followed by dialysis against 1M NaCl-0.2 M Tris-Cl (pH 8.5)-0.02 M EDTA-70% formamide (Hyman and Summers, 1972), for 16 hr at room temperature. During the latter dialysis, the left-strand DNA renatures with a portion of the right-strand DNA to form T7 DNA homoduplex molecules.

The remaining right-strand DNA was separated from the T7 homoduplex by passage of the mixture over a 4-ml column of hydroxylapatite. The column was equilibrated with 0.14 M sodium phosphate (pH 6.8) and the T7 DNA pool was dialyzed against the same buffer. An aliquot of ^{32}P-labeled T7 DNA was added to the dialyzed DNA and the sample was applied to the column. The right-strand DNA was eluted with 0.14 M sodium phosphate (pH 6.8). The duplex DNA was eluted with 1 M sodium phosphate (pH 6.8).

The DNA containing fractions were localized by the absorption at 260 nm and by scintillation counting of the ^{32}P-labeled T7 duplex DNA marker. Less than 10% of the duplex marker DNA was found in the right-strand single-strand DNA fractions. This results in about a 1-2% contamination of the right-strand DNA by T7 homoduplex. The right-strand DNA was dialyzed for 16 hr at 4° against 0.1 M Tris-Cl (pH 8)-0.15 M NaCl-1 mM EDTA, and stored at −20° in small aliquots.

RNA-DNA Hybrid Construction. The hybrid consisting of unfractionated T7 late RNA made *in vitro* with T7 RNA polymerase and T7 right-strand DNA was constructed according to the method of Hyman and Summers (1972). T7 late RNA was added to right-strand DNA in a sixfold mass excess. The sample was dialyzed against 1 M NaCl-0.2 M Tris-Cl (pH 8.5)-0.02 M EDTA-70% formamide, overnight at room temperature. The reaction was terminated by dialysis into 0.1 M Tris-Cl (pH 8.5)-1 mM EDTA and the hybrid was stored in aliquots at −20°.

Electron Microscopy. The basic protein film technique of Davis *et al.* (1971) was employed to visualize the single- and double-stranded regions of the duplex. Circular ϕX174 RF II and single-strand ϕX174 circular DNA molecules (a gift from Dr. G. N. Godson) were employed as length standards. Prior to spreading, the hybrid was treated with pancreatic ribonuclease (boiled for 3 min to remove deoxyribonuclease), 1 μg/ml, for 20 min at 0° to remove the nonhybridized RNA which otherwise forms large clumps and obscures the hybrids. The hybrid sample was diluted from 0.1 to 0.5 μg/ml in a 40% formamide solution and the sample was spread on a 10% formamide hypophase. The DNA containing film was picked up onto a parlodion coated grid, stained with uranyl acetate, and shadowed with 80% platinum-20% palladium. The hybrids were observed with an Hitachi 7S electron microscope. For data analysis, photographs of *selected* (see below) molecules were taken.

Analysis of Heteroduplex Molecules. Hybrid molecules were chosen which appeared large in relation to the ϕX174 DNA standards, and exhibited an apparent single-strand region somewhere along the DNA. About 50 molecules were photographed and the single- and double-stranded regions were traced using a Nikon Microcomparator. The lengths of the DNA were determined by comparison to the ϕX174 DNA standards. For a given molecule, single- and double-stranded lengths were added starting from one end of the hybrid. The lengths of the single-stranded DNA portions of the hybrids were not corrected to that of the double-stranded DNA because the ratio of the lengths of the RF II DNA to the single-stranded ϕX174 DNA was 0.98, and this deviation was less than the error for the technique. Molecules which did not mea-

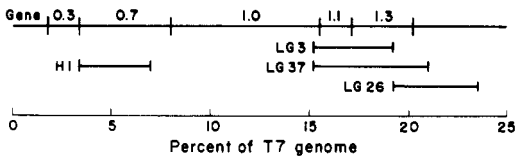

FIGURE 1: Genetic map of the early region of the T7 DNA. Distances are measured in per cent of genome length from the left end of the map. Gene numbers and their order are according to Simon and Studier (1973). Gene 1 is T7 RNA polymerase and gene 1.3 is DNA ligase. Positions of deletions H1, LG3, LG37, and LG26 are indicated.

sure at least 22 × 10^6 daltons were considered fragments and disregarded. Of the molecules measured, three appeared to be full sized T7 homoduplexes and probably represent contamination from the left-strand DNA. The remainder all had some single-strand region at one end.

For a comparison of the lengths of the single-strand regions, all molecules could be aligned with the single-stranded end to the left. Of the 39 molecules which appeared full length, only two had single-stranded portions at both ends.

To calculate the percentage of the 39 hybrid molecules which appeared single stranded over a given length, the number of molecules which were single stranded over each 1% length of the DNA was divided by the total number of molecules analyzed. The data have been grouped into units of DNA length which equal 4% of the length of total T7 DNA.

Results

The objective of this investigation was to find which regions of the T7 DNA were transcribed by the T7 RNA polymerase *in vitro* and *in vivo*. We focused on the early region for the hybridization enrichment experiments by using deletion mutants of T7. T7 H1 (2.9–7.0%), T7 LG3 (15.2–19.2%), T7 LG37 (15.2–21.0%), and T7 LG26 (19.1–23.5%) are represented in Figure 1. The numbers in parentheses represent the positions of the deletions measured from the left end of the DNA by DNA heteroduplex mapping with the electron microscope (Simon and Studier, 1973).

The hybridization enrichment procedure (Bϕvre and Szybalski, 1971) depletes *in vivo* or *in vitro* synthesized T7 late mRNA of all sequences except those complementary to the regions deleted in the T7 mutants. For example, if we wish to deplete the T7 late RNA of all sequences except those which could have been transcribed from 15.2 to 19.2%, we incubate the RNA solution with a nitrocellulose filter to which denatured DNA from T7 LG3 has been fixed. The RNA hybridizes to the filter with an efficiency of 50–80%. Since the RNA was transcribed from wild type T7, however, a portion of the RNA may have been transcribed from 15.2 to 19.2%. The RNA fragments from 15.2 to 19.2% would not hybridize to the LG3 DNA because the LG3 DNA lacks the sequences between 15.2 and 19.2%, and they would remain in solution after the other RNA species were bound to the filter. The result of such a prehybridization step is to partially purify the T7 late RNA that was transcribed from 15.2 to 19.2% on the wild type genome.

After four to six successive prehybridization steps, only a few per cent of the total radioactivity in the enriched RNA solution bound to the filter containing LG3 DNA. At this point, we determined whether or not the enriched T7 late RNA included sequences complementary to the region from 15.2 to 19.2% of wild type T7. To do this, we divided the enriched RNA solution in two and incubated half of it with a T7 LG3 DNA filter and the other half with a wild type T7 filter. The only difference between the LG3 DNA filter and the T7$^+$ DNA filter is the exclusion of sequences from 15.2 to 19.2% in the mutant. Therefore, if the wild type DNA filter binds significantly more of the enriched T7 late RNA than the deletion DNA filter does, then there was RNA which had been transcribed from 15.2 to 19.2% present in the total RNA sample before prehybridization enrichment.

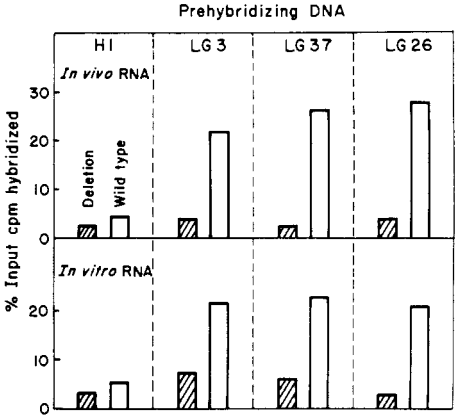

FIGURE 2: RNA–DNA hybridization of [^{3}H]RNA which had been prehybridized to T7 DNA carrying various deletions. Cross-hatched bars show per cent of input radioactivity which bound to filters containing the same mutant DNA with which prehydrization enrichment was performed. Empty bars show hybridization of the various enriched RNAs to wild type DNA. Control filters containing no DNA reproducibly bound 0.5% of the input radioactivity. Prehybridization to each of the four mutant DNAs was performed on RNA samples containing 10^6 initial counts per minute. The input radioactivities of the *in vitro* RNA samples after prehybridization to the following mutant DNAs were: 0.9 × 10^4 cpm for H1, 1.9 × 10^4 for LG3, 3.2 × 10^4 for LG37, and 6.1 × 10^4 for LG26. The input radioactivities of the *in vivo* RNA samples were: 2.3 × 10^4 for H1, 7.5× 10^4 for LG3, 7.8 × 10^4 for LG37, and 9.7 × 10^4 for LG26. The input counts per minute include nonhybridizable material.

Figure 2 presents the results of annealing prehybridized RNAs (*i.e.*, those complementary to the regions absent in the mutants) with wild type DNA filters and deletion DNA filters. Wild type DNA filters bound significantly more RNA in this last step for all enriched RNAs except those enriched with T7 H1 DNA. We interpret this to mean that there was no significant amount of RNA transcribed from 2.9 to 7.0% in the total late *in vitro* or *in vivo* T7 RNA samples, but RNA was present that was transcribed from 15.2 to 23.5%. In other words, an initiation site for late transcription lies to the right of 7.0% and to the left of, or within, the LG3 deletion (15.2–19.2%).

To ensure that the LG3 DNA was not contaminated with DNA having deletions to the right of 19%, *in vivo* RNA complementary to the LG3 deletion was hybridized to filters containing H1, LG3, LG26, and LG37 DNAs. The results showed that LG3 RNA hybridizes only to the region of the DNA deleted in LG3 and the overlapping LG37 deletion.

To ensure that the *in vivo* RNA labeled from 4 min after the addition of rifampicin to 200 μg/ml is essentially all late T7 RNA, the kinetics of synthesis of this RNA was compared with the synthesis of *E. coli* RNA and early T7 RNA, synthesized in the absence of late T7 RNA by infection with T7 *am* 342, which carries an *amber* mutation in gene *1* (T7 RNA polymerase). The incorporation directed by *E. coli* RNA polymerase from 4 min after rifampicin addition was less than 5% that directed by T7 RNA polymerase.

Heteroduplex Analysis. The lengths of the heteroduplex molecules were determined by a comparison with lengths of ϕX174 RF II DNA and single-stranded ϕX174 DNA present on the same grid. A histogram of the distribution of the lengths of the hybrids is presented in Figure 3. It can be seen that there

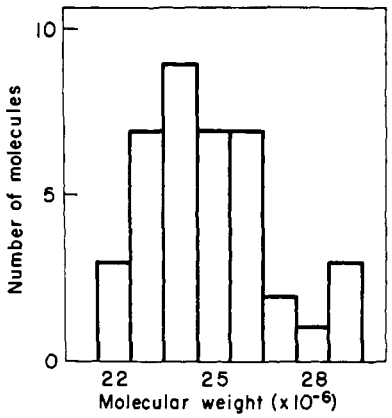

FIGURE 3: The lengths of the T7 *in vitro* late RNA-right-strand DNA heteroduplex molecules were determined by a comparison to the ϕX174 RF II DNA and single-stranded ϕX174 DNA. The single- and double-stranded lengths were summed from one end of the hybrid molecule. The mean molecular weight is calculated to be 25×10^6 daltons.

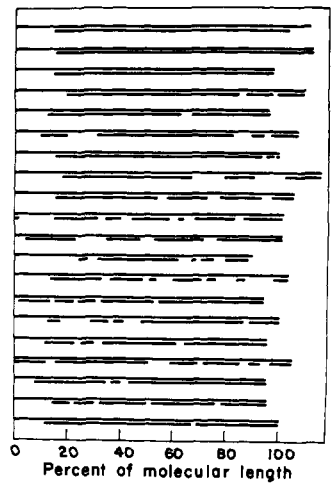

FIGURE 4: A line diagram is presented of 20 of the 39 molecules used in the heteroduplex analyses. Double-stranded regions are represented by a double line. The molecules with single-stranded ends are aligned with the single-stranded ends placed at the left. The distribution of the single-stranded regions of the other 19 molecules is the same as the molecules presented in this figure.

is a narrow distribution of lengths with a mean of 25×10^6 daltons molecular weight. This is in good agreement with the molecular weight of T7 DNA reported by Studier (1965) and Davis and Hyman (1971).

The results of Hyman (1971) and Siegel and Summers (1970) demonstrate that *in vivo* late T7 RNA is synthesized from the rightmost 80% of the T7 DNA. In addition, further evidence presented in this paper indicates that no detectable T7 late RNA is synthesized from the region of the H1 deletion, 2.9–7% from the left end of the T7 DNA. Therefore, the heteroduplex molecules with predominantly single-stranded ends were placed with the single-stranded end to the left. Figure 4 represents 20 molecules of the 39 molecules measured.

Quantitation of the lengths of the single-stranded ends of all 39 molecules is presented in Figure 5. In this figure, the per cent of the molecules which are single stranded over each 4% length of the DNA is plotted against the length of T7 DNA. It can readily be observed that from 0 to 12% from the left end of the DNA molecules, the hybrid is single stranded in 75–90% of the molecules measured. From 12 to 16% from the left end, 55% show single-stranded regions. To the right of 16%, the hybrid is rarely single stranded (between 10 and 15%). This residual single-stranded character over the range of 16–100% is probably the result of incomplete hybridization over random stretches of the DNA and the inherent difficulty in being able to unambiguously determine single- from double-stranded regions along the DNA. In Figure 4, it can be seen that the regions from 16 to 100% are mostly duplex and that the single-stranded regions usually account for DNA lengths of less than 10% of any single DNA molecule.

From Figures 4 and 5, two conclusions are readily apparent. First, the leftmost late RNA initiation site lies between 12 and 16% from the left end of the T7 DNA, and second, the total region of the DNA is transcribed *in vitro*. Both of these observations are in agreement with the hybridization competition data of Siegel and Summers (1970), and the hybridization enrichment experiments presented in this article.

Discussion

The results of these experiments show that at least one initiation site on T7 DNA for T7 RNA polymerase exists in the early region of the T7 genome (within the first 20% of the DNA molecule). This initiation site, designated PL1, is to the right of the H1 deletion (7%), and either within or to the left of the LG3 deletion (15.2%–19.2%). Since DNA ligase, but not T7 RNA polymerase, is made at late times (Studier, 1972), it is likely that PL1 is beyond the start of gene *1* (polymerase), but before the start of gene 1.3 (ligase). More precise localization of PL1 by the RNA-DNA hybridization enrichment methods described here requires deletions in the 7–15% region. Such deletions may not be viable either because essential portions of gene *1* (T7 polymerase) would be lost, or because deletion of PL1 might eliminate the production of essential late mRNA.

Analyses of the hybrid molecules formed between T7 late *in vitro* RNA and T7 right-strand DNA confirm the results obtained by the hybridization enrichment technique. Of the molecules analyzed, 75–90% of the RNA–DNA hybrid to the left of 12% is single stranded. Between 12 and 16% from the left end of the hybrid molecules is 55% single stranded (Figures 4 and 5). These data strongly indicate that the strongest leftmost initiation point for late transcription is located between 12 and 16% from the left end of the T7 DNA. This is within the early region, and to the left of the ligase gene.

In addition, the data presented in Figures 4 and 5 clearly indicate that the RNA products transcribed *in vitro* by T7 RNA polymerase cover the whole late region of the genome. Since the hybridization was done in sixfold mass excess of RNA to

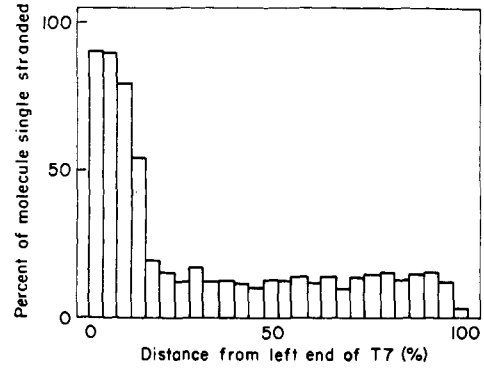

FIGURE 5: Distribution of single-stranded regions along the length of the T7 DNA. The percentage of molecules which are single stranded along a 1% length of the T7 DNA was determined by dividing the number of molecules which appear single stranded by the total number of molecules analyzed. The data are presented in arbitrary intervals of 4% of the length of the T7 DNA for convenience.

DNA, it does not imply that the six major RNA species observed by polyacrylamide gel electrophoresis (Golomb and Chamberlin, 1974a,b; Niles *et al.*, 1974) are unique transcripts covering the whole late region. Golomb and Chamberlin (1974a) have recently presented convincing evidence that at least four of the major transcripts initiate at different sites to the right of 50% of the DNA and terminate at the same point at 99% of the T7 DNA. It is possible that minor RNA species, not readily observed by the gel technique, may account for some of the hybrid regions. This possibility is being investigated by heteroduplex analysis of hybrids formed between T7 right-strand DNA and the isolated *in vitro* transcripts.

Previous studies have clearly shown that the ligase gene was transcribed by the *E. coli* RNA polymerase and was thus an early gene. R. Condit (manuscript in preparation) has used T7 RNA polymerase and DNAs from various T7 *amber* mutants in an *in vitro* protein synthesizing system to demonstrate that all of the T7 late proteins are synthesized. In addition, R. Condit and E. G. Niles (manuscript in preparation) have programmed an *in vitro* protein synthesizing system with T7 late RNA made *in vitro* from wild type and mutant DNAs. They have shown that the late transcripts can be translated to produce each of the T7 late proteins including ligase. The results of the present study show that the ligase region is also transcribed by the T7 RNA polymerase, both *in vivo* and *in vitro*. By this criterion, it is also a late gene.

It is perhaps curious that DNA ligase, which is dispensable in the presence of host cell ligase, is both an early and a late gene. However, protracted synthesis of ligase may be of benefit to the virus. T7 induces several nucleolytic activities, which may be antagonized by adequate ligase levels (Sadowski, 1974). In addition, ligase may be needed early in the initiation of replication and may function late in the formation of recombinant molecules, once replication is well under way. This would require an early burst of ligase and perhaps even more at late times.

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References

Bϕvre, K., and Szybalski, W. (1971), *Methods Enzymol. 21,* 350.

Chamberlin, M., McGrath, J., and Waskell, L. (1970), *Nature (London) 228,* 227.

Davis, R. W., and Hyman, R. W. (1971), *J. Mol. Biol. 62,* 287.

Davis, R. W., Simon, M. N., and Davidson, N. (1971), *Methods Enzymol. 21,* 413.

Dunn, J., and Studier, F. W. (1973a), *Proc. Nat. Acad. Sci. U. S. 70,* 1559.

Dunn, J., and Studier, F. W. (1973b), *Proc. Nat. Acad. Sci. U. S. 70,* 3296.

Golomb, M., and Chamberlin, M. J. (1974a), *Proc. Nat. Acad. Sci. U. S. 71,* 760.

Golomb, M., and Chamberlin, M. J. (1974b), *J. Biol. Chem. 249,* 2858.

Hyman, R. W. (1971), *J. Mol. Biol. 61,* 369.

Hyman, R. W., and Summers, W. C. (1972), *J. Mol. Biol. 71,* 573.

Nikolaev, N., Silengo, L., and Schlessinger, D. (1973), *J. Biol. Chem. 248,* 7967.

Niles, E. G., Conlon, S. W., and Summers, W. C. (1974), *Biochemistry 13,* 3904.

Sadowski, P. D. (1974), *J. Virol. 13,* 226.

Siegel, R. B., and Summers, W. C. (1970), *J. Mol. Biol. 49,* 115.

Simon, M. N., and Studier, F. W. (1973), *J. Mol. Biol. 79,* 249.

Studier, F. W. (1965), *J. Mol. Biol. 11,* 373.

Studier, F. W. (1972), *Science 176,* 367.

Studier, F. W. (1973), *J. Mol. Biol. 79,* 237.

Summers, W. C. (1970), *Anal. Biochem. 33,* 459.

Summers, W. C., Brunovskis, I., and Hyman, R. W. (1973), *J. Mol. Biol. 74,* 291.

Summers, W. C., and Siegel, R. B. (1970), *Nature (London) 228,* 1160.

Summers, W. C., and Szybalski, W. (1968), *Virology 34,* 9.

Yamamoto, K. R., Alberts, B. M., Benziger, R., Lawhorne, L., and Trieber, G. (1970), *Virology 40,* 734.