Proc. Nat. Acad. Sci. USA
Vol. 71, No. 2, pp. 329–333, February 1974

Size Distribution and Molecular Polarity of Newly Replicated DNA in *Escherichia coli*

(DNA biosynthesis/discontinuous replication/DNA·DNA hybridization//*polA*$^-$ mutant/nascent DNA)

JEAN-MICHEL LOUARN* AND ROBERT E. BIRD

(Département de Biologie Moléculaire, Université de Genève, 1211-Genève 4, Switzerland

Communicated by Richard B. Setlow, September 12, 1973

ABSTRACT **Newly synthesized DNA, in *E. coli* lysogenic for the phage λ, was labeled by short pulses of [^{3}H]-thymidine, isolated, and separated on the basis of size by alkaline sucrose density gradient centrifugation. The molecular polarity of this DNA was determined by hybridization with each of the separated strands of λ DNA. The results show that, in the 3′ to 5′ direction, replication proceeds by synthesis of short chains that are subsequently joined to long DNA. This is true for both a *polA*$^+$ and a *polA*$^-$ strain. (The *polA* locus codes for DNA polymerase I.) In the 5′ to 3′ direction, replication proceeds continuously, by addition of nucleotides to long DNA, for the *polA*$^+$ strain. In the *polA*$^-$ strain, however, replication in the 5′ to 3′ direction is also discontinuous, but the discontinuities are 1–40 times less frequent than in the other direction.**

In bacteria, at least some of the newly synthesized DNA appears as short chains (Okazaki fragments) that are subsequently joined to long molecules (1–3). Whether such discontinuous replication occurs on both strands or only on one is an open question. Evidence, recently reviewed by Klein and Bonhoeffer (4), has been introduced for both possibilities.

In order to resolve this question, in the case of *Escherichia coli* replication, we have studied the molecular polarity of newly synthesized DNA at a given point on the *E. coli* chromosome, the prophage lambda. The passive replication (5) of this prophage by the chromosomal machinery occurs in a known direction (6), the polarity of integration is known (7, 8) and the DNA strands can be easily separated (9). These properties have allowed us to study, by DNA·DNA hybridization, the size distribution and the molecular polarity of the DNA made during exposure to short pulses of tritiated thymidine.

EXPERIMENTAL DESIGN

Fig. 1 shows two possible models for the manner in which the new DNA strands are formed (1). In model *A*, (continuous–discontinuous replication) synthesis occurs continuously in the 5′ → 3′ direction by addition of nucleotides, to long DNA chains, at the 3′ end. Replication in the 3′ → 5′ direction occurs by synthesis of short chains (5′ → 3′, i.e., backward relative to the movement of the replication fork) that are subsequently joined to long DNA.

In model *B* (discontinuous replication) both strands are synthesized first as short chains then are joined to long DNA.

We have isolated the radioactive short fragments made during a short pulse of radioactive thymidine in a strain lysogenic for λ, and hybridized these fragments with the separated strands of the phage DNA. Model *B* predicts that radioactive fragments will hybridize to both strands of phage λ, whereas model *A* predicts that fragments will hybridize only to strand *l* of λ and long DNA will hybridize to strand *r*.

We have found that replication occurs according to model *A* in our *polA*$^+$ strain. In a *polA*$^-$ strain two size classes of fragments are formed: the smaller class, 10 S, corresponds to the fragments found in the *polA*$^+$ strains; the other class, 12–50 S, corresponds to the strand that is elongated continuously in *polA*$^+$ strains.

MATERIALS AND METHODS

Bacterial and Bacteriophage Strains. The *E. coli* K12 strain CB 0129 (F$^-$ Thy$^-$Leu$^-$ B$_1^-$) was used. Its replication pat-

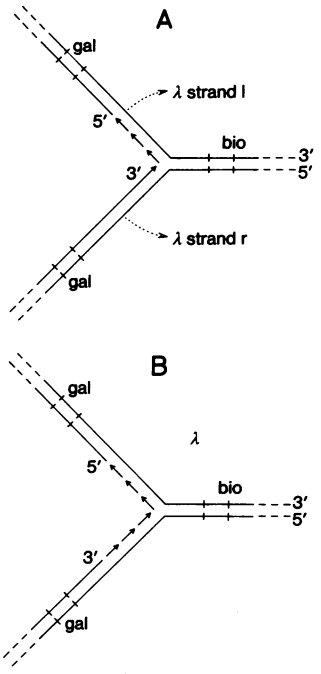

FIG. 1. Models for DNA replication. Two possible models for the mechanism by which replication forks pass the prophage λ on *E. coli* chromosomes.

Abbreviation: SSC, standard saline–citrate solution (0.15 M sodium chloride–0.015 M sodium citrate, pH 7); 6 × SSC means that the concentration of the solution used is 6 times that of the standard saline–citrate solution.

* Present address: Centre de Recherche de Biochimie et de Génétique cellulaires du CNRS, 118, route de Narbonne, 31-Toulouse, France.

TABLE 1. *Control hybridizations*

DNA on filters	Radioactive DNA (cpm bound per filter)				
	CB 0129 total label	LC 434 total label	λ DNA total label	LC 434 (*polA*$^+$) 30-sec pulse	LC 450 (*polA*$^-$) 30-sec pulse
Blank	50	157	660	45	66
Calf-thymus	312	489	1,490	159	229
λ strand-*r*	322	1,010	28,200	434	573
λ strand-*l*	358	1,220	34,600	627	837
Input per filter, cpm	36,700	80,000	99,600	105,300	104,300

The method used for extraction of DNA from fully labeled bacteria and bacteriophage has been described (6). The DNAs were sonicated, denatured, and hybridized to the indicated DNA on filters. The results are the average of duplicate or triplicate samples. All hybridization samples were counted to an error of 2% or less.

tern is now well known (6). LC 434 is CB 0129 rendered doubly lysogenic for λ and λimm434; it is *polA*$^+$. It has a low level of spontaneous induction (free phage/bacterium $\leq$ 1%). Strain LC 450 is P 3478 *polA1*$^-$ (10), made lysogenic for bacteriophage λ and λimm434. It is deficient in DNA polymerase I. The number of spontaneously released phages was somewhat higher (5–10 times) than in the *polA*$^+$ strain; however, the number of induced cells was probably never higher than 1% of the total cell number at any time. Furthermore, the quantity of λ-specific DNA labeled during a pulse was not increased in this strain.

Phage stocks were prepared from thermally induced bacteria lysogenic for λc_I857 S7.

Media and Reagents. Cultures for the isolation of newly replicated DNA were grown in M9 medium (11) supplemented with 0.5% casamino acids (Difco vitamin-free) and 4 μg/ml of thymine. L-broth (12) was used for phage stocks. Radioactive isotopes were purchased from New England Nuclear, poly(U,G) from Miles, and calf-thymus DNA from Worthington.

Separation of λ Strands. Phage stocks were purified by CsCl centrifugation, then dialyzed against 5 mM $MgSO_4$.

λ strands were separated by isopycnic centrifugation of denatured λ DNA treated with poly(U,G) (9). Fractions corresponding to the strands *r* or *l* were pooled, brought up to 6 × SSC, incubated 4 hr at 65° in order to reanneal contaminating strands, and loaded onto filters.

The purity of each strand was determined by hybridization to early RNA from λc_I857 x13 [transcript of the *l* strand only, (13)] and to late RNA from λc_I857 S7 [where strand *r* is transcribed 5- to 10-fold more than strand *l*, (14)]. By these criteria strand *r* preparations were judged to be pure; strand *l* preparations contained less than 10% of strand *r*.

Labeling and Isolation of Newly Replicated DNA. All pulse labeling was done at 20°. Cultures at 2 to 4 × 10^8 cells per ml were rapidly poured into a beaker containing tritiated thymidine (45 Ci/mmol) so that the final [^{3}H]dT concentration was 8 μCi/ml. The culture medium always contained thymine, 4 μg/ml. The incorporation was stopped in one of two ways: (*1*) the cells were poured onto crushed frozen medium at −50°, or (*2*) they were poured onto crushed frozen medium containing 10% (v/v) pyridine and 1 mM KCN (15). The second method gave about 2/3 the incorporated radioactivity obtained with the first for a 30-sec pulse. Therefore, pulses made with the first method are probably longer than indicated. The cultures were washed with either cold buffer or buffer plus pyridine, depending on the method employed to stop incorporation.

The cells were lysed in 0.1 N NaOH, 1 mM EDTA. The lysates were kept on ice for several hours, incubated in some cases for 30 min at 37°, and centrifuged 15 min at 6,000 × *g* to remove debris and unlysed cells. Sedimentation was performed in alkaline 5–20% sucrose gradients (0.2 N NaOH, 1 mM EDTA, 0.8 M NaCl) on top of a cushion of saturated CsCl in alkaline 20% sucrose.

Fractions were collected from the bottom of the tubes. Aliquots were precipitated with cold 5% trichloroacetic acid in the presence of 10 μg of calf-thymus DNA, then collected on glass-fiber filters (Whatman GF/C), and rinsed once with water and once with ethanol. The filters were then dried and the radioactivity measured in a Packard Tricarb liquid scintillation spectrometer (6).

The DNA from the gradient fractions was purified by equilibrium centrifugation in CsCl (40 hr at 40,000 rpm in a Spinco Ti 50 rotor) followed by dialysis against 4 × SSC.

DNA · DNA Hybridization. Native DNA was denatured in 0.15 N NaOH for 10 min on ice and neutralized with HCl. All DNA was loaded on membrane filters in 6 × SSC. These filters were dried, desiccated overnight, and heated at 80° for 2 hr. Before hybridization, filters were preincubated at 65° for 6 hr in 0.02% bovine-serum albumin, 0.02% Ficoll, and 0.02% polyvinylpyrrolidone in 6 × SSC. Hybridizations were made in 4 × SSC at 65° for 18–24 hr (16). Radioactive DNA was sonicated with a Branson Sonifier with a micro-tip for 30 sec at maximum output.

The method for extracting total cell DNA has been described (6).

RESULTS

The first experiments were designed to demonstrate that λ-specific DNA can be detected in DNA extracted from lysogens.

Controls. In a first experiment, radioactive DNA from the nonlysogenic strain, a lysogenic strain, phage lambda, and pulse-labeled lysogenic strains has been hybridized to blank filters, and to filters supporting calf-thymus DNA or separated strands *r* or *l* of lambda (Table 1).

DNA from the nonlysogenic strain, CB 0129, binds to the separated strands of λ and to heterologous DNA from calf thymus with approximately the same low efficiency; the binding to blank filters is considerably lower. The low level binding of DNA from nonlysogenic cells to the strands of lambda could be caused by partial homologies or by nonspecific associations that are not removed in the washing of the filters. Since the nonspecific binding to calf-thymus DNA

approximated that to strands of lambda, the amount of binding to calf-thymus DNA was taken as a measure of background in our experiments. More radioactive DNA from a lysogenic strain (Table 1, column 2) binds to strands of lambda than to either blank filters or filters supporting calf-thymus DNA. This difference is at least 2-fold. It should be noted that slightly more radioactivity binds to the strand-*l* than to strand-*r*. This difference, also found using purified lambda DNA (column 3), is probably due to contaminating strand-*r* in the strand-*l* preparations.

Pulse-labeled DNA from lysogenic strains (columns 4 and 5) binds significantly more radioactivity to the separated strands of lambda than to the calf-thymus control. This amount of radioactivity is sufficient for establishing the molecular polarity of newly replicated DNA.

These results indicate that DNA·DNA hybridization, using pulse-labeled DNA from strains lysogenic for λ, is sufficiently sensitive to allow the study of molecular polarity of newly synthesized DNA.

Size Distribution of Newly Replicated DNA. Fig. 2*A* shows the distribution, in an alkaline sucrose gradient, of radioactive DNA from the *polA*$^+$ strain pulse-labeled with [^{3}H]-thymidine for 30 sec at 20°. Approximately half of the radioactivity is at the top of the gradient in the short fragment peak of about 10 S. The remainder is on the CsCl shelf at the bottom of the tube. Polyoma DNA (^{14}C) was added to provide sedimentation markers (17). We shall call "short fragments" the material found between 20 S and the top of the tube, and "long DNA" the material with sedimentation coefficient greater than 53 S.

In a *polA*$^-$ strain the distribution is quite different from that in the *polA*$^+$ (Fig. 2*B*). The peak at approximately 10 S is still present but has now a broad shoulder, from 20 S to greater than 53 S. No radioactivity is found on the CsCl shelf at the bottom of the tube (long DNA).

Molecular Polarity of Newly Replicated DNA. Short fragments and long DNA from pulse-labeled cultures were isolated on alkaline sucrose gradients and hybridized with each of the isolated DNA strands of phage lambda. The data are presented in Table 2. All are corrected for background (amount of label binding to calf-thymus DNA filters).

For 6-, 15-, and 60-sec pulses, it was found that the labeled short fragments bind preferentially to λ strand-*l* and little or not all to strand-*r*. Long DNA shows a reverse specificity of binding to the two λ strands after a 6-sec pulse of label; for longer pulses it binds nearly equally to both strands.

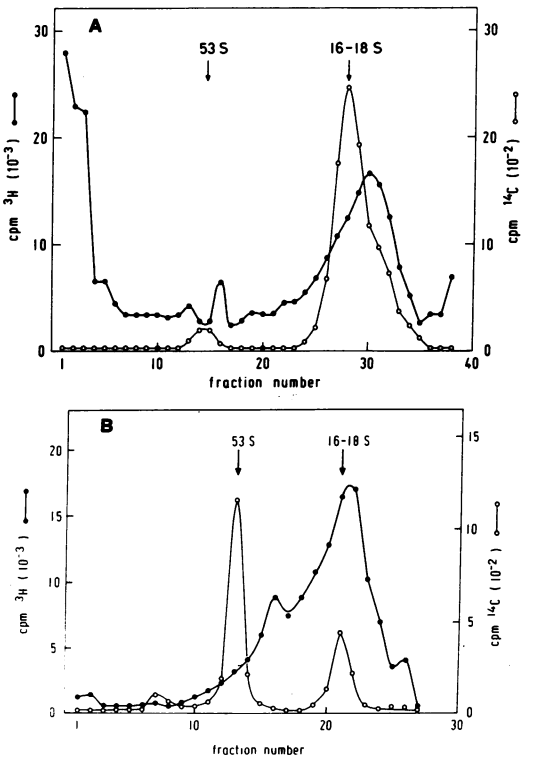

FIG. 2. Size distribution of DNA labeled in a short pulse. (*A*) The lysate from a 10-ml culture of LC 434 pulse-labeled for 30 sec with [^{3}H]dT was centrifuged for 16 hr at 22,000 rpm on an alkaline 5–20% sucrose gradient in the SB-110 rotor in an IEC B-60 ultracentrifuge. (*B*) A culture of LC 450 was pulsed for 30 sec with [^{3}H]dT. DNA was extracted and centrifuged on alkaline sucrose for 16 hr at 22,000 rpm in the SB 283 rotor. Polyoma [^{14}C]DNA, a generous gift of Dr. Thomas Seebeck, was mixed with the lysates prior to centrifugation. The covalently twisted circle of polyoma DNA has a sedimentation coefficient of 53 S, which corresponds to a molecular weight of 32×10^6 for a linear single-stranded DNA molecule. The nicked circles provide one linear single strand, 16 S, and one circular single strand, 18 S (17). ○ = polyoma [^{14}C]DNA; ● = *E. coli* [^{3}H]DNA. The numbers on the ordinates have been obtained by multiplying the experimentally derived values by the factors given in parentheses.

TABLE 2. *Strand specificity of the various classes of newly replicated DNA*

	Radioactive DNA (cpm bound per filter)				
	LC 434 *polA*$^+$ 6-sec pulse		LC 434 *polA*$^+$ 15-sec pulse		LC 434 *polA*$^+$ 60-sec pulse
DNA on filters	10S fragments	Long DNA	10S fragments	Long DNA	10S fragments
λ strand-*r**	0	33	28	33	24
λ strand-*l**	94	2	90	31	146
Calf-thymus	58	51	45	31	92
Input per filter, cpm	12,100	5,200	9,320	10,850	10,700

Short DNA fragments and long DNA (defined in the text) were purified as described in *Materials and Methods*. These fractions were sonicated and hybridized to the same type filters used in Table 1. The data have not been corrected for any bias due to contamination of strand-*l*.

* Corrected for background (DNA bound by calf-thymus DNA on the filter, cpm).

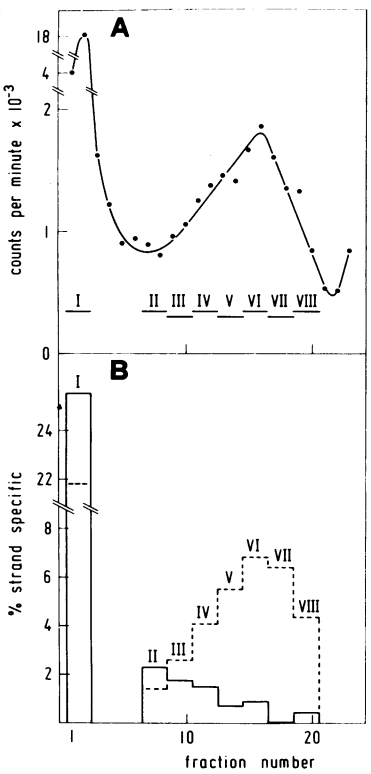

FIG. 3. Strand specificity of pulse-labeled DNA as a function of size in *polA*$^+$ cultures. A large culture (200 ml) of LC 434 was pulse-labeled for 30 sec with [^{3}H]dT, lysed, and centrifuged on two alkaline gradients (16 hr at 22,000 rpm in the SB110 rotor, in an Internaticnal B-60). Fractions were assayed for radioactivity and corresponding fractions from each gradient were pooled pairwise as indicated (*A*, I-VIII). The pooled fractions were purified by banding in CsCl. The DNA-containing fractions were pooled, sonicated, and hybridized against blank filters, calf-thymus DNA, and lambda strands. The percent strand specific DNA (panel *B*) was calculated by the equation: % strand specific = (cpm bound to one strand)/(cpm bound to both strands) × 100 × fraction of total radioactivity in each pair of fractions. Note that the radioactivity binding to strand-*l* was corrected for the bias found for purified λ DNA (see Table 1), and calf-thymus DNA was used as background. (– – –) = material hybridizing to strand-*l*; (——) = material hybridizing to strand-*r*.

To analyze the size and molecular polarity of newly replicated DNA in more detail, a 200-ml culture of the *pol A*$^+$ strain was pulsed with [^{3}H]dT for 30 sec, and the DNA was extracted and sedimented in alkaline sucrose. The distribution of radioactivity is shown in Fig. 3*A*. Fractions were pooled as indicated (I, II,..., VIII) and purified by isopycnic centrifugation in CsCl. Each pooled fraction was hybridized with filters containing calf-thymus DNA or the isolated strands of λ DNA. The profile of radioactivity hybridized with each strand is shown in Fig. 3*B*.

The bulk of the radioactivity hybridizing to λ strand-*r* is found in fraction I. Some radioactivity hybridizing to strand-*l* is found in all fractions but more of it is in fractions II–VIII. Only a small amount of radioactivity, in these fractions, hybridizes to strand-*r*, and practically none is found in the center of the short-fragment peak (fractions V–VIII).

A similar experiment for the *polA*$^-$ strain is given in Fig. 4. The distribution of radioactivity appears in Fig. 4*A* and the

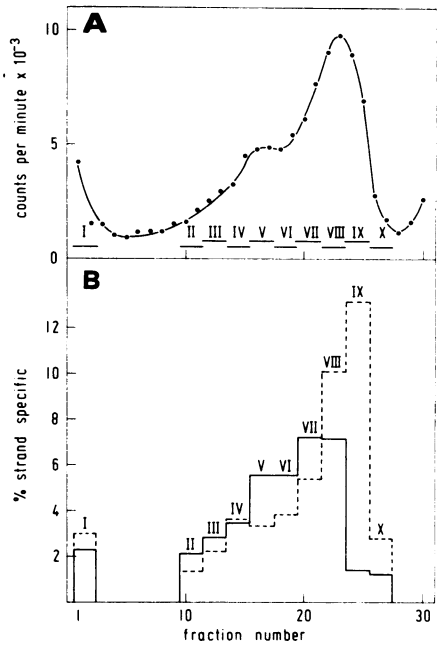

FIG. 4. Strand specificity of pulse-labeled DNA as a function of size in *polA*$^-$ cultures. The experiment in Fig. 3 was repeated for strain LC 450. The centrifugation was for 3 hr at 22,000 rpm followed by 10 hr at 26,000 rpm in the SB 110 rotor in an International B-60. (*A*) Radioactivity in the alkaline sucrose gradients. (*B*) Strand specificity: (– – –), strand-*l*; (——), strand-*r*.

distribution of strand specificity in Fig. 4*B*. The bulk of the radioactivity that hybridizes to strand-*r* is seen as a broad peak from approximately 20–55 S (fractions II–VIII). The strand-*l* specific DNA is found primarily in fractions VII–X (short fragments). Hence, there are two size classes of fragments in the *polA*$^-$ strain. The smaller fragments hybridize to λ strand-*l*, the longer fragments to λ strand-*r*.

DISCUSSION

Summary of Results. We have demonstrated that the two strains used in this study, one *polA*$^+$ and the other *polA*$^-$, have different size distributions of newly replicated DNA. In the *polA*$^+$, one strand appears to grow continuously in the 5′ to 3′ direction; whereas the other strand grows discontinuously in the 3′ to 5′ direction (Fig. 1*A*). In the *polA*$^-$ strain, both strands appear to grow discontinuously. The discontinuities on the strand in the 5′ to 3′ direction are 1–40 times less frequent than in the other direction. This fits the model shown in Fig. 1*B;* however, the fragments growing 5′ to 3′ are longer.

Other Results with polA$^+$ *Strains.* Okazaki and collaborators (1, 18) have concluded that DNA replication in *E. coli* is totally discontinuous (see Fig. 1*A*). The basis for their conclusions was that most of the radioactivity from a very short pulse of [^{3}H]dT was found in the short fragment peak (whereas our results would predict a maximum of 50%). The amount of incorporation for a pulse of 5 sec or less at 20° is very low; thus the determination of radioactivity in long DNA is difficult, as this radioactivity is spread over many fractions. We have always found at least 40% of the radioactivity in long DNA (greater than 15 S) for pulses of 5 sec (data not shown). This is in agreement with the results of others (19).

Other Results with polA⁻ Strains. Experiments with *polA*⁻ strains have indicated that newly synthesized fragments are joined more slowly than in the wild type (20, 21). This indicates a possible role for DNA polymerase I in the sealing of short DNA fragments.

The *in vitro* experiments of Olivera and Bonhoeffer (22) have shown two size classes of fragments from *polA*⁻ strains. It was further shown that these two classes were distinct; each class does not self-anneal but the two classes cross-anneal (23). Our results are in complete agreement with this result and give the molecular polarity of such fragments.

Strand Specificity of Short Fragments in Other Systems. Experiments made to test the strand specificity of fragments from bacteriophages λ and T4 of *E. coli* (18, 24) and SPP 1 of *Bacillus subtilis* (25) have shown that fragments bind to both of the isolated strands in each of these systems. However, λ (26) and possibly T4 (27) replicate bi-directionally. If fragments occur only on one template strand at each replication fork there would be fragments produced that would bind to either strand. Moreover bacteriophage P2 replicates only in one direction (28) and the newly synthesized DNA fragments hybridize to only one of the separated strands of P2 DNA (29).

Other Mutations Affecting Size of Pulse-Labeled DNA. It should be noted that other mutations, for example those in the gene for DNA ligase (*lig*), affect the profile of pulse-labeled DNA (15, 30). The molecular polarity as a function of size has not been measured in strains carrying such mutations.

Over-Replication of One Strand During a Short Pulse. We have consistently found more radioactivity incorporated into strand-*r*, that is into material hybridizing with the strand-*l* of λ, during short pulses (see Table 2 and Figs. 3 and 4). The amount of this over-replication varied from 20 to 80%.

The source of this over-replication is still not clear; however, if there are more growing chains on one strand this strand may be over-replicated by a momentary increase in the rate of chain elongation caused by the pulse of [³H]dT.

Conclusions. The simplest model to be derived from our results is that replication, in a wild-type (*polA*⁺) strain, is continuous in the 5′ to 3′ direction and discontinuous in the 3′ to 5′ direction. In the case of a deficiency in DNA polymerase I, (*polA*⁻), replication is discontinuous in both molecular directions. In the 3′ to 5′ direction the fragments in a *polA*⁻ are similar in size to those found in the wild-type strain, while in the 5′ to 3′ direction they are much longer. One possible explanation for the size difference could be that nicks are introduced in the DNA of the *polA*⁻ strain, on the 5′ to 3′ direction, in connection with the passage of the replication fork (since the 20–50S fragments are not produced with similar frequency in the total DNA). A more likely explanation is that initiation of a new DNA chain can occur in both directions, but much less frequently in the 5′ to 3′ direction than in the other. The appearance of the 20–50S fragments in the DNA newly synthesized by the *polA*⁻ strain would only be due to a sealing of nicks in long DNA slower than in the wild type.

Note Added in Proof. Rudolf Werner (personal communication) has found that, in thymine-requiring *E. coli*, short pulses of radioactive thymine or thymidine are incorporated exclusively into short polynucleotide chains (about 5 S) if the pulses are preceded by a short incubation in the absence of thymine to deplete the pools.

We wish to thank Dr. L. Caro for support and for critically reading the manuscript in its many forms. This work was supported by a grant from the Swiss National Foundation for Scientific Research to Dr. Caro. J.-M.L. was a Postdoctoral Fellow of the European Molecular Biology Organization.

1. Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K., Kainuma, R., Sugino, A. & Iwatsuki, N. T. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 129–143.
2. Sugino, A., Hirose, S. & Okazaki, R. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1863–1867.
3. Sugino, A. & Okazaki, R. (1972) *J. Mol. Biol.* **64**, 61–85.
4. Klein, A. & Bonhoeffer, F. (1972) *Annu. Rev. Biochem.* **41**, 301–332.
5. Eberle, H. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 467–474.
6. Bird, R. E., Louran, J., Martuscelli, J. & Caro, L. (1972) *J. Mol. Biol.* **70**, 549–566.
7. Campbell, A. (1959) *Virology* **9**, 293–305.
8. Wu, R. & Kaiser, A. D. (1967) *Proc. Nat. Acad. Sci. USA* **57**, 170–177.
9. Szybalski, W., Kubinski, H., Hradecna, Z. & Summers, W. C. (1971) in *Methods in Enzymology XXI*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), pp. 383–413.
10. de Lucia, P. & Cairns, J. (1969) *Nature* **224**, 1164–1166.
11. Adams, M. H. (1959) in *Bacteriophages* (Interscience Publishers, Inc., New York), p. 446.
12. Lennox, E. S. (1955) *Virology* **1**, 190–206.
13. Kourilsky, P., Bourguignon, M.-F., Bouquet, M. & Gros, F. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 305–314.
14. Lozeron, H. A. & Szybalski, W. (1969) *Virology* **39**, 373–388.
15. Jacobson, M. K. & Lark, K. G. (1973) *J. Mol. Biol.* **73**, 371–396.
16. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
17. Weil, R. & Vinograd, J. (1963) *Proc. Nat. Acad. Sci. USA* **50**, 731–738.
18. Sugimoto, K., Okazaki, T., Imae, Y. & Okazaki, R. (1969) *Proc. Nat. Acad. Sci. USA* **63**, 1343–1350.
19. Iyer, V. N. & Lark, K. G. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 629–636.
20. Kuempel, P. L. & Veomett, G. E. (1970) *Biochem. Biophys. Res. Commun.* **41**, 973–980.
21. Okazaki, R., Arisawa, M. & Sugino, A. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2954–2957.
22. Olivera, B. M. & Bonhoeffer, F. (1972) *Nature New Biol.* **240**, 233–235.
23. Herrman, R., Huf, J. & Bonhoeffer, F. (1972) *Nature New Biol.* **240**, 235–237.
24. Ginsberg, B. & Hurwitz, J. (1970) *J. Mol. Biol.* **52**, 265–280.
25. Polsinelli, M., Milanesi, G. & Ganesan, A. T. (1969) *Science* **166**, 243–245.
26. Schnös, M. & Inman, R. B. (1970) *J. Mol. Biol.* **51**, 61–73.
27. Delius, H., Howe, C. & Kozinski, A. W. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3049–3053.
28. Schnös, M. & Inman, R. B. (1971) *J. Mol. Biol.* **55**, 31–38.
29. Okazaki, R., Sugino, A. Hirose, S., Okazaki, T., Imae, Y., Kainuma-Kuroda, R., Ogawa, T., Arisawa, M. & Kurosawa, Y. *DNA Synthesis in vitro*, eds. Wells, R. D. & Inman, R. B. (University Park Press, Baltimore, Md.), in the press.
30. Gottesman, M. M., Hicks, M. L. & Gellert, M. (1973) *J. Mol. Biol.* **77**, 531–547.