MECHANISM OF DNA CHAIN GROWTH, I. POSSIBLE DISCONTINUITY AND UNUSUAL SECONDARY STRUCTURE OF NEWLY SYNTHESIZED CHAINS

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In vivo studies[1–7] of chromosome replication have led to the inference that both daughter strands of chromosomal DNA grow continuously, the direction of synthesis being 3′ to 5′ on one strand and 5′ to 3′ on the other (Fig. 1*A*). No enzymatic mechanism for the biosynthesis of deoxypolynucleotide in the 3′ to 5′ direction has been demonstrated, although 5′ to 3′ *in vitro* synthesis of DNA is accomplished by DNA polymerase.[8] If discontinuous synthesis of DNA could occur *in vivo* (Figs. 1*B–D*), short stretches could be synthesized by a reaction in the 5′ to 3′ direction and subsequently connected to the growing polynucleotide chain by formation of phosphodiester linkages.

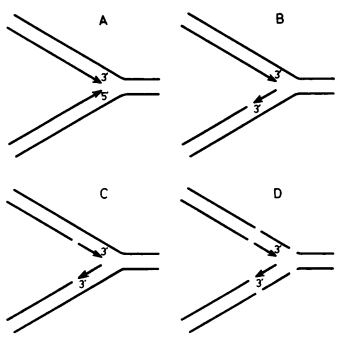

FIG. 1.—Models for the possible structure and reaction in the replicating region of DNA.

It is possible to distinguish between continuous and discontinuous chain growth by elucidating the structure of the most recently replicated portion of the chromosome; that is, that portion selectively labeled by an extremely short radioactive pulse. If the chromosome replicates discontinuously by one of the mechanisms shown in Figures 1*B*, *C*, or *D*, a large portion of the radioactive label would be found in unconnected short chains which can be isolated, after denaturation, from the large DNA chains derived from the other portion of the chromosome. No such difference in the molecular size between the pulse-labeled and bulk DNA would be expected from a mechanism of continuous synthesis (Fig. 1*A*).

Our results to be described here, together with those reported previously,[9] indicate that in a variety of bacterial systems and in one bacteriophage system most of the recently synthesized portion of the chromosome can be obtained after denaturation as small DNA molecules with a sedimentation coefficient of about 10*S*. This supports the prediction of those mechanisms by which two daughter strands are synthesized in a discontinuous fashion (Fig. 1*C* or *D*). It is also shown that the secondary structure of the chromosomal region containing these newly synthesized chains may differ from that of ordinary double-stranded DNA.

Materials and Methods.—Organisms used were as follows: *Escherichia coli* strains B, 15T⁻, W3110, and 1100 (endonuclease I-deficient strain provided by Dr. H. Hoffman-

Berling), *Bacillus subtilis* strain SB 19, and bacteriophages T4 (wild-type) and δA (provided by Dr. I. Watanabe).[10]

Reagents: The following commercial products were used: H^3-thymidine and C^{14}-thymidine (New England Nuclear); crystalline pancreatic DNase and RNase and egg-white lysozyme (Worthington); Pronase P (Kaken Chemical). *E. coli* exonuclease I was a gift of Dr. I. R. Lehman. *B. subtilis* nuclease was fraction I-A described previously.[11] Bacterial α-amylase was provided by Dr. F. Fukumoto. Hydroxylapatite was prepared according to Miyazawa and Thomas.[12] C^{14}-*E. coli* DNA used as standard substrate for DNase was prepared as described previously.[11] DNA from phage δA was obtained by phenol extraction.[10]

Culture media: Medium *A:* glucose salt medium containing 0.1 *M* potassium phosphate buffer, pH 7.3, 1 mM $MgSO_4$, 0.02 *M* $(NH_4)_2SO_4$, 0.002 mM $Fe(NH_4)(SO_4)_2$ and 1% glucose; medium *B:* medium *A* supplemented with 0.5% casamino acids, 0.01% cysteine and DL-tryptophan, and 1.2×10^{-5} *M* thymidine; medium *C:* M9 synthetic medium supplemented with 0.5% casamino acids. Media *A*, *B*, and *C* were used for experiments with *E. coli* B, *E. coli* $15T^-$, and T4 phage-infected *E. coli* B, respectively. Medium *B* containing no thymidine was used for *E. coli* W3110 and 1100.

Pulse labeling: To pulse label the bacteria with no thymine requirement or T4 phage-infected cells, H^3-thymidine (14 mc/μmole) was added to the stirred culture (5×10^8 cells/ml) at 20° (at 30° with *B. subtilis*) to a concentration of 10^{-7} *M*. After allowing the cells to incorporate H^3-thymidine for a desired time, the culture was poured onto crushed ice and KCN (to 0.02 *M*), and the cells were collected by centrifugation at 0°. To pulse label *E. coli* $15T^-$, cells grown in medium containing thymidine were precipitated and resuspended in a small volume of fresh medium at 0° containing no thymidine. The cell suspension was poured into a larger volume of stirred medium at 20°. H^3-thymidine (10^{-7} *M*) was added and the reaction was stopped by KCN and ice.

Extraction of DNA: (a) *Extraction of native DNA by the Thomas procedure*[13] (*Figs. 3, 4, 7, and 8; Table 1*): This was carried out as described previously[9] except that in some experiments sodium dodecyl sulfate (SDS) treatment was at 37° and the DNA solution was concentrated by filtration through a collodion membrane. DNA from 1 ml of culture was finally obtained in a volume of 0.5–1 ml. In *E. coli* B, recovery of DNA labeled by various lengths of pulse was greater than 90%. With *E. coli* $15T^-$, recovery varied from 30 to 60% but no systematic difference was found between the pulse- and uniformly labeled DNA's in parallel experiments.

(b) *Extraction of denatured DNA by NaOH-EDTA* (*Figs. 2, 5, and 6*): The cells were suspended in ice-cold 0.1 *N* NaOH containing 0.01 *M* ethylenediaminetetraacetic acid (EDTA) at a concentration of 5×10^9 cells/ml. The suspension was incubated at 37° for 20 min with occasional gentle stirring with a glass rod, and the insoluble material was removed by low-speed centrifugation. More than 80% of *E. coli* DNA and 50–80% of pulse-labeled DNA from T4 phage-infected cells were recovered by this procedure.

Denaturation of DNA: DNA extracted in the native state was denatured by incubation in 0.1 *N* NaOH containing 1 mM EDTA at room temperature for 20 min.

Zone sedimentation in sucrose gradients: (a) *Alkaline sucrose gradient:* Either the SW25.1 or SW25.3 rotor of a Spinco L or L2 centrifuge was used. With the SW25.1 rotor, 1 ml of DNA sample in 0.1 *N* NaOH containing 0.01 *M* EDTA was layered on a 29-ml 5–20% linear sucrose gradient containing 0.1 *N* NaOH, 0.9 *M* NaCl, and 1 m*M* EDTA. With the SW25.3 rotor, the volumes of the sample and gradient were 0.3 and 16 ml, respectively. Five mμmoles of DNA from bacteriophage δA was added to each sample as internal reference. After centrifugation, fractions were collected from the bottom of the tube. Radioactive DNA in each fraction was counted in a Tri-Carb liquid scintillation spectrometer after repeated precipitation with cold 5% trichloroacetic acid (TCA) and solubilization by 5% TCA at 90°. Distribution of δA DNA among fractions was determined by assaying aliquots for infectivity in *E. coli* protoplasts.[10] Distance of sedimentation was shown relative to the distance from the meniscus to the band of δA

DNA. Sedimentation coefficients were calculated from the value of 19*S* for this marker DNA, obtained by boundary sedimentation in 0.1 *N* NaOH-0.9 *M* NaCl.

(b) *Neutral sucrose gradient:* Centrifugation was carried out in the SW25.1 rotor, layering 1 ml of DNA sample over a 29-ml 5–20% sucrose gradient, pH 7.0, containing 0.15 *M* NaCl, 0.015 *M* sodium citrate, and 1 m*M* EDTA.

Recovery of DNA from alkaline and neutral sucrose gradients was more than 90%.

Other methods: Chromatography of DNA on hydroxylapatite was carried out according to Bernardi.[14] Recovery of DNA from the column was 60–65%. Formation of acid-soluble product by enzymatic degradation of labeled DNA was measured as described by Lehman.[15]

Results.—Nature of the replicating region as revealed by alkaline sucrose gradient sedimentation: To facilitate labeling of a small portion near the growing end of the daughter strands, all the pulse-labeling experiments with *E. coli* (normal or T4 phage-infected) were carried out at 20°. The rate of macromolecular synthesis at 20° is estimated to be about one sixth of the rate at 37°, since at 20° the generation time (and doubling time of DNA) of *E. coli* is about 3 hours and the lysis by T4 phage occurs about 140 minutes after infection.

In the experiment presented in Figure 2, growing cells of *E. coli* B were exposed

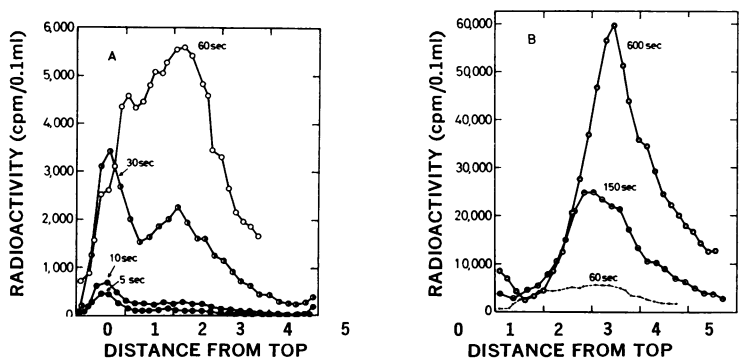

FIG. 2.—Alkaline sucrose gradient sedimentation of pulse-labeled DNA from *E. coli* B. Cells were grown at 37° to a titer of 3 × 10^8 cells/ml and then at 20° to 5 × 10^8 cells/ml. and the 10-ml culture was pulse-labeled with 10^{-7} *M* H^3-thymidine at 20° for the indicated time. DNA was extracted by NaOH–EDTA treatment and sedimented in the SW25.3 rotor for 10 hr at 22,500 rpm and 4°. Distance from top is relative to that of infective DNA from phage δA (19*S*, reference).

to H^3-thymidine for various times. DNA was extracted in the denatured state by the NaOH–EDTA treatment and sedimented in alkaline sucrose gradients. Infectious DNA from phage δA used as internal reference had a sedimentation coefficient of 19*S* in 0.1 *N* NaOH–0.9 *M* NaCl. Most of the radioactivity incorporated into DNA during the five-second pulse was recovered in a distinct component with an average sedimentation rate of 11*S*. Some radioactivity was found in material sedimenting at faster rates. Increasing the pulse time to 10 or 30 seconds increased the radioactivity in the "11*S* component" as well as the radioactivity in the fast-sedimenting DNA. Further increasing the pulse time resulted in large increases of the radioactivity in the fast-sedimenting DNA with little or no increase in the radioactive "11*S* component." The presence of the

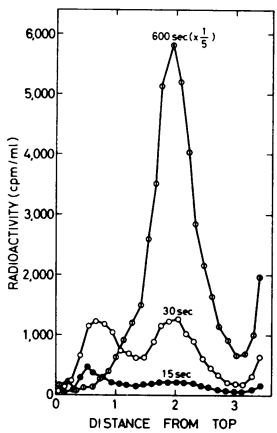

FIG. 3.—Alkaline sucrose gradient sedimentation of pulse labeled DNA from *E. coli* B. A 25-ml culture was pulse-labeled as in Fig. 2. DNA was extracted by the Thomas method. An aliquot was denatured in alkali and sedimented in the SW25.1 rotor for 15 hr at 20,500 rpm and 8°.

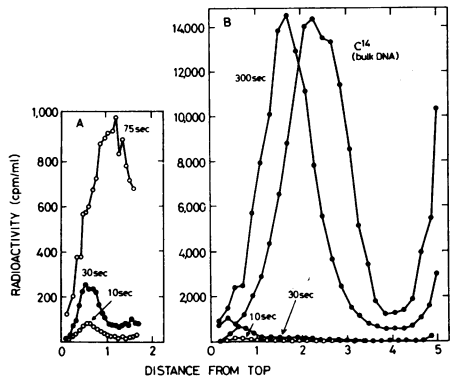

FIG. 4.—Alkaline sucrose gradient sedimentation of pulse-labeled DNA from *E. coli* 15T⁻. A 5-ml culture was pulse-labeled at 20° for the indicated period. To the 300-sec sample, a small amount of culture uniformly labeled by C^{14}-thymidine was added before DNA extraction by the Thomas method. Sedimentation was carried out in the SW25.1 rotor at 8° and 20,500 rpm for (*A*) 25 hr or (*B*) 10 hr.

latter was obscure after the 150- or 600-second labeling because of the possible trailing of the high molecular DNA containing a large amount of radioactivity. The average sedimentation rate of the fast-sedimenting component increased gradually and was about 50*S* after the ten-minute pulse.

Essentially the same result was obtained by using the Thomas method for DNA extraction (Fig. 3).

Similar results were also obtained with other *E. coli* strains, i.e., *E. coli* 15T⁻, W3110, and 1100 (endonuclease I-deficient) (Figs. 4 and 5), and *B. subtilis* strain SB 19. The initial label of H^3-thymidine always appeared in the DNA component with an average sedimentation rate of 10–11*S*.

That the "11*S* component" is really DNA was substantiated by several facts. It is degraded by the action of pancreatic DNase or by *E. coli* exonuclease I at the same rate as the denatured *E. coli* DNA routinely used as standard DNase substrate.[11] It is also completely degraded by *B. subtilis* nuclease[11] but not by alkali, pancreatic RNase, or bacterial α-amylase.

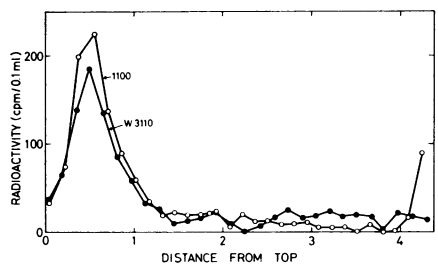

FIG. 5.—Alkaline sucrose gradient sedimentation of a 10-sec pulse DNA of *E. coli* W3110 and 1100. Experiments were carried out as in Fig. 2.

Figure 6 shows a result obtained with T4 phage-infected *E. coli* B. Cells were pulse-labeled after 70 minutes of infection at 20°, when phage DNA is being synthesized actively. After a two-second pulse the radioactive label incorporated

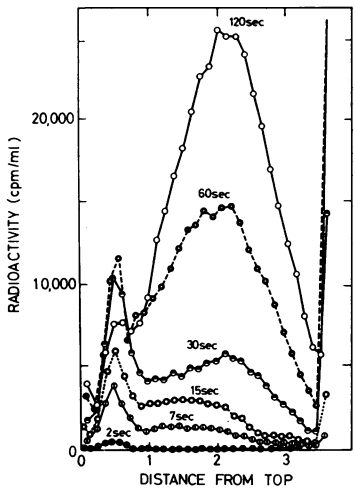

FIG. 6.—Alkaline sucrose gradient sedimentation of pulse-labeled DNA from T4 phage-infected *E. coli* B. Cells grown at 37° to 5 × 10^8 cells/ml were suspended in M9 medium containing no glucose at 10^9 cells/ml and incubated for 15 min at 37°. Following addition of DL-tryptophan (40 μg/ml), the cells were infected with T4 phage (MOI = 10). After 5 min at 37°, the culture was cooled to 20° and an equal volume of M9 medium containing twice as much glucose and casamino acids as medium *C* was added. After incubation with stirring at 20° for 70 min, the 10-ml culture was pulse-labeled with H^3-thymidine for the indicated time. DNA was extracted by NaOH–EDTA treatment and sedimented in the SW25.1 rotor for 15 hr at 20,500 rpm and 8°.

was recovered almost exclusively in DNA component with a sedimentation coefficient of 9*S*. After a longer period of labeling, the radioactivity was found also in faster-sedimenting material. The radioactivity in the "9*S* component" increased quickly and reached a maximum in about 30 seconds, whereas the radioactivity in the fast-sedimenting component increased almost linearly and in two minutes attained a level ten times higher than the radioactivity in the "9*S* component." The sedimentation rate of the fast component increased gradually as in growing bacterial cells. The average rate was about 40*S* after the two-minute pulse. In other experiments average rates of 45 and 50*S* were obtained for five- and ten-minute pulse DNA, respectively.

In these experiments the pulse labeling was stopped by KCN and ice, cells were precipitated, and denatured DNA was obtained by either (*a*) extraction by the Thomas method followed by alkali denaturation, or (*b*) extraction with NaOH-EDTA. The following changes in these procedures did not alter the essential feature of the results: (1) omission of the phenol step from (*a*), (2) addition of a pretreatment with lysozyme to (*b*), (3) directly adding NaOH-EDTA to the culture with or without prior addition of KCN and ice in (*b*), (4) denaturation with formamide in (*a*), and (5) extraction by the method of Nomura *et al.*[16] followed by alkali denaturation.

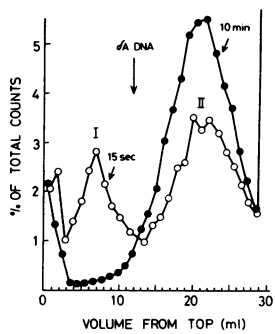

FIG. 7.—Neutral sucrose gradient sedimentation of pulse-labeled DNA from *E. coli* B. Native DNA samples of Fig. 3 were sedimented for 15 hr at 20,500 rpm and 8°.

Secondary structure of the replicating region: Pulse-labeled DNA, isolated by the Thomas procedure but not subjected to denaturation, was analyzed by sedimentation in neutral sucrose gradients. A result obtained with *E. coli* B is shown in Figure 7. While most of the DNA isolated from the cells labeled with H^3-thymidine for ten minutes sedimented at a rate

faster than δA DNA, having a sedimentation coefficient of 29*S* in 0.5 *M* NaCl, pH 7.0, a considerable fraction of 15-second pulse DNA was recovered in a band sedimenting at a much slower rate. It was shown in other experiments that the fraction of the radioactivity found in the slowly sedimenting band decreases with increasing pulse time.

On the other hand, a large fraction of the DNA labeled by a short pulse was found to be susceptible to degradation by *E. coli* exonuclease I, which specifically hydrolyzes single-stranded DNA[17] (Table 1). Approximately the same fraction of the labeled DNA was eluted from hydroxylapatite at the relatively low phosphate concentration expected for single-stranded DNA and was found to be completely susceptible to the action of exonuclease I (Fig. 8 and Table 1). The susceptibility of unfractionated pulse DNA to exonuclease I and the fraction eluted from hydroxylapatite at low phosphate concentrations decrease with

TABLE 1. *Susceptibility of pulse-labeled DNA to E. coli exonuclease I prior to denaturation treatment.*

	Extent of Degradation by Exonuclease I (%)				
				Neutral Sucrose	
Pulse time	Unfractionated	Hydroxylapatite Fraction I	Hydroxylapatite Fraction II	Gradient Fraction I	Gradient Fraction II
5 Sec	45				
10 Sec	32*			77*	24*
15 Sec	30	96	24		
30 Sec	24, 24*	96, 88*	18, 15*		
10 Min	4, 0*	78	2		

E. coli B was pulse-labeled as in Fig. 2. Extraction and fractionation of labeled DNA were carried out as in Figs. 3, 7, and 8. SDS treatment for DNA extraction was 37°* or at 60°. Hydroxylapatite fractions I and II are shown in Fig. 8, and neutral sucrose gradient fractions I and II in Fig. 7.

For susceptibility to exonuclease, the 150-μliter reaction mixture, containing 10 μmoles glycine–KOH buffer, pH 9.2, 1 μmole $MgCl_2$, 0.15 μmole 2-mercaptoethanol, 60-μliter DNA sample (300–12,000 cpm), and 3 units of *E. coli* exonuclease I (DEAE-cellulose fraction), was incubated at 37°. After 60 min, 3 units of enzyme were added to the mixture and the incubation was continued for another 60 min. Acid-soluble and insoluble counts were determined at 0, 60, and 120 min. More than 85% of the radioactive DNA degraded during the 120-min period was already acid soluble at 60 min.

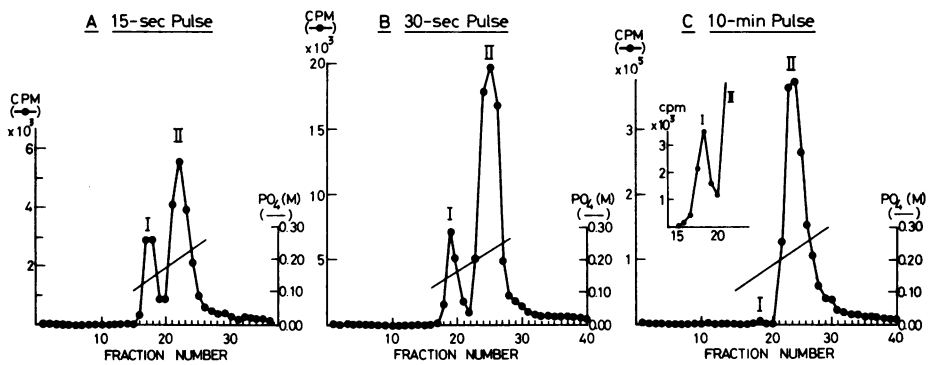

FIG. 8.—Hydroxylapatite chromatography of pulse-labeled DNA of *E. coli* B. The native DNA samples of Fig. 3 were dialyzed against 0.01 *M* potassium phosphate buffer, pH 6.8. Elution was achieved with a linear 0.01–0.7 *M* gradient of the same buffer (total vol 140 ml). Fractions of 2.5 ml were collected at 30-min intervals.

FIG. 9.—Schematic illustration of a possible structure of the daughter strand in the vicinity of the growing end.

increasing pulse time (Table 1 and Fig. 8). Furthermore, the slowly sedimenting component of pulse DNA recovered from the neutral sucrose gradient was shown to be highly susceptible to exonuclease I, while the fast-sedimenting component had a low susceptibility to the enzyme (Table 1).

Thus an appreciable fraction of the newly synthesized material as isolated appears to be single-stranded, and this fraction is sedimented slowly in the neutral sucrose gradient.

Discussion.—Average chain growth rate of *E. coli* chromosome is estimated to be about 400 nucleotides per second at 20°. Therefore, a 5 second pulse would label the stretches of about 2000 nucleotides or a 0.05 per cent portion of the whole chromosome. Our experiments show that the portion of the chromosome, labeled by such a short radioactive pulse is separable in alkali from the bulk of chromosomal DNA as small molecules. Observations described in this and a previous paper[9] indicate that this represents an intermediary state in the formation of chromosomal DNA. This result conforms to the prediction from the replication mechanisms by which two daughter strands are synthesized discontinuously (Figs. 1*C* and *D*). The replication mechanism by which only one of the two daughter strands is synthesized discontinuously (Fig. 1*B*) is less likely, because virtually all the label is recovered in the slowly sedimenting component after the very short pulse. The sedimentation coefficient of the initially labeled material is 10–11*S* in various bacterial systems and 8–9*S* in the T4 phage system, suggesting that the length of the "unit" may be 1,000–2,000 nucleotides. This corresponds to the dimension of cistron.

Figure 9 illustrates a possible structure of the daughter strands in the vicinity of the growing end. "Units" synthesized at the growing point would be joined together by phosphodiester bonds to form longer strands located in the nonterminal position. The number of "units" and of chains with intermediate lengths would be determined by the relative rates of synthesis and of joining.

An alternative interpretation of our results is that artificial breaks may be introduced selectively in the newly replicated region during DNA extraction. This possibility, which in any case suggests selective weakness in the newly replicated region, is diminished by the fact that similar results are obtained using different methods in a number of different systems (including an endonuclease I-deficient *E. coli* strain).

Our results on native DNA do not distinguish clearly between the two mechanisms for discontinuous chain growth shown in Figures 1*C* and *D*. Although a fraction of the pulse-labeled DNA sediments at a much slower rate than the bulk of DNA in the neutral sucrose gradient, this material proved to be single-stranded. The remaining portion, which is in a duplex form, is not separated from the bulk DNA by sedimentation. The fact that an appreciable fraction of the pulse DNA is isolated in the single-stranded form would imply either that most of the newly formed "units" exist as single strands in the cell or that the secondary structure of the replicating region containing these "units" is abnormally un-

stable. It may represent a unique state during replication or might indicate functioning of the newly synthesized "units" or the complementary portions of the parental strands as templates for RNA synthesis.

Our hypothesis of discontinuous DNA chain growth is encouraged by the discovery of polynucleotide-joining enzyme (ligase) in normal and T4 phage-infected *E. coli*.[18-21] The enzyme is encoded in one of the T4 genes previously implicated as a structural gene controlling DNA synthesis.[22] It has been used in *in vitro* synthesis of biologically active circular DNA in conjunction with DNA polymerase.[23-24] The synthesis and joining of the "units" assumed in our hypothesis may be carried out by DNA polymerase and polynucleotide ligase, respectively. A similar idea has recently been suggested by Kornberg and co-workers.[24, 25] Further support for such hypotheses will await proof of the following: (1) the "units" are synthesized in the cell only by a reaction in the 5′ to 3′ direction; (2) the "units" are joined in the cell by the ligase reaction.

Note added in proof: Recent studies indicate that cells infected with temperature-sensitive mutants of T4 phage defective in ligase accumulate a large amount of the newly synthesized short DNA chains at 42°.

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[1] Cairns, J., *J. Mol. Biol.*, **6**, 208 (1963).

[2] Cairns, J., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 43.

[3] Nagata, T., these PROCEEDINGS, **49**, 551 (1963).

[4] Yoshikawa, H., and N. Sueoka, these PROCEEDINGS, **49**, 559 (1963).

[5] *Ibid.*, **49**, 806 (1963).

[6] Bonhoffer, F. B., and A. Gierer, *J. Mol. Biol.*, **7**, 534 (1963).

[7] Lark, K. G., T. Repko, and E. J. Hoffman, *Biochim. Biophys. Acta*, **76**, 9 (1963).

[8] Mitra, S., and A. Kornberg, *J. Gen. Physiol.*, **49**, 59 (1966).

[9] Sakabe, K., and R. Okazaki, *Biochim. Biophys. Acta*, **129**, 651 (1966).

[10] A filamentous bacteriophage specific to male strains of *E. coli*. The DNA extracted from this phage with phenol has a single-stranded circular structure and is infective to *E. coli* protoplasts (Okazaki, R., M. Morimyo, and K. Sugimoto, in preparation).

[11] Okazaki, R., T. Okazaki, and K. Sakabe, *Biochim. Biophys. Res. Commun.*, **22**, 611 (1966).

[12] Miyazawa, Y., and C. A. Thomas, Jr., *J. Mol. Biol.*, **11**, 223 (1965).

[13] Thomas, C. A., Jr., K. I. Berns, and T. J. Kelley, Jr., in *Procedures in Nucleic Acid Research* (New York: Harper and Row, 1966), p. 535.

[14] Bernardi, *Nature*, **22**, 779 (1965).

[15] Lehman, I. R., *J. Biol. Chem.*, **235**, 1479 (1960).

[16] Nomura, M., K. Matsubara, K. Okamoto, and R. Fujimura, *J. Mol. Biol.* **5**, 535 (1962).

[17] Lehman, I. R., and A. L. Nussbaum, *J. Biol. Chem.*, **239**, 2628 (1964).

[18] Gellert, M., these PROCEEDINGS, **57**, 148 (1967).

[19] Olivera, B. M., and I. R. Lehman, these PROCEEDINGS, **57**, 1426 (1967).

[20] Gefter, M. L., A. Becker and J. Hurwitz, these PROCEEDINGS, **58**, 241 (1967).

[21] Weiss, B., and C. C. Richardson, these PROCEEDINGS, **57**, 1021 (1967).

[22] Fareed, G. C., and C. C. Richardson, these PROCEEDINGS, **58**, 665 (1967).

[23] Goulian, M., and A. Kornberg, these PROCEEDINGS, **58**, 1723 (1967).

[24] Goulian, M., A. Kornberg, and R. L. Sinsheimer, these PROCEEDINGS, **58**, 2321 (1967).

[25] Mitra, S., P. Richard, R. B. Inman, L. L. Bertsch, and A. Kornberg, *J. Mol. Biol.*, **24**, 429 (1967).