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Replication of Colicin E1 Plasmid DNA in Cell Extracts

(initiation/rifampicin/semiconservative/intermediates)

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ABSTRACT **Cell extracts were prepared from *Escherichia coli* carrying colicin E1 plasmid. The DNA in extracts was almost exclusively closed-circular DNA of the plasmid. Labeled deoxyribonucleotides were incorporated into DNA in extracts. DNA of colicin E1 plasmid was the sole DNA product, and was composed of completely replicated molecules and a class of replicative intermediates. The intermediates carried an average of approximately two pieces of DNA fragments that had a sedimentation coefficient of approximately 6 S and were not covalently attached to the parental DNA strands. The replication was initiated on closed-circular molecules and the complete molecules were synthesized semiconservatively. The DNA synthesis depended on the four ribonucleoside triphosphates and was sensitive to rifampicin. A round of DNA replication, once initiated, was completed in the presence of rifampicin, indicating that RNA synthesis is involved in the initiation of replication of the plasmid DNA. Most of the replicated molecules were isolated in super-coiled structures. These results indicate that this soluble system is capable of carrying out a complete round of replication of colicin E1 plasmid DNA.**

DNA synthesis *in vitro* has been observed in several systems (1–3), but a system in which the whole process of replication of double-stranded DNA is carried out has not been established. Such a system should show, at least, initiation of DNA synthesis at a specific site, continuation of replication in a semiconservative manner, and formation of progeny molecules by segregation. In this report we describe a soluble system in which closed-circular DNA molecules initiate and complete a round of semiconservative replication.

The DNA used in this study is colicin E1 plasmid (Col E1) an *Escherichia coli* plasmid that directs the production of an antibiotic protein. The plasmid exists as a closed-circular duplex DNA with a molecular weight of 4.2×10^6 (4). It has been shown by *in vivo* experiments that the majority of Col E1 DNA molecules replicate in circular structures (5) and that replication depends on the *dnaA* (6) and *polA* (7) functions but not on the *dnaE* function (8). Molecules of Col E1 DNA replicate extensively in the absence of net protein synthesis (9) but the replication is inhibited by rifampicin (10), which is known to block initiation of RNA synthesis.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* YS10 *thr leu thi str minA end* (Col E1) is a derivative of P678-54 (5) to which the *end* marker and Col E1 were introduced from EB5004 Hfr *end* and 193 F+ *cys his str* (Col E1), respectively. P678-54 was provided by J. Inselburg. The other strains and A745 *met thy* (Col E1) were in S. E. Luria's stock.

Chemicals. The four deoxyribonucleoside and ribonucleoside triphosphates (dNTPs and rNTPs), nicotinamide adenine dinucleotide (NAD), and 2′-deoxyadenosine were obtained from Sigma. Bromodeoxyuridine triphosphate (BrdUTP) was from PL Biochemicals, Inc. [α-^{32}P]dTTP and [α-^{32}P]dATP were obtained from New England Nuclear Corp. and International Chemical Nuclear Corp., respectively. [*Methyl*-^{3}H]- and [*methyl*-^{14}C]thymine and [*methyl*-^{3}H]thymidine were obtained from Schwarz Mann. Rifampicin and chloramphenicol were obtained from Calbiochem and Sigma, respectively. Brij 58 was from Sigma.

Preparation of Extracts. YS10 was cultured at 37° with shaking in 750 ml of a Casamino acids medium (OC medium) which contained 10.6 g of K_2HPO_4, 4.0 g of KH_2PO_4, 0.05 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of $(NH_4)_2SO_4$, 0.47 g of Na_3-citrate$\cdot 2H_2O$, 2 mg of thiamine, 5 g of Casamino acids (Difco), and 2.5 g of glucose per liter of H_2O. When the cell number reached 2×10^8/ml, chloramphenicol (180 μg/ml) was added and cultivation was continued for an additional 2 hr. The culture side triphosphates (dNTPs and rNTPs), nicotinamide adenine dinucleotide (NAD), and 2′-deoxyadenosine were obtained from Sigma. Bromodeoxyurine triphosphate (BrdUTP) was was then centrifuged at 10,000 rpm in a Sorvall GSA rotor for 10 mm at 4°. The cells were resuspended with 1 ml of 10% sucrose–50 mM potassium phosphate buffer (pH 7.4), frozen in a dry ice–isopropanol bath, and stored at −20°. The frozen cells were used usually within 4 hr after harvesting. The frozen cells were thawed in an ice-water bath, and the total volume was adjusted to 1.9 ml with 10% sucrose–50 mM phosphate buffer (pH 7.4). Seventy-five microliters of 2 M KCl, 200 μl of 4 mg/ml of lysozyme in 50 mM phosphate buffer (pH 7.4), and 50 μl of 5% Brij 58 were successively added to the cells, which were then incubated at 0° for 30 min. The resulting lysate was centrifuged at 25,000 rpm for 20 min at 2° in a Beckman rotor SW50.1. The supernatant provided the enzymes and DNA for the soluble system. Extracts contained 13–15 mg/ml of protein. The extent of lysis differed depending on the particular strain used.

DNA in the extract consisted almost exclusively of closed-circular Col E1 DNA as described later (Fig. 1). Based on the specific activity of [^{3}H]thymidine, approximately 2×10^{12} molecules per ml were present (Fig. 1). The amount of DNA was approximately 15% of the total amount of cellular closed-circular DNA, as determined by ethidium bromide–CsCl

Abbreviation: Col E1, colicin E1 plasmid.

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density gradient centrifugation of the cell lysate. The latter was approximately 6% of the total cellular DNA. When Col E1 DNA in the extract was treated with 0.3 N NaOH–0.2 M K_2HPO_4 solution at 40° for 60 min to convert closed-circular molecules carrying ribonucleotide bonds in the DNA strands to an open-circular form (11), less than 5% conversion was observed. When the extract was treated with 0.5% sodium dodecyl sulfate for 10 min at 37° to convert closed-circular DNA-protein relaxation complexes to an open-circular form (12), the conversion was not significant.

Assay of DNA Synthesis. The standard reaction mixture (300 μl) contained 7.5 mM $MgCl_2$, 25 μM each of the four dNTPs, 200 μM each of the four rNTPs, 2 mM NAD, 25 mM potassium phosphate buffer (pH 7.4), 67 mM KCl, and 100 μl of the extract. The extract contained approximately 100 mM KCl, 6.7% sucrose, 33 mM phosphate buffer, and 0.17% Brij 58. The specific activity of [α-^{32}P]dTTP or dATP was approximately 0.1 Ci/mmol for kinetic analysis and 1 Ci/mmol for product analysis. The mixture was incubated at 30°. Incorporation of a labeled nucleotide into an acid-insoluble fraction was followed by addition of cold 10% trichloroacetic acid–0.1 M PP_i solution and 20 μg of salmon-sperm DNA to each sample, which was filtered through a Whatman GF/C glass filter (2.4 cm) and washed with cold trichloroacetic acid–PP_i solution and then with cold 0.01 N HCl. The filters were dried and the radioactivity was measured. When the properties of DNA were to be analyzed, the reaction was stopped by the addition of EDTA (20 mM) and sodium dodecyl sulfate (0.25%) followed by incubation at 30° for 5 min. After the addition of one-tenth volume of 1.5 M NaCl–0.15 M Na_3-citrate, the mixture was vigorously shaken with an equal volume of chloroform–isoamyl alcohol 24:1 (v/v). After centrifugation at 12,000 rpm for 10 min, the aqueous phase was dialyzed against PENT buffer (11) consisting of 50 mM K_2HPO_4, 5 mM EDTA, 50 mM NaCl, and 50 mM Tris·HCl (pH 8.0). More than 90% of DNA was recovered.

Preparation of Reference Col E1 DNA. A745 was cultured at 37° in OC medium supplemented with 2 μg/ml of thymine. When the cell number reached 2 × 10^8/ml, chloramphenicol (180 μg/ml) was added. After an additional 3 hr of cultivation, 5 μCi/ml of [^{3}H]- or [^{14}C]thymine was added and cultivation was continued for an additional 15 hr. The cells were lysed and closed-circular Col E1 DNA was isolated by CsCl–ethidium bromide density gradient centrifugation (11). In alkaline sucrose gradient analysis the reference DNA was pretreated with 0.3 N NaOH–0.2 M K_2HPO_4 solution at 40° for 30 min to convert approximately half of the closed-circular molecules to the open-circular form (11).

Sedimentation Analysis. Sucrose solutions were made up in 0.15 M NaCl–0.015 M Na_3-citrate containing 5 mM EDTA or in 0.3 N NaOH–1.0 M NaCl containing 5 mM EDTA. Samples of 0.1–0.2 ml were layered on a 4.4 ml of 5–20% sucrose gradient with 0.3 ml underlayer of CsCl-saturated 20% sucrose. The samples were centrifuged, unless otherwise stated, at 45,000 rpm for 150 min at 10° or for 105 min at 5° for neutral or alkaline sucrose gradient analysis, respectively, in a SW50.1 rotor. For neutral CsCl density gradient analysis, a sample was mixed with 0.15 M NaCl–0.015 M Na_3-citrate containing 5 mM EDTA and the density was ajdusted to 1.72 g/cm^3 with CsCl. For an alkaline CsCl density gradient analysis, the sample was treated with 0.3 N NaOH–0.2 M K_2HPO_4 solution at 90° for 5 min, mixed with 0.15 M NaCl–0.015 M Na_3-citrate containing 5 mM EDTA, 0.1 N NaOH, and 0.03% sodium lauroyl sarkosinate and the density was adjusted to 1.76 g/cm^3. The final volume was 4.5 ml. The samples were centrifuged at 36,000 rpm for 60 hr at 20° in a SW50.1 rotor. Fractions were collected from the tube bottom.

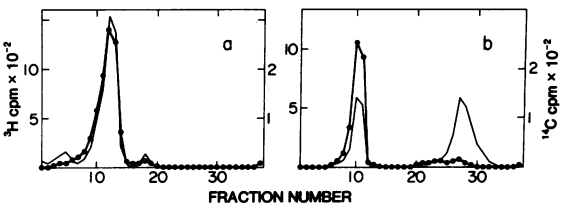

FIG. 1. Sedimentation analysis of DNA in the extract. YS10 was grown at 37° to 1 × 10^8 cells per ml, and [^{3}H]thymidine (2 μCi, 12.5 μg/ml) was added with 250 μg/ml of 2′-deoxyadenosine. When the cell number reached 2 × 10^8/ml, chloramphenicol (180 μg/ml) was added and cultivation was continued for an additional 2 hr. The extract was prepared, and DNA was extracted. Then the DNA was analyzed by neutral (*a*) and alkaline (*b*) sucrose gradient centrifugation. In (*b*), the reference Col E1 DNA was pretreated with alkali. (——) ^{14}C-labeled reference DNA; (●—●) ^{3}H-labeled DNA.

RESULTS

Col E1 DNA Synthesis in Extracts. DNA in extracts consisted almost exclusively of closed-circular molecules of Col E1 DNA (Fig. 1). The extracts incorporated labeled dTMP into high-molecular-weight substances (Fig. 2). The rate of incorporation was approximately 0.7 pmol of dTMP/min per mg of protein at 30° and continued more than 90 min (Fig. 2). The synthesis was inhibited by nalidixic acid (Table 1). The labeled product obtained at 60 min was converted to acid-soluble materials by treatment at 37° for 30 min with DNase I (20 μg/ml) but not with a mixture of RNase A (125 μg/ml) and RNase T1 (50 μg/ml). More than 90% of the labeled materials was hybridizable to Col E1 DNA (data not shown). These results indicate that labeled dTMP was incorporated into Col E1 DNA.

The incorporation increased proportionally as the extract concentration increased from 5 to 50% of the reaction mix-

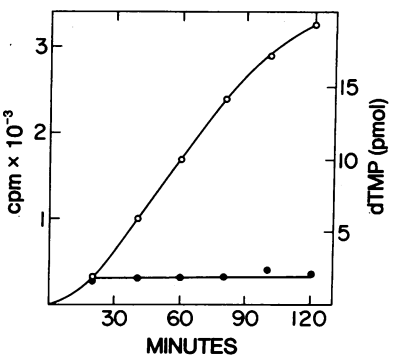

FIG. 2. Kinetics of incorporation of dTMP and effect of rifampicin. The extract was incubated in the standard assay condition with [α-^{32}P]dTTP in the presence (●) and absence (○) of rifampicin (10 μg/ml). Fifty μl of each was withdrawn at the time indicated.

TABLE 1. *Requirements for ribonucleoside triphosphates and inhibition by nalidixic acid and rifampicin*

Additions or omissions	Deoxyribonucleotide incorporated	
	cpm	ratio
Complete	2454	1.00
+ nalidixic acid (200 μg/ml)	580	0.24
+ rifampicin (3 μg/ml)*	568	0.23
− rCTP	1336	0.54
− rUTP	898	0.37
− rGTP	1082	0.44
− rCTP, rUTP	586	0.24
− rCTP, rGTP	608	0.25
− rUTP, rGTP	496	0.20
− rCTP, rUTP, rGTP	473	0.19
− rCTP, rUTP, rGTP, rATP	284	0.12

The extract was dialyzed at 0° with three changes against 50 mM potassium phosphate buffer (pH 7.4) containing 10% sucrose, 0.1 M KCl, 0.1 mM dithiothreitol, and 0.5 mM EDTA and used in 0.1 ml of standard assay mixtures (187 cpm/pmol of [α-^{32}P]-dTTP) from which the indicated rNTPs were omitted. The mixtures were incubated for 45 min.

* The incorporation did not significantly change at concentrations of 10, 30, and 100 μg/ml.

ture. The optimum concentration of Mg^{++} was approximately 7.5 mM, and more than 12.5 mM was inhibitory. Ten millimolar EDTA completely blocked the incorporation. The optimum concentration of KCl was approximately 100 mM, and more than 150 mM was inhibitory. Incorporation with NAD (0.3–3 mM) was 1.2–1.5 times that without the added NAD. Addition of dithiothreitol (0.3–3 mM) had no effect.

Synthesis of Col E1 DNA was dependent on the four rNTPs and inhibited by rifampicin (Table 1 and Fig. 2). The optimum concentrations of rNTPs were approximately 200 μM each, and more than 1 mM rATP was inhibitory. Rifampicin-sensitive DNA synthesis was also observed with extracts from cells carrying Col E1 that were not treated with chloramphenicol, although the activity per mg of protein was approximately one-fifth of that from the cells treated with chloramphenicol for 2 hr.

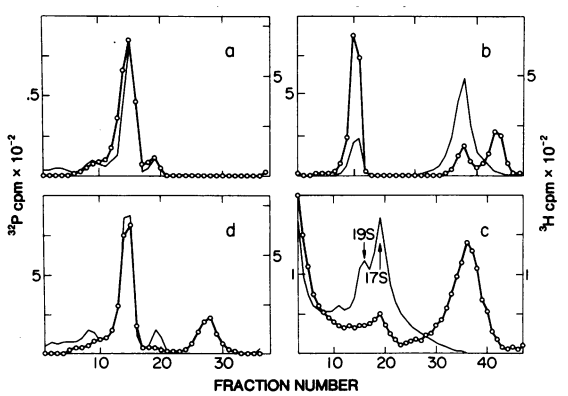

FIG. 3. Characterization of Col E1 DNA synthesized in the extract. The extract was incubated for 60 min in the standard assay condition with [α-^{32}P]dTTP. DNA was analyzed by neutral sucrose gradient centrifugation before (*a*) and after heating at 90° for 90 sec in PENT buffer. (*d*), and by alkaline sucrose gradient centrifugation for 105 min (*b*) and 300 min (*c*). In (*c*), the reference DNA was not pretreated with alkali. (——) ^{3}H-labeled reference DNA; (O——O) ^{32}P-labeled DNA.

Synthesis of Closed-Circular Molecules. The DNA in the sample taken at 60 min sedimented with the reference closed-circular Col E1 DNA in neutral sucrose gradient centrifugation although the band has a significant skew in the leading position (Fig. 3*a*). In an alkaline sucrose gradient, approximately 65% and 15% of the DNA sedimented with closed-circular and open-circular or linear molecules of the reference DNA, respectively, and small DNA molecules accounted for approximately 20% (Fig. 3*b*). The average size of the small DNA molecules was approximately 6 S, which corresponds to approximately one-fifteenth of a complete single strand of Col E1 DNA (Fig. 3*c*). Since small molecules were not detected in a neutral sucrose gradient (Fig. 3*a*), they seem to have been attached to the parental molecules before alkaline denaturation. To confirm this, the DNA was heated at 90° for 90 sec before sedimentation in a neutral sucrose gradient. As shown in Fig. 3*d*, the skew in the main band and the small band near the position of open-circular molecules observed with the unheated sample (Fig. 3*a*) disappeared and, instead, super-coiled closed-circular molecules and small molecules of approximately 6 S were observed. The DNA extracted after incubation for 30 and 90 min gave sedimentation patterns in both the neutral and alkaline sucrose gradient analysis similar to those obtained with the 60-min sample.

Semiconservative Synthesis of Closed-Circular Molecules. To examine the mode of replication, properties of newly synthesized density-labeled DNA were examined. An extract from [^{3}H]thymidine-labeled cells was incubated in the presence of BrdUTP, [α-^{32}P]dATP, dGTP, and dCTP. The amount of DNA synthesized in 60 min was approximately 80% of that in the presence of dTTP in the same duration. Alkaline sucrose gradient analysis of the DNA (Fig. 4*a*) gave almost the same pattern as that of the DNA synthesized in the presence of dTTP (Fig. 3*a*). In a neutral CsCl density gradient, the newly synthesized BrdU-labeled DNA formed a band with a shoulder on the light side of the peak (Fig. 5*a*). The DNA in the peak fraction of Fig. 5*a* was recentrifuged in both a neutral CsCl density gradient and an alkaline CsCl gradient after the alkali treatment to open the closed-circular form. As shown in Fig. 5*b* and *c*, the difference in density of the [^{32}P]DNA and the light [^{3}H]DNA in the alkaline gradient was twice that in the neutral gradient. The presence of ^{3}H and ^{32}P radioactivities in the middle region between the light and heavy positions in the alkaline gradient was expected since a fraction of closed-circular molecules remained after the alkaline treatment. These results show that the DNA at the peak in the first neutral CsCl density gradient centrifugation (Fig. 5*a*) consisted of hybrid molecules of a heavy ^{32}P-labeled strand and a light ^{3}H-labeled strand. In addition, the hybrid molecules were shown to be completely replicated molecules (Fig. 4*b*). These results indicate that closed-circular Col E1 DNA molecules were synthesized semiconservatively. From the results of the neutral CsCl density gradient analysis, it was estimated that approximately 5% of [^{3}H]DNA in the extract completed replication. No significant amount of ^{32}P radioactivity was observed in the full-heavy position under these conditions (Fig. 5*a*).

The hybrid peak of newly synthesized DNA showed a shoulder on the light side (Fig. 5*a*). Recentrifugation of DNA

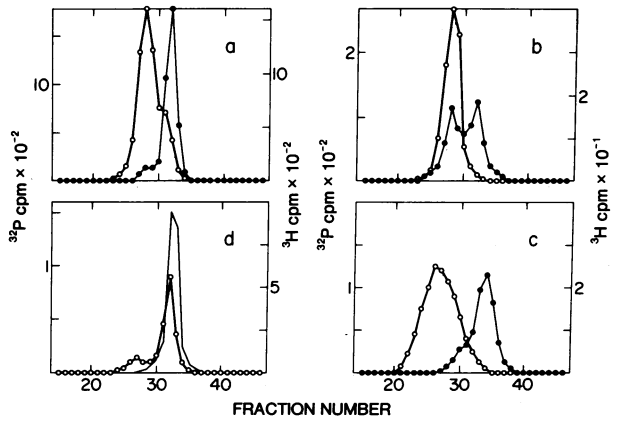

FIG. 4. Sedimentation analysis of newly synthesized BrdU-labeled Col E1 DNA. The extract from the cells labeled with [^{3}H]thymidine, as described in the legend to Fig. 1, was incubated for 60 min in the standard assay condition, except that 50 μM each of BrdUTP, dCTP, dGTP, and [α-^{32}P]dATP were added instead of the four dNTPs. Total DNA (*a*) and fractions no. 27 and 28 of the CsCl density gradient in Fig. 5*a* (*b*) were analyzed in alkaline sucrose gradient centrifugation. DNA at fraction no. 31 of Fig. 5*a* was analyzed by alkaline (*c*) and neutral (*d*) sucrose gradient centrifugation. The underlayer in gradients was omitted except in (*a*); ^{14}C-labeled reference DNA was added in (*d*) to obtain the positions of 23S and 17S peaks. (●—●) ^{3}H-labeled parental DNA; (O—O) ^{32}P-labeled newly formed DNA. BrdU-labeled DNA sedimented slightly faster than the light DNA of the same class.

at the shoulder produced a sharp major band which was displaced from the light position by roughly one-tenth of the distance between the light and hybrid positions (Fig. 5*d*). The molecules at the shoulder carried newly synthesized 6S DNA which was not covalently attached to the parental DNA

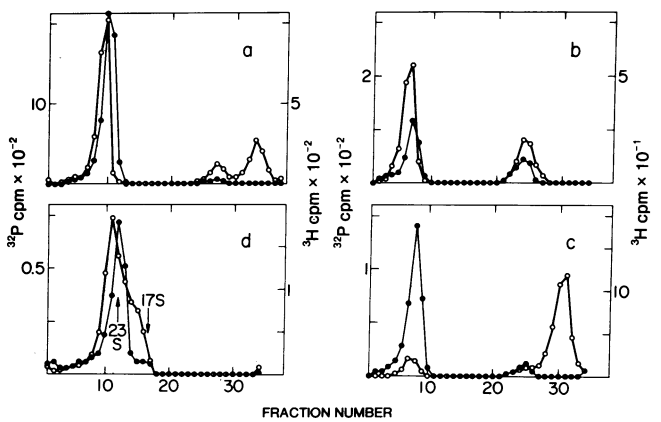

FIG. 5. Equilibrium CsCl density gradient centrifugation of BrdU-labeled Col E1 DNA. The BrdU-labeled Col E1 DNA, prepared as described in the legend to Fig. 4, was centrifuged in a neutral CsCl density gradient (*a*). DNA at fractions no. 27 and 28 were recentrifuged in neutral (*b*) and alkaline (*c*) gradients. DNA at fraction no. 31 of (*a*) was recentrifuged with excess ^{3}H-labeled reference DNA in a neutral gradient (*d*). The density differences between the light [^{3}H]DNA and the [^{32}P]DNA at their peaks were approximately 25 mg/cm^3 in (*b*) and 50 mg/cm^3 in (*c*). Because of the presence of endogenous dTTP, substitution of dTMP by BrdUMP was not extensive. (●—●) ^{3}H-labeled parental DNA; (O—O) ^{32}P-labeled newly formed DNA; (——) ^{3}H-labeled reference DNA.

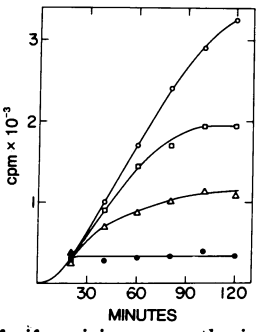

FIG. 6. Effect of rifampicin on synthesis of Col E1 DNA. The assay condition was as described in the legend to Fig. 2. Rifampicin (10 μg/ml) was added at 0 min (●), 10 min (△), and 20 min (□) after incubation. (O) Without rifampicin.

(Fig. 4*c*) and sedimented in a neutral gradient in two bands, one of which sedimented slightly faster than the closed-circular reference DNA and the other slightly faster than the open-circular reference DNA. The DNAs located at the positions of these two bands in Fig. 3*a* disappeared after the heating at 90° for 90 sec (Fig. 3*d*).

Effect of Rifampicin on Col E1 DNA Synthesis. The synthesis of Col E1 DNA in extracts required rNTPs and was sensitive to rifampicin (Table 1 and Fig. 2). The amount of DNA synthesized was dependent on the time of addition of rifampicin with considerably greater amounts synthesized if the addition was delayed (Fig. 6). A great majority of labeled DNA extracted after the incubation with rifampicin, which was added at 10 min (Fig. 7) as well as 0 min (data not shown), was found in the completely replicated molecules of Col E1 DNA, indicating that synthesis of Col E1 DNA was able to continue to complete a round of replication in the presence of rifampicin. Therefore the process which was blocked by rifampicin is a preliminary process involved in the initiation of synthesis of Col E1 DNA. The final amount of DNA synthesized in the presence of rifampicin would then be determined by the number of molecules that had finished this preliminary process.

DISCUSSION

Extracts from cells carrying Col E1 were shown to synthesize Col E1 DNA as the sole DNA product. Approximately 80% of DNA synthesized during 60 min was found in the form of completely replicated monomeric molecules (Fig. 3*a*). The analysis of BrdU-labeled DNA (Fig. 5) shows that these

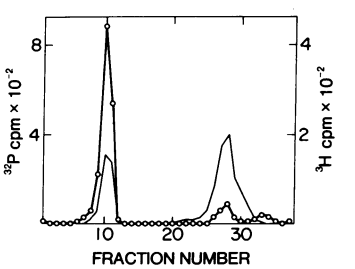

FIG. 7. Sedimentation analysis of Col E1 DNA synthesized in the presence of rifampicin. Rifampicin (30 μg/ml) was added at 10 min to the standard reaction mixture, and incubation was continued for an additional 75 min. DNA was analyzed by alkaline sucrose gradient centrifugation. (——) ^{3}H-labeled reference DNA; (O——O) ^{32}P-labeled DNA.

molecules were synthesized semiconservatively. Approximately 5% of the DNA molecules initially present in the extract completed a round of replication within 60 min. The absence of a significant amount of full-heavy DNA shows that replication of those molecules that had completed the first round of replication was not preferentially reinitiated, which is consistent with the *in vivo* observation (13).

In addition to the completely replicated molecules, a class of molecules that carried 6S DNA pieces, the length of which was approximately one-fifteenth of a unit length of molecule of Col E1 DNA, was synthesized. If every one of the molecules is assumed to carry two pieces of the 6S DNA, the difference in density of the molecules carrying BrdU-labeled 6S DNA from the light DNA is expected to be approximately one-eighth of that between the hybrid and the light DNA. The value of the observed difference (Fig. 5*d*) was not much different from the expected value. The amount of 6S DNA synthesized accounted for approximately 20% of the total DNA synthesized (Fig. 4*a*). Therefore, the number of molecules carrying 6S DNA synthesized in 60 min is calculated to be approximately twice that of completely replicated molecules or approximately four times that of the parental molecules that completed a round of replication during the time. Therefore, the fraction of preexisting molecules that participated in replication in 60 min is estimated to be approximately 25%. The molecules of Col E1 DNA in extracts consisted almost exclusively of closed-circular molecules (Fig. 1) and, thus, it is evident that closed-circular molecules did indeed participate in replication in extracts. The molecules carrying the 6S DNA are replicative intermediates and the 6S DNA is located at a specific region, at approximately 20% from a single cleavage site by restriction endonuclease *Eco*R1, on Col E1 DNA (manuscript in preparation).

The synthesis of Col E1 DNA in extracts required rNTPs and was sensitive to rifampicin. The DNA synthesis, once initiated, completed a round of replication even in the presence of rifampicin (Fig. 7). This result indicates that RNA synthesis is involved in the initiation of synthesis of Col E1 DNA. Association of RNA to the 6S DNA has been observed (manuscript in preparation). RNA-primed initiation of synthesis of DNA strands complementary to single-stranded phage DNAs in soluble systems has been reported (3, 14). The initiation is sensitive to rifampicin in the M13 system (3), while that in the ϕX174 system is resistant (14). Synthesis of lambda phage DNA, *in vitro*, is reduced by rifampicin (15). The initiation of replication of *E. coli* chromosomes is known to be sensitive to rifampicin (16). In contrast, RNA-primed synthesis of short pieces of DNA in propagation of *E. coli* chromosomes is relatively resistant to rifampicin (17). Chain growth of Col E1 DNA in the soluble system occurred by a discontinuous mechanism (manuscript in preparation) which is resistant to rifampicin.

Molecules of Col E1 DNA completed a round of replication by segregating out monomeric closed-circular molecules. Most molecules were isolated as a super-coiled structure (Fig. 3*a*) with a superhelix density similar to Col E1 DNA isolated from bacteria (manuscript in preparation). Completely replicated half-heavy molecules were visualized as twisted circular forms by electron microscopy and showed biological activity in a transfection assay (manuscript in preparation). In addition, extracts supported replication of exogenously supplied closed-circular Col E1 DNA (manuscript in preparation). These observations complete the evidence that this soluble system is capable of carrying out the entire process of Col E1 DNA replication.

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