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Replication of Colicin E1 Plasmid DNA In Vivo Requires No Plasmid-Encoded Proteins

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A derivative of bacteriophage λ containing a colicin E1 plasmid replicon was constructed by recombinant DNA techniques. This phage, λ*col100*, has two functional modes of DNA replication; it can replicate via either plasmid or phage replication systems. λ*col100* has been used to introduce the colicin E1 plasmid replicon into *Escherichia coli* previously treated with chloramphenicol to block protein synthesis. Under these conditions, λ*col100* DNA is replicated normally as a colicin E1 plasmid. This suggests that colicin E1 plasmid replication in vivo does not require any plasmid-encoded proteins.

Colicin E1 plasmid (ColE1) is a plasmid of *Escherichia coli* that has received wide interest both for study of its mode of replication and for use as a recombinant DNA vector. About 4.2×10^6 daltons in size, ColE1 exhibits the following properties: (i) although non-self-transmissible, ColE1 can be mobilized by conjugative plasmids such as F; (ii) it codes for the protein colicin E1; (iii) it codes for a protein which confers upon the host bacterium immunity to colicin E1; and (iv) it replicates independently of the chromosomal DNA as a covalently closed circular DNA molecule (4, 9, 11, 13).

Mini-ColE1, also designated pVH51, refers to a deletion of a ColE1 derivative that retains approximately 2.0×10^6 daltons of the parent plasmid (10). Mini-ColE1 is not efficiently mobilized by conjugative plasmids, nor does it produce colicin E1. Nonetheless, mini-ColE1 still codes for immunity to colicin E1 and remains capable of autonomous replication.

Replication of ColE1 and mini-ColE1 DNA continues under conditions where protein synthesis is inhibited by chloramphenicol (CAM). In the presence of CAM, initiation of *E. coli* chromosomal replication ceases, yet these plasmids continue to replicate for more than 10 h, and eventually may represent as much as 40% of the total cellular DNA (7, 10). The molecular mechanism of this amplification in the presence of CAM is not yet fully understood.

In a soluble in vitro system, ColE1 DNA replication appears to depend entirely upon bacterial functions (25, 31). On the other hand, this observation is seemingly in conflict with the existence of ColE1 mutants that are temperature sensitive for replication (9, 15, 16). Thus, the possible role of any plasmid-encoded proteins in ColE1 replication in vivo has remained unclear.

To clarify this contradiction, we have inserted mini-ColE1, using its unique *Eco*RI site, into a phage λ vector. This recombinant DNA molecule has two functional modes of replication—phage or plasmid. The phage-encoded replication functions (gene products *O* and *P*) can initiate replication at the λ DNA replication origin, or alternatively, other replication functions can initiate at the ColE1 DNA replication origin. Replication via the phage λ functions can be experimentally manipulated through the use of a thermolabile phage repressor. This recombinant phage, designated λ*col100*, may be thought of as mini-ColE1 cloned in a bacteriophage vector or as phage λ cloned in a plasmid vector.

λ*col100* differs significantly from other derivatives of lambda which can exist as plasmids. For example, the replication of λN^- as a plasmid depends upon a low level of expression of genes *O* and *P* and occurs regardless of whether functional repressor is present (18, 27). λ*col100* is incapable of this form of plasmid replication, however, as it is N^+ and carries the *nin5* (*N*-independent) deletion, which blocks plasmid formation even by N^- phage (N. Kleckner, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1974). Another example of a lambda derivative that replicates as a plasmid is λ*dv*, which is a deletion mutant of λ retaining only the region from *cI* to *P* (21). In contrast to λN^- and λ*dv*, which replicate as plasmids using λ-encoded functions, λ*col100* replicates extrachromosomally by virtue of its ColE1 replicon and requires a functional repressor to repress lytic lambda functions.

Since phage λ particles will inject their DNA

into bacteria in the absence of protein synthesis (30, 35), λ*col100* permits the introduction of a ColE1 replicon into cells in which protein synthesis has been arrested. Phage λ replication, dependent upon genes *O* and *P*, is abolished by treatment with inhibitors of protein synthesis (1, 30), and, thus, any synthesis of λ*col100* DNA under such conditions must arise from the ColE1 replicon. We have used this approach to clarify the origin of those proteins required for the replication of ColE1 DNA in vivo.

MATERIALS AND METHODS

Bacteria and bacteriophage. *E. coli* strains C600 r_k^- m_k^- and Q1 were obtained from R. W. Davis and M. S. Fox, respectively. Q1 is a *thi*$^+$ derivative of C600 (28); C600 has the genotype *thr-1 leu-6 thi-1 supE44 lacY1 tonA21* λ$^-$ (3). λ*gt*-λ*C* (29) was obtained from R. W. Davis.

In this section, recombinant DNA phages are named following the convention of Thomas et al. (29) whereby λ*gt* refers to the left and right *Eco*RI fragments of their generalized transducing derivative of phage λ. *Eco*RI fragments inserted into λ*gt* are then listed after a hyphen. For convenience, we have used the trivial name λ*col100* to refer to the phage λ*gt-Km-ex mini-ColE1* λ*-RI-C*.

λ*col100* has the following origin: DNAs from λ*gt vir Jam27 Zam718-*λ*BC* (8) and the plasmid pML21 (19) were mixed and treated sequentially with *Eco*RI and T4 DNA ligase, and transfected into C600 r_k^- m_k^-. The phage λ*gt vir Jam27 Zam718-Km*^{r} *mini-ColE1* λ*-RI-C* was isolated as one of many phages scoring positive for transfer of kanamycin resistance, as already described by Donoghue and Sharp (8). The *Eco*RI fragments designated *Km*^{r} and *mini-ColE1* are both *Eco*RI digestion products of the plasmid pML21. The *Jam27* and *Zam718* markers were removed from this latter phage by a first cross with λ*gt*-λ*C*, and the *vir* marker was removed by a second cross with λ*gt*-λ*C*. The resulting phage, λ*gt-Km*^{r} *mini-ColE1* λ*-RI-C*, proved to be unstable at 39°C because of its oversized genome (107% of wild type). A spontaneously occurring derivative was isolated which had deleted 1.6 × 10^6 daltons and no longer contained the inverted repeat sequence $\eta\theta$ (23) nor the antibiotic resistance gene. Thus, the 4.6 × 10^6-dalton *Km*^{r} fragment was replaced by an excision product of 3.02 × 10^6 daltons, designated *Km-ex*, which is devoid of any known genetic function. The genome structure of λ*gt-Km-ex mini-ColE1* λ*-RI-C*, hereafter referred to as λ*col100*, has been confirmed by restriction enzyme mapping and by heteroduplex analysis in the electron microscope (D. J. Donoghue and P. A. Sharp, manuscript in preparation). This phage has already been partially described elsewhere (D. J. Donoghue and P. A. Sharp, ICN-UCLA Symposia on Molecular and Cellular Biology, in press).

Phage infections were carried out using twice-CsCl-banded phage stocks at a multiplicity of five phage per bacterium. Bacterial cultures were used in mid-log phase (3 × 10^8 cells/ml) for all experiments described.

Media. YM broth, containing 1% tryptone–0.25% NaCl–0.01% yeast extract, supplemented with 1 mM $MgCl_2$, 0.2% maltose, and adjusted to pH 7.2, was routinely used. CAM, used at a concentration of 200 μg/ml, was purchased from Sigma Chemical Co. When CAM was added to bacterial cultures, they were also supplemented with 0.4% glucose because of the uncertain effect of CAM upon maltose utilization.

Pulse-labeling of cell DNA. To monitor the rate of total DNA synthesis, 0.5 ml of cells was added to 5 μCi of [*methyl*-^{3}H]deoxyribosylthymine (dT) (New England Nuclear, NET-027E, dried, and resuspended in water before use) with shaking for 10 min at 32°C. To stop labeling, an equal volume of cold 25% trichloroacetic acid was added, and precipitates were collected on nitrocellulose filters (Millipore Corp.).

Ethidium bromide-cesium chloride (EtBr-CsCl) density gradient centrifugation. Samples for EtBr-CsCl gradients were prepared by labeling cells with [^{3}H]dT as above, except that at the end of the labeling period, a 2-h chase with cold dT (4,000-fold excess) was performed. Determination of total trichloroacetic acid precipitable radioactivity during the chase period showed that incorporation of radioactivity was reduced more than 95%. At the conclusion of the chase period, cells were collected by centrifugation and disrupted by lysozyme, sodium dodecyl sulfate-Pronase, and gentle shearing, as described by Womble et al. (34). Solid CsCl was added to a refractive index of 1.3905 (1.599 g/ml), and ethidium bromide was added to 250 μg/ml. In addition, approximately 5 μg of unlabeled plasmid DNA was added to each gradient as carrier DNA; pCR1, which is a ColE1-*Km*^{r} hybrid plasmid derived from pML2 (11), was used for this purpose and consisted of a mixture of forms I and II. The samples were centrifuged for 48 h in a Beckman type 65 rotor at 42,000 rpm and 20°C. Gradients were collected by dripping through a Hoefer Scientific Products gradient-collecting device at a rate of less than 0.5 ml/min. Aliquots of fractions were trichloroacetic acid precipitated and collected on Millipore nitrocellulose filters.

Agarose gel electrophoresis. Before *Eco*RI digestion, ethidium bromide was removed (when necessary) by dialysis at 20°C against Bio-Rad AG 501-X8 mixed bed resin in 1 M NaCl–10 mM tris(hydroxymethyl)aminomethane (Tris)–1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.5, for 24 h, followed by dialysis against 10 mM Tris–1 mM EDTA, pH 8.5, at 4°C for an additional 24 h. *Eco*RI was prepared and used as previously described (8). Electrophoresis through 1.4% agarose gels and subsequent staining with ethidium bromide was carried out as described by Sharp et al. (26). Fluorography to detect [^{3}H]DNA was performed as described by Laskey et al. (17).

Pulse-labeling of cells with [^{3}H]leucine. Cells were grown in an N-salts medium containing 0.047 M Na_2HPO_4–0.022 M KH_2PO_4–0.018 M $(NH_4)_2SO_4$–2 μM $FeCl_3$, supplemented with 0.2% vitamin-free Casamino Acids, 1 mM $MgCl_2$, 10 μg of thiamine-HCl per ml, and 0.4% glucose. At different times after addition of 200 μg of CAM per ml, 0.5 ml of cells was added to 100 μCi of L-[4,5-^{3}H]leucine (New England Nuclear, NET-135L). After 5 min of labeling, incorporation was stopped by addition of 0.5 ml of 5-mg/ml leucine–2.5% sodium dodecyl sulfate–0.2 M NaOH–25 mM EDTA.

Samples were boiled for 10 min, precipitated with 3 ml of cold 25% trichloroacetic acid, and then collected on filters for counting.

Biohazard containment. The recombinant DNA experiments described in this work were carried out under P1 EK1 conditions in accordance with the National Institutes of Health guidelines (22).

RESULTS

Description of λ*col100*. The genome structure of λ*col100* is shown in Fig. 1. λ*col100* is the product of an in vitro recombination event between λ vector DNA and the plasmid pML21 (19).

The left and right ends of λ*col100* are identical to those of the generalized transducing phage λ*gt* constructed by Thomas et al. (29), and encode all the necessary functions for lytic growth of phage λ. The genome of λ*gt* has been designed so as to allow the insertion of foreign DNA through *Eco*RI ends into the nonessential region of the vector. Two *Eco*RI sites have been genetically mutated in the right arm of λ*gt*, so that *Eco*RI cleavage sites no longer exist in regions of essential genetic information. In addition, Thomas et al. (29) introduced the *nin5* deletion (*N* independent) which deletes 1.66×10^6 daltons of DNA between genes *P* and *Q*; this permits a larger fragment of foreign DNA to be introduced into the λ*gt* vector. By themselves, the λ*gt* ends are too small to be efficiently packaged; thus, there is a strong selection for phage carrying an inserted segment of DNA. The right arm of the phage genome also carries a thermolabile repressor, designated *cI857*.

In addition to the essential left and right ends of the phage genome, λ*col100* contains three inserted *Eco*RI fragments: (i) the λ-*RI-C* fragment is the *Eco*RI fragment of phage λ DNA that codes for λ general and site-specific recombination functions. (ii) The *mini-ColE1* fragment is one of two *Eco*RI fragments contained in the plasmid pML21. Also designated pVH51 (10), the *mini-ColE1* fragment carries information for ColE1 replication and for immunity to colicin E1. (iii) The *Km-ex* fragment is an excision product of the 4.6×10^6-dalton fragment of pML21 that codes for resistance to the antibiotic

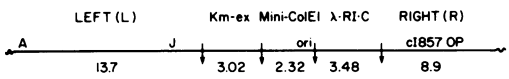

FIG. 1. *EcoRI cleavage sites and partial genetic map of λcol100. EcoRI restriction sites are designated by vertical arrows; the numbers below the fragments represent their molecular weights in megadaltons (Donoghue and Sharp, in press). In addition to some genetic markers of phage λ, the ColE1 origin of replication is also shown (ori).*

kanamycin (*Km*^{r}). Although the entire *Km*^{r} fragment was originally present in the phage, this resulted in an oversized phage genome. A spontaneous internal deletion of 1.6×10^6 daltons removed the inverted repeat sequence $\eta\theta$ (23) and the antibiotic resistance gene, leaving the *Km-ex* fragment devoid of any known genetic functions.

Propagation of λ*col100* as phage or plasmid. Since λ*col100* can be replicated by two different DNA replication systems, it can be grown either as a phage or as a plasmid. At 39°C, the *cI857* repressor is inactivated and λ*col100* is replicated and packaged primarily by lambda functions. Figure 2A shows an *Eco*RI digest of λ*col100* DNA propagated in this way; the DNA was extracted from phage particles after purification in CsCl equilibrium gradients. The left (L) and right (R) arms of the phage DNA are seen, as well as the three inserted *Eco*RI fragments. Alternatively, at 32°C, the lambda repressor is active and lytic lambda functions are repressed. In its lysogenic state, lambda integrates into the *E. coli* chromosome at the *att*λ site; in addition, λ*col100* can replicate extrachromosomally by virtue of the ColE1 replicon. When grown in this way, λ*col100* DNA can be prepared as a covalently closed supercoiled molecule in EtBr-CsCl gradients. After treatment with CAM, amplification occurs and greater yields of λ*col100* DNA per cell are obtained. Figure 2B shows an *Eco*RI digest of λ*col100* DNA prepared in this latter fashion; the left (L) and right (R) arms are now covalently joined at the cohesive ends, so that the bands migrating with the mobility of bands L and R are not observed and a new band migrating with the mobility of L + R is observed.

Replication of λ*col100* in CAM-treated cells. A concentration of 200 μg of CAM per ml was used in all experiments described below. This antibiotic has been widely used when complete inhibition of protein synthesis is desired (12, 24, 33). In strain C600 r_k^- m_k^-, Fig. 3 shows that the rate of protein synthesis, as measured by the incorporation of [^{3}H]leucine, decreases to the extent of 98 to 99% within 5 min of CAM addition.

At a coarse level, ColE1 replication may be monitored in CAM-treated cells by determining the incorporated radioactivity following a pulse-label with [^{3}H]dT (2). Within several hours of CAM treatment, chromosomal DNA replication slows to a greatly reduced rate, whereas ColE1 replication continues at a significant level. Figure 4A shows the incorporation of [^{3}H]dT during 10-min pulse-labels at 32°C in different strains over an extended period of time. A lysogen containing λ*col100*, Q1(λ*col100*), synthesizes DNA

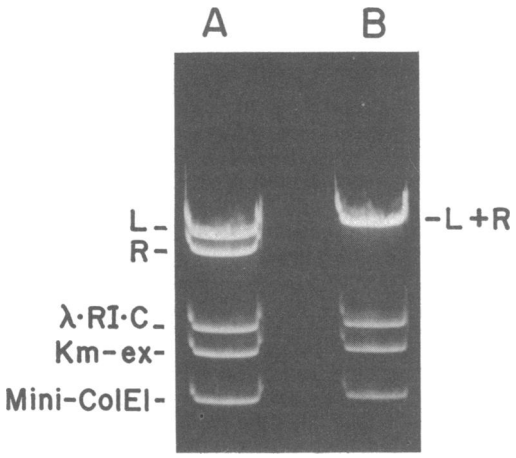

FIG. 2. *Agarose gel electrophoresis of EcoRI-digested λcol100 DNA. (A) λcol100 DNA prepared from phage particles; (B) λcol100 DNA prepared as a plasmid from Q1(λcol100) after CAM amplification. Both samples were heated to 68°C for 5 min before loading to dissociate the λ cohesive ends. Note in (B) that the left (L) and right (R) ends are covalently joined to produce one larger fragment (L + R).*

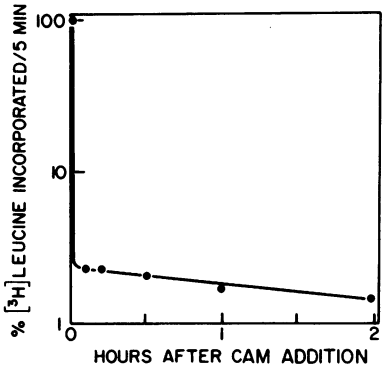

FIG. 3. *Inhibition of protein synthesis by CAM in C600 r_k^- m_k^-. Cells were pulse-labeled for 5 min at 32°C with [3H]leucine at different times after addition of CAM to a concentration of 200 μg/ml. Trichloroacetic acid-precipitable radioactivity of duplicate samples was determined as described in the text. The maximum incorporated radioactivity (100%) was 9.0 × 10^5 cpm just before addition of CAM.*

at a significant rate in the presence of CAM, whereas synthesis in the control, C600 r_k^- m_k^-, falls off sharply. Q1(λ*gt*-λ*C*), which lacks a ColE1 replicon in the lambda prophage, also ceases incorporation rapidly.

Figure 4B shows the rate of incorporation of [3H]dT, determined in a similar fashion, in cells infected with the phage λ*col100* before or after treatment with CAM. As before, the rate of DNA synthesis in uninfected C600 r_k^- m_k^- decreases rapidly after CAM addition. However, if infected with λ*col100* at a multiplicity of five phage per cell, either 15 min before or 10 min after the CAM block, [3H]dT incorporation continues at a significant rate above the control. Indeed, even when infected with λ*col100* at 2.5 h after the CAM block, these cells synthesize DNA at a greater rate than uninfected cells.

These data suggest that ColE1 replication proceeds in the presence of CAM regardless of when the ColE1 replicon is introduced into the cell: (i) it may be preexisting in a lysogenized cell (Fig. 4A); (ii) it may enter the cell via phage infection prior to the CAM block (Fig. 4B); or (iii) it may enter the cell via phage infection subsequent to the CAM block (Fig. 4B). Data presented below confirm directly that the increased rate of [3H]dT incorporation seen is indeed ColE1 specific.

EtBr-CsCl density gradient centrifugation of pulse-labeled DNA. ColE1 replication gives rise to covalently closed circular molecules (form I DNA) that can be isolated by EtBr-CsCl density gradient centrifugation. If CAM-treated cells are subsequently infected wih λ*col100* and then pulse-labeled with [3H]dT at different times after infection, the labeled DNA can be examined by this technique, as shown in Fig. 5A, B, and C. In these experiments, the DNA was labeled for 10 min at 32°C, followed by a 2-h chase with cold dT, so that molecules only partially replicated at the end of the labeling period would mature during the chase period and appear in the form I position. The samples shown in Fig. 5A, B, and C were labeled at 25 min, 3 h, and 6 h, respectively, after addition of CAM. Although no radioactivity is observed at the form I position immediately after λ*col100* infection (Fig. 5A), by 6 h the form I peak represents approximately one-third of the pulse-labeled DNA (Fig. 5C).

In a parallel experiment (Fig. 5D, E, and F), CAM-treated cells were infected with λ*gt*-λ*C*. As expected, even at 6 h after infection, no form I peak of pulse-labeled DNA was observed (Fig. 5F). The radioactivity in the main band position of Fig. 5D, E, and F represents residual labeling of *E. coli* chromosomal DNA. This is consistent with the residual labeling of uninfected cells seen in Fig. 4 after treatment with CAM, and does not represent replication of λ*gt*-λ*C* DNA.

The fractions in the form I and upper band positions of Fig. 5C were separately pooled as indicated, and the DNA samples were digested with *Eco*RI and subjected to electrophoresis through a 1.4% agarose gel. The pattern of the [3H]DNA is shown in the fluorograph in Fig. 6. All of the labeled material from the form I peak is found in λ*col100* DNA restriction fragments (Fig. 6A). When the DNA from the upper band

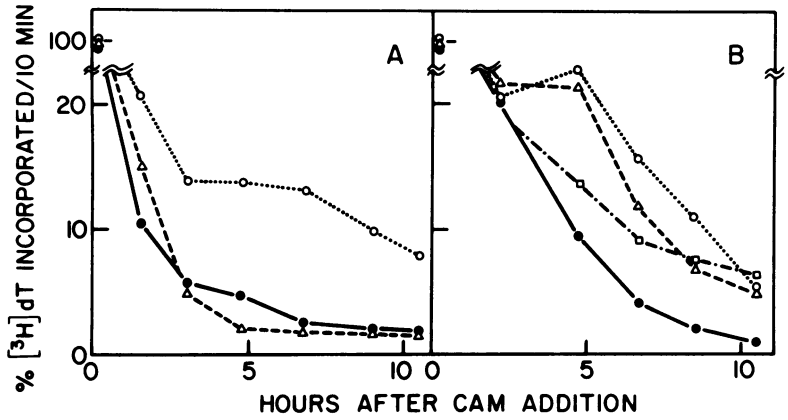

FIG. 4. *Pulse-labeling of cells with [^{3}H]dT at different times after CAM addition. Labeling of duplicate samples was for 10 min at 32°C as described in the text. (A)* ●, *incorporation of C600 r_k^- m_k^-;* △, *incorporation of Q1(λgt-λC);* ○, *incorporation of Q1(λcol100). The incorporated radioactivity just before the addition of CAM (100%) was 1.2 × 10^6 cpm for C600 r_k^- m_k^-, 1.0 × 10^5 cpm for Q1(λgt-λC), and 8.0 × 10^4 cpm for Q1(λcol100). (B) Incorporation of uninfected C600 r_k^- m_k^- or infected with λcol100 is presented:* (●) *uninfected cells;* (△) *cells infected 15 min before addition of CAM;* (○) *cells infected 10 min after addition of CAM;* (□) *cells infected 2.5 h after addition of CAM. All infections with λcol100 were performed at a multiplicity of five phage per bacterium. The incorporated radioactivity just before addition of CAM (100%) was 1.3 × 10^5 cpm for all curves shown in (B).*

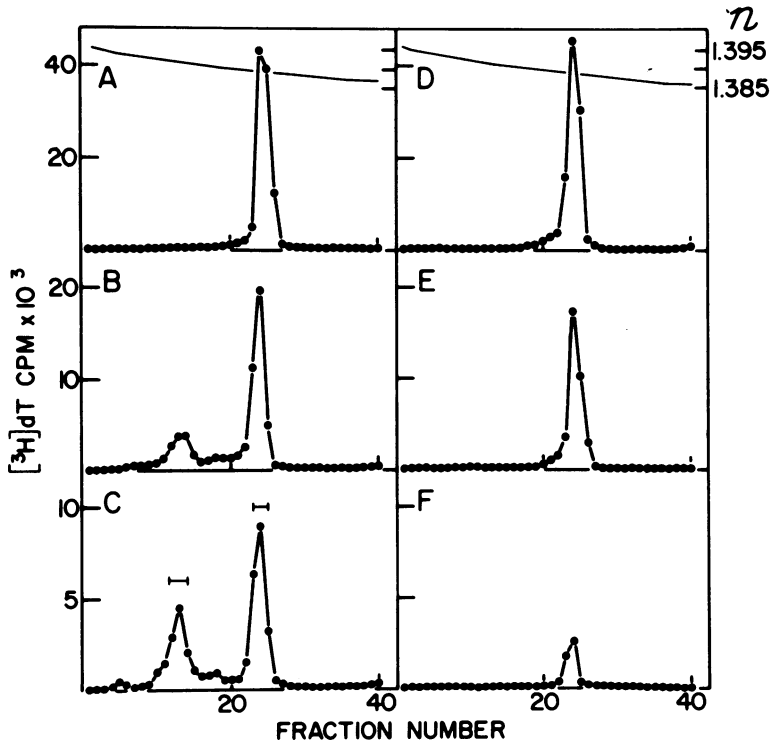

FIG. 5. *EtBr-CsCl density gradient centrifugation of pulse-labeled DNA from phage-infected cells. One sample of C600 r_k^- m_k^- was infected with λcol100 10 min after addition of CAM and pulse-labeled as described in the text at 25 min (A), 3 h (B), and 6 h (C). Another sample of C600 r_k^- m_k^- was infected in parallel with λgt-λC 10 min after addition of CAM and pulse-labeled at 25 min (D), 3 h (C), and 6 h (F). The form I position was n = 1.3918 (1.614 g/ml), and the upper band position was n = 1.3887 (1.579 g/ml).*

is similarly examined, there is some labeled chromosomal DNA which migrates heterogeneously; nonetheless, greater than one-half of the radioactivity appears in λ*col100* DNA restriction fragments (Fig. 6B). This material might be in the upper band position for several reasons; for example, some form I molecules might have become nicked during the sample preparation, or some partially replicated molecules may require more than 2 h to chase into covalently closed circular molecules. When the radioactively labeled DNA from the main band position of Fig. 5F was similarly examined after *Eco*RI cleavage, no phage-specific bands were observed in the fluorograph (Donoghue and Sharp, unpublished data), consistent with the CAM sensitivity of phage λ replication.

Thus, λ*col100* DNA is replicated even if the ColE1 replicon is introduced after the CAM block. Is this replication due to the slightly leaky synthesis of ColE1-encoded proteins? If so, then by infecting cells with λ*col100* 15 min before the CAM block, thereby permitting protein synthesis for a brief period of time, one might expect λ*col100* replication to proceed at an initially increased rate. Figure 7 presents two parallel sets of EtBr-CsCl gradients of λ*col100*-infected cells, which were pulse-labeled at 1, 2, and 4 h after addition of CAM. In Fig. 7A, B, and C, cells were infected 15 min before the CAM block, whereas in Fig. 7D, E, and F, cells were infected at 10 min after the CAM-block. At each of the times examined, a comparable fraction of the total pulse-labeled DNA appears in the form I position; this again demonstrates that λ*col100* replication occurs regardless of whether any protein synthesis is permitted.

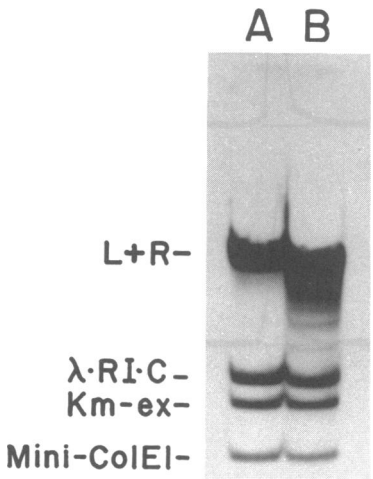

FIG. 6. *EcoRI digestion and fluorography of [³H]DNA from λcol100-infected cells pulse-labeled at 6 h. Form I and upper band peaks of the EtBr-CsCl gradient of Fig. 5C were pooled separately, as indicated, and digested with EcoRI after removal of ethidium bromide. An identical amount of radioactivity (about 2,000 cpm) was loaded onto each slot of a 1.4% agarose gel. (A) EcoRI digestion of DNA from the form I peak of the gradient shown in Fig. 5C. (B) EcoRI digestion of DNA from the upper band of the same gradient.*

DISCUSSION

ColE1 DNA appears to replicate by similar mechanisms in vivo and in soluble in vitro systems. For example, in both cases, replication is semiconservative and proceeds unidirectionally from a fixed origin of replication (7, 13, 20, 25, 32). In addition, rifampin-sensitive RNA synthesis is required in both systems (5, 25).

In vitro, ColE1 DNA can be added exogenously to an extract prepared from cells that do not carry ColE1, and replication appears to proceed normally in the absence of protein synthesis. The conclusion that ColE1 DNA replication in vitro depends entirely upon bacterial functions (31), however, appears to contradict the existence of temperature-sensitive mutants of ColE1 defective in replication (9, 15, 16). Several explanations may be invoked to resolve this apparent dilemma. For example, one well-characterized mutant of ColE1 ceases replication at the nonpermissive temperature. The lesion in this case resides in one of the proteins of the relaxation complex, for it has been shown that shifting to the nonpermissive temperature induces relaxation of the DNA-protein complex of this mutant (9). The existence of this mutant demonstrates that the relaxation-complex proteins may, under certain conditions, interfere with ColE1 DNA replication; nevertheless, it does not necessarily follow that these proteins are required for replication.

The experiments reported in this work clarify the above dilemma and suggest that, in vivo, there are no ColE1-encoded proteins essential for plasmid replication. Thus, replication of ColE1 DNA in the presence of CAM appears to require only host-specified proteins, which must be capable of recognizing various sites in the nucleotide sequence of the plasmid. There may, however, exist ColE1-encoded proteins that regulate copy number or otherwise modulate ColE1 DNA replication, and ColE1 may nevertheless encode proteins that are required for normal plasmid replication, though not required in the abnormal situation where protein synthesis is blocked.

The mechanism of ColE1 DNA replication is a separate question from that of the regulation of this replication. Several models have been

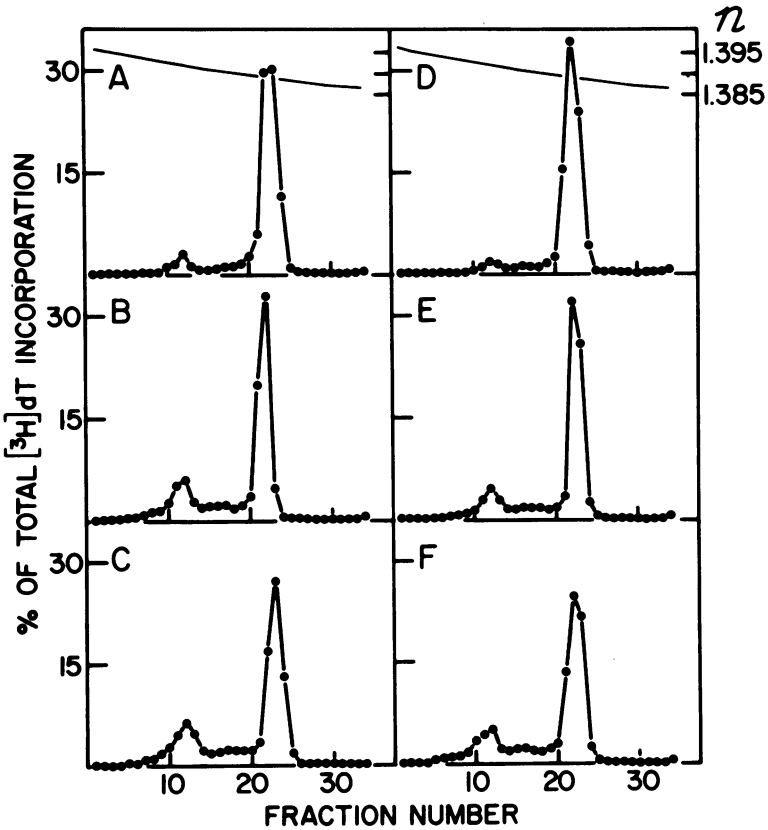

FIG. 7. *EtBr-CsCl density gradient centrifugation of pulse-labeled DNA from λcol100-infected cells. One sample of C600 r_k^- m_k^- was infected with λcol100 15 min before addition of CAM and pulse-labeled as described in the text at 1 h (A), 2 h (B), and 4 h (C). Another sample of cells was infected with λcol100 10 min after addition of CAM and pulse-labeled at 1 h (D), 2 h (E), and 4 h (F). The form I position was n = 1.3918 (1.614 g/ml), and the upper band position was n = 1.3887 (1.579 g/ml). Data is presented as the percentage of total incorporated radioactivity, which was 1.3 × 10^5 cpm for (A), 1.1 × 10^5 cpm for (B), 8.7 × 10^4 cpm for (C), 1.1 × 10^5 cpm for (D), 1.0 × 10^5 cpm for (E), and 6.0 × 10^4 cpm for (F).*

proposed to explain how plasmid copy number is controlled. Jacob et al. (14) postulated a mechanism for F-*lac* regulation whereby a positively acting "initiator" is produced in limiting amounts. This model has been previously challenged for ColE1 replication (6) and appears yet more unlikely given the ability of ColE1 to replicate in the absence of any ColE1-encoded proteins. A second regulatory model presupposes a negatively acting repressor compound that is diluted out during cell growth and division, thereby allowing a new cycle of plasmid replication. Indeed, this model has recently received support from a study of a pSC101-ColE1 composite plasmid, named pSC134 (6). One might adopt, however, a third model for the regulation of ColE1 replication whereby no ColE1-encoded proteins need be involved at all. By this model, replication would be controlled by limiting amounts of an essential host-specified protein. This model reduces to that of Jacob et al. (14), except that the structural gene controlling synthesis of the initiator would now reside in the chromosomal DNA rather than in the plasmid DNA.

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