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Isolation of Minicircular Deoxyribonucleic Acids from Wild Strains of *Escherichia coli* and their Relationship to other Bacterial Plasmids

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Supercoiled minicircular deoxyribonucleic acid (DNA) molecules with molecular weights of 1.8×10^6 and 2.3×10^6 have been isolated from two wild strains of *Escherichia coli*. DNA-DNA hybridization experiments indicate that these DNA molecules share extended homologies with the minicircular DNA of *E. coli* 15. The DNA of the colicinogenic factor E1 (ColE1) also hybridizes to a large extent with minicircular DNA of *E. coli* 15. In contrast, no hybridization could be detected with various large extrachromosomal DNA elements such as the colicinogenic factor V (ColV), the beta-hemolytic factor (Hly), or the P1-like DNA of *E. coli* 15. Two different insertion DNA species of *E. coli* integrated into λdg-DNA (λdg UP_{in} 128, λdg UP_{in} 308) do not show any annealing with minicircular DNA of *E. coli* 15.

Minicircular, supercoiled deoxyribonucleic acid (DNA) in bacteria was first isolated by Cozzarelli, Kelly, and Kornberg (7) from *Escherichia coli* 15 T^-. This plasmid DNA, whose function is completely unknown, seems to be common to all *E. coli* 15 strains. Meanwhile, DNA molecules with similar molecular weights have also been found in other bacteria: *Shigella paradysenteriae* (26), *Micrococcus lysodeikticus* (22), and *Enterobacter cloaceae* (28). There are no indications whether these small bacterial plasmids, which may carry the genetic information of about two to four genes, are related to each other and may code possibly for common proteins.

In this paper we describe the isolation of two other small plasmid DNA species from wild strains of *E. coli*. The molecular weight of these DNA species range between those of the minicircular DNA of *E. coli* 15 and the colicinogenic factor E1 (ColE1). Hybridization experiments indicate that all these small plasmid DNA species share extended homologous nucleotide sequences.

MATERIALS AND METHODS

Bacterial strains. *E. coli* 15 THU^-, *E. coli* JC411 (ColE1) and *E. coli* C600 (ColE1, V) (K30) were given to us by D. R. Helinski. *E. coli* SC78, *E. coli* SC79, and *E. coli* SC52 are wild strains which were isolated from ox intestines. These strains were kindly provided by P. Hummel.

Media and growth conditions. The two *E. coli* wild strains SC78 and SC79 were grown in phosphate-buffered minimal medium (9). *E. coli* 15 THU^- was cultivated in the same medium supplemented with 4 μg of thymine/ml, 20 μg of uracil/ml, and 40 μg of histidine/ml or in tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride-buffered minimal medium supplemented with the same substrates. All strains were grown at 37 C to a cell density of 5×10^8 cells/ml.

Labeling conditions. For labeling of the DNA of the wild strains, [*methyl*-^3H]thymidine (5 μCi/ml) was added to the culture medium in the presence of deoxyadenosine (250 μg/ml). *E. coli* 15 THU^- was labeled with [*methyl*-^3H]thymidine (5 μCi/ml) or with carrier-free $H_3{}^{32}PO_4$ (20 μCi/ml).

Counting of radioisotopes. Samples were added to squares of filter paper and washed successively with cold 10% trichloroacetic acid, 95% ethanol, and ether. The dried filters were immersed in scintillation vials containing 10 ml of a scintillation mixture [5 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene in 1 liter of toluene] and counted in an Intertechnique SL30 or SL40 liquid scintillation counter.

Sources of reagents and DNA species. The radioisotopes, [*methyl*-^3H]thymidine and $H_3{}^{32}PO_4$, were purchased from The Radiochemical Centre, Amersham (GB). Cesium chloride was obtained from Merck AG (Germany), ethidium bromide from Calbiochem, and ficoll (approximate molecular weight, 400,000) and polyvinylpyrrolidone (molecular weight 360,000) from Sigma Chemical Co. (St. Louis). High-molecular-weight salmon sperm DNA was obtained from EGA-Chemie (Germany). The two insertion

DNA species, λdg UP_{in} 128 and λdg UP_{in} 308, and λdg DNA were kindly provided by Dr. Saedler.

Isolation of extrachromosomal DNA. Supercoiled plasmid DNA was isolated as previously described (15). To obtain larger amounts of extrachromosomal DNA for hybridization, the bacteria were grown in 500-ml cultures to the log phase. The cells were harvested by centrifugation (10,000 × *g*; 10 min; 4 C; J 21-centrifuge, Beckman) and suspended in 7 ml of 25% sucrose in 0.01 M Tris-hydrochloride buffer, *p*H 7.4. Lysis of the cells was performed with lysozyme (50 μg/ml) and Brij 58 (0.5% final concentration). The viscous lysate was centrifuged at 8,000 × g for 10 min at 4 C (J21 centrifuge, Beckman). The clear supernatant fluid (10 ml) was extracted twice with a mixture of 10 ml of phenol and 5 ml of chloroform, to remove the protein. The aqueous layer, containing most of the extrachromosomal DNA and the remainder of the chromosomal DNA was directly subjected to a dye-buoyant density centrifugation (25). The mixture contained 10 ml of phenol-extracted supernatant fluid, 3 ml of ethidium bromide solution (1 mg/ml), 14.6 g of cesium chloride, and 2 ml of ^{3}H-labeled cleared lysate of the same bacteria grown in a 30-ml culture and labeled with ^{3}H-thymidine. The solution was thoroughly mixed and placed in a polyallomer tube. The remainder of the tube was filled with mineral oil and centrifuged in a Spinco Ti60 rotor at 44,000 rev/min and 20 C for 48 hr (Spinco L2-65B). Fractions (0.4 ml) were collected from the bottom of the tube in small test tubes. The fractions containing the supercoiled DNA were pooled and dialyzed against dilute SSC (0.015 M NaCl, 0.0015 M sodium citrate, *p*H 7.0) and 0.005 M ethylenediaminetetraacetic acid (EDTA). The DNA was further purified by centrifugation through a linear sucrose gradient.

Sucrose gradient centrifugation conditions. Sucrose gradient centrifugations were performed in a Spinco L50 or L2-65B ultracentrifuge at 45,000 rev/min and 20 C by using a SW50 or a SW65 swinging-bucket rotor. For the purification of larger amounts of DNA, a SW25.1 rotor was used, and centrifugation was carried out at 20,000 rev/min and 20 C. Samples were layered on top of 5 to 20% (5 ml) or 15 to 50% (30 ml) linear sucrose gradients in TES buffer (0.05 M Tris-hydrochloride, 0.05 M NaCl, 0.005 M EDTA, *p*H 8.0). Alkaline sucrose contained 0.2 M NaOH and 0.7 M NaCl.

DNA-DNA hybridization. DNA-DNA hybridization was performed according to the membrane filter method of Denhardt (8). DNA solutions, containing 2 μg of ^{3}H-labeled supercoiled DNA per ml, were adjusted to 0.1 N NaOH and heated for 15 min at 100 C. This procedure opens most of the supercoiled DNA and denatures the relaxed DNA molecules (3). The samples were quickly cooled in ice, neutralized with 0.1 N HCl in 12 × SSC (1.8 M NaCl, 0.18 M sodium citrate, *p*H 7.0) and diluted with ice-cold 6 × SSC to the desired concentration. DNA in a final volume of 5 ml was slowly applied to membrane filters (HAWG, 0.45 μm, Millipore Corp.), which were prewashed with 6 × SSC. The filters were washed with 5 ml of 6 × SSC and dried at room temperature for 4 to 8 hr, then for 12 hr in a desiccator, and finally for 2 hr in a vacuum oven. Each filter was preincubated for 6 hr at 65 C in a vial containing 2 ml of a mixture of 0.02% ficoll and 0.02% polyvinylpyrrolidone in 3 × SSC. Filters were then incubated for 22 hr at 65 C with 1.2 ml of the annealing mixture containing 10 μg of salmon sperm DNA per ml and ^{32}P-labeled minicircular DNA of *E. coli* 15. This DNA was degraded by sonic treatment (5 amp, 5 × 30 sec, Branson sonic oscillator with microprobe) to DNA fragments with an average molecular weight of 5×10^5 and denatured by heating for 15 min at 100 C in 6 × SSC, by the method of Denhardt (8). The denatured sample was quickly cooled in ice. The desired concentration was adjusted by diluting with 6 × SSC. After annealing, the filters were slowly washed on both sides with 40 ml of 6 × SSC, and dried. The filters were placed in scintillation vials, and the radioactivity was measured.

Electron microscopy. DNA samples containing 1 to 2 μg of DNA per ml were diluted 10-fold with 0.02% cytochrome *c* solution (in 1 M ammonium acetate, *p*H 6.0). One-milliliter samples of these DNA solutions were mixed with 0.05 ml of 0.25% formaldehyde solution and spread onto a water surface. Grids were prepared by the method of Kleinschmidt (21). Electron microscopy was performed with a Siemens I electron microscope. Photographs of the circular DNA molecules were enlarged, and the contour lengths were measured.

RESULTS

Isolation and characterization of minicircular supercoiled DNA from wild strains of E. coli (SC78 and SC79). Two wild strains of *E. coli* (SC78 and SC79), isolated from ox intestines, do not exhibit any character of known extrachromosomal inheritance, like colicinogeny, drug-resistance, or hemolysin production. Phage production is not observed upon induction with UV light or mitomycin C.

Cells of these strains, grown to the log phase, were gently lysed with lysozyme and Brij 58 (5). Most of the chromosomal DNA (>95%) was pelleted by centrifugation (8,000 × *g*; 15 min; 4 C). The cleared lysates, which contain the extrachromosomal DNA and the rest of the chromosomal DNA, were centrifuged to equilibrium in the presence of the dye ethidium bromide. Figures 1A and 2A demonstrate that the DNA in both lysates is separated into two bands with different buoyant densities which are characteristic of supercoiled, and linear or open circular DNA. The fractions of the denser band containing supercoiled DNA were further analyzed on sucrose gradients under neutral and alkaline conditions to differentiate between supercoiled and open circular DNA (29).

These analyses revealed the presence of unusual small supercoiled DNA molecules in both *E. coli* strains. *E. coli* SC78 contains one

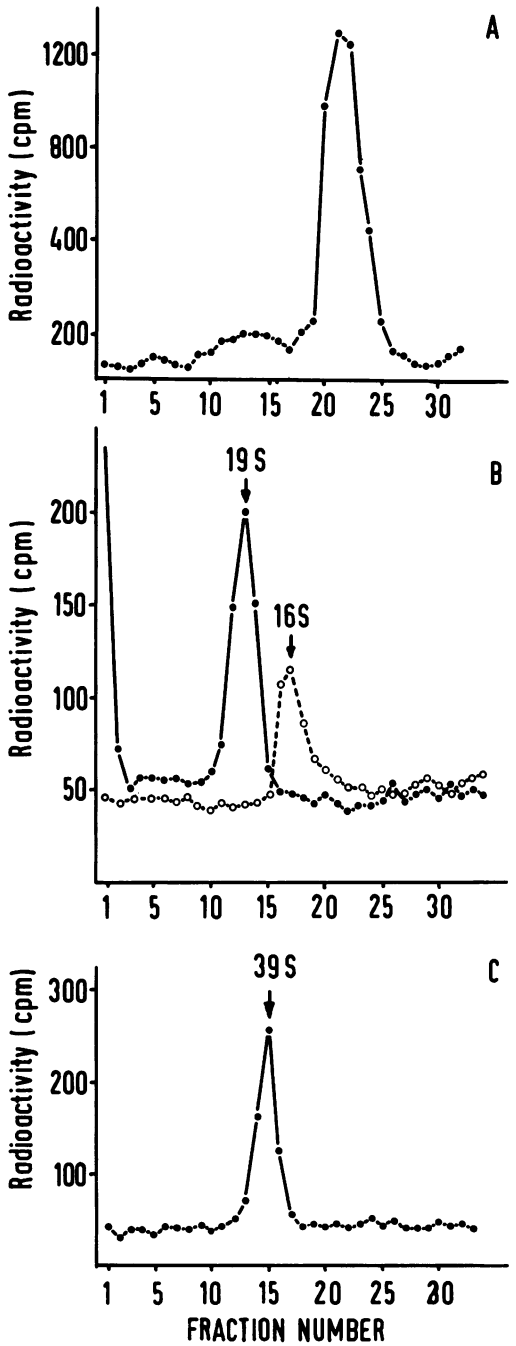

FIG. 1. *A, Dye-buoyant density centrifugation of a cleared lysate of E. coli SC78. A culture (30 ml) of this strain was grown in the presence of ^{3}H-thymidine to the log phase. A cleared lysate was prepared by the lysozyme-Brij 58 procedure and centrifuged, after addition of CsCl and ethidium bromide, for 16 hr at 2 C and 44,000 rev/min in a Spinco fixed-angle rotor type 50. Fractions (15 drops) were collected from the bottom of the tube in small vials. A sample of each fraction (0.02 ml) was spotted on filter discs and assayed for radioactivity as described in Materials and Methods. B, Sucrose gradient analysis of the supercoiled DNA of E. coli SC78. Supercoiled DNA obtained from the fractions of the heavy satellite band of the cesium chloride-ethidium bromide equilibrium centrifugation was dialyzed as described in Materials and Methods. A portion (0.2 ml) was layered on a neutral 5 to 20% sucrose gradient and centrifuged for 210 min (at 20 C; 45,000 rev/min; Spinco SW65). ^{32}P-labeled minicircular DNA of E. coli 15 was used as internal marker. Fractions (10 drops) were collected from the bottom of the tube directly on filter squares, which were assayed for radioactivity as described in Materials and Methods. Symbols: ●, ^{3}H-labeled supercoiled DNA of E. coli SC78; ○, ^{32}P-labeled minicircular DNA of E. coli 15. C, Alkaline sucrose gradient analysis of the supercoiled DNA of E. coli SC78. A portion (0.2 ml) of the dialyzed supercoiled DNA fraction was layered on an alkaline 5 to 20% sucrose gradient and centrifuged for 90 min at 20 C and 45,000 rev/min in a Spinco SW50 rotor. Fractionation was carried out as described in Fig. 1B. Supercoiled ^{3}H-labeled ColE1 DNA was used as external marker.*

species of extrachromosomal DNA with a sedimentation coefficient of 19*S*, related to the supercoiled minicircular DNA of *E. coli* 15 THU^{-} (16*S*) as an internal marker (Fig. 1B). As expected for a DNA molecule with supercoiled conformation, the *S* value increases to 39*S* when the sedimentation is performed under alkaline conditions (Fig. 1C).

The most prominent supercoiled DNA of *E. coli* SC79 sedimenting at 17*S* under neutral (Fig. 2B) and at 33*S* under alkaline conditions (Fig. 2C) represents supercoiled DNA molecules with a low molecular weight.

The amount of minicircular DNA in *E. coli* SC78 is 0.15% of the total DNA, which corresponds to about two copies per chromosome, whereas minicircular DNA in SC79 amounts to roughly 0.5 to 0.8% or 6 to 10 copies per chromosome. These calculations represent a minimal number of copies since we cannot exclude some loss of small extrachromosomal DNA with the isolation procedure used.

Both strains SC78 and SC79 contain in addition to the minicircular DNA larger supercoiled DNA molecules of unknown function. These plasmids will not be further described here. In the sucrose gradients shown in Fig. 1B and 2B, these DNA molecules pellet at the bottom of the gradient.

Electron microscopy supports the results, described above, of the molecular properties of these DNA molecules. As shown in Fig. 3, supercoiled and open circular DNA molecules with contour lengths of 0.95 ± 0.05 μm and 1.2

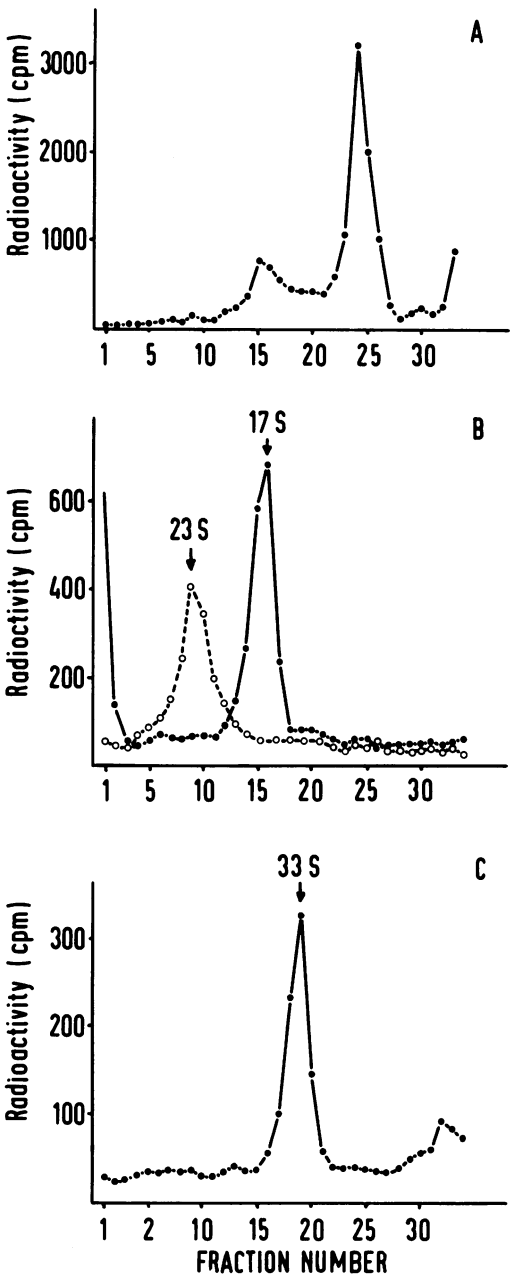

FIG. 2. *A, Fractionation of a cleared lysate of E. coli SC79 after cesium chloride-ethidium bromide equilibrium centrifugation. The preparation of the lysate and the centrifugation were performed as described in Fig. 1A. B, Neutral sucrose gradient centrifugation of supercoiled DNA of E. coli SC79. The supercoiled DNA was purified by dye-buoyant density centrifugation and dialyzed. Centrifugation conditions were the same as described in Fig. 1B. ^{32}P-labeled ColE1 DNA (23S) was used as internal marker. Symbols: ●, ^{3}H-labeled supercoiled DNA of E. coli SC79; ○, ^{32}P-labeled supercoiled ColE1 DNA. C, Alkaline sucrose gradient analysis of supercoiled DNA of E. coli SC79. The centrifugation was performed as described in Fig. 1C. Supercoiled ^{3}H-labeled ColE1 DNA was used as external marker.*

± 0.05 μm are observed. The molecular weights are calculated as 1.8×10^6 for the minicircular DNA of *E. coli* SC79 and 2.3×10^6 for the minicircular DNA of *E. coli* SC78. [Calculations of the molecular weights are based on the sedimentation-coefficients and the contour lengths of these DNA molecules (6). The values given above represent an average of both determinations.]

Hybridization of minicircular DNA of E. coli SC78 and SC79 with minicircular DNA of E. coli 15. DNA molecules with sizes similar to those reported here have been previously isolated from *E. coli* 15 strains (7, 23). It was therefore of considerable interest to examine whether these different minicircular DNA species are related in their nucleotide sequences. To detect sequence homologies, DNA-DNA hybridization was performed with the purified minicircular DNA of *E. coli* SC78 and SC79 and purified minicircular DNA of *E. coli* THU$^-$ by the method of Denhardt (8). Supercoiled minicircular DNA of strains SC78 and SC79 was opened and denatured as previously described (3) by heating the DNA solution for 15 min at 100 C in the presence of 0.1 N NaOH. This treatment converts practically all of the supercoiled DNA to open circular DNA as demonstrated by sucrose gradient analysis. After the heat treatment, the DNA solution was neutralized with 0.1 N HCl to *p*H 7.0 as described above. The minor amount of DNA which remains in the supercoiled conformation after this treatment passes through the filter during the filtration and washing procedure. Increasing amounts of the denatured ^{3}H-labeled minicircular DNA of strains SC78, SC79, and 15 THU$^-$ were fixed on filters, which were incubated with constant amounts of heat-denatured ^{32}P-labeled minicircular DNA of *E. coli* 15 THU$^-$ by the method of Denhardt (8). The latter DNA was degraded by sonic treatment to DNA fragments sedimenting at about 8*S*. Denaturation of the fragmented minicircular DNA by alkali with subsequent neutralization yielded the same results (Table 1).

Figure 4 shows the hybridization saturation curves obtained. At saturation, 50% of the ^{32}P-labeled minicircular DNA of *E. coli* 15, bound to ^{3}H-labeled minicircular DNA of *E. coli* 15, fixed on the membrane filter. This value was taken as 100% homology, and the amount of

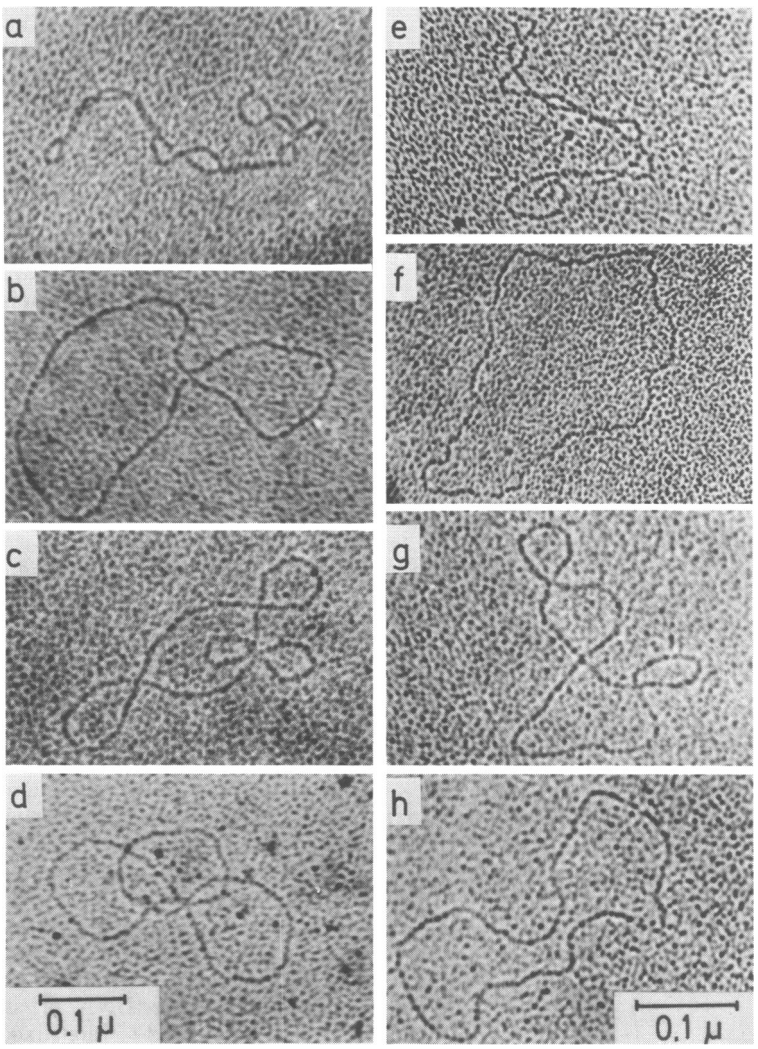

FIG. 3. *Electron micrographs of supercoiled and open circular molecules of minicircular DNA extracted from E. coli SC78 (a–d) and from E. coli SC79 (e–h).*

hybridization of ^{32}P-minicircular DNA with the other DNA species was related to this value (Fig. 4 and 5; and Tables 1 and 2). Minicircular DNA of *E. coli* 15 yielded a large amount of hybridization with the minicircular DNA species of *E. coli* SC78 and SC79. According to the saturation plateaus, as much as 45% of the minicircular DNA of *E. coli* 15 shows homology with the small DNA of strain SC78, whereas as much as 30% anneals with minicircular DNA of strain SC79. (Fig. 4). Virtually no hybridization was observed when chromosomal DNA of *E. coli* K-12 or 15 THU$^-$ was fixed on the filter (Table 1). The slightly higher amount of hybridization with chromosomal DNA *E. coli* 15 THU$^-$ may be due to some contamination with minicircular DNA.

Hybridization of minicircular DNA of E. coli 15 with ColE1 DNA and larger plasmid DNA species. To examine whether the high degree of hybridization of minicircular DNA of *E. coli* 15 with the isolated small DNA of strains SC78 and SC79 is a particular property of these minicircular DNA species or may be common to all or most of the extrachromosomal DNA elements, DNA-DNA hybridization experiments were performed with several other purified plasmid DNA species. Among those tested [P1-like plasmid DNA of *E. coli* 15 (19), ColE1 DNA (1), ColV (K30) DNA (11),

TABLE 1. *Hybridization of minicircular DNA of E. coli 15 with various large plasmid DNA species and with chromosomal DNA*

Source of DNA	Amount of DNA fixed on the filter (μg)	^{32}P-labeled minicircular DNA of *E. coli* 15		^{32}P bound after subtraction of ^{30}background (counts/min)	Relative binding (%)
		2×10^5 counts per min per μg	10^5 counts per min per μg		
Minicircular DNA of *E. coli* 15	3	10,000		4,703	100
Hly DNA of *E. coli* SC52	5[b]	10,000		20	0.42
ColV (K30) DNA of *E. coli* C600 (ColV, E)	8[b]	10,000	3	24	0.51
P1-like DNA of *E. coli* 15	4[b]	10,000		27	0.57
Salmon sperm DNA	50	10,000		24	0.51
	100	10,000		21	0.44
Chromosomal[c] DNA of *E. coli* K-12	32	10,000		25	0.53
	68	10,000		19	0.4
Chromosomal[c] DNA of *E. coli* 15	47	10,000		120	2.5
Minicircular DNA of *E. coli* 15	2		3,000	1,610	100
	0.5		3,000	1,417	87.5
	0.12		3,000	920	57.0
ColE1 DNA of *E. coli* JC411 (ColE1)	4		3,000	585	36.2
	1		3,000	379	23.5
	0.25		3,000	191	11.8
Minicircular DNA of *E. coli* SC78	2		3,000	717	43.9
	1		3,000	597	37.0
	0.5		3,000	463	28.7
Minicircular DNA of *E. coli* SC79	1.2		3,000	453	28.1
Chromosomal DNA of *E. coli* K-12	70		3,000	20	1.24

[a] The DNA was sonically treated to 8*S* fragments. The solution was adjusted to 0.1 N NaOH and heated for 5 to 10 min at 100 C. The samples were quickly cooled in ice and neutralized with 0.1 N HCl in 12 × SSC. Annealing was performed as described in Fig. 4.

[b] DNA-DNA hybridization was performed as described in Fig. 4. The values represent the highest amount of the corresponding plasmid DNA species fixed on the filter in the hybridization experiment shown in Fig. 4.

[c] DNA-DNA hybridization was performed as described in Fig. 4. Phenol-extracted DNA was purified on neutral sucrose gradients. For the hybridization experiment, the DNA sedimenting between 30 to 60*S* was taken.

and Hly DNA (15)] only ColE1 DNA showed a high degree of hybridization with minicircular DNA of *E. coli* 15 THU$^-$ (Fig. 5 and Table 1). The hybridization saturation curve in Fig. 5 indicates that as much as 40% of the nucleotide sequence of minicircular DNA of *E. coli* 15 may be homologous to ColE1 DNA.

Failure of minicircular DNA of E. coli 15 to hybridize with insertion DNA of E. coli. Strong polar mutations in the galactose operon in *E. coli* have been described and characterized both genetically and physiologically (24, 27). These mutations arise through the random insertion of foreign DNA into a structural gene, which may exist in the host in a plasmid state. Since the size of the insertion DNA is similar to that of minicircular DNA, we have examined whether a relationship exists between these DNA species. Two different insertion DNA species (λdg PU_{in} 128 and λdg PU_{in} 308) were hybridized, as described, with minicircular DNA of *E. coli* 15. As shown in Table 2, no annealing is observed between these insertion DNA species and minicircular DNA of *E. coli* 15.

To determine the limits of detection of hy-

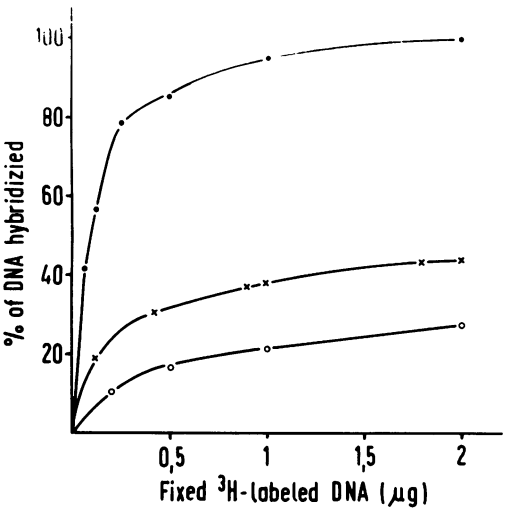

FIG. 4. *DNA-DNA hybridization of ^{32}P-labeled minicircular DNA of E. coli 15 with various fixed amounts of ^{3}H-labeled minicircular DNA of E. coli 15 (●), of E. coli SC78 (×), and of E. coli SC79 (○). Annealing was performed as described in Materials and Methods in a total volume of 1.2 ml containing approximately 0.05 µg of ^{32}P-labeled DNA (2 × 10^{5} counts per min per µg of DNA). The percent values are normalized as described in the text.*

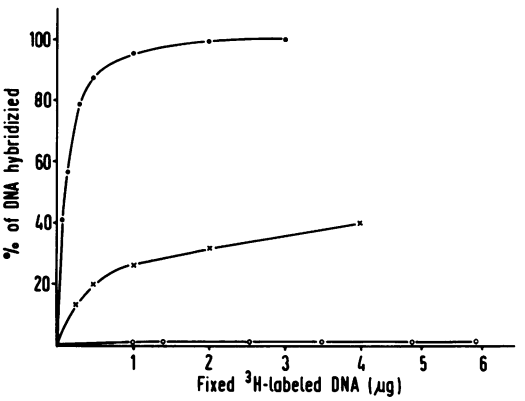

FIG. 5. *DNA-DNA hybridization of ^{32}P-labeled minicircular DNA extracted from E. coli 15 THU^{-} with fixed ^{3}H-labeled minicircular DNA of E. coli 15 THU^{-} (●), with ColE1 DNA from E. coli JC411 (ColE1) (×), and with ColV (K30) DNA (○). (Hybridization of minicircular DNA of E. coli 15 with Hly DNA and P1-like DNA gave essentially the same values as shown for ColV [K30] DNA). Annealing was performed as described in Fig. 4. Percent values are normalized as described in the text.*

bridization of this procedure, a reconstruction experiment was performed. Denatured ^{3}H-labeled ColE1 DNA and unlabeled chromosomal DNA of *E. coli* K-2 at a weight:weight ratio of 5,000:1 were fixed on a filter and hybridized with ^{32}P-labeled ColE1 DNA as described. Table 3 indicates that the degree of hybridization was about the same regardless whether the large excess of chromosomal DNA was present or not. This result demonstrates that this procedure should be capable of detecting one or a few copies of minicircular DNA even in the larger DNA species. (Weight:weight ratios are about 2,000:1 for one copy of minicircular DNA per chromosome of *E. coli*, and about 25:1 for one copy of minicircular DNA per λdg insertion DNA.)

TABLE 2. *Hybridization of minicircular DNA of E. coli 15 with two insertion DNA species*[a]

Source of DNA	Amount of DNA fixed on the filter (µg)	Input ^{32}P-labeled minicircular DNA (10^{5} counts per min per µg)	^{32}P bound after subtraction of 30 background (counts/min)	Relative binding (%)
Minicircular DNA of *E. coli* 15 THU^{-}	2	6,500	1,000	100
λ dg	1	6,500	30	3.0
λ dg UP$_{in}$ 128	1	6,500	45	4.5
λ dg UP$_{in}$ 308	1	6,500	28	2.8

[a] DNA-DNA hybridization was performed as described in Fig. 4. Each value represents an average of two hybridization experiments.

TABLE 3. *Hybridization of ColE1 DNA in the presence of a large excess of chromosomal DNA of E. coli K-12 with ColE1 DNA*

Source of DNA	Amount of DNA fixed on the filter (µg)	Input ^{32}P-labeled DNA (1.6 × 10^{4} counts per min per µg	^{32}P bound[a] after subtraction of background (counts/min)
Chromosomal DNA of *E. coli* K-12	188	4,000	34
ColE1 DNA of *E. coli* JC411 (ColE1)	0.04	4,000	340
ColE1 DNA and chromosomal DNA of *E. coli* K-12	0.04 + 188	4,000	400

[a] Each value represents an average of three hybridization experiments.

[b] Thirty counts per minute were subtracted as background counts.

DISCUSSION

Minicircular DNA species represent interesting genomes since their genetic information is limited to a very small number of genes. The minicircular DNA of *E. coli* 15 with a molecular weight of 1.45×10^6 (23) may contain about three to four genes, the functions of which are completely unknown. The small plasmid DNA species described here have been isolated from two different wild strains of *E. coli*. The molecular weights of these new minicircular DNA species are somewhat higher than that of minicircular DNA *of E. coli* 15 (1.8×10^6 and 2.3×10^6, respectively.) However, hybridization experiments with these plasmid DNA species have revealed a large extent of homology between them. The extended sequence homology between these DNA molecules may indicate common function(s) or a common origin, or both, for these small plasmid DNA species. Several models may be considered for the formation of such small plasmids.

(i)Disintegration of certain chromosomal genes may result in the formation of circular DNA. This DNA, once disintegrated, could replicate as an autonomous plasmid, when the disintegrated genes contain the information for function(s) necessary to maintain a state of autonomous replication. The opposite event of insertion of small DNA into the chromosomal DNA seems to occur in *E. coli* (24, 27). Since no homologies seem to exist between the minicircular DNA of *E. coli* 15 and chromosomal DNA, the formation of the described minicircular DNA by disintegration appears unlikely. Likewise, no relationship seems to exist between the various insertion DNA species and the minicircular DNA of *E. coli* 15.

(ii) Amplification of certain genes of the chromosome could also result in the formation of small DNA molecules which may circularize. Amplification of ribosomal genes is well established in various systems (4, 10, 12). However hybridization of minicircular DNA of *E. coli* 15 with 16*S* and 23*S* ribosomal DNA has failed to demonstrate such a function (23). The origin of *E. coli* 15 minicircular DNA as an event due to gene amplification has been further weakened by the failure of minicircular DNA to hybridize with *E. coli* chromosomal DNA.

(iii) A third possibility is that minicircular DNA species originate from larger plasmids by eliminating part of the genes, leaving behind mainly those which are essential for maintaining an autonomous state of replication. In this connection it is interesting to notice the high degree of sequential relationship between minicircular DNA of *E. coli* 15 and the larger ColE1 DNA, whereas no such similarities are observed between minicircular DNA and several other large plasmids. Previous studies on the mode of replication of these two plasmids have also indicated a close relationship. (i) Both plasmids contain several copies per chromosome [10–15 for ColE1 (18) and about 15 for minicircular DNA (23)]. (ii) They share a common mode of replication, i.e., some of the copies are replicated twice, while an equal number of copies is not replicated at all during one generation time (2, 17). (iii) They are replicated semiconservatively even at the restrictive temperature in certain temperature-sensitive replication mutants of *E. coli* (13, 17). (iv) There are indications that DNA polymerase I may be involved in the maintenance or replication of both plasmids (16, 20, and *unpublished results*). (v) Plasmid DNA replication mutants of an *E. coli* strain containing both plasmids have been isolated which show a strict modulation in the replication of both plasmids (W. Goebel and W. Schroen, *unpublished data*). In all these respects minicircular DNA and ColE1 DNA are different from the larger plasmids, which are present in the cell in one or a few copies per chromosome. With these latter plasmids ColE1 DNA and the minicircular DNA species do not seem to share any nucleotide sequence homologies. It is therefore tempting to speculate that the sequential relationship between minicircular DNA and ColE1 DNA may represent a common gene(s) involved in the replication of the DNA of these plasmids which is absent on the DNA of the other plasmids.

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