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Complex ColE1 DNA in *Escherichia coli* and *Proteus mirabilis*

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Summary. Incubation of the colicinogenic *Escherichia coli* strain JC 411 (ColE1) at elevated temperatures (47–49°) leads to the accumulation of catenated molecules and replicative intermediates of this plasmid. Mature supercoiled ColE1 DNA molecules synthesized under these conditions have an increased number of tertiary turns as shown by electron microscopy. The monomeric tightly supercoiled molecules possess a slightly slower sedimentation rate and a higher binding capacity for ethidium bromide than supercoiled monomers synthesized at lower temperatures. Recombination deficient mutants of *E. coli recA*, *recB* and *recC*, which carry the ColE1 plasmid, form about the same amount of catenated molecules at the elevated temperature as a rec$^+$ strain.

In addition, we have observed by electron microscopy a small percentage (~5% of the circular DNA molecules) of minicircular DNA molecules in all preparations of JC 411 (ColE1). They are homogenous in size, with a molecular weight of 1.4×10^6 daltons.

Addition of chloramphenicol to a culture of *Proteus mirabilis* (ColE1) leads to an increased amount of higher multiple circular oligomers and to a stimulated accumulation of catenated ColE1 DNA molecules of varying sizes. ColE1 DNA synthesis is more thermosensitive than chromosomal DNA replication in *P. mirabilis*. Plasmid replication stops completely at temperatures above 43°C.

Introduction

In cells containing extrachromosomal small genetic systems, like bacteriophages, animal viruses, mitochondria, chloroplasts or bacterial plasmids, often DNA structures, termed oligomeric or complex forms, were found in addition to the monomeric circular unit (for a summary, see Helinski and Clewell, 1971). These forms are either circular or catenated oligomeric DNA molecules. This DNA may be formed by recombination, (Hudson and Vinograd, 1967; Rush and Warner, 1968) by replication (Goebel and Helinski, 1968; Nass, 1969, 1970; Goebel, 1971; Meinke and Goldstein, 1971; Jaenisch and Levine, 1972; Brack *et al.*, 1972) or by a combination of both events (Benbow *et al.*, 1972; Jaenisch and Levine, 1973). With the bacterial plasmid ColE1, we have previously shown that the formation of circular multiple forms can be enhanced in *Proteus mirabilis* by the inhibition of protein synthesis (Goebel and Helinski, 1968). Similar observations have been made with mitochondrial DNA (Nass, 1969, 1970) virus DNA (Jaenisch and Levine, 1972; Bourgaux, 1973) and phage DNA (Benbow *et al.*, 1972). In *Escherichia coli* inhibition of protein synthesis has a negligeable effect on the formation of complex ColE1 DNA molecules. Such forms are however synthesized in *E. coli* in various temperature-sensitive DNA replication mutants (*dnaB*, *C*, *E*), which are able to maintain at least in part a semiconservative synthesis of the ColE1 plasmid at the restrictive temperature (Goebel, 1970, 1971;

Goebel, 1972). More recently we have found that wild strains of *E. coli* carrying the colicinogenic factor E1 (ColE1) accumulate preferentially replicative and catenated DNA molecules when the cultures are kept at elevated temperatures (Goebel and Kreft, 1972).

We report here the results of further investigations on the effect of elevated temperatures and chloramphenicol on the formation of complex ColE1 DNA molecules in *E. coli* and *P. mirabilis*.

Materials and Methods

Bacterial Strains and Growth Conditions

E. coli JC 411 (ColE1) and *P. mirabilis* (ColE1) have been previously described (Goebel and Kreft, 1972; Goebel and Helinski, 1968). Recombination-deficient mutants *E. coli* JC 2926 *recA13*, JC 5743 *recB21* and JC 5489 *recC22* were kindly provided by Dr. T. J. Foster.

Transfer of ColE1 plasmid into the rec-mutants were performed with HfrH (ColE1) as donor strain. Colicinogenic recipients were counterselected on agar plates containing 100 μg/ml streptomycin and 200 units colicin E1/ml.

Cultures of the strains were grown in phosphate-buffered minimal medium containing in 1 l solution; 1.0 g NH_4Cl, 1.5 g KH_2PO_4, 3.5 g Na_2HPO_4, 5 g casamino acids (Difco), 0.2 g $MgSO_4$, 2 g glucose and 0.2 g thiamine.

Broth contained 8 g nutrient broth (Difco), 5 g bactopeptone (Difco), 1.5 g KH_2PO_4, 3.5 g Na_2HPO_4, 5 g NaCl and 1 g glucose. Broth plates were made up to 1,5% with Bacto-agar (Difco).

Isolation of Plasmid DNA

Plasmid DNA from *E. coli* cells was extracted by the lysozyme/Brij 58 procedure (Clewell and Helinski 1969; Goebel 1970). Plasmid DNA from *P. mirabilis* was extracted by a modified procedure of Vapnek and Rupp, 1971. Cells from a 20 ml culture were resuspended in 0.1 M Tris · HCl, pH 8.0, containing 25% sucrose, and treated at room temperature for 10 min with lysozyme (1 mg/ml) and 0.05 M EDTA (ethylendiaminetetraacetic acid-disodiumsalt) pH 7.5. The mixture was cooled in ice and made 0.5% on sodiumdodecylsulfate. After 10 min, solid CsCl was added to the viscous lysate to a concentration of 1 M CsCl. The whole mixture was allowed to stand in ice for at least 10 h. Then it was centrifuged at 20.000 × *g* for 30 min (4°C). The supernatant containing most of the plasmid DNA is referred to as cleared lysate. The pellet was discarded. Plasmid DNA was further isolated by CsCl-ethidium bromide equilibrium centrifugation (Radloff, Bauer and Vinograd, 1967) and separated on sucrose gradients as previously described (Goebel, 1971).

Ultracentrifugation Procedures

The experimental conditions used in neutral sucrose gradients are described in the legends to the Figures. CsCl equilibrium centrifugation in the presence of ethidium bromide was performed according to Radloff *et al.*, 1967, using the Spinco Ti 50 rotor. Centrifugation was carried out for 18–20 h at 44000 rpm and 2°C.

Electron Microscopy

The grids for the electron microscopy were prepared by the technique of Kleinschmidt and Zahn, 1959. DNA-containing fractions from the CsCl-ethidium bromide gradient were pooled. The dye was removed by extraction with isopropanol. CsCl and residual isopropanol were removed by dialysis against diluted (0.1) SSC (0.015 M sodium citrate, 0.15 M NaCl). The dialyzed fractions were diluted 1:5 with 0.02% cytochrome C in 1 M ammonium acetate. The hypophase was bidistilled water. The grids were positively stained with uranylacetate, rotary

shadowed with platinum-palladium (Pt-Pd) and examined in a Siemens Elmiskop 1 (magnification 20000 ×). Contour lengths were measured on large scale prints.

Sources of Materials

Lysozyme and ethidium bromide were obtained from Merck (Germany). ^{3}H-thymidine (specific ativity 23 Ci/mmol) was purchased from the Radiochemical Centre (Amersham, England).

Results

1. ColE1 DNA Structures Formed at Elevated Temperatures in JC 411 (ColE1)

We have previously demonstrated that the replication of the small non-transmissible plasmid, colicinogenic factor E1 (ColE1) continues at elevated temperatures of 45–49° C, whereas the replication rate of the bacterial chromosome decreases rapidly above 45° C and stops completely above 47° C (Goebel and Kreft, 1972). Fig. 1 shows two interesting properties of the ColE1 DNA synthesized at these elevated temperatures. First, the buoyant density in CsCl-ethidium bromide of the supercoiled DNA (band I) decreases with increasing temperature, which indicates that the superhelix density (Eason and Vinograd, 1971) of the supercoiled ColE1 DNA synthesized under these conditions increases. Second, above 45° C an increasing amount of a DNA species is synthesized, which bands in the CsCl-ethidium bromide gradient at an intermediate buoyant density (band II).

Sucrose gradient centrifugation of the DNA of band I under neutral conditions shows the appearance of faster sedimenting DNA species at 47–49° C with sedimentation coefficients (31 S, 37 S) corresponding to supercoiled dimeric and trimeric ColE1 DNA (Fig. 2). Electron microscopy of the DNA of band I reveals above 47° C, the presence of mainly monomeric supercoiled molecules with an increasing number of superhelical turns (Fig. 3), which is highest at 49° C.

The supercoiled dimeric and trimeric molecules seen are most likely catenates consisting of interlocked tightly twisted supercoiled monomers, as no open circular dimer or trimer ColE1 DNA molecule could be observed in these preparations. It is interesting to notice that the supercoiled monomeric ColE1 DNA isolated from a 49° C culture sediments slightly slower than a supercoiled marker DNA synthesized at 30° C (Fig. 2). The high superhelical density probably causes a rather rigid, rod-like shape of these molecules, which may cause the observed decrease in the sedimentation rate.

A detailed analysis of the DNA of band II by sedimentation centrifugation on neutral sucrose gradients and electron microscopy (Goebel and Kreft, 1972) has revealed that this band contains mainly replicative intermediates with two untwisted branches and one twisted branch (Jaenisch *et al.*, 1971 b; Fuke and Inselburg, 1972) and catenated molecules with interlocked supercoiled and open circular rings. Most if not all of these catenates consist of two or more unit length molecules (Fig. 4). No higher circular oligomer as part of these catenates was observed. There is still a substantial amount of monomers in this band with a high number of tertiary turns, which suggests that the superhelix density of the supercoiled ColE1 DNA synthesized at the elevated temperature may be rather heterogeneous.

It has been shown in various instances that the inhibition of protein synthesis leads to a stimulated synthesis of circular and catenated oligomeric molecules

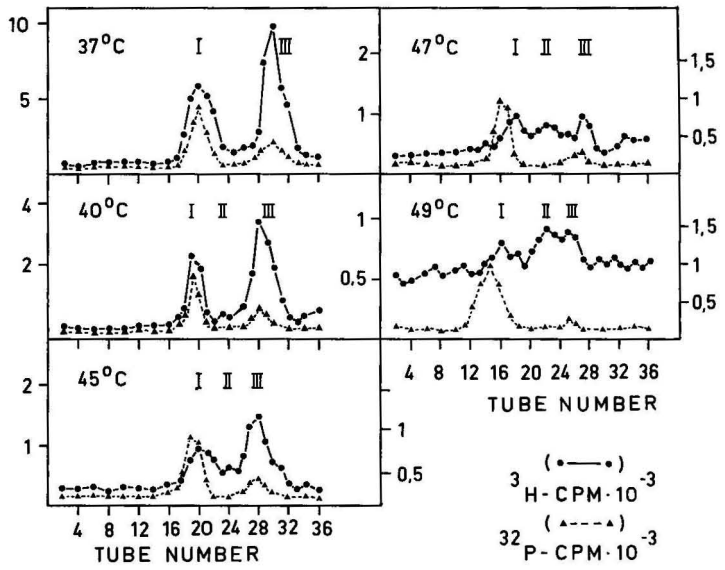


Fig. 1. Analysis of ColE1 DNA synthesized at various temperatures by dye-buoyant density centrifugation. A culture of *E. coli* JC 411 was grown to a density of 2–3 × 10^8 cells/ml at 30° C. Then it was divided into five equal parts which were incubated for 1 h at the indicated temperatures and labeled during this time with [Me-^{3}H]-thymidine (10 μCi/ml). ColE1 DNA was extracted by the lysozyme-Brij procedure (Clewell and Helinski, 1969). Cleared lysates were centrifuged to equilibrium in CsCl-ethidium bromide gradients. ^{32}P-labeled supercoiled DNA isolated from a culture of JC 411 grown at 30° C was added to each gradient as an internal marker. Fractions (10 drops) were collected from the bottom of the centrifuge tubes in small vials. Aliquots (20 μl) of these fractions were taken for determining the radioactivity as described (Goebel, 1970)

(Goebel and Helinski, 1968; Nass, 1969, 1970; Jaenisch and Levine, 1972; Bourgaux, 1973). To examine whether the inhibition of protein synthesis by chloramphenicol may further stimulate the formation of higher oligomeric ColE1 DNA molecules in *E. coli* at elevated temperatures, we have added the drug in concentrations of 20–200 μg/ml for 5 h to a culture of JC 411 (ColE1) which was kept at 49° C. Whereas low concentrations of the antibiotic (20–80 μg/ml) do not change the pattern of the replication products synthesized at 49° C, higher concentrations of the drug cause a drastic inhibition of the incorporation of ^{3}H-thymidine into ColE1 DNA, without significantly affecting the qualitative composition of the replication products.

2. Synthesis of Complex ColE1 DNA in Recombination Deficient Mutants at Elevated Temperatures

The incubation of a culture of JC 411 (ColE1) at 49° favors the formation of catenated DNA molecules without generating substantial amounts of multiple circular molecules. It has been claimed that both structures may not be synthesized by the same mechanism (Benbow, Eisenberg and Sinsheimer, 1972; Jaenisch and Levine, 1973).

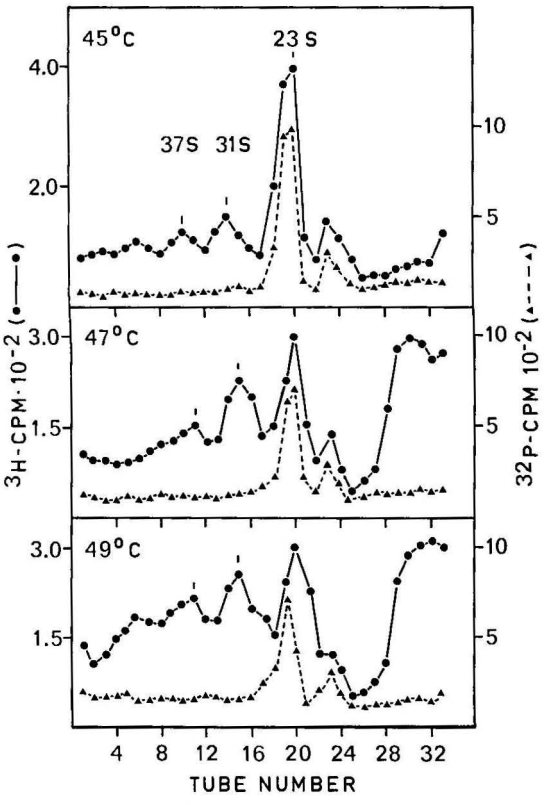


Fig. 2. Sucrose gradient analyses of the supercoiled DNA (band I) synthesized at 45°C, 47°C and 49°C. Fractions with the highest buoyant density (band I) of the CsCl-ethidium bromide gradients from lysates of cells grown at 45°C, 47°C and 49°C were pooled. The dye was removed by repeated extractions with isopropanol. The fractions were then extensively dialyzed to remove CsCl and the excess of isopropanol. Samples were layered on top of neutral 5–20% sucrose gradients and centrifuged at 45000 rev/min for 2 h at 20°C. Fractions (10 drops) were directly collected from the bottom of the tubes on filter paper squares

To examine whether recombination may be responsible for the generation of catenated forms at the elevated temperature, we have transferred the ColE1 factor into *recA*, *recB* and *recC* mutants and analyzed the ColE1 DNA synthesized in these mutants at 49°C by CsCl-ethidium bromide centrifugation and on neutral sucrose gradients. Fig. 5A-C, demonstrates that all three mutants yield ColE1 DNA at 49°C which forms three bands with different buoyant densities in an CsCl-ethidium bromide gradient. The DNA peak I bands at a slightly lower buoyant density than supercoiled ^{32}P-ColE1 DNA added as an internal marker. It represents mainly supercoiled monomeric ColE1 DNA with increased tertiary turns and some tightly supercoiled catenated dimers. The DNA peak II can be resolved on a neutral sucrose gradient into 2–3 DNA species, which besides monomeric supercoiled DNA (23 S) probably represent catenated molecules with one supercoiled molecule and one open molecule (27 S, Wang, 1970), with one super-

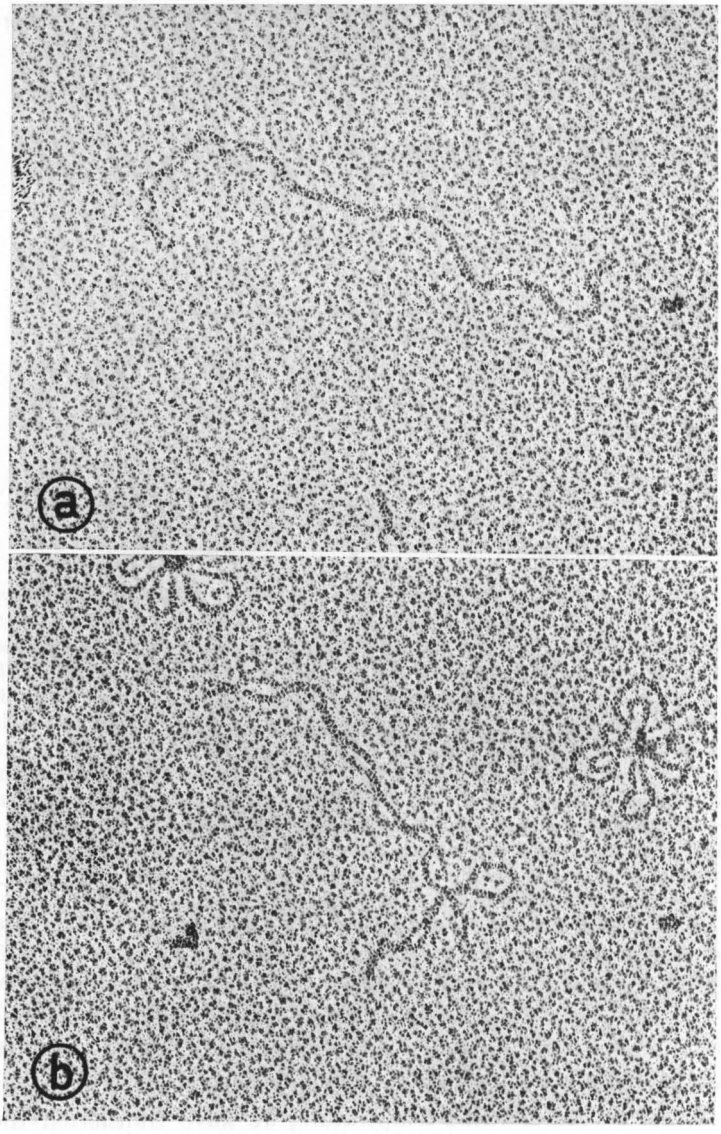


Fig. 3a—d. Electronmicrographs of ColE1 DNA molecules from *E. coli* JC 411. a and b grown at 49° C. c and d grown at 47° C. The bar represents 0.5 μm

coiled molecule and two open circular molecules (33 S) and with two supercoiled molecules and one open circular molecule (36 S). The DNA peak III contains mostly catenates with open circular molecules and some monomeric open circular molecules.

The generation of these complex molecules is basically the same in all three recombination deficient mutants. The minor quantitative differences in the pat-

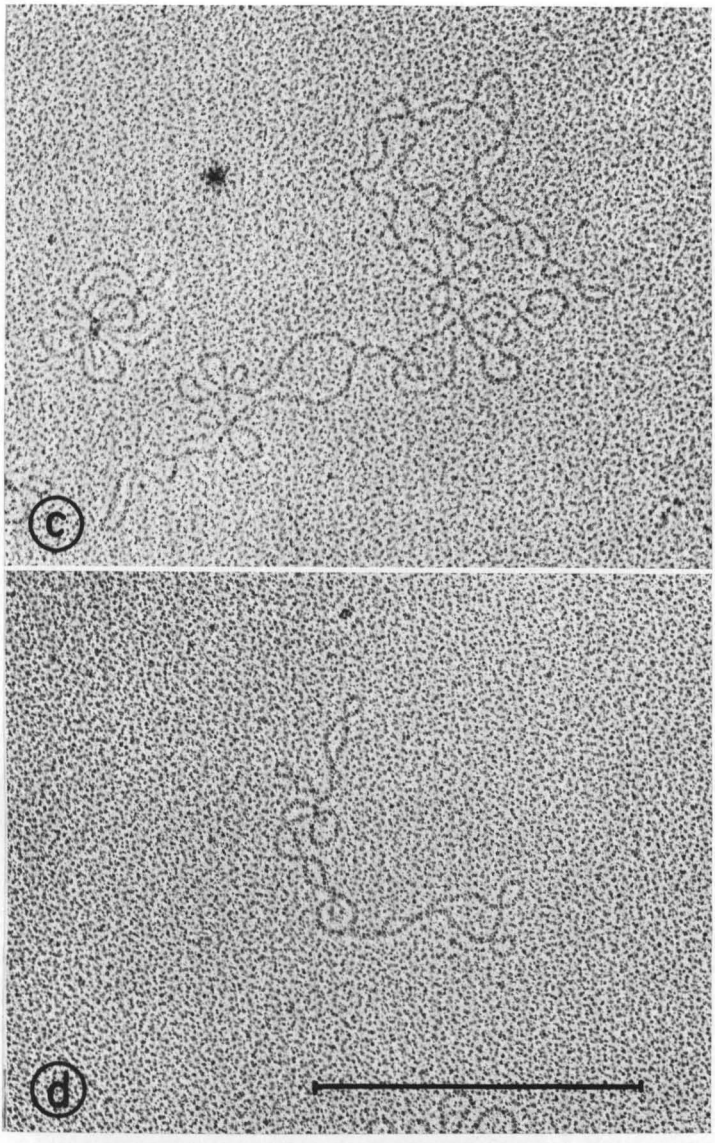
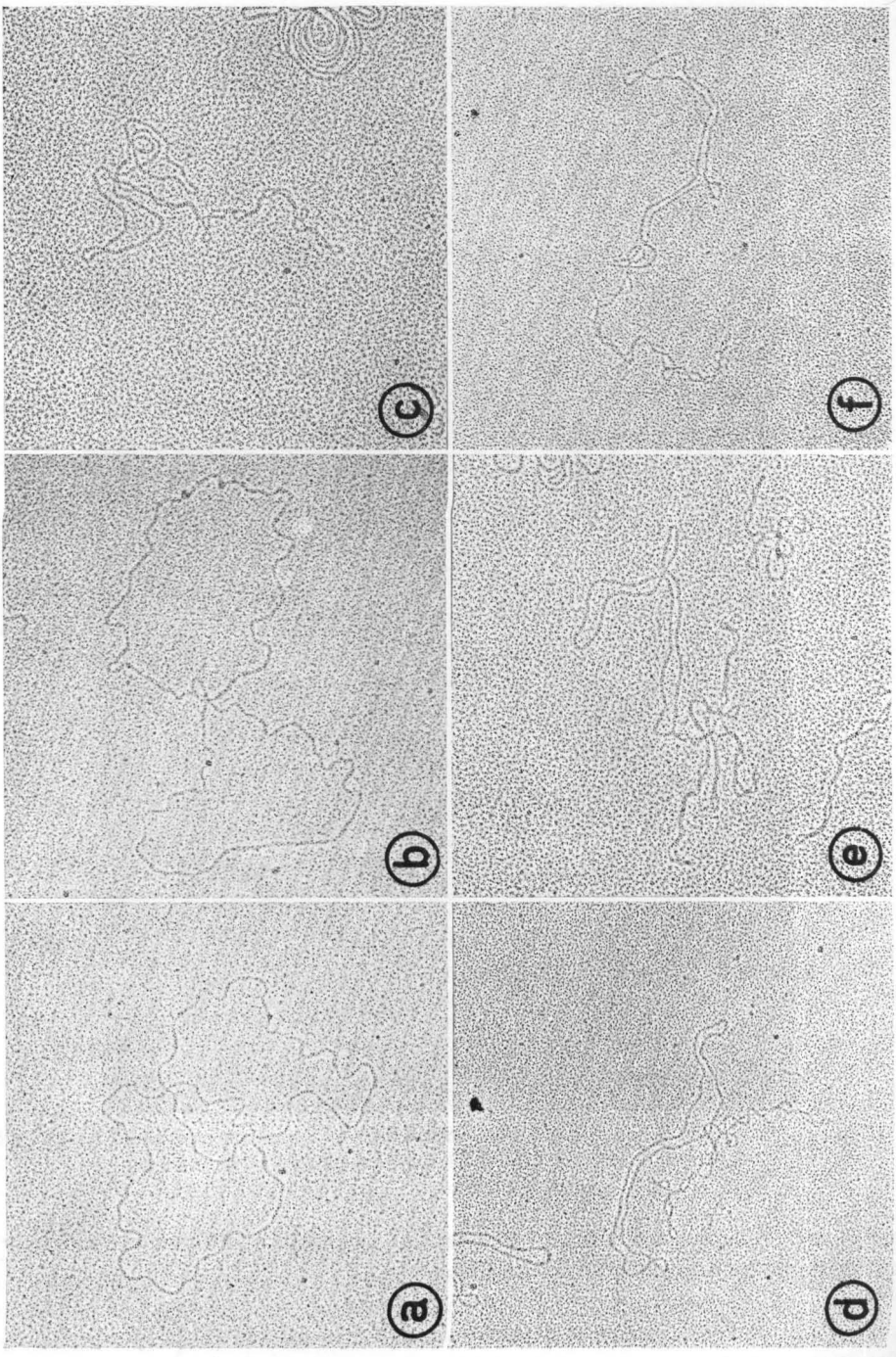


Fig. 3 c and d

terns do probably not indicate substantial qualitative differences but may rather represent experimental fluctuations.

3. Identification of Minicircular DNA in JC 411 (ColE1)

During the investigation of the ColE1 DNA preparations from JC 411 (ColE1) by electron microscopy we have observed in all DNA samples obtained from the high-buoyant-density and the intermediate-density bands of the CsCl-ethidium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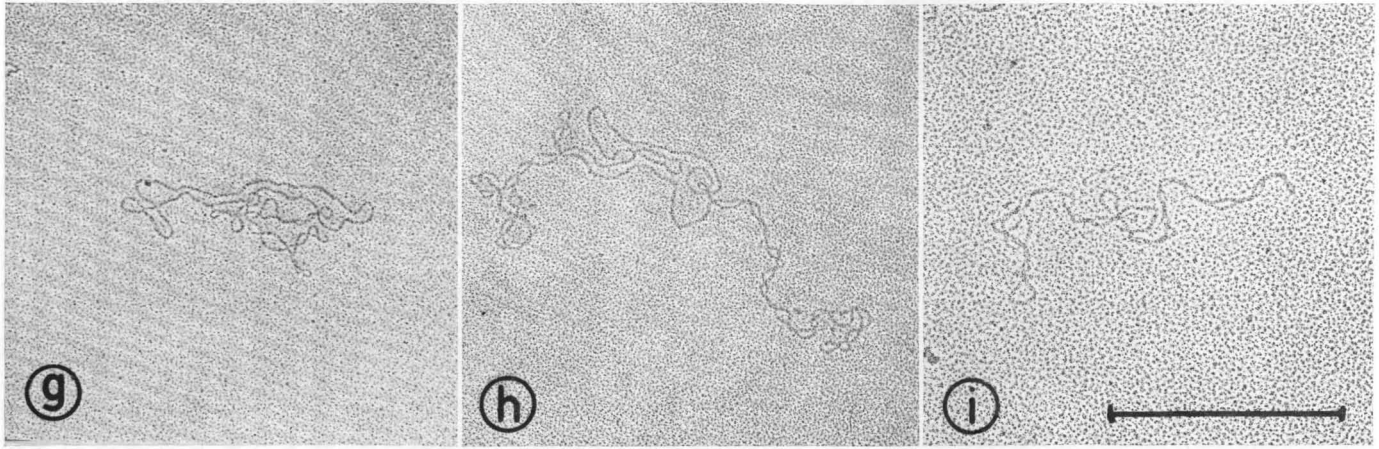


Fig. 4a—i. Electronmicrographs of catenated ColE1 DNA from *E. coli* JC 411 consisting of a and b two open monomers, c—g one open circular and one supercoiled monomeric molecule, h one open circular and two supercoiled monomers, i two supercoiled monomeric parts. The bar represents 1 μm

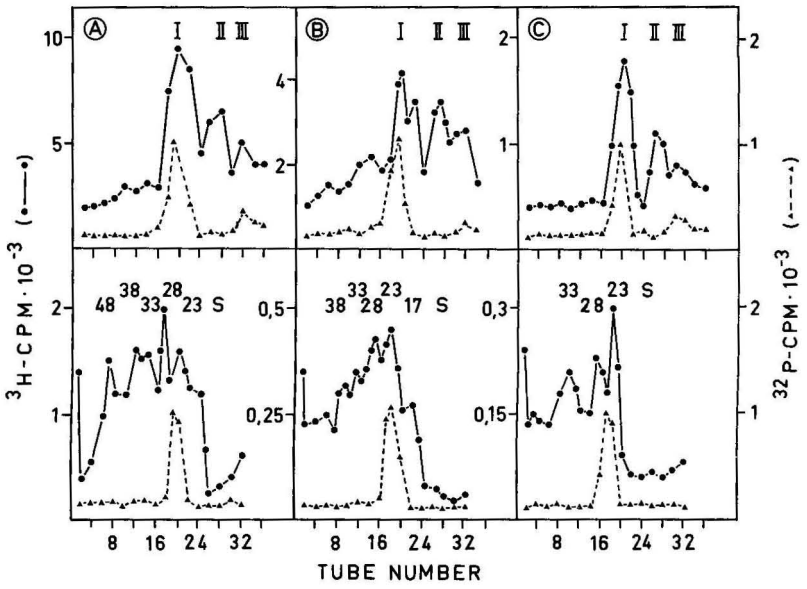


Fig. 5A—C. Analysis of ColE1 DNA, synthesized at 49°C in three recombination-deficient mutants of *E. coli*, by dye-buoyant-density centrifugation and sucrose gradient centrifugation. Cultures of JC 2926 *recA* (ColE1), JC 5743 *recB* (ColE1) and JC *recB* (ColE1) were grown at 30°C to 2–3 × 10^8 cells/ml and then incubated further for 1 h at 49°C. Cultures were labeled during this time with ^{3}H-thymidine (30 μCi/ml). ColE1 DNA was extracted and centrifuged in CsCl-ethidium bromide gradients as described in Fig. 1 (top). The DNA fractions with intermediate buoyant density (band II) were further analyzed on neutral 5–10% sucrose gradients as described in Fig. 2 (bottom). (A) JC 2926 *recA*; (B) JC 5743 *recB*; (C) JC 5489 *recC*

bromide gradients, a small percentage of DNA molecules, which are considerably smaller in size than ColE1 DNA (Fig. 6). They are rather homogenous in size with a mean contour length of 0.7 μ, which corresponds to a molecular weight of 1.4 × 10^6 daltons. They represent roughly 5% of the total number of circular molecules in the supercoiled fraction. We do not believe that these molecules are microscopical artefacts, since we have observed besides open circular and supercoiled forms a few molecules which have the structure of replicative intermediates, (Fig. 6) which indicates that these molecules are replicating entities.

In addition, we have carefully analyzed a preparation of supercoiled DNA from JC 411 (ColE1) cells grown at 30°C on alkaline sucrose gradients. (Under neutral conditions the expected sedimentation value for these small supercoiled DNA molecules of 16–17 S (Bazaral and Helinski, 1968) would be identical with the open circular form of ColE1 DNA). As shown in Fig. 7 there is indeed a small, but well reproducible peak at around 36 S between the fast sedimenting supercoiled ColE1 DNA (48 S) and the denatured ColE1 DNA (16–17 S). This peak amounts also to about 5% of the total supercoiled DNA. This sedimentation coefficient agrees quite well with the value expected for a supercoiled DNA molecule of this size under alkaline conditions.

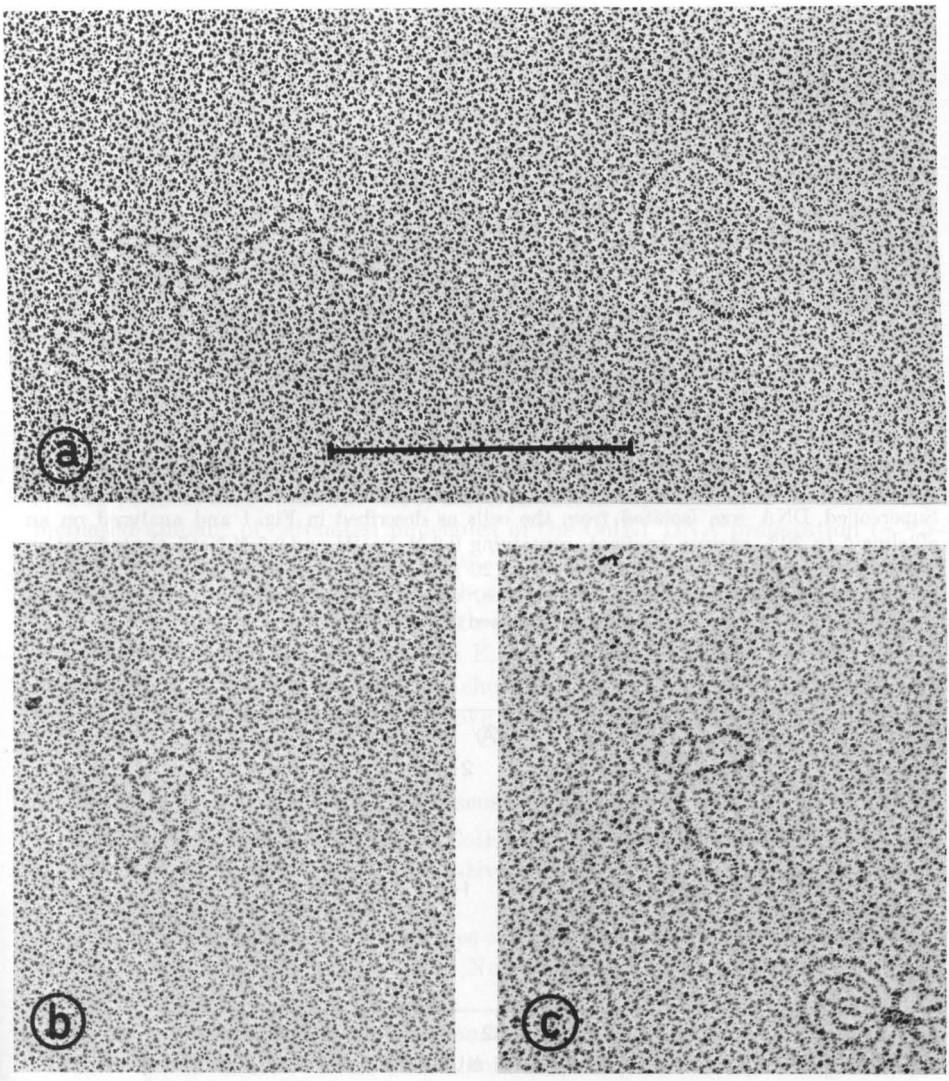


Fig. 6a—c. Electronmicrographs of minicircular DNA from *E. coli* JC 411 (ColE1) a open circle. At the left side supercoiled ColE1 DNA, b supercoiled form, c replicative form of minicircular DNA. The bar represents 0.5 μm

4. Formation of Complex DNA Molecules in P. mirabilis

In *P. mirabilis*, ColE1 DNA exists as multiple circular DNA forms under normal growth conditions (Bazaral and Helinski, 1968). In addition, it has been shown that the inhibition of protein synthesis in *Proteus mirabilis*, carrying the ColE1 factor, leads to an increased accumulation of these forms (Goebel and Helinski, 1968). The existence of catenated molecules in *P. mirabilis*, has not been shown in these studies. If, however, catenated DNA molecules are connected with DNA replication as suggested by the previous data, one may expect to find more

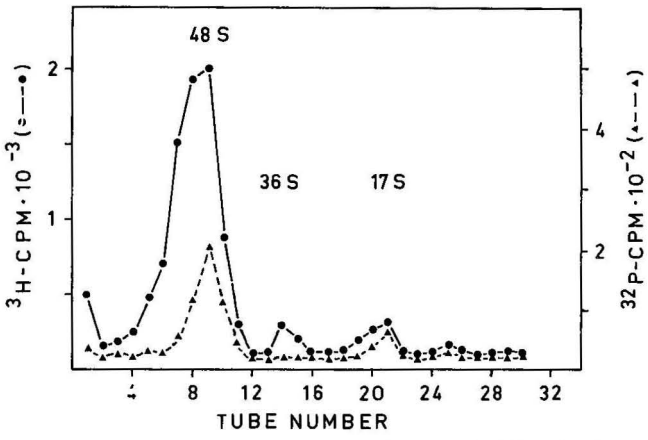


Fig. 7. Alkaline sucrose gradient of supercoiled DNA of JC 411 (ColE1) grown at 37°C. A culture of JC 411 (ColE1) was grown at 37°C and labeled with ^{3}H-thymidine (5 μCi/ml). Supercoiled DNA was isolated from the cells as described in Fig. 1 and analyzed on an alkaline 5 to 20% sucrose gradient, containing 0.2 M NaOH and 0.7 M NaCl. Centrifugation was carried out for 90 min at 45000 rev/min (20°). Fractions were collected from the bottom of the tube directly on filter paper squares, which were counted in a liquid scintillation counter as described (Goebel, 1970)

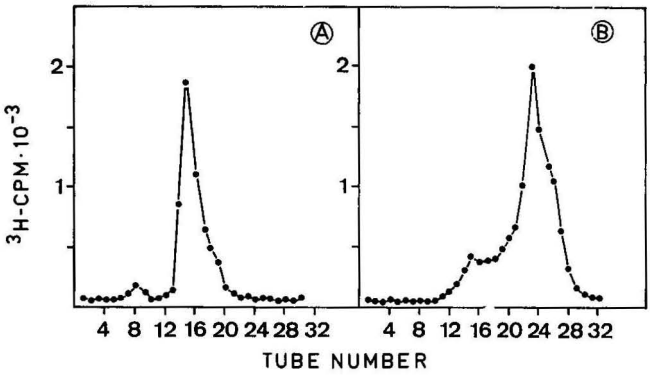


Fig. 8A and B. Analysis of ColE1 DNA, synthesized in the presence and absence of chloramphenicol, in *Proteus mirabilis* (ColE1) by dye-buoyant density centrifugation and sucrose gradient centrifugation. A culture of *P. mirabilis* (ColE1) was grown at 37°C to 5×10^8 cells/ml. Then it was divided into two equal parts. One part was kept at 37°C and labeled with ^{3}H-thymidine (10 μCi/ml) for 1 h. To the other part, chloramphenicol (200 μg/ml) was added. The treated culture was labeled with ^{3}H-thymidine (10 μCi/ml). ColE1 DNA was extracted by a modified procedure of Vapnek and Rupp, 1971 (see Materials and Methods) and further purified by dye-buoyant density centrifugation. *Top:* (A) without chloramphenicol, (B) with chloramphenicol

complex catenated ColE1 DNA molecules as possible replicative intermediates of the circular oligomers in this microorganism. To test, whether such catenated ColE1 DNA molecules are present in *P. mirabilis* (ColE1) cells and whether their

formation is enhanced by a stop of protein synthesis, we have prepared cleared lysates of *P. mirabilis*, untreated and treated with chloramphenicol (150–200 μg/ml) by a modified procedure of Rupp and Vapnek, 1971, and analyzed them by equilibrium centrifugation in CsCl-ethidium bromide gradients. Although there is no apparent intermediate band in the CsCl-ethidium bromide gradient (Fig. 8A) of the untreated lysate, which would indicate the presence of catenated molecules with an open circular and a supercoiled partner, we have pooled fractions 6–13 and examined them by electron microscopy. This revealed the presence of catenates of varying composition (Fig. 9). About 1.5% of the total cellular DNA is ColE1 DNA. The amount of catenates in this DNA population is about 8–10%. *P. mirabilis* cells were treated with 200 μg/ml chloramphenicol for 14 h at 30°C, and a cleared lysate was analyzed by CsCl-ethidium bromide centrifugation as before (Fig. 8B). Fractions 12–20 were pooled and analyzed on neutral sucrose gradient and by electron microscopy. Whereas a sucrose gradient analysis showed an increased amount of higher sedimenting forms in agreement with previous results (Goebel and Helinski, 1968) electron microscopy revealed in addition an increased amount of catenated molecules in the ColE1 DNA preparation (~15–20% of the total ColE1 DNA) (Fig. 9). Thus the inhibition of protein synthesis leads to an increased production of higher multiple rings and catenated molecules.

Since elevated temperatures lead to a pronounced increase in the formation of catenated ColE1 DNA molecules in E. *coli*, we have performed similar experiments with *P. mirabilis*. However, as shown in Table 1, ColE1 DNA synthesis is severely inhibited at temperatures above 43°C and stops faster than the chromosomal DNA synthesis.

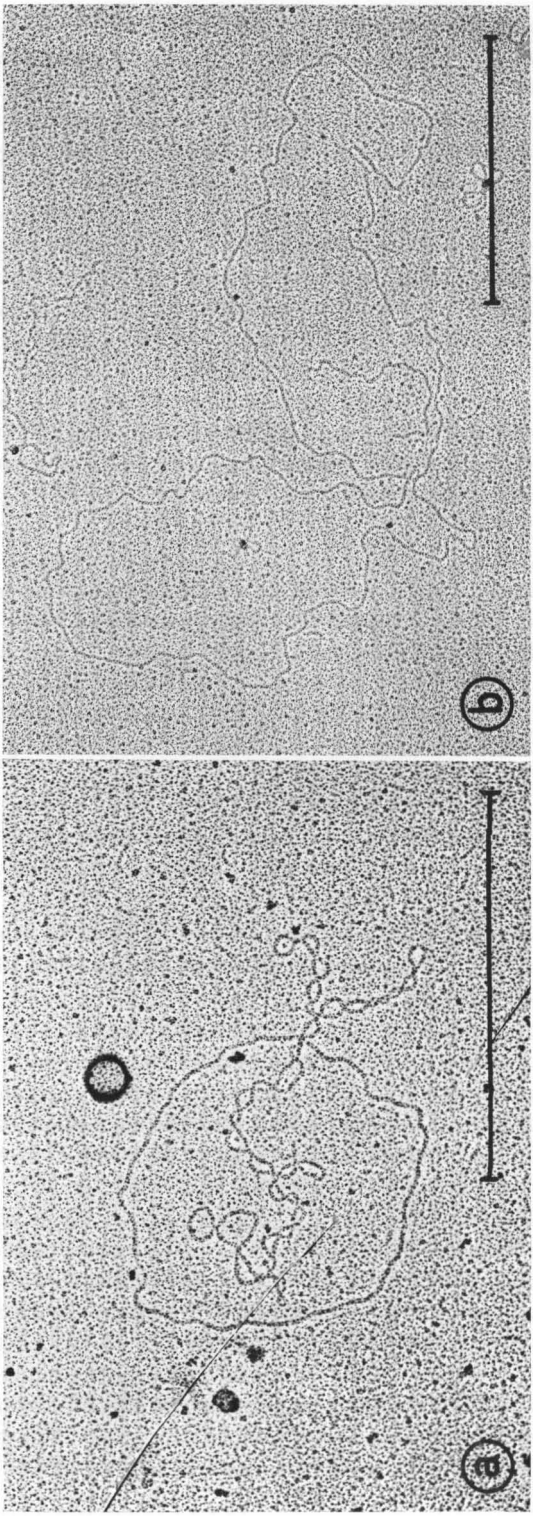
Discussion

Synthesis of the bacterial plasmid ColE1 in *Escherichia coli* at elevated temperatures of 48–49°C leads to two qualitative changes in the plasmid DNA which is produced:

1. A high percentage of the molecules consists of catenates, which are composed of two or more unit length molecules. No substantial amount of multiple length circular oligomers is formed.

2. The supercoiled molecules show an anomalous high number of tertiary turns.

Presumably an alteration of the balance of various factors regulating the replication and the structure of ColE1 DNA leads to conditions which favor the formation of these structures. The observation that the formation of the catenates is independent of the recombination functions *recA*, B and C favors the interpretation, that the generation of these forms is caused by DNA replication. The formation of catenated molecules by replication has been claimed for many genetic systems (Nass, 1969, 1970; Goebel, 1971; Meinke and Goldstein, 1971; Jaenisch and Levine, 1972, 1973; Bourgaux, 1973) although there is so far only circumstantial evidence for this assumption. It remains to be seen whether the formation of the supercoiled ColE1 DNA molecules at 49°C, which have an unusual high number of tertiary turns, is caused solely by the temperature. Although ColE1 DNA molecules undergoing ring closure at elevated temperatures might well have a higher superhelical density, it is also conceivable that the superhelical density of the intracellular DNA is altered as a result of the effects of the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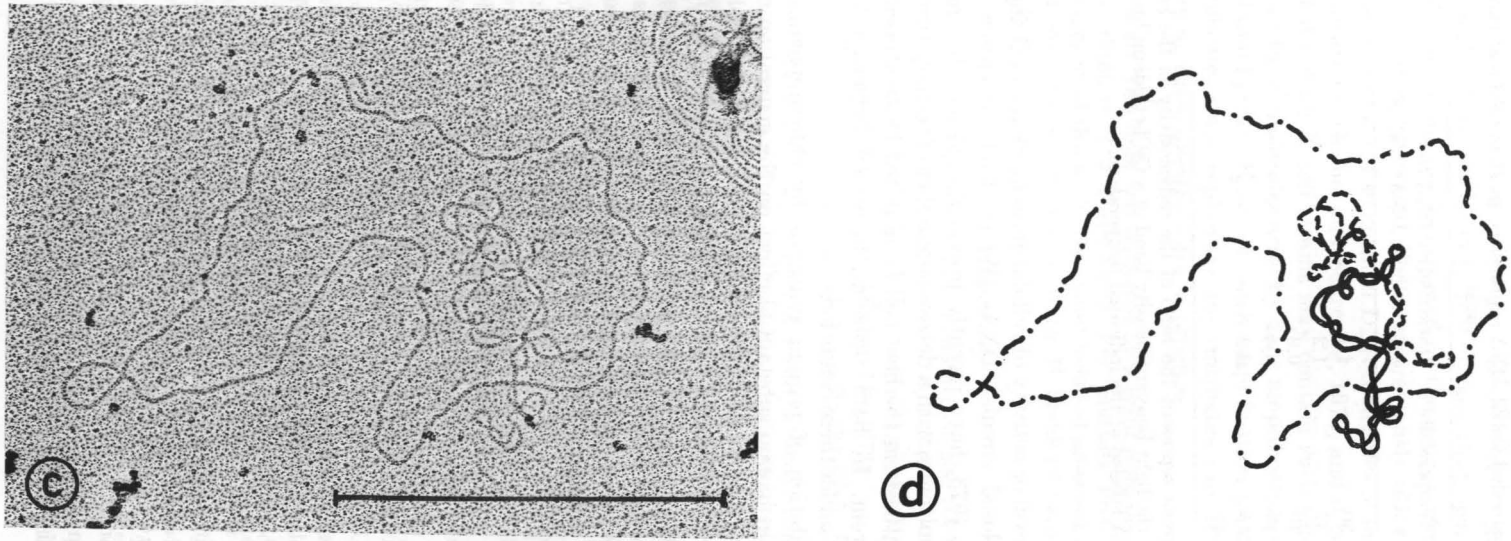

Fig. 9a—d. Electronmicrographs of catenated ColE1 DNA from *P. mirabilis* (ColE1) treated with chloramphenicol (100 μg/ml). a Open circular monomer and supercoiled dimer, b two open circular dimers, c two supercoiled monomers and one open circular dimer, d schematic representation of the complex molecule from c. The bar represents 1 μm

Table 1. Amount of supercoiled ColE1 DNA synthesized in *P. mirabilis* at elevated temperatures

Temperature (° C)	Ratio: ColE1 DNA to total cellular DNA[a] (%)
30	1.1
37	1.2
40	0.9
43	0.6
45	0.2
47	—

[a] The percent values given represent the ratio of the radioactivity in the heavy bouyant-density band to that of the light bouyant-density band of a CsCl-ethidium bromide gradient of the total cellular DNA labeled at the indicated temperature.

temperature on the level or activity of cellular proteins that can change the superhelical density of closed circular DNAs (Wang, 1971). Jaenisch and Levine, 1973 and Bourgaux, 1973, have recently provided evidence that mature SV 40 and polyoma DNA molecules with a decreased number of tertiary turns are formed when the protein synthesis in the host cell is inhibited by treatment with cycloheximide or puromycin. In both cases an increased formation of catenated molecules is observed under these conditions.

Whereas the inhibition of protein synthesis by chloramphenicol in a colicinogenic *E. coli* strain has no substantial effect on the generation of oligomeric forms of ColE1 DNA, it induces the formation of higher circular oligomers and catenated oligomers in the related enterobacterium *Proteus mirabilis*. This indicates that both types of complex DNA structures may arise by the same mechanism, probably by an unbalanced replication process. If this assumption is correct, the present and previous results (Goebel and Helinski, 1968; Bazaral and Helinski, 1968) indicate, that in contrast to *E. coli*, the replication of ColE1 DNA in *P. mirabilis* is unbalanced even under normal growth conditions (37° C) and leads to a rather high background of oligomeric DNA.

There is another profound difference in the replication of ColE1 DNA in *E. coli* and *P. mirabilis*. Whereas ColE1 DNA synthesis in *E. coli* proceeds at elevated temperatures of 48–49° C, where no chromosomal DNA synthesis takes place anymore, replication of ColE1 DNA in *P. mirabilis* stops above 43° C prior to the chromosomal DNA synthesis.

It has been demonstrated that DNA polymerase I is involved in *E. coli* in the replication of this plasmid (Kingsbury and Helinski, 1970; Goebel, 1972). Assuming a similar dependence on DNA polymerase I in *P. mirabilis* it is conceivable that the early stop of ColE1 DNA synthesis is caused by a thermolability of this enzyme. In vitro studies with DNA polymerase I of *P. mirabilis* (unpublished results) seem to indicate that this enzyme is indeed more thermolabile than DNA polymerase I of *E. coli*.

The small circular DNA molecules found in all DNA preparations of JC 411 (ColE1) have the same size as the minicircular plasmid DNA present in all *E. coli* 15 strains (Cozzarelli *et al.*, 1968). However, the number of copies in the latter

strain is 10–15 per cell, whereas a rough estimate would give only about 1–2 copies per cell in *E. coli* JC 411 (ColE1). It is obvious that such a low concentration of this small plasmid would not be detected by the usual techniques if it would be present in all *E. coli* K 12 strains. We have previously observed in other *E. coli* wild strains the occurrence of such small DNA molecules of unknown functions in varying concentrations (Goebel and Schrempf, 1972) and it is tempting to speculate that most if not all *E. coli* strains may contain such small DNAs. It is also conceivable that these molecules arise by gene amplification either of some chromosomal or plasmid genes. However, such amplification products would have to be able to replicate, since replicative intermediates can also be seen. Investigations concerning these questions are in progress.

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References

Bazaral, M., Helinski, D. R.: Characterization of multiple circular DNA forms of colicinogenic factor E1 from *Proteus mirabilis*. Biochemistry **7**, 3513–3519 (1968)

Benbow, R. M., Eisenberg, M., Sinsheimer, R. L.: Multiple Length DNA Molecules of Bacteriophage ϕx 174. Nature (Lond.) New Biol. **237**, 141–144 (1972)

Bourgaux, P.: On the origin of oligomeric forms of polyoma DNA. J. molec. Biol. **77**, 197–206 (1973)

Brack, C., Delain, E., Riou, G.: Replicating, covalently closed, circular DNA from Kinetoplasts of *Trypanosoma cruzi*. Proc. nat. Acad. Sci. (Wash.) **69**, 1642–1646 (1972)

Clewell, D. B., Helinski, D. R.: Supercoiled circular DNA-protein complex in *Escherichia coli*: Purification and induced conversion to an open circular DNA form. Proc. nat. Acad. Sci. (Wash.) **62**, 1159–1166 (1969)

Cozzarelli, N. R., Kelly, R. B., Kornberg, A.: A minute circular DNA from *Escherichia coli* 15. Proc. nat. Acad. Sci. (Wash.) **60**, 992–999 (1968)

Eason, R., Vinograd, J.: Supercoiled heterogeneity of SV40 DNA. J. Virol. **7**, 1–7 (1971)

Fuke, M., Inselburg, J.: Electron microscopic studies of replicating and catenated colicin factor E1 DNA isolated from minicells. Proc. nat. Acad. Sci. (Wash.) **69**, 89–92 (1972)

Goebel, W.: Studies on extrachromosomal DNA. Replication of the colicinogenic factor *Col* E1 in two temperature-sensitive mutants of *Escherichia coli* defective in DNA replication. Europ. J. Biochem. **15**, 311–320 (1970)

Goebel, W.: Formation of complex *Col* E1 DNA by replication. Biochim. biophys. Acta (Amst.) **232**, 32–42 (1971)

Goebel, W.: Replication of the DNA of the colicinogenic factor E1 (*Col* E1) at the restrictive temperature in a DNA replication mutant thermosensitive for DNA polymerase III Nature (Lond.) New Biol. **237**, 67–70 (1972)

Goebel, W., Helinski, D. R.: Generation of higher multiple circular DNA forms in bacteria. Proc. nat. Acad. Sci. (Wash.) **61**, 1406–1413 (1968)

Goebel, W., Kreft, J.: Accumulation of replicative intermediates and catenated forms of the colicinogenic factor E1 in *E. coli* during the replication at elevated temperatures. Biochem. biophys. Res. Commun. **49**, 1699–1708 (1972)

Goebel, W., Schrempf, H.: Isolation of minicircular deoxyribonucleic acids from wild strains of *E. coli* and their relationship to other bacterial plasmids. J. Bact. **111**, 696-704 (1972)

Helinski, D. R., Clewell, D. B.: Circular DNA. Ann. Rev. Biochem. **40**, 899–942 (1971)

Hudson, B., Vinograd, J.: Catenated circular DNA molecules in HeLa cell mitochondria. Nature (Lond.) **216**, 647–652 (1967)

Jaenisch, R., Levine, A.: DNA replication in SV 40 infected cells VI. The effect of cycloheximide on the formation of SV 40 oligomeric DNA. Virology **48**, 373–379 (1972)

Jaenisch, J., Levine, A.: DNA replication of SV 40 infected cells VII. Formation of SV 40 catenated and circular dimers. J. molec. Biol. **73**, 199–212 (1973)

Jaenisch, R., Mayer, A., Levine, A.: Replicating SV 40 molecules containing closed circular template DNA strands. Nature (Lond.) Biol. **233**, 72–75 (1971b)

Kingsbury, D. T., Helinski, D. R.: DNA polymerase as a requirement for the maintenance of the bacterial plasmid colicinogenic factor E 1. Biochem. biophys. Res. Commun. **41**, 1538–1544 (1970)

Kleinschmidt, A. K., Zahn, R. K.: Über Desoxyribonucleinsäure-Molekeln in Protein-Mischfilmen. Z. Naturforsch. B **14**, 770–779 (1959)

Meinke, W., Goldstein, D. A.: Studies on the structure and formation of polyoma DNA replicative intermediates .J. molec. Biol. **61**, 543–563 (1971)

Nass, M. M. K.: Reversible generation of circular dimer and higher multiple forms of mitochondrial DNA. Nature (Lond.) **223**, 1124–1129 (1969)

Nass, M. M. K.: Abnormal DNA patterns in animal mitochondria: ethidium bromide-induced breakdown of closed circular DNA and conditions leading to oligomer accumulation. Proc. nat. Acad. Sci. (Wash.) **67**, 1926–1933 (1970)

Radloff, R., Bauer, W., Vinograd, J.: A dye-buoyant density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. Proc. nat. Acad. Sci. (Wash.) **57**, 1514–1521 (1967)

Rush, M. G., Warner, R. C.: Multiple-length rings of ϕx 174 and S 13 replicative forms III. A possible intermediate in recombination. J. biol. Chem. **243**, 4821–4826 (1968)

Vapnek, D., Rupp, W. D.: Identification of individual sex factor DNA strands and their replication during conjugation in thermosensitive DNA mutants of *Escherichia coli*. J. molec. Biol. **60**, 413–424 (1971)

Wang, J. C.: Interlocked DNA rings. II. Physicochemical studies. Biopolymers **9**, 489–502 (1970)

Wang, J. C.: Interaction between DNA and an *Escherichia coli* protein ω. J. molec. Biol. **55**, 523–533 (1971)

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