Proc. Natl. Acad. Sci. USA
Vol. 74, No. 5, pp. 1965–1968, May 1977
Biochemistry

Role of DNA gyrase in φX replicative-form replication *in vitro*

(DNA gyrase/supertwisted φX replicative form/φX gene *A* protein)

K. J. MARIANS, JOH-E IKEDA, SAMUEL SCHLAGMAN, AND JERARD HURWITZ

Department of Developmental Biology and Cancer, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

Contributed by Jerard Hurwitz, March 10, 1977

ABSTRACT **Preparations containing DNA gyrase activity [Gellert, M., Mizuchi, K., O'Dea, M. H. & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. USA* 73, 3872–3876] have been extensively purified from *Escherichia coli*. Such fractions, in the presence of ATP and Mg^{2+}, catalyze supertwisting of relaxed circular double-stranded DNA replicative forms of a number of DNAs that results in the formation of superhelical replicative forms. Relaxed φX174 replicative form (φX RFIV) is not attacked by the A protein endonuclease coded for by the φX DNA genome. After exposure to preparations of DNA gyrase, the relaxed φX174 replicative form is converted to φX RFI which can then be attacked by the φX gene *A* protein and participate in replication of duplex φX DNA.**

Cell-free preparations which replicate φX RFI have been described (1, 2). The initial product formed is an RFII structure that is then converted to an RFI structure identical to the supertwisted RFI structure formed *in vivo* (1). The replication of φX RFI both *in vivo* (3) and *in vitro* (2, 4) is completely dependent on the φX gene *A* product coded for by the φX genome. This protein, purified to homogeneity (4), was shown to be an endonuclease (5, 6) which acted specifically at the *A* gene region of the viral (+) strand of φX RFI to form a stable RF·*A* protein complex containing a blocked 5′-terminus and a free 3′-OH end. The φX gene *A* protein acted only on supertwisted φX RFI and was inactive with φX RFIV (4). Crude fractions, which can carry out complete replication of φX RFI, utilized φX RFIV only after a lag in time. In contrast, φX RFI was utilized efficiently and without a lag. The lag, evident when using φX RFIV, was followed by a rapid rate of DNA synthesis which mirrored the rate of φX RFI formation from φX RFIV. Further studies led to the isolation of an activity capable of supertwisting relaxed DNA. During the course of our studies, Gellert *et al.* (7, 8), proceeding from the observation that integrative recombination of phage λ *in vitro* required superhelical DNA (9), isolated an enzyme activity (DNA gyrase) from *Escherichia coli* in the presence of ATP and Mg^{2+} resulted in the supertwisting of RFIV structures. This activity, as reported by Gellert *et al.* (8), was inhibited by novobiocin and coumermycin, two known inhibitors of *E. coli* DNA replication (10, 11) and φX RF replication *in vitro* (1). In this report we demonstrate that DNA gyrase activity is responsible for converting φX RFIV to φX RFI, and is thus a prerequisite for the action of the φX gene *A* protein.

MATERIALS AND METHODS

***E. coli* Strains, and Preparation of DNA and Proteins.** All *E. coli* and bacteriophage φX174 strains used were those described (1, 4). Ammonium sulfate fractions from uninfected *E. coli* H560, fraction II from *E. coli* H514 infected with φX174 H90, and φX174 RFI DNA were prepared as described (1, 12). The φX gene *A* protein was prepared according to Ikeda *et al.* (4). Colicin E1 DNA was prepared from *E. coli* strain JC411 (Col E1) as described by Kupersztoch-Portnoy *et al.* (13). φX174, Col E1 and PM2 RFIV DNAs were prepared either by introducing nicks in the DNA with pancreatic DNase followed by sealing with T4 DNA ligase, or by treating the DNA with a nicking–closing enzyme isolated from calf thymus nuclei (14). In either case, enzymes were inactivated by heating at 55° for 12 min and the DNA was purified by phenol extraction and ethanol precipitation. These methods resulted in relaxed DNA contaminated with approximately 25% RFII structure; the latter form of DNA did not interfere in the assay described below. When necessary, RFII was removed by propidium diiodide/cesium chloride ($PrdI_2$/CsCl) density gradient centrifugation (4).

Assay for DNA Replication. Unless otherwise indicated, all procedures were as described by Sumida-Yasumoto *et al.* (1) and by Ikeda *et al.* (4).

Assay of DNA Gyrase Activity. The conversion of RFIV to RFI by DNA gyrase preparations was measured by agarose gel electrophoresis. Standard reaction mixtures (20 μl) contained 25 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 1.25 mM ATP, 1 mg/ml of bovine-serum albumin, 270 μg/ml of *E. coli* tRNA, 350 pmol of RFIV DNA, and the enzyme as indicated. The mixture was incubated at 30° for 30 min and reactions were halted with 5 μl of a solution containing 5% sodium dodecyl sulfate ($NaDodSO_4$) or sarkosyl, 50 mM EDTA, 50% glycerol (vol/vol), and 1 mg/ml of bromphenol blue. The entire mixture was applied to either a 14 × 13 × 0.15 cm or a 20 × 20 × 0.3 cm slab gel of 0.8–1.0% agarose (Miles Co. or Seakem Co.) followed by electrophoresis at 100 V for 1–3 hr at 25° with 50 mM Tris·HCl (pH 7.9), 40 mM sodium acetate, and 1 mM EDTA as the running buffer. Gels were then stained in a solution of 2 μg/ml of ethidium bromide in water for 20 min after running. Fluorescent bands were photographed by shortwave ultraviolet light and negatives were traced by using a Joyce-Loebl microdensitometer. One unit of DNA gyrase activity is defined as the amount of enzyme which fully supertwists 1 nmol (as nucleotides) of relaxed DNA under the above conditions.

RESULTS

Isolation of DNA Gyrase. DNA gyrase activity was purified from *E. coli* HMS-83 which was grown in Hershey broth to an OD_{595} of 0.7. Cells were resuspended in an equal volume of solution containing 50 mM Tris·HCl (pH 7.5) and 25% sucrose and were frozen at −70° until used. All steps were at 0–4° unless otherwise indicated. Cells (130 g of wet weight) after thawing were made 1 mM with dithiothreitol and EDTA and diluted to 600 ml with a solution containing 50 mM Tris·HCl (pH 7.5)

Abbreviations: RF, duplex, replicative form of DNA (RFI is double-stranded, covalently closed, circular, and superhelical; RFII, double-stranded DNA of circular replicative form with a discontinuity in at least one strand; RFIII, full-size linear DNA form of double-stranded RF DNA; RFIV, relaxed form identical to RFI but not supertwisted); $NaDodSO_4$, sodium dodecyl sulfate; $PrdI_2$/CsCl, propidium diiodide/cesium chloride.

and 1 mM each of dithiothreitol and EDTA. Cells were disrupted in two 300-ml batches by sonic irradiation in a Branson W185 sonic-disrupter by using a microtip at full power for 15 min. During this period, the temperature was not allowed to rise above 10°. The suspension was then centrifuged at 100,000 × *g* for 90 min. The supernatant fraction was assayed for DNA gyrase activity; the activity was detected only upon high dilution of the supernatant (fraction 1). Because of contaminating nucleases it was difficult to measure DNA gyrase activity in early fractions; only after phosphocellulose chromatography, as described below, could the DNA gyrase activity be quantitatively assessed. Fraction 1 was treated with 5% (wt/vol) "Polymin P," (Gallard and Schlesinger, lot no. 1895410) that had been neutralized to pH 7.9 with HCl; this solution was added over a period of 30 min to rapidly stirring fraction 1 to give a final concentration of "Polymin P" of 0.55% (wt/vol). The solution was stirred an additional 20 min and then centrifuged at 10,000 × *g* for 15 min. The pellet was resuspended in 250 ml of a solution of 50 mM Tris·HCl (pH 7.5) and 1 M NaCl; the suspension was stirred for 30 min, and centrifuged at 10,000 × *g* and the pellet discarded. The supernatant (fraction 2) was adjusted to 65% with solid ammonium sulfate (0.4 g/ml) which was added over a 15 min period. The pH of the solution was kept above 7 by addition of 1 M NH_4OH. The suspension was stirred an additional 15 min. The pellet was collected by centrifugation and dissolved in 75 ml of buffer D [20 mM Tris·HCl (pH 7.5), 1 mM dithiothreitol, 1 mM EDTA, and 15% (vol/vol) glycerol]. This solution (fraction 3) was dialyzed for 3 hr against two changes of 2 liters of buffer D.

When fraction 3 was applied to a DEAE-cellulose column and eluted with a linear gradient of NaCl, DNA gyrase activity was difficult to assay and the peak was extremely broad. Therefore, fraction 3 was batch-eluted from DEAE-cellulose as follows: fraction 3 was slowly diluted with 2 liters of buffer D. An additional precipitate which formed at this stage was ignored. DEAE-cellulose (750 ml to 1 liter of Whatman, DE-52), prewashed with 1 M NaCl and equilibrated with buffer D, was slowly added to the solution and the volume adjusted to 4 liters with buffer D. After stirring for 12 hr the suspension was filtered through a 3-liter sintered-glass funnel and the resin washed successively with 2 liters of buffer D and 0.1 M NaCl in buffer D. Protein was eluted with 0.25 M NaCl in buffer D. Fractions (75 ml) were collected and their OD at 280 nm was determined. The protein peak (fraction 4) was pooled and concentrated by addition of solid $(NH_4)_2SO_4$ to 65% saturation; after centrifugation, the pellet was dissolved in 50 ml of buffer E [20 mM potassium phosphate (pH 7.0), 1 mM dithiothreitol, 1 mM EDTA, and 20% (vol/vol) glycerol]. After dialysis for 2 hr against 2 liters of buffer E, fraction 4 was diluted with buffer E to a protein concentration of 0.8 mg/ml and applied to a 200 ml column of phosphocellulose (Whatman, P-11, previously equilibrated with buffer E), at a rate of 60 ml/hr. The column was washed with 400 ml of buffer D and protein was eluted with a 1500-ml linear gradient of KCl from 0 to 0.5 M in buffer E, at 100 ml/hr. DNA gyrase activity eluted between 0.2 and 0.25 M KCl. The protein was precipitated by addition of solid $(NH_4)_2SO_4$ to 70% (0.44 g/ml) and collected by centrifugation at 100,000 × *g* for 1 hr. The pellet was dissolved in 2 ml of buffer F [20 mM potassium phosphate (pH 6.8), 1 mM dithiothreitol, 0.1 mM EDTA, and 20% (vol/vol) glycerol] and dialyzed overnight against 1 liter of the same buffer. Fraction 5 (15 mg of protein) was diluted to 1 mg/ml and applied to a 10 ml column of hydroxylapatite (Biogel HTP) at a rate of 7.5 ml/hr. The column was washed with 20 ml of buffer F and then eluted with a 100-ml linear gradient of potassium phosphate from 20 to 300 mM at a rate of 30 ml/hr. One-milliliter fractions were collected and DNA gyrase activity was eluted between 175 and 190 mM potassium phosphate. Fractions were pooled and adjusted to 70% with solid $(NH_4)_2SO_4$. After centrifugation, the pellet was dissolved in buffer F (50 mM Tris·HCl (pH 7.5), 1 mM dithiothreitol, 1 mM EDTA, and 25% glycerol (vol/vol)]) at a concentration of 0.5 mg/ml (total 520 μg) and stored frozen at −80° (fraction 6).

Table 1. Requirements for DNA gyrase activity

Additions	RFI DNA formed pmol/30 min
Complete	263
−ATP	<10
−ATP + dATP	<10
$-Mg^{2+}$	<10
+ *N*-Ethylmaleimide (1 mM)	<10
+ Novobiocin (1 μg/ml)	<10
+ Nalidixic acid (500 μg/ml)	263
+ Fraction 6, heated at 55° for 12 min	<10

Reaction mixtures were as described in *Materials and Methods* with 1 mM ATP (or any other rNTP or dNTP), 350 pmol of Col E1 RFIV (containing 25% RFII), and 0.5 unit of DNA gyrase (fraction 6). Negatives were traced by using a microdensitometer. In the above reaction, there was quantitative conversion of Col E1 RFIV to RFI. *N*-Ethylmaleimide sensitivity was determined as follows: a reaction mixture (10 μl) containing 1 unit of DNA gyrase activity (fraction 6) was incubated with 1 mM *N*-ethylmaleimide at 25° for 10 min. This was followed by the addition of dithiothreitol (final 2 mM) and the mixtures were incubated for 2 min at 25°. DNA gyrase activity was then determined. As a control, DNA gyrase preparations were incubated with a mixture of 1 mM *N*-ethylmaleimide plus 2 mM dithiothreitol; no loss of DNA gyrase activity was detected in the latter case.

As mentioned above, it was difficult to assign any units to the fractions 1 through 4. It was possible to determine the specific activity of more purified fractions (5 and 6); fraction 5 contained 2200 units with a specific activity of 150 units/mg of protein; fraction 6 contained 600 units with a specific activity of 1060 units/mg of protein.

Requirements and Properties of DNA Gyrase. The conversion of Col E1 RFIV DNA to RFI required ATP and Mg^{2+} and was inhibited by low concentration of novobiocin, in accord with the observation of Gellert *et al.* (7, 8). As summarized in Table 1, no other nucleoside triphosphate (at 1 mM) replaced ATP. In addition, the formation of Col E1 RFI was completely inhibited by *N*-ethylmaleimide and heating, but was unaffected by nalidixic acid. These properties of the system were identical when φX or PM2 RFIV was used in place of Col E1 RFIV. Purified DNA gyrase activity (fraction 6 as described in legend to Table 1) was stable. There was no detectable loss of activity after three months storage at −80° with repeated freezing and thawing.

Role of DNA Gyrase in Replication of φX174 RFI. The φX gene *A* protein is a highly specific endonuclease which acts only at the cistron *A* region of φX DNA that codes for it (3, 4) resulting in formation of a break only in the viral strand. A complex containing φX RFII and φX gene *A* protein is formed and can be isolated by glycerol gradient centrifugation. The φX gene *A* protein is active only on supertwisted φX RFI DNA (Fig. 1*B*) (4). It is likely that the actual *in vivo* conversion of single-stranded circular φX174 DNA to duplex DNA yields φX RFIV. If this were the case, φX RFIV must be converted to supertwisted φX RFI DNA in order for the φX gene *A* protein

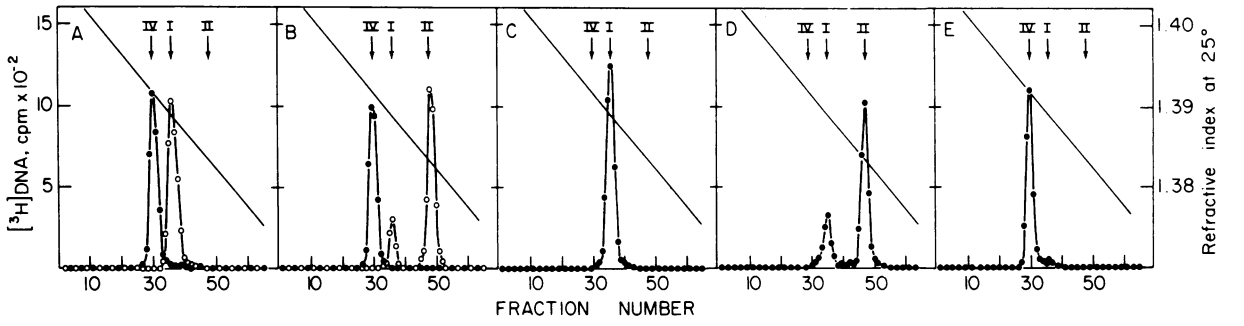

FIG. 1 Analysis of DNA products formed by DNA gyrase, and the ϕX gene *A* protein: effect of DNA replication inhibitors. ϕX RFIV DNA (3 nmol) was treated with DNA gyrase under a variety of conditions. Reaction mixtures (0.2 ml) were increased 10-fold over that described in *Materials and Methods;* where indicated, the following additions were made: DNA gyrase (fraction 6), 25 units/ml; novobiocin, 300 μg/ml; and nalidixic acid, 100 μg/ml. After incubation at 30° for 30 min, reactions were stopped by addition of EDTA to 50 mM. An equal volume of 1 M Tris·HCl (pH 7.5) was then added and solutions were extracted successively with an equal volume of water-saturated $CHCl_3$, and phenol. The aqueous layer was isolated each time and residual phenol was removed with ether. DNA was precipitated by the addition of 3 volumes of ice-cold ethanol and was dissolved in a solution containing 10 mM Tris·HCl (pH 7.5), and 1 mM EDTA. Two nanomoles of each substrate DNA was brought to 40 μl in a buffer containing 50 mM Tris·HCl (pH 7.5) at 30°, 2 mM dithiothreitol, 10 mM $MgCl_2$, 0.1 M NaCl, and 40 μg/ml of bovine-serum albumin. The ϕX gene *A* protein (0.6 μg) was added as indicated, followed by incubation at 30° for 15 min. An aliquot (500 pmol of DNA) was transferred to the system capable of replicating ϕX RFI (Fig. 2). The remainder of each mixture was brought to 10 mM EDTA and the products were analyzed by $PrdI_2$/CCl density gradient centrifugation. (*A*) ϕX RFI (○) and ϕX RFIV (●). The two DNAs were carried through the above procedure but were not treated with any inhibitors or proteins. (*B*) ϕX RFI (○) and ϕX RFIV (●) treated with ϕX gene *A* protein as described above. (*C*) ϕX RFIV treated with DNA gyrase preparation as above. (*D*) ϕX RFIV treated with DNA gyrase and then with ϕX gene *A* protein as described above. (*E*) ϕX RFIV treated with DNA gyrase in the presence of novobiocin and isolated as described above. In all the above gradient profiles, a background of ϕX RFII DNA was subtracted from the profile (RFII in RFIV, 25%; in ϕX RFI, 10%). Arrows in the figures denote the positions for ϕX RFI (I), ϕX RFII (II), and ϕX RFIV (IV).

to initiate replication. The following experiments demonstrate that DNA gyrase preparations perform this function. ϕX RFIV DNA was treated with DNA gyrase under a variety of conditions as described in Fig. 1, followed by incubation with the ϕX gene *A* protein. The DNA products were analyzed by $PrdI_2$/CsCl density gradient centrifugation (Fig. 1) and for their ability to support replication *in vitro* (Fig. 2). ϕX RFI (isolated from phage ϕX174 infected cells) supported DNA replication without a lag in contrast to ϕX RFIV which supported DNA synthesis with a distinct lag. This lag corresponded to the time required to convert ϕX RFIV to RFI* (Fig. 2*A*). When ϕX RFIV DNA was converted to supertwisted RFI by DNA gyrase and then nicked by the ϕX gene *A* protein, DNA synthesis occurred without a lag (Fig. 2*A*).

Novobiocin (at a concentration of 300 μg/ml) inhibited ϕX RFI replication 80% (Fig. 2*B*) (4). Gellert *et al.* (8) have shown that DNA gyrase activity is markedly inhibited by novobiocin at very low concentrations (1–2 μg/ml) and we have obtained identical results (Table 1).

When ϕX RFIV DNA was incubated with purified DNA gyrase fractions in the presence of 300 μg/ml of novobiocin, no supertwisted DNA was formed (Fig. 1*E*). Furthermore, when this DNA was isolated (as described in Fig. 1) and used as a template for DNA synthesis *in vitro*, a distinct lag was found as expected for RFIV DNA (Fig. 2*B*). In contrast, ϕX RFI DNA incubated with novobiocin and isolated as described (see legend to Fig. 1), supported DNA synthesis in an identical manner as ϕX RFI DNA never exposed to novobiocin. Nalidixic acid, another inhibitor of ϕX RFI replication (1), inhibited DNA synthesis more than 80% at a level of 100 μg/ml. However, under the conditions described above, DNA gyrase activity was unaffected by this inhibitor (Fig. 2*B*). These results suggest that DNA gyrase is an essential component of the DNA replication machinery and a necessary prerequisite for the action of the ϕX gene *A* protein.

* DNA gyrase activity is present only in the ammonium sulfate fraction II isolated from ϕX174-infected cells (1). Such preparations were isolated from DEAE-cellulose columns used to remove nucleic acid. No detectable DNA gyrase activity was found in extracts which were treated with high levels of streptomycin sulfate to remove nucleic acids.

DISCUSSION

Studies on the three stages of ϕX174 DNA replication *in vitro* indicate that novobiocin has no influence on the conversion of ϕX174 DNA to ϕX RFII (1) but inhibits replication of ϕX RFI (1) as well as the synthesis of ϕX174 DNA from ϕX RFI (C. Sumida-Yasumoto, unpublished results). In the studies described above, DNA gyrase, an activity capable of introducing superhelical turns into relaxed covalently-closed, double-stranded DNA molecules, has been extensively purified from extracts of *E. coli*. The DNA gyrase activity is essential for replication of ϕX RFIV DNA. This form of DNA must be supertwisted before the ϕX gene *A* protein can act. The DNA gyrase activity fulfills this function, thus allowing the ϕX gene *A* protein to specifically attack the viral strand of ϕX RFI resulting in the initiation of ϕX RFI replication.

There is a marked difference in the concentration of novobiocin required to inhibit DNA gyrase activity and Col E1 DNA-dependent DNA synthesis (9) (1–2 μg/ml) compared to the amount required to inhibit ϕX RFI replication *in vitro* (1) (100–300 μg/ml). This discrepancy raises many questions. It is possible that the ϕX RFI replication system includes an additional novobiocin-sensitive factor or that DNA gyrase exists as a complex with other replication factors and is, in some manner, desensitized to the effects of novobiocin. These results suggest that DNA gyrase activity (and/or another novobiocin-sensitive protein) is essential for the polymerization of dNTPs rather than solely involved in supertwisting of the end products of these reactions. It is also possible that DNA gyrase activity is fashioned to keep the replication fork clear of DNA that is not undergoing replication at that moment.

The mechanism of action of DNA gyrase in catalyzing supertwisting is presently unknown. The fact that ATP is required in the reaction suggests multiple steps. In some way the enzyme

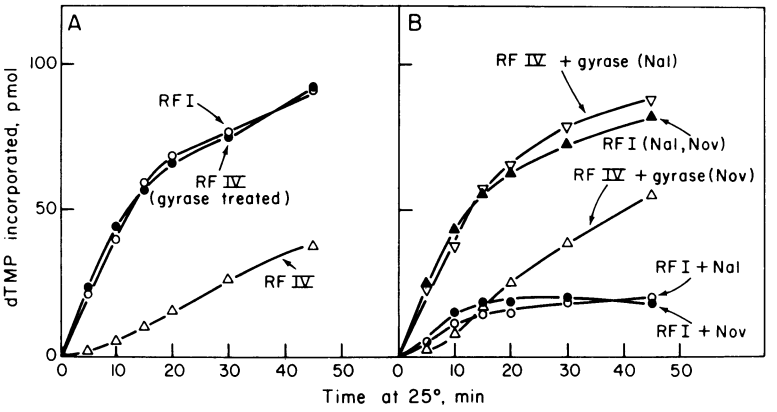

FIG. 2. Replication of different forms of ϕX RF; effect of inhibitors on DNA replication. Various ϕX RF substrates prepared as described in the legend to Fig. 1, were examined for their ability to support ϕX RF replication. DNA synthesis was measured as described in *Materials and Methods*. (*A*) ϕX RFI (○), ϕX RFIV treated with DNA gyrase (●), ϕX RFIV (△). (*B*) ϕX RFI pretreated with novobiocin (Nov) or nalidixic acid (Nal (▲), ϕX RFIV treated with DNA gyrase in the presence of nalidixic acid (▽) or novobiocin (△), ϕX RFI in the presence of nalidixic acid (100 μg/ml) (○) and novobiocin (300 μg/ml) (●) added at zero time followed by incubation as described above.

must span the two strands of relaxed DNA and twist as well as nick and close one strand. We have observed that RFIV is converted to RFII form after incubation with DNA gyrase preparations under certain limiting conditions (0.05 μM ATP and 0.05 unit of DNA gyrase activity). It remains to be proven that this nicking activity is due to DNA gyrase. In addition, we have found that the assay of DNA gyrase activity shows marked sigmoidicity. This observation has made the assay of crude fractions extremely difficult to quantitate.

Recent studies indicate an important role for supertwisted circular DNA structures. The integrative recombination of bacteriophage λ DNA *in vitro* has been shown to depend upon supertwisted λ RFI DNA; no recombination was noted when λ RFII or λ RFIV were used in place of the λ RFI DNA (7). The studies of Gellert *et al.* (9), and those presented here, indicate that the RFIV forms of Col EI and ϕX DNAs (4) are not replicated; such DNA preparations are replicated only after conversion to RFI forms by DNA gyrase preparations. Furthermore, transcription of DNA catalyzed by *E. coli* RNA polymerase has been shown to increase rapidly with increasing negative superhelical density (15–19); in addition, the introduction of superhelical turns in DNA can markedly alter its interaction with a variety of enzymes and small molecules (19, 20).

The above observations indicate that DNA gyrase activity [which yields negative superhelical RFI (8) (K. J. Marians, J.-E Ikeda, S. Schlagman, and J. Hurwitz, unpublished results)] is an important factor in regulating the role that DNA can play in recombination, replication and transcription. A similar and perhaps antagonistic role may be played by the *E. coli* ω protein (15) that catalyzes the relaxation of negative (but not positive) superhelical turns in DNA. It is interesting to note that crude extracts used to replicate ϕX RFI contain *E. coli* ω protein (K. J. Marians, J.-E Ikeda, S. Schlagman, and J. Hurwitz, unpublished observation) and lead to supertwisted RFI products. This suggests that DNA gyrase activity predominates over the relaxing activity catalyzed by *E. coli* ω protein. The elucidation of the role these two antagonistic activities play in controlling the above systems will serve to answer many intriguing questions.

This work was supported by the National Institutes of Health, The National Science Foundation, and the American Cancer Society. A grant from the American Cancer Society provided postdoctoral fellowship support for K.J.M.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

1. Sumida-Yasumoto, C., Yudelevich, A. & Hurwitz, J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1887–1891.
2. Eisenberg, S., Scott, J. F. & Kornberg, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1594–1597.
3. Tessman, E. S. (1966) *J. Mol. Biol.* **17**, 218–236.
4. Ikeda, J., Yudelevich, A. & Hurwitz, J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2669–2673.
5. Henry, T. J. & Knippers, R. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1549–1553.
6. Franke, B. & Ray, D. S. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 475–479.
7. Gellert, M., Mizuchi, K., O'Dea, M. H. & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3872–3876.
8. Gellert, M., O'Dea, M. H., Itoh, T. & Tomizawa, J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4474–4478.
9. Mizuchi, K. & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3524–3528.
10. Smith, D. H. & Davis, B. D. (1967) *J. Bacteriol.* **93**, 71–79.
11. Ryan, M. J. (1976) *Biochemistry* **15**, 3769–3777.
12. Wickner, S., Wright, M., Berkower, I. & Hurwitz, J. (1974) in *DNA Replication*, ed. Wickner, R. B. (Marcel-Dekker, Inc., New York), pp. 195–215.
13. Kuperztoch-Portnoy, Y., Livett, M. & Helinski, D. (1974) *Biochemistry* **13**, 5484–5496.
14. Vosberg, H. P. & Vinograd, J. (1976) *Biochem. Biophys. Res. Commun.* **68**, 456–484.
15. Wang, J. C. (1971) *J. Mol. Biol.* **55**, 523–533.
16. Hayashi, Y. & Hayashi, M. (1971) *Biochemistry* **10**, 4212–4218.
17. Puga, A. & Tessman, I. (1973) *J. Mol. Biol.* **75**, 83–97.
18. Westphal, H. (1970) in *2nd Lepetit Colloquia on Biology and Medicine*, ed. Silvestri, L. (North Holland, Amsterdam), pp. 77–87.
19. Wang, J. C. (1974) *J. Mol. Biol.* **87**, 797–816.
20. Holloman, W. K., Weigand, R., Hoessli, C. & Radding, C. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2394–2398.