Interaction between DNA Gyrase and Its Cleavage Site on DNA*

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Alan Morrison,‡ N. Patrick Higgins,§ and Nicholas R. Cozzarelli††

From the ‡Departments of Biochemistry and of §Biophysics and Theoretical Biology, University of Chicago, Chicago, Illinois 60637

DNA gyrase negatively supercoils DNA. When the requisite breaking and resealing of the DNA are uncoupled by gyrase inhibitors, the DNA becomes cleaved at specific sites. ATP, the cofactor for supercoiling, changes the sites of DNA cleavage. The mechanism of this effect was studied at sites in ColEl and ΦX174 DNA at which ATP strikingly enhanced cleavage. The following results showed that this increase in cleavage did not require movement of gyrase to these sites. 1) Stimulation of cleavage was unaffected by excess competitor DNA and therefore did not require recruitment of free gyrase. 2) Movement of gyrase from other sites along the DNA was ruled out since stimulation of cleavage still occurred in small DNA fragments containing only one prominent cleavage site and in a DNA fragment large enough (176 base pairs) to bind only a single gyrase molecule. 3) ATP increased cleavage of small DNA fragments without increasing the amount of gyrase bound to the DNA. We conclude that gyrase remains stationarily bound at the discrete locations where it cleaves the DNA and that ATP changes the conformational state of the enzyme, thus altering the proportion of bound gyrase that cleaves.

The stability of gyrase binding to DNA varied site-specifically, depending on DNA tertiary structure, and decreased with diminishing DNA size. Of three DNA fragments containing the same cleavage site, a 509 base pair fragment bound gyrase much more stably than a 176 base pair fragment and a 77 base pair fragment showed no detectable interaction. Gyrase bound very tightly at some sites on linear or relaxed DNA, dissociating with half-lives of several hours at 30°C. However, gyrase bound much less well to negatively supercoiled DNA. Thus, addition of ATP to the complex between gyrase and relaxed, circular DNA triggered processive cycles of supercoiling followed by rapid release of the enzyme from the DNA.

DNA gyrase catalyzes the introduction of negative superhelical turns into closed, duplex DNA at the expense of ATP (1). The enzyme participates in many DNA metabolic processes in Escherichia coli, including DNA replication, transcription, repair, and recombination (2, 3). Gyrase is composed of two subunits: the A subunit which is the target protein of the related inhibitors, nalidixic acid and oxolinic acid (4–6), and the B subunit which is the target of the related drugs novobiocin and coumermycin A₁ (7–9). In the absence of ATP, gyrase relaxes negatively supercoiled DNA. The introduction and removal of supercoils both require the concerted breakage and resealing of the DNA backbone.

Gyrase interacts with DNA to form two types of complex that are retained by a nitrocellulose filter (10). The first complex, which we call E·DNA, involves no covalent linkage between enzyme and DNA and is disrupted by protein denaturants. Gyrase binds to a 140 bp³ segment of DNA thus protecting it from nuclease digestion (11). This DNA segment is apparently wrapped around the enzyme in a positive coil (12). A second complex, which we call E·DNA, forms when breakage and resealing of the DNA are uncoupled by oxolinic (or nalidixic) acid, and a protein denaturant, usually SDS, is subsequently added (4, 5). In this aborted reaction, both DNA strands are cleaved at points staggered by 4 bp and the A subunit¹ of gyrase remains covalently attached to the newly created protruding 5′-DNA termini (13). The E·DNA complex also forms at low efficiency in the absence of drugs,³ and the breakage of both DNA strands is intrinsic to the mechanism of DNA supertwisting by gyrase (14).

Gyrase cleaves DNA at specific sites, making cuts that are unique at the nucleotide level (4, 5, 13). The sites that have been sequenced thus far possess no common DNA sequence except for the dinucleotide TG which straddles the cut on one DNA strand (13). ColEl, ΦX174, and SV40 DNAs are cleaved by gyrase at multiple sites with a hierarchy of preferences; each of these DNAs, however, contains one or two major sites of cleavage (10). Intriguingly, ATP or its nonhydrolyzable analogue, App(NH)p, alters the distribution of cleavage sites. Thus, addition of ATP to the complex between gyrase and circular DNA triggered processive cycles of supercoiling followed by rapid release of the enzyme from the DNA.

Four simple models that could explain the ATP-induced

³ The abbreviations used are: bp, base pairs; SDS, sodium dodecyl sulfate; App(NH)p, adenyly-5′-yl-imidodiphosphate; RFI DNA, repliative form I DNA.

† Present address, Division of Biochemistry, University of Wyoming, Box 3944, University Station, Laramie, Wyo. 82071.

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§ Present address, Division of Biochemistry, University of Wyoming, Laramie, Wyo. 82071.

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shift in cleavage sites are depicted in Fig. 1, y is a site (or set of sites) at which cleavage is diminished by ATP, and x is a site (or sites) at which cutting is increased by ATP. Models c, d, and e envision that gyrase remains statically bound at both sites and that ATP alters the efficiency of cleavage in a site-dependent manner. Models b, c, and d are dynamic models in which ATP stimulates net recruitment of gyrase to the x site from a site(s) where it binds silently without cleaving; indeed, the DNA cleavage patterns might be poorly representative of where gyrase is actually bound to the DNA.

Using a nitrocellulose filter-binding assay, we found that, in the presence of ATP, 80 to 90% of the noncovalent E-DNA complexes could be converted to the SDS-resistant E-DNA complexes. Therefore, most of the gyrase that is bound noncovalently to DNA can participate in the cleavage reaction. To test the models explaining the cleavage site switch, we focused on the enhancement of cleavage at two prominent donor sites [CoEI b and the χ174 a sites (Fig. 2)]. Models b to d demand that ATP stimulate recruitment of gyrase at these sites, and models c and d further require a donor site(s) on the same DNA molecule. The shift in cleavage sites was unaffected by a large excess of competitor DNA, eliminating hopping (Fig. 1b). Furthermore, ATP still stimulated cleavage at the CoEI b and χ174 a sites contained in small DNA fragments lacking other major cleavage sites, even when one such fragment could accommodate only a single gyrase molecule. These results rule out the translocation models of Fig. 1, c and d. Finally, ATP did not increase the amount of gyrase bound noncovalently at the CoEI b or χ174 a sites, but instead increased the efficiency of cleavage, results that directly support the quiescent model of Fig. 1a.

The static association between gyrase and DNA was further manifested by the stable binding of gyrase to DNA fragments that contained major cleavage sites. However, gyrase did turn over in the supercoiling reaction since the stable complex formed between gyrase and relaxed, closed, duplex DNA dissociated rapidly after the addition of ATP provoked supercoiling of the DNA. The ability of gyrase to bind or cleave DNA fragments containing the CoEI b site decreased with diminishing DNA size and, in sufficiently small DNA fragments, the presence of the cleavage site was insufficient to allow a detectable interaction.

**EXPERIMENTAL PROCEDURES**

Nucleic Acids—CoEI DNA and χ174 RFI DNA were prepared as described (4). CoEI [3H]DNA was the gift of S. Rashbaum of this laboratory. SV40 RFI DNA and phage λ DNA were from Bethesda Research Laboratories, Inc. Calf thymus DNA (type I) was from Sigma Chemical Co. Yeast tRNA was the gift of P. Sigler of this laboratory. Relaxation of plasmid DNA by DNA Gyrase

![Fig. 1. Models depicting the ATP-driven switch in the primary sites of cleavage of DNA by gyrase. The enzyme (C) is bound to DNA (—) at cleavage sites x or y. ATP causes cleavage to decrease at y and increase at x.](http://www.jbc.org/content/2212/6/2212/F1/F1.large.jpg)
Cleavage of DNA by DNA Gyrase

Effect of ATP on Cleavage Sites in ColEl, φX174, and SV40 DNAs—The effect of ATP on site-specific cleavage of ColEl, φX174, and SV40 DNAs by gyrase was illustrated using linear DNA substrates produced by restriction enzyme digestion. Linear DNA was incubated with gyrase in the absence or presence of ATP, and cleavage was induced by successive additions of oxolinic acid and SDS. There was an average of less than one gyrase cut per DNA molecule, and each cleavage event produced two bands, labeled a and a’, b and b’, etc. (Fig. 2). ATP enhanced cleavage at the φX174 a and a’ sites (Fig. 2, Lanes 1 and 2) while reducing it at the φX174 d and e sites (Lanes 1 and 2) and the SV40 a site (Lanes 3 and 4). ATP had little effect on cleavage at the SV40 c site (Fig. 2, Lanes 3 and 4). As observed earlier (15), ATP shifted the primary cleavage event in ColEl DNA from the a site to the b site (Fig. 2, Lanes 5 and 6).

To enable isolation of the major cleavage sites of ColEl and φX174 DNAs in small DNA fragments, these sites were mapped using different restriction enzymes to produce the linear DNA substrate for gyrase (10). The diagram below Fig. 2 shows the positions of the mapped sites and several restriction enzyme-generated DNA fragments used in this report.

The arrangement of the primary cleavage sites in ColEl DNA, i.e. two major sites located about 400 bp apart, one mutated and the other enhanced by ATP, is not clearly preserved in φX174 DNA (Fig. 2) or in SV40, or G4 DNAs. Thus, the relative spatial arrangement of cleavage sites is probably not mechanistically relevant.

Enhancement of Cleavage by ATP Does Not Require Recruitment of Free Gyrase—If the enhancement of cleavage at the ColEl b site requires recruitment of gyrase either by hopping or by simple acquisition of free enzyme, it should be prevented by an excess of competitor DNA. 32P-labeled, linear ColEl DNA was preincubated with gyrase for 30 min before addition of a 30-fold excess of unlabeled, relaxed ColEl DNA. ATP was then added where appropriate, followed by successive additions of oxolinic acid and SDS (Fig. 3). ATP stimulated cleavage at the b site to the same extent whatever competitor DNA was present (Fig. 3, d and e) or not present (Fig. 3, b and c), thus eliminating the hopping model of Fig. 1b. There was sufficient competitor DNA to bind all added enzyme since addition of competitor before the start of the reaction abolished cleavage of the 32P-labeled DNA (Fig. 3f).

For reasons that are unclear, cleavage at the ColEl a site in the absence of ATP (Fig. 3b) was considerably lower than expected.

Efficiency of Cleavage—The elimination of the hopping model simplifies consideration of the ATP-induced switching of cleavage sites since only those enzymes originally associated with a DNA molecule can cleave. The proportion of noncovalently bound enzyme that participates in cleavage, i.e. the efficiency of cleavage, is an important parameter that relates the site specificity of cleavage to the site specificity of noncovalent DNA binding. The efficiency of cleavage was determined using the nitrocellulose filter-binding assay to measure the amount of E-DNA (the noncovalent complex between gyrase and DNA) and E-DNA (the covalent complex between the cleaved DNA and the denatured A subunit of gyrase); cleavage efficiency is simply E-DNA/E-DNA. 32P-labeled Eco RI-linearized ColEl DNA was incubated with various

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5 J. Tomizawa, personal communication.
To determine whether the stimulation of cleavage at a given site reflects recruitment of enzyme or, as required by the quiescent model, an increase in the efficiency of cleavage, we first used the 376 bp φX174 Mbo II-F fragment which contains the prominent, ATP-stimulated site (Fig. 2). The E-DNA and E-DNA complexes were measured by the nitrocellulose filter-binding assay as a function of gyrase concentration and in the presence or absence of ATP. ATP did not affect formation of the noncovalent E-DNA complex with this DNA fragment (Fig. 5a) but increased the amount of covalent E-DNA complex 3- to 4-fold (Fig. 5b). The efficiency of cleavage in the presence of ATP averaged 87% over the range of gyrase concentrations used. Gel electrophoresis showed that cleavage

amounts of DNA gyrase in the presence of oxolinic acid. The adsorption to a nitrocellulose filter was determined either without prior addition of SDS (to measure E-DNA) or after addition of 1% SDS (to measure E-DNA). The inclusion of oxolinic acid did not affect the estimation of E-DNA and enabled both E-DNA and E-DNA to be measured conveniently in samples from a single reaction. ATP increased the proportion of E-DNA that could be converted to E-DNA and thus increased the efficiency of cleavage (Fig. 4, a and b).

The proportion of bound gyrase that cleaved the DNA increased with gyrase concentration, perhaps because the ColE1 DNA sites that bind gyrase at low enzyme concentrations have a relatively low efficiency of cleavage. When 50% of the DNA was complexed as E-DNA, the efficiency of cleavage was 84 and 43% in the presence and absence of ATP, respectively (Fig. 4). An almost identical result was obtained with relaxed, circular ColE1 DNA, for which the corresponding values of the efficiency of cleavage were 84 and 52%, respectively. Efficiencies of cleavage in the range of 80 to 100% in the presence of ATP were also observed for nicked, circular ColE1 DNA and several DNA fragments tested: the Mbo II-F and -G fragments of ColE1 DNA and the Mbo II-E and -F fragments of φX174 DNA. The generally high efficiency of cleavage in the presence of ATP means that cleavage is a representative property of DNA gyrase and not restricted to a subclass of altered or moribund enzymes.

ATP Changes the Efficiency of Cleavage at a Single Site—

Fig. 4. Efficiency of gyrase cleavage. Twelve-microliter reactions contained 1.2 fmol of 32P-labeled, Eco RI-linearized ColE1 DNA, 0 to 24 units of DNA gyrase, 0.2 mM oxolinic acid, and no ATP (a) or 1.3 mM ATP (b). After 40-min incubation, 5-μl aliquots of each reaction were subjected to nitrocellulose filter-binding without (●) or with (○) prior addition of 1% SDS.

Fig. 5. Effect of ATP on efficiency of cleavage at the φX174 a site. The 15-μl reactions contained 15 fmol of 32P-labeled φX174 Mbo II-F DNA fragment, 0.2 mM oxolinic acid, 0 to 30 units of DNA gyrase, with (●) or without (○) 1.4 mM ATP. After incubation for 30 min, 5-μl samples were adsorbed to nitrocellulose filters without prior addition of SDS (a). The remaining 10 μl of each reaction was made 1% in SDS, one-half of this was assayed by filter binding to measure the covalent E-DNA complex (b), and the remainder was treated with proteinase K and the cleaved DNA displayed by electrophoresis in an acrylamide gel. An autoradiograph of the gel, with a scale in base pairs, is shown in c. Reactions in c contained the following amounts of gyrase: none (Lanes 1 and 7); 1.25 units (Lanes 2 and 8); 2.5 units (Lanes 3 and 9); 3.75 units (Lanes 4 and 10); 5 units (Lanes 5 and 11); and 10 units (Lanes 6 and 12). Reactions in Lanes 1 to 6 contained no ATP, and reactions in Lanes 7 to 12 contained 1.4 mM ATP. The fragments resulting from cleavage at the φX174 gyrase a site are labeled a and a'.
occurred almost exclusively at the α site to produce the expected α and α′ partner fragments (Fig. 5c). Densitometer tracings demonstrated that the increase in cleavage at the α site was the same as the increase in formation of the covalent E-DNA complex as measured by nitrocellulose filter-binding. ATP stimulated cleavage at the ΦX174 α site in this small DNA fragment at least as efficiently as with whole ΦX174 DNA. Since the ATP-mutated d site, which neighbors the α site in ΦX174 DNA (Fig. 2), is absent from the ΦX174 Mbo II-E DNA fragment, the d site is not required to feed stimulation of cleavage at the α site.

A more striking activation of quiescent enzyme was demonstrated in a kinetic experiment using the 509 bp ColEl Mbo II-E fragment which contains the ColEl b site but no other major gyrase cleavage site. The DNA fragment was incubated with DNA gyrase and the E-DNA and E-DNA complexes were monitored by filter binding (Fig. 6a). Excess calf thymus DNA (120-fold, w/w) was added 10 min after the start of the reaction to prevent further binding of gyrase to the DNA fragment. ATP added at 37 min reduced binding slightly but increased cleavage about 6.5-fold. Fig. 6b displays the cleaved DNA at various times in the experiment, showing that cleavage occurred only at the b site immediately before and after ATP addition. This result rules out involvement of the ATP-mutated ColEl a site in the mechanism of enhancement of cleavage at the b site by ATP since the a site is absent from the Mbo II-E fragment. Fig. 6 also shows that binding of gyrase to the b site fragment was extremely stable in the presence of a large excess of competitor DNA. The efficiency of cleavage of this DNA fragment of 60% in the presence of ATP was clearly less than the average of about 80% for whole ColEl DNA.

Cleavage of a 176 bp Fragment Containing the ColEl b Site—The experiments in Figs. 5 and 6 rule out translocation of gyrase to the ATP-stimulated ΦX174 a and ColEl b sites from other prominent sites of cleavage. However, movement of gyrase from weak cleavage sites or sites where gyrase binds silently without cleaving is still possible. Liu and Wang (11) have shown that gyrase binds to a 140 bp segment of DNA. The scale in b is numbered in base pairs. The fragments resulting from cleavage at the ColEl b site are labeled b and b′; their unequal intensities result from differential labeling of the two 5′ termini of the Mbo II-E DNA fragment by T4 polynucleotide kinase. (The designation of gyrase cleavage sites in the ColEl Mbo II-E and Hae III-J fragments as the ColEl b site is based on their approximate map positions and has not been confirmed by DNA sequencing.)
that each cleavage event marks the position of a different gyrase molecule. The simultaneous action of several gyrase molecules on a single DNA molecule therefore leads to multiple cutting of the DNA fragment. The 176 bp ColEI Hae III-J fragment and, as a control, the 509 bp ColEI Mbo II-E fragment were cleaved by gyrase at low and very high levels of enzyme (Fig. 9). Densitometer tracings of the resulting cleavage products show extensive multiple cutting of the \( b \) band of the 509 bp Mbo II-E fragment at the high enzyme level (Fig. 9a), indicating more than one cleavage per DNA molecule. In contrast, the cleavage pattern of the 176 bp ColEI Hae III-J fragment was essentially insensitive to enzyme concentration (Fig. 9, a and b). Thus, although this DNA fragment possesses several sites of cleavage, only one site on any DNA molecule can accommodate a gyrase molecule. This rules out all but very limited movement of gyrase relative to the cleavage site.

The difference in the rates of dissociation of gyrase from the ColEI Hae III-J and Mbo II-E DNA fragments, both of which contain the ColEI \( b \) site, implies that stable binding requires a lower DNA size limit. Consistent with this, we found that the 77 bp Hae II-F fragment of ColEI DNA, which contains the ColEI \( b \) site near one end (Fig. 2), displayed no detectable interaction with gyrase as monitored by the binding or cleavage assays. (The \( b \) site apparently lies about 10 bp from the left end of the Hae II-F fragment, based on measurements of the sizes of the gyrase and Hae II cleavage products of the Hae III-J fragment on a denaturing gel (22).)

**Gyrase Turnover**—Our results establish the quiescent model shown in Fig. 1a, and reveal that the interaction between gyrase and a sufficiently large DNA fragment containing the ColEI \( b \) site is an extremely stable one. Since the \( b \) site is the major binding site for gyrase on ColEI DNA, this highly stable binding is not readily compatible with previous observations that a single enzyme is able to completely supertwist many ColEI DNA molecules (6, 8). An escape from this conundrum would be provided if supercoiling allowed turnover. An experiment that tests this is shown in Fig. 10. Gyrase was prebound to relaxed \([\text{3H}]\)ColEI DNA circles in the absence of ATP. A 35-fold excess of competitor DNA was added and the amount of E.DNA complex was assayed by filter binding at various times later. To distinguish the effects of supercoiling from those of ATP, the reaction also contained

\[ ^{32}\text{P}-\text{labeled, linear ColEI DNA which cannot be supercoiled. Addition of ATP provoked rapid supercoiling of the circular DNA and changed the turnover rate of gyrase from this DNA from a half-life of many hours to one initially of about 5 min (Fig. 10). Concomitantly, ATP increased the turnover rate of gyrase from \(^{32}\text{P}\)-linear DNA to a half-life of about 2 h. The curve for the circular DNA substrate displayed a second component that decayed with a half-life of about 2 h. This more stable component corresponds to 25 to 30\% of the bound \([\text{3H}]\)DNA, and presumably represents dissociation of the enzyme from nicked, circular DNA which comprised 25\% of the \([\text{3H}]\)ColEI DNA. We conclude that the E.DNA complex between gyrase and ColEI DNA is destabilized both by ATP and by negative supercoiling but that supercoiling has the more profound influence and effectively allows turnover of the enzyme. Furthermore, excess competitor DNA did not prevent supercoiling of the circular ColEI DNA and, therefore, gyrase, once bound to DNA, supertwists it processively.

**Site-specific Variation in Stability of Gyrase-DNA Interaction**—The E.DNA complex between gyrase and the ColEI Mbo II-E fragment (containing the ColEI \( b \) site) was very stable, dissociating in most experiments with a half-life of about 5 h when ATP was present. Gyrase also formed stable complexes with the 392 bp ColEI Mbo II-F fragment containing the ColEI a site and with the 378 bp \( \phi X174\ Mbo \) II-F fragment containing the prominent \( \phi X174\ a \) site; gyrase dis-
ATP. At containing ATP was added. The amounts of circular [H]ColEl measured for gyrase binding. The linear DNA microliter samples were also subjected to electrophoresis through a linearized ColEI DNA measured as described under “Experimental Procedures.” FIG. 11. Differential stability of cleavage sites in ColEI Mbo 4.1-E DNA fragment. The 0.14-ml reaction contained 75 fmol of [P]labeled ColEI Mbo II-E DNA fragment, 75 units of DNA gyrase, and no ATP. At 10 min, 6 μg of calf thymus DNA was added, followed at 36 min by 2 mM ATP. Ten-microliter samples were incubated with 0.4 mM oxolinic acid for 3 min and then mixed with 1% SDS at the times indicated. After incubation with proteinase K, the samples were subjected to electrophoresis through a polyacrylamide gel which was then autoradiographed. The fragments arising from cleavage at the ColEI 6 site are labeled b and b’. (The enhancement of the b band after addition of competitor DNA resulted from a recession of double cutting of the DNA fragment as the secondary cleavages waned.)

**Figure 10.** Effect of ATP and supercoiling on the stability of gyrase binding. The 0.23-ml reaction contained 320 fmol of relaxed [H]ColEI DNA (70 cpm/fmol), 17 fmol of 32P-labeled, Eco RI-linearized ColEI DNA (107 cpm/fmol), 50 units of DNA gyrase, and no ATP or spermidine. At 10 min, 0.115 ml of reaction mixture containing 41 μg of phage λ DNA was added, and at 30 min 1.2 mM ATP was added. The amounts of circular [H]DNA (Φ) or 32P-labeled, linear DNA (◊) retained by gyrase on a nitrocellulose filter were measured for 15-μl samples taken at the indicated times. Fifteen-microliter samples were also subjected to electrophoresis through a 1% agarose gel and the percentage of DNA supercoiled (△) was measured as described under “Experimental Procedures.”

**Figure 11.** Differential stability of cleavage sites in ColEI Mbo II-E fragment. The 0.14-ml reaction contained 75 fmol of 32P-labeled ColEI Mbo II-E DNA fragment, 75 units of DNA gyrase, and no ATP. At 10 min, 6 μg of calf thymus DNA was added, followed at 36

Sociated from these two DNA fragments with half-lives of about 2 h and 30 min, respectively. However, some secondary sites of cleavage bound gyrase much less stably. Fig. 11 shows the time course of cleavage of the ColEI Mbo II-E fragment before and after addition of competitor DNA. The experiment is similar to that in Fig. 6b, except that a higher gyrase to DNA ratio was used so that cleavage now also occurred at several secondary sites. After addition of competitor DNA at 10 min, the secondary cleavages diminished with an average half-life of about 5 min. Cleavage at the b site remained, and was again stimulated by ATP added at 37 min (Fig. 11). Comparable results were obtained with the 766 bp Hinf-C fragment of SV40 DNA. This fragment displayed multiple cleavage sites at which cutting decayed with half-lives varying from less than 5 to over 60 min after competitor DNA was added. Cleavage at the SV40 a site, contained in the Hinf-C fragment, diminished with a half-life of about 30 min. We conclude that the stability of the interaction between gyrase and DNA varies site-specifically, and that the prominent sites of cleavage generally bind gyrase most stably.

**Discussion**

The ATP-dependent supertwisting of DNA by gyrase requires movement of the DNA relative to the enzyme, and some models have proposed translocation of the enzyme along the DNA (8, 12). While such translocation could explain the ATP-driven rearrangement of cleavage sites on the DNA, the evidence is now excellent that the quiescent model is correct. The quiescent model envisions that enzyme remains stationary bound at the discrete locations where it cleaves the DNA, and that the proportion of enzyme in the conformation competent for cleavage is sensitive to both ATP and local DNA sequence.

The evidence against the movement models and supporting the quiescent model of Fig. 1 may be listed as follows. 1) The ATP-driven stimulation of cleavage at the ColEI b site was unaffected by the presence of competitor DNA (Figs. 3, 6, and 7). This eliminates the hopping model of Fig. 1b and the simple acquisition of enzyme from a free pool in solution. 2) If ATP incites translocation of gyrase to the ColEI b site from other sites on the DNA, the most likely donor site is the major a site at which ATP usually diminishes cleavage (15). Yet, cleavage at the ColEI b site was stimulated 6-fold by ATP in a 509 bp fragment lacking the ColEI a site. 3) The latter result was also obtained using a 176 bp fragment that could only accommodate a single gyrase molecule, ruling out all but very limited movement of gyrase relative to the cleavage site. 4) Increased cleavage at the ColEI b site in the 509 bp fragment was not paralleled by any increased binding of gyrase since this was prevented by excess competitor DNA (Fig. 6). 5) Cleavage at the ΦX174 a site was stimulated by ATP in a 376 bp fragment lacking the ATP-mutated d site and any other major cleavage site (Fig. 5). Again, this stimulation of cleavage was not paralleled by any increased binding of gyrase to the DNA fragment.

While we used two major, ATP-stimulated cleavage sites for our proof of the quiescent model, this model also seems applicable to other ATP-stimulated cleavages. The hopping mechanism was eliminated at such secondary sites since the

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inclusion of competitor DNA immediately before the additions of ATP, oxolinic acid, and SDS failed to prevent stimulation of cleavage. The translocation models of Fig. 1, c and d, probably do not operate at secondary, ATP-stimulated cleavage sites since such sites occurred in the ColEl Hae III-J DNA fragment which was too small to allow appreciable movement (Fig. 7).

We were unable to test rigorously the quiescent model with the ATP-muted SV40 a and ColEl a cleavage sites because the diminution of cleavage by ATP at these sites was not always reproducible (see Figs. 2 and 3) and because small DNA fragments containing these sites also contained ATP-stimulated cleavage sites. The diminution of cleavage by ATP at a site might represent a decrease in the efficiency of cleavage or release of gyrase from the site. However, diminution of cleavage at some sites is not coupled to enhancement at others since we have shown that the converse is not true.

The quiescent model increases our understanding of the role of ATP. Both ATP and its nonhydrolyzable analogue App(NH)p change the cleavage pattern (though not always in the same manner) and promote negative supercoiling of the DNA (15). Sugino et al. (15) proposed that binding of ATP or App(NH)p induces a conformational change that allows one cycle of supercoiling and that fresh cycles require ATP hydrolysis. No direct evidence for the conformational change was presented. With the establishment of the quiescent model, it is hard to avoid this conclusion. Gyrase may be envisaged as existing in several conformational modes during the supercoiling process, including a transient state in which the DNA is cleaved. At sites where ATP stimulates cleavage, ATP binding may switch the enzyme to a conformer which is competent to cleave the DNA. Since the effects of ATP vary site-specifically, the conformational state of gyrase or the competence to cleave is also sensitive to local DNA sequence.

The sites at which gyrase cleaves DNA in the presence of ATP are a good, though not perfect, indicator of where gyrase binds and presumably acts to superwarp DNA. This follows, since we have shown that gyrase remains statically associated with the DNA at the cleavage site and since more than 80% of the gyrase that was bound to DNA also cleaved the DNA with the DNA at the cleavage site and since we have shown that gyrase remains statically associated with the DNA at the cleavage site.

The stability of the interaction between gyrase and DNA fragments containing the ColEl b site depended on the size of the fragment. Gyrase formed an extremely stable complex with the 509 bp Mbo II-E fragment which contains the b site about 130 bp from its right terminus and a much less stable complex with the 176 bp Hae III-J fragment in which the b site is about 50 bp from the right terminus, and showed no detectable interaction with the 77 bp Hae II-F fragment which contains the b site about 10 bp from its left terminus. Liu and Wang (11) found that gyrase wraps a 140 bp segment of DNA, thus protecting it from nuclease digestion. The ability of the 176 bp Hae III-J fragment of ColEl DNA to bind only a single gyrase molecule is compatible with E-DNA being a complex between gyrase and a 140 bp stretch of DNA. The relative instability of the complex between gyrase and this fragment suggests that the cleavage site is specifically located relative to the 140 bp DNA segment and that a part of this segment needed for stable binding of gyrase is absent from the fragment. Simply, gyrase may require that the DNA flanking the cleavage site be of a minimum size before forming a stable complex. An alternative explanation, that gyrase must interact with specific DNA sequences distant from the cleavage site, is not ruled out, but DNA sequence elements common to different gyrase cleavages have been observed only at the cleavage site itself (13). It is clear, however, that gyrase interacts with a relatively large piece of DNA, and the presence of the cleavage site alone is insufficient to allow an interaction that is detectable by the cleavage or filter-binding assays.

All properties of gyrase tested varied site-specifically. These include the preference for cleavage at any site, the stability of DNA binding, the efficiency of cleavage, and the effects on cleavage of ATP and App(NH)p. The significance of these variations is unclear, but they must ultimately stem from differences in local DNA sequence. The only DNA sequence common to different gyrase cleavages is the dinucleotide TG which straddles the gyrase cut on one DNA strand. Other DNA sequences must be involved, but the way in which gyrase recognizes sites on the DNA is not presently understood.

The dissociation rate of the E-DNA complex was extremely sensitive to the superhelicity of the DNA. The tight binding of gyrase at some sites on linear DNA initially presented a paradox since gyrase turns over during the supercoiling process (6, 8). However, the stable complex formed between gyrase and relaxed, circular ColEl DNA dissociated with a half-life of about 5 min when supercoiling was permitted (Fig. 10). Other observations show that relaxed DNA is the superior substrate both for the initial binding of gyrase and for the DNA-dependent ATPase of gyrase (8). This is both mechanistically and teleologically explicable. Gyrase wraps DNA in a positive coil (12), and the accompanying introduction of compensatory negative superwarpst into the unbound DNA domain is less energetically favorable if the DNA is initially negatively supercoiled. Furthermore, the greater affinity of gyrase for its substrate (relaxed DNA) than for its product (negatively supercoiled DNA) provides a mechanism both for turnover and for limiting the final superwarp density of the DNA.

The interactions between gyrase and DNA defined by the present work can be summarized as follows. The enzyme does not bind randomly but remains closely associated with the cleavage site on the DNA. In the absence of ATP or supercoiling, this association can be extremely stable having a half-life of many hours at 30°C. ATP effects a conformational change in the enzyme. This conformational change is observed as an alteration in the proportion of bound enzyme that cleaves and becomes covalently attached to the DNA following additions of oxolinic acid and SDS. When supercoiling is permissible, gyrase rapidly superwarpst the DNA in a processive manner (Fig. 10) and then dissociates from the DNA with an average half-life of about 5 min.

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