type originated by Budyko (*30*) and Sellers (*51*). The same experimental strategy has been used with general circulation models capable of simulating the atmospheric response in three dimensions (*52*). So far, only the simpler, zonal models have been used in an alternative strategy designed to simulate the climatic response to orbital variations. These experiments are discussed in the following section.
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48. G. R. North, *J. Atmos. Sci.* **32**, 2033 (1975).
49. R. S. Lindzen and B. Farrell, *ibid.* **34**, 1487 (1977).
50. R. E. Newell, *Quat. Res. (N.Y.)* **4**, 117 (1974); B. Saltzman and A. D. Vernekar, *ibid.* **5**, 307 (1975).
51. W. D. Sellers, *J. Appl. Meteorol.* **8**, 392 (1969).
52. J. Williams, R. G. Barry, W. M. Washington, *ibid.* **13**, 305 (1974); W. L. Gates, *J. Atmos. Sci.* **33**, 1844 (1976); *Science* **191**, 1138 (1976); S. Manabe and D. G. Hahn, *J. Geophys. Res.* **82**, 3889 (1977).
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54. R. G. Johnson and B. T. McClure, *Quat. Res. (N.Y.)* **6**, 325 (1976).
55. The need for a differential model has been recognized since the time of Milankovitch (*3*). Although an extensive literature exists on what is called the retardation problem [F. E. Zeuner, *The Pleistocene Period* (Hutchinson, London, 1959), pp. 200–202; R. M. Fairbridge, *Ann. N.Y. Acad. Sci.* **95**, 542 (1961); W. S. Broecker (*35*)], only recently has a time-dependent model of the astronomical theory been formulated in terms of differential equations describing the system response.
56. J. Weertman, *J. Glaciol.* **6**, 145 (1964).
57. Weertman (*56*) used a physical model to estimate that the characteristic growth times of large ice sheets are on the order of 15,000 to 30,000 years. See also J. T. Andrews and M. A. W. Mahaffy, *Quat. Res. (N.Y.)* **6**, 167 (1976).
58. G. E. Birchfield, *J. Geophys. Res.* **82**, 4909 (1977); _____ and J. Weertman, *ibid.* **83**, 4123 (1978).
59. N. Calder, *Nature (London)* **252**, 216 (1974).
60. J. Smagorinsky, in *Meteorological Challenges: A History*, D. P. McIntyre, Ed. (Information Canada, Ottawa, 1972), p. 29.
61. For most insolation curves the error is less than 1 percent. The exceptions are in high latitudes, where there are seasons of continual darkness or light.
62. Weertman (*56*) attributes the difference to two effects. First, even if the accumulation rate on a growing ice sheet equals the ablation rate on a shrinking ice sheet, the rate of change in ice volume will be greater on the shrinking sheet than on the growing sheet because the shrinking sheet is more spread out. Second, the accumulation rate is expected to be smaller than the ablation rate.
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64. However, there is evidence that ice sheets of modest size, such as those represented by certain substages of isotopic stages 5 and 7, can grow rapidly.
65. Expressed in terms of half-response times, the parameters T_m, T_c, and T_w are 11,800, 29,500, and 7,300 years, respectively.
66. W. S. Broecker, *Science* **189**, 460 (1975); J. M. Mitchell, Jr., "Carbon dioxide and future climate," *EDS (Environ. Data Serv.)* (March 1977), p. 3.
67. W. Q. Chin and V. Yevjevich, *Colo. State Univ. (Fort Collins) Hydrol. Pap. 65* (1974).
68. Although the character of climatic records is fairly constant over the past 600,000 years (*7*, *38*), older Pleistocene records are quite different. For example, isotopic records in the interval from 600,000 to 2 million years ago have much reduced amplitudes and lack the 100,000-year cycle (*41*). Because the nature of orbital variation is thought to have remained constant over the past 2 million years, we conclude that to understand these long climatic records, it may be necessary to use models whose parameters vary with time (*69*).
69. V. Y. Sergin, *Oregon State Univ. Clim. Res. Inst. Rep. 2* (1978).
70. J. M. Mitchell, Jr. (*45*), for example, doubts the existence of climatic resonance. But Sergin (*69*) finds that there is a tendency to oscillate at periods on the order of several tens of thousands of years in a complex model of the climate system. See also E. Källén, C. Crafoord, M. Ghil, *J. Atmos. Sci.* **66**, 2292 (1979).
71. System functions of this kind are discussed in E. Eriksson, *Meteorol. Monogr.* **8**, 68 (1968).
72. E. N. Lorenz, *ibid.* **8**, 1 (1968); *Tellus* **3**, 289 (1969).
73. Other possible deterministic sources of variability will also have to be explored (*45*). However, since orbital variation is now the only forcing function that can be specified exactly, it is best to exhaust the explanatory power of the astronomical theory first.
74. Building stochastic models to understand the variations unexplained by deterministic models is a relatively undeveloped field for the range of frequencies under consideration in this article (*12*). A model proposed by Hasselmann (*43*) generates a red-noise oceanic response to white-noise atmospheric forcing. Almost-intransitive models (*72*) provide another potentially fruitful avenue of research.
75. Two input parameters, corresponding to the choice of latitude and season (or α and ϕ), are assigned to models that use a single input curve. If the scale of the input is important, then a third parameter is assigned. All other parameters are counted in c_t. For the linear model there is one time constant only; for the nonlinear model there are warming and cooling time constants; for Calder's model there is a ratio of warming and cooling response rates and a critical value of the input; and for Weertman's model there are accumulation and ablation rates, basal shear stress, slope of the snow line, and a critical value of the input.
76. J. Iversen, *Dan. Geol. Unders. 5* (1973); J. C. Bernabo and T. Webb. *Quat. Res. (N.Y.)* **8**, 64 (1977); H. E. Wright, Jr., *Annu. Rev. Earth Planet. Sci.* **5**, 123 (1977).
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78. K. J. Mesolella, R. K. Matthews, W. S. Broecker, D. L. Thurber, *J. Geol.* **77**, 250 (1969).
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80. All spectra have been calculated by using standard autocorrelation procedures identical to those described in (*5*). Let N be the number of sample points, m be the number of lags, Δt be the sampling interval in K years, and C be the constant of a first-difference filter. For the model input and output spectra in Fig. 9, A and B, N = 501, m = 199, and Δt = 2K. For the eccentricity spectrum in Fig. 9A, N = 551, m = 199, and Δt = 2K. In Fig. 9C, N = 157, m = 50, Δt = 3K, and C = 0.998 for the prewhitened spectrum. In Fig. 9D, N = 122, m = 61, and Δt = 6K for the unprewhitened spectrum and N = 122, m = 47, Δt = 6K, and C = 0.998 for the prewhitened spectrum. In Fig. 9E, N = 367, m = 133, and Δt = 3K. Confidence intervals and bandwidths have been calculated by procedures given in G. M. Jenkins and D. G. Watts [*Spectral Analysis and its Applications* (Holden-Day, San Francisco, 1968)].
81. Supported by NSF grants OCD75-14934 and ATM77-07755 to Brown University. K. Bryan, I. M. Held, J. M. Mitchell, Jr., T. C. Moore, Jr., W. F. Ruddiman, T. Webb III, and J. Weertman critically read an earlier version of this article and made valuable suggestions for improving it. We thank R. M. Mellor and T. A. Peters for preparing the typescript. This paper will be presented as a Richard Foster Flint lecture at Yale University, New Haven, Conn., on 4 March 1980.

DNA Gyrase and the Supercoiling of DNA

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Virtually all duplex DNA exists naturally in a negatively supertwisted form. This supertwisting is an important facet of the processes of DNA replication, transcription, and recombination. Just 4 years ago, a bacterial enzyme that introduces negative supertwists into DNA was discovered by Gellert *et al.*, who christened it DNA gyrase (*1*). This article describes how the rapidly advancing studies of gyrase have traversed a spectrum of topics of contemporary interest including the mechanism of supercoiling, the energetics of macromolecular movement, the conversion of DNA into complex topological forms, the site-specific binding of enzymes to DNA, the reversible association of enzyme subunits, and the mechanism of inhibitors of DNA synthesis. It is a propitious time for a review of gyrase, since critical features of the enzyme can now be explained satisfactorily by the recently proposed mechanism termed sign inversion (*2*). This article focuses on the enzyme from *Escherichia coli* about which most is known (*3*).

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Background

The two polynucleotide strands of duplex DNA wind around each other in a right-handed helix containing about one helical turn every ten base pairs. Supercoiling, the coiling of the helix axis itself, was discovered by Vinograd *et al.* in a study of closed, circular DNA (*4*). The sense of supercoiling can, in principle, be either the same or opposite to that of the helical twists which, by convention, are designated as positive. Remarkably, all supercoiled DNA isolated from natural sources is twisted opposite to the direction of the double helix, with about one negative supercoil per 15 double helical twists (*5*). There is a fundamental topological constraint on any DNA molecule, such as closed, circular DNA, whose polynucleotide strands cannot rotate freely about the helix axis: the algebraic sum of the number of supercoils and the number of double helical turns is a constant, the linking number (*6*). Thus, unwinding of the double helix must be compensated by an equivalent winding up—that is, removal—of negative supercoils. Since the supercoiled configuration is strained, the removal of supercoils is highly energetically favorable. Negative supercoiling thereby promotes the binding of any protein that locally denatures (unwinds) DNA; this occurs with a number of enzymes of DNA metabolism (*7*).

Wang initiated the enzymology of supercoiling with the discovery of *E. coli* ω protein (*8*). Requiring no nucleotide cofactor, ω removes (that is, relaxes) most of the negative supercoils in DNA. Since the linking number is changed in this reaction, a transient break in the DNA is required. The ω protein is the archetype of the important class of enzymes known as topoisomerases, which catalyze the interconversion of DNA molecules that differ only in linking number (*9*). Thus *E. coli* ω protein has been designated Eco DNA topoisomerase I (topo I).

The ω protein relaxes positively supercoiled DNA very poorly (*8, 10*). This may seem a trivial deficiency since DNA in the cell is negatively supercoiled. It is, in fact, important in the cell to maintain negative supertwists and to relieve positive twisting stress that is generated by processes, such as DNA replication, that unwind the double helix. Champoux and Dulbecco discovered a topoisomerase in mouse cells, which relaxes positively and negatively supercoiled DNA with equal facility (*11*). Similar topoisomerases have since been identified in many eukaryotic organisms, and enzymes with the restricted relaxation pattern of ω are limited to bacteria where their function remains an enigma (*12*).

Summary. Negative supercoiling of bacterial DNA by DNA gyrase influences all metabolic processes involving DNA and is essential for replication. Gyrase supercoils DNA by a mechanism called sign inversion, whereby a positive supercoil is directly inverted to a negative one by passing a DNA segment through a transient double-strand break. Reversal of this scheme relaxes DNA, and this mechanism also accounts for the ability of gyrase to catenate and uncatenate DNA rings. Each round of supercoiling is driven by a conformational change induced by adenosine triphosphate (ATP) binding; ATP hydrolysis permits fresh cycles. The inhibition of gyrase by two classes of antimicrobials reflects its composition from two reversibly associated subunits. The A subunit is particularly associated with the concerted breakage-and-rejoining of DNA and the B subunit mediates energy transduction. Gyrase is a prototype for a growing class of prokaryotic and eukaryotic topoisomerases that interconvert complex forms by way of transient double-strand breaks.

DNA gyrase (topo II) is the only known topoisomerase that catalyzes supercoiling of DNA. It was discovered in a remarkably logical way. Purified *int* protein from bacteriophage λ requires negatively supercoiled DNA to promote recombination in vitro (*13*). However, relaxed DNA sufficed with a crude preparation of *int* if adenosine triphosphate (ATP) was provided (*14*). The search for an ATP-dependent supercoiling activity contaminating the *int* preparation was rewarded by the discovery of DNA gyrase (*1, 14*). The importance of DNA gyrase in both DNA replication and in the introduction of negative supercoils was soon established. Gellert *et al.* found that novobiocin and coumermycin A_1, two closely related inhibitors of nucleic acid synthesis in vivo, were inhibitors of DNA gyrase in vitro (*15*). Moreover, coumermycin A_1 inhibited the supercoiling of circular λ DNA in vivo. Both of these effects were abolished by a mutation in the *cou* gene, which controls the cellular level of sensitivity to these drugs (*16*).

My involvement with DNA gyrase resulted from a study of the mechanism of a different pair of inhibitors of DNA replication—nalidixic acid and the more potent analog, oxolinic acid. The target protein for these drugs in *E. coli*, the *nalA* gene product, was purified (*17*) by means of a complementation assay for φX174 DNA replication (*18, 19*). The discovery that gyrase from wild-type but not *nalA* mutant cells was inhibited by nalidixic acid (*17, 20*) established the surprising link between the *nalA* protein and DNA gyrase.

Structure of Gyrase

The physical basis for the segregation of gyrase inhibitors into two classes is the construction of the enzyme from two subunits, A and B, which are the target proteins for nalidixic acid and novobiocin, respectively (*21, 22*). *Micrococcus luteus* gyrase subunits α and β are functionally analogous to A and B, respectively (*23, 24*). Establishment of the composition of gyrase was hampered by low purification yields until independent studies on the very different microorganisms *E. coli* (*25*) and *M. luteus* (*23*) revealed that only a small proportion of the subunits are associated in extracts. Mixing of the subunits under appropriate conditions efficiently reconstitutes gyrase activity and provides a convenient assay that has been used in the preparation of both subunits in physically homogeneous form (*25, 26*). There is perhaps ten times more subunit A than subunit B in cells (*25*); as discussed below, subunit A is also part of another topoisomerase. Subunit A is a homodimer of 105,000-dalton *nalA* protomers and subunit B consists of 95,000-dalton *cou* protomers (*25*). The small amount of constituted enzyme in *E. coli* cell extracts has been purified as such and contains just the same two polypeptides (*25, 27*).

Convincing evidence for the structural gene assignments led to the renaming (*28*) of *nalA* and *cou* as *gyrA* and *gyrB*. First, by two-dimensional electrophoresis under denaturing conditions, subunit A and radioactive *gyrA* gene product are identical (*21*), as are subunit B and authentic *gyrB* gene product (*28*). Second, subunit A from a *gyrA* mutant conferring nalidixic acid resistance and subunit B from a novobiocin-resistant *gyrB* mutant each reconstitute a gyrase with the expected drug resistance (*25*). Third, subunit A purified from a *gyrA* temperature-sensitive mutant is thermolabile (*29, 30*). Fourth, attachment of a reactive ATP derivative to subunit B of gyrase is specifically inhibited by novobiocin (*27*).

Although the protomer structure of gyrase is not rigorously established, it almost surely is of the form $\alpha_2\beta_2$. There is good evidence that gyrase contains two

gyrA protomers, each of which has an active site (*21, 31*), and that gyrase contains equivalent amounts of *gyrA* and *gyrB* protomers (*27, 31*). Equal molar amounts of subunits A and B reconstitute gyrase activity, and subunit A, at least, is a homodimer (*17, 25*). Finally, the best estimate for the molecular weight of the holoenzyme is 400,000, the value expected for an $\alpha_2\beta_2$ structure (*27, 32*).

Activities of DNA Gyrase

Gyrase remains bound to its DNA substrate, supercoiling it processively (*33*) and catalytically (*25, 27*). One molecule introduces about 100 supertwists per minute at 30°C. Under standard reaction conditions, the supercoiling of Col El DNA reaches a limit at one and a half times the density of Col El DNA supercoiled in vivo (*1*). Both positively supercoiled and relaxed DNA can be negatively supercoiled (*21, 24*).

The supercoiling reaction has two components. Since the linking number of the DNA is changed, there must be a transient break of one or both strands (*5*). In addition to this concerted breakage-and-reunion activity (*34*), gyrase must have a coupled energy transduction component since supercoiling is an endergonic reaction. Some of the reactions of gyrase described below isolate one of these components uncomplicated by the other. All the reactions have been important in understanding the enzyme.

Adenosine triphosphatase (ATPase). Like many enzymes of DNA metabolism (*35*), gyrase hydrolyzes ATP to adenosine diphosphate (ADP) and P_i in the presence of DNA (*19, 26, 27, 36*). The reaction is highly specific for ATP among naturally occurring nucleotides, as is supercoiling. Duplex DNA is about a ten times better effector than single-stranded DNA in accord with the preferential binding of gyrase to duplex DNA. A simple interpretation of the effector activity of DNA is that its binding stabilizes an enzyme conformation that has ATPase activity. The ATP hydrolysis is not necessarily coupled to supertwist formation and continues after maximum coiling of a DNA effector. The potent inhibition of ATPase by novobiocin but not by oxolinic acid makes it likely that the energy transduction component is associated intimately with the B subunit of gyrase (*37*).

Relaxation. Gyrase spontaneously relaxes negative supercoils in the absence of ATP (*17, 20*) but cannot relax positive supercoils (*23, 24*). This asymmetry may reflect the importance of handedness in the sign inversion mechanism for gyrase. The rate of relaxation is an order of magnitude lower than the rate of supertwisting (*25*) and therefore underestimates the breakage-and-reunion capacity of gyrase. A plausible explanation is that breakage-and-reunion is usually coupled to supertwist formation and thus is stimulated by ATP. Relaxation is inhibited by nalidixic acid but not by novobiocin, just the opposite to results with ATPase. Therefore, subunit A is particularly important for the breakage-and-reunion component of DNA gyrase.

Cleavage. Nalidixic acid inhibits gyrase by interfering with the breakage-and-reunion component of supercoiling. Addition of a protein denaturant to a nalidixic acid–treated gyrase reaction results in breakage of both polynucleotide strands and concomitant attachment of enzyme to the cleaved DNA. Gyrase disrupts the DNA strands at points staggered by four base pairs, creating termini that each possess a free 3′ hydroxyl group and a 5′ extension attached covalently to a *gyrA* protomer (*31, 38*). These termini therefore provide a template-primer for DNA polymerases but are completely resistant to 5′ terminal labeling with bacteriophage T4 polynucleotide kinase (*38*). Gyrase thus contains two *gyrA* protomers each of which makes up a critical portion of the breakage-and-reunion active sites. The covalent interaction between gyrase and DNA can be understood in terms of Wang's model (*8*) for the way in which topoisomerases, unlike classical DNA ligases (*39*), reseal transient breaks in DNA without infusion of energy from a nucleotide cofactor. Instead of irreversible hydrolysis of a phosphodiester bond, it is proposed that strand breakage occurs in a transfer reaction in which the enzyme itself becomes attached covalently to the broken end. Resealing in the reverse transfer reaction releases free enzyme. This scheme is supported by the general observation that various conditions uncouple breakage from reunion by topoisomerases and result in covalent joining of enzyme to DNA (*12*). The discovery of breakage of both DNA strands by gyrase (*17, 20*) set it apart from other topoisomerases that nick only one strand, and implies that the mechanism of gyrase is fundamentally different.

A further distinction of gyrase from nearly all other topoisomerases is that cleavage is highly site-specific (*17, 20, 38*). With less than one gyrase molecule per DNA substrate of about 4 to 5×10^6 daltons, most often cleavage is at one or a few sites. The balance occurs with widely varying frequency at a number of other positions so that potential cleavage sites occur, on the average, about once per 100 base pairs (*38*). At each site, the cuts are specific at the nucleotide level. Cleavage can be induced by reaction perturbants other than nalidixic or oxolinic acid and is observed at low frequency in the unperturbed reaction (*21, 24, 40*). All these different conditions result in cleavage at the same sites (*24*). These sites are also the ones cut by *M. luteus* and *Bacillus subtilis* gyrases, by a hybrid gyrase constructed from *M. luteus* and *E. coli* subunits, and by *E. coli* topo II′, an enzyme related to gyrase (*24, 41*). This conservation suggests that site specificity plays a critical role in supercoiling (*42*).

The cohesive end sequences of six gyrase cleavage sites have been determined (*38, 43*). The dinucleotide TG (T, thymine; G, guanine), straddling the gyrase cut on one of the DNA strands, provided the only common bases within a 250 base-pair region surrounding the sites. Analysis of other sites showed that cutting between a TG doublet is common to nearly all gyrase cleavages. Other bases common to some of the sequenced sites are clustered nonrandomly around the TG doublet and may be variable components of the cleavage sequence, but no simple set of related sequences has been identified that unfailingly predicts sites of cleavage. This pattern of site specificity in the absence of a unique determining sequence is emerging as a common feature in nucleic acid–protein recognition.

In contrast to the conservation of sites of cleavage, the degree of cleavage at any site varies with reaction conditions. ATP specifically incites a redistribution of cleavage pattern, stimulating cleavage at some sites while reducing it at others (*36*). This phenomenon might have suggested movement of gyrase between cleavage sites, either by solution or by translocation along the DNA. Such movement was ruled out (*33*), however, since cleavage site redistribution was unaffected by competitor DNA and since ATP still stimulated cleavage at individual sites isolated in small (176 to 509 base pairs) DNA fragments. Furthermore, ATP did not alter the binding of gyrase to small DNA fragments although it stimulated cleavage by as much as an order of magnitude. Gyrase is thus envisioned as remaining stationarily bound to DNA at discrete locations; ATP effects a conformational change in the enzyme that alters, in a fashion dependent on the local DNA sequence, the proportion of bound gyrase that cleaves its substrate.

Binding to DNA. The binding of gyrase to radioactively labeled DNA is easily measured by the resulting retention of the label by nitrocellulose filters (*21, 44*). This complex of gyrase with DNA, un-

like that formed by cleavage, is stabilized by noncovalent bonds that are disrupted by high ionic strength or protein denaturants. It is nonetheless a stable complex, since gyrase dissociates from linear Col E1 DNA with a half-life of days at 23°C. Neither gyrase subunit by itself binds detectably to DNA.

Gyrase binds to DNA site specifically. The sites at which gyrase cleaves DNA are generally a faithful reflection of where it binds, since almost 90 percent of the enzyme bound noncovalently can also cleave the DNA (*33*). Site-specific binding implies that gyrase also acts in vivo only at certain sites. The limited cleavage following oxolinic acid addition in vivo is consistent with this conclusion and may provide an opportunity to map the sites of gyrase action in the cell (*45*). Given that gyrase acts at discrete chromosomal locations, there may be regional differences in processes that gyrase facilitates, depending on the location and strength of both gyrase binding sites and the barriers that delineate supercoiling domains in the cell (*46*).

Gyrase binds less tightly to DNA that is negatively supercoiled than to relaxed DNA (*44*), and thus the stable complex with relaxed DNA dissociates rapidly when addition of ATP provokes supercoiling (*33*). This feedback loop provides a mechanism for turnover of an otherwise stably bound enzyme and for limiting the final supertwist density of DNA.

Liu and Wang (*23, 47*) have proposed that when DNA gyrase binds to DNA the DNA becomes wrapped around the enzyme in a positive coil. This was based initially on the *increase* in linking number imparted by bound DNA gyrase to nicked circular DNA converted to the covalently closed form by DNA ligase. Supporting evidence for the coiling of DNA around gyrase is provided by the similarity in the pattern of protection from nucleases afforded by gyrase to that obtained with nucleosomes where wrapping has been established.

Catenation and uncatenation. The most recently discovered of the gyrase reactions are catenation—the interlocking of duplex DNA rings—and the resolution of catenanes into component circles (*40*). Catenanes are physiologically important structures that are the constant companion of circular DNA molecules and are sometimes the predominant cellular species; they have frequently been suggested as intermediates in recombination or replication (*48, 49*). As in supercoiling and relaxation, the linking number is changed in these reactions but at the level of quaternary rather than tertiary structure. Catenation and uncatenation provide the first direct evidence that gyrase makes a double-strand break, passes the DNA through the break, and reseals the break in an efficient, concerted reaction. The alternative of progressive invasion of one ring by another seems unlikely in view of the double-strand breaks resulting from cleavage. The analogous reaction—removal of knots from duplex DNA circles, catalyzed by several topoisomerases, including gyrase—is also interpretable in terms of a transient double-strand break (*50, 51*).

Catenation and uncatenation are the most fastidious of the gyrase reactions, and this helps explain why they were missed in earlier work. There is a sharp ionic strength optimum, and ATP, Mg^{2+}, and spermidine are required. However, the reactions are surprisingly effective since most of the substrate can be catenated or decatenated. Negatively supercoiled DNA is more efficiently interlocked than nicked, circular DNA. Homology between donor and acceptor DNA is not required since catenanes are formed between unrelated DNA molecules. The number of rings linked and unlinked progressively changes with time, and molecules containing as many as thousands of rings have been observed.

Summary of activities. The activities of DNA gyrase and their sensitivity to the two families of drugs are summarized in Fig. 1. Only those reactions requiring ATP (supercoiling, ATPase, catenation, and decatenation) are inhibited by novobiocin, and the reactions that require breakage-and-reunion (supercoiling, relaxation, catenation, and decatenation) are selectively inhibited by oxolinic acid. DNA is either a substrate or an effector for each reaction. Reconstitution of all the activities requires an equal amount of subunits A and B. The need for both subunits for binding to DNA is sufficient to explain this requirement for all the other reactions.

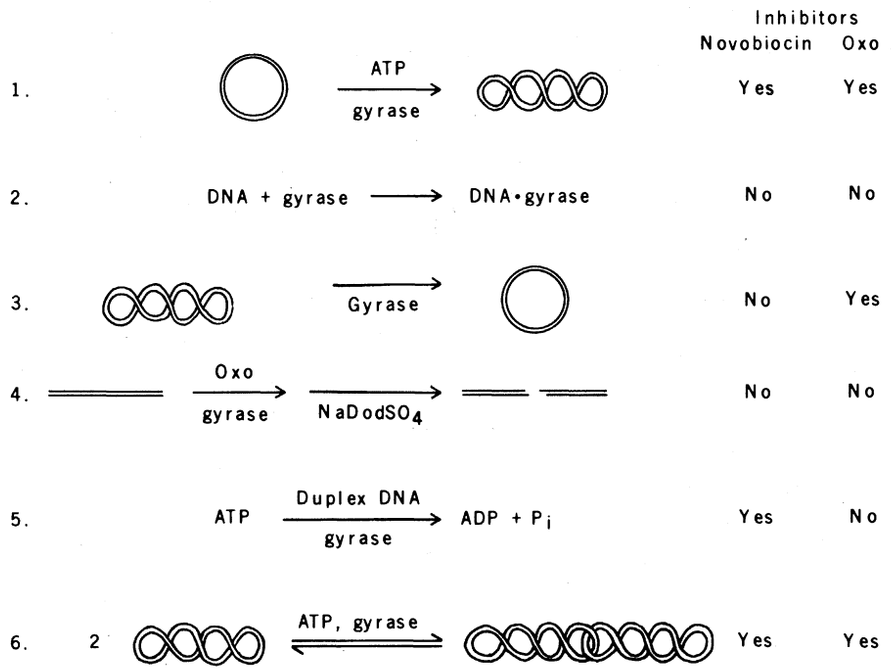


Fig. 1. The activities of DNA gyrase. The reactions depicted are supercoiling (reaction 1), binding (reaction 2), relaxation (reaction 3), cleavage (reaction 4), ATPase (reaction 5), and catenation and uncatenation (reaction 6). The DNA substrate is shown as relaxed, duplex, circular DNA for reaction 1, negatively supercoiled DNA for reactions 3 and 6, and as linear DNA for reaction 4. Oxolinic acid is abbreviated *Oxo* and sodium dodecyl sulfate as $NaDodSO_4$. Nalidixic acid has the same inhibition spectrum as oxolinic acid, and coumermycin A_1 has the same spectrum as novobiocin. Second-order effects, such as the low stimulatory activity of single-stranded DNA for ATPase and the inhibition of ATPase by oxolinic acid under certain conditions (*26*), are not presented.

Energy Coupling and the Mechanism of Action of Novobiocin

The key feature of the mechanism of energy coupling by gyrase was discovered serendipitously (*36*). A nonhydrolyzable analog of ATP, adenyl-5′-yl-imidodiphosphate [App(NH)p], caused a shift in cleavage pattern similar to that evoked by ATP. This was surprising because hydrolysis of ATP was known to be required for other gyrase reactions, such as supercoiling (*36*). However, DNA gyrase can turn over in the super-

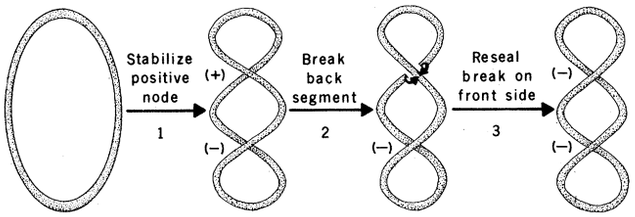
coiling reaction (*25, 27*) but not in the cleavage reaction, which requires enzyme denaturation. The paradox would be resolved, then, if the binding of ATP [or of App(NH)p] leads to a single round of supercoiling, but ATP hydrolysis is needed for enzyme turnover. This strong prediction was confirmed; with substrate levels of gyrase, App(NH)p led to supercoiling stoichiometric with the number of enzyme molecules (*36*). The derived stoichiometry of a little less than one supercoil per gyrase molecule is a lower limit since it neglects inactive enzyme and the back reaction of relaxation.

To explain these results, it is proposed that binding of ATP or of App(NH)p results in a conformational transition that drives a crucial part of the supercoiling cycle (*36*). For gyrase to return to its original conformation, that is, for turnover, nucleotide desorption is required, and this is facilitated by hydrolysis of ATP to the less tightly bound ADP.

This type of energy coupling was originally proposed to explain chemical energy-fueled movement in other processes, such as muscle contraction, transfer RNA translocation, active transport, and oxidative phosphorylation (*52-55*). It should be contrasted with the bioenergetic pattern first conceived by Lipmann (*56*) and Kalckar (*57*) in which a high-energy intermediate is formed that consists of part of a nucleoside triphosphate covalently bonded to a substrate of the endergonic reaction, such as aminoacyl adenosine monophosphate in protein synthesis. A monetary analogy clarifies a major difference between the two bioenergetic schemes. The conformational coupling pathway is like using a credit card since one can "buy now" (supercoil) but "pay later" (hydrolyze ATP); the Lipmann-Kalckar mechanism has the parsimony of pay-as-you-go. Recent experiments suggest that the T4 topoisomerase (*51*) and the *E. coli recA* protein (*58*) also employ credit-card energetics. There are many other ATP-dependent enzymes in DNA metabolism (*35*), and these may also follow the gyrase model.

Novobiocin selectively interferes with the energy transduction component of gyrase; reactions independent of ATP are immune (Fig. 1). The basis for this specificity was suggested by the finding that novobiocin prevents reorientation of the cleavage pattern by ATP or by App(NH)p (*36*). Thus, the antibiotic must act prior to ATP hydrolysis. Competitive inhibition with respect to ATP in both the supercoiling and ATPase reactions showed that the sensitive step is ATP binding (*36*). The same conclusion was reached from the prevention by novobiocin of labeling of subunit B by an ATP analog (*27*). Novobiocin is an impressive inhibitor—the K_i (inhibition constant) of $10^{-8}M$ is several orders of magnitude less than the K_m (Michaelis constant) for ATP of 0.3 mM (*36*). Despite the selective and competitive effect of novobiocin on ATP binding, there is no obvious structural similarity between them. Novobiocin may block ATP access without sharing binding sites, or the enzyme conformations competent for binding ATP and novobiocin could be incompatible.

Fig. 2. The sign inversion model for enzymatic supercoiling of DNA. The enzyme maintains and acts at the upper node; the (+) and (−) symbols refer to the sign of the nodes.



Mechanism of Supercoiling

Early models for gyrase postulated that negative supercoils are introduced in two steps. An initial segregation of positively and negatively supercoiled regions of a DNA molecule is followed by selective relaxation of the positive supercoils, leaving just the negative supercoils (*17, 23, 27*). The critical observation concerning supercoil segregation is that the binding of gyrase per se generates positive supercoils in the absence of ATP; these are adjacent to the enzyme since they are immune to relaxing enzymes (*21, 23, 47*). Compensating negative supercoils result in other regions of the molecule since the linking number must be unchanged. Addition of App(NH)p not only causes limited negative supercoiling stoichiometric with the number of gyrase molecules but also an equivalent reduction in protected positive supercoils (*21*). An explanation for this correlation is a causal link between removal of the positive supercoils of binding and introduction of negative supercoils.

The second step in supercoiling, selective relaxation of positively supercoiled regions, has usually been described in terms of the traditional topoisomerase model of nicking of one strand, rotation about the helix axis to dissipate the positive stress, and resealing of the nick (*59*). Recent work, however, has established a categorically different mechanism called "sign inversion" because the positive supercoils are not relaxed but actively inverted to negative supercoils (*2*). This mechanism is illustrated in Fig. 2 and consists of the following steps.

1) Gyrase binds to a DNA molecule such that the bound segments cross to form a right-handed node (the upper node in Fig. 2). This stabilizes a positive supercoil and induces a counterposing negative supercoil represented by the lower (−) node.

2) Gyrase introduces a double-strand break in the DNA at the back of the right-handed node and passes the front segment through the break, inverting the handedness and thus the sign of the node.

3) The break is resealed on the front side of the now left-handed node.

Gyrase could then release one of the two crossing segments of the negative node, the negative supercoils would distribute along the DNA, and the cycle could begin again. The net result of the steps illustrated in Fig. 2 is to reduce the linking number of the DNA by two (*60*). Reversal of these steps, that is, starting with a left-handed node and inverting it to a right-handed one, increases the linking number by two and is the proposed path for relaxation of negative supercoils by gyrase.

The unique prediction of the model has been verified; both supercoiling and relaxation of DNA change the linking number in steps of two (*2*). Since no odd-numbered changes in linking number were observed, the ends of the double-strand break in the intermediate do not rotate relative to one another and this ensures that supercoils are not dissipated. Catenation, unknotting, and uncatenation substantiate the other characteristic feature of the model, passing of DNA through a transient double-strand break (*40, 51*). These three related reactions are, in turn, readily explicable by the sign-inversion model. Catenation, for example, results when the two crossing segments of the node are contributed by different DNA molecules.

The following somewhat speculative elaboration of the sign inversion mechanism incorporates the gyrase energy transduction scheme and features of gyrase as revealed by studies of DNA binding and cleavage. Gyrase binds to DNA at specific DNA sequences, forming a

right-handed node. A positive coiling of the DNA around the enzyme is a plausible means for ensuring the proper handedness (*23, 47*), but the sequence of the crossing segments could provide the necessary information (*2*). The subsequent binding of ATP by each of the *gyrB* protomers changes the conformation of gyrase to one that stabilizes a left-handed node. The resultant strain is relieved by passing the front segment of DNA through a double-strand break made in the back segment. Remarkably, this is accomplished while gyrase holds both ends of the break so that they cannot rotate. The *gyrA* protomers are attached by covalent bonds to each 5′-phosphoryl terminus of the break. The energy of the broken phosphodiester bonds is conserved in this covalent complex and is used to reseal the break after sign inversion. The cohesive ends of the break aid the proper alignment for resealing. Gyrase then catalyzes hydrolysis of ATP, and the segment that was passed through the break is released so that resetting is possible. The desorption of the loosely bound ADP causes the enzyme to return to its initial conformation, the more stable one in the absence of a nucleotide effector. Gyrase remains bound at the same specific DNA sites (those at which it cleaves), acting processively through cycles of sign inversion until binding is sufficiently weakened by the increased negative supertwist density so that the enzyme is released.

New Topoisomerase Activities Related to Gyrase

Equimolar amounts of subunits A and B are needed to reconstitute both the supertwisting and relaxing activities of gyrase. It was therefore puzzling that the ratio of oxolinic acid–sensitive relaxation activity to supertwisting activity declined (to a nonzero plateau value) with increasing purity of the enzyme (*17, 21, 25*). One explanation for this apparent paradox would be contamination of less pure preparations with a relaxing enzyme distinct from gyrase but sharing the *gyrA* product that controls oxolinic acid sensitivity.

Such an activity has been identified and is designated topo II′ (*24*). It is constructed from subunit A and a 50,000-dalton protein called ν. Since subunit B and ν peptide maps are quite similar, ν may be a processed form of subunit B or derived from a transcript of part of *gyrB*. Whatever the ontogeny of ν, available evidence implies that topo II′ exists normally in the cell rather than being derived from subunit B during purification. Four separate purifications each detected an order of magnitude more ν than subunit B (*24*), and similar levels of ν are found in the dissimilar *E. coli* K and B strains (*61*). Among *E. coli* topoisomerases, topo II′ alone relaxes positive supercoils as efficiently as it relaxes negative supercoils. Topo II′ has no negative supercoiling activity and is not affected by either ATP or novobiocin but closely resembles gyrase in its other properties, including sensitivity to oxolinic acid, stabilization of positive supercoils, double-strand cleavage at the same DNA sites, and catenation and uncatenation of DNA rings. If the structural homology of ν and subunit B is confirmed, then it appears that subunit B is divided into functional domains. One domain represented by ν is sufficient for binding subunit A and reconstitution of breakage and reunion. The other domain contains the ATP binding site or allows its expression in energy-requiring reactions. Its absence in topo II′ somehow uncouples positive node inversion from ATP binding and allows relaxation of positive supercoils.

The discovery of a novel topoisomerase in T4-infected cells explains some puzzling results (*50, 62*). DNA synthesis directed by almost all other bacteriophages is strikingly reduced by inhibition of topo II and topo II′, but the reduction in T4-infected cells was only about fivefold and sometimes less (*30, 63*). However, mutants in genes 39, 52, or 60, so-called DNA delay genes, are totally dependent on the host gyrase by the same tests (*64*). The products of these three genes are the protomers of a T4 topoisomerase that requires ATP not to supertwist DNA but to relax it, whether it is negatively or positively supercoiled (*50, 62, 65*). Very high levels of T4 topoisomerase catenate supercoiled DNA intramolecularly (that is, knot it) in an ATP-independent reaction; in the presence of ATP the same enzyme can untie the knot catalytically, even after relaxation or nicking (*51*).

Gyrase and T4 topoisomerase can probably perform the same function in T4 metabolism since each enzyme spares the requirement for the other. The lack of supertwisting by the phage enzyme is therefore intriguing. Perhaps what is crucial for the phage is an activity shared by both topoisomerases, such as uncatenation or relief of positive twisting stress. Alternatively, there is substantial circumstantial evidence that genes 39, 52, and 60 are required for proper initiation of T4 DNA replication (*66*). It has been suggested (*48*) that the T4 enzyme might be an origin-specific gyrase and thus supertwist such DNA.

Whereas no eukaryotic enzyme has yet been shown to supercoil DNA, gyrase and the T4 enzyme are prototypes of the new class of topoisomerases that interconvert complex topological forms of DNA and make transient double-strand breaks. It was in an extract of a eukaryote (*Xenopis laevis*) that a catenation activity was actually first identified several years ago by Attardi *et al.* (*67*); this activity has been shown to be ATP dependent (*68*). A topoisomerase from early embryos of *Drosophila* can catenate DNA (*69*) and untie knots produced by T4 topoisomerase in circular DNA (*51*); both reactions require ATP. A vaccinia virus–induced enzyme and trypanosome extracts can resolve the giant catenated networks of trypanosome kinetoplast DNA (*70*).

Functions of Gyrase

The physiological role of gyrase has been studied with the use of specific drugs, structural gene mutations, and in vitro DNA replication and recombination systems. The isolation of *gyrA* and *gyrB* conditional lethal alleles demonstrated that DNA gyrase is an essential enzyme for *E. coli* growth. The emerging conclusion is of a widespread importance of the enzyme in DNA metabolism.

There is now good evidence that gyrase is responsible for introducing at least most of the negative supertwists in vivo. Gyrase inhibitors block supertwisting of infecting phage λ DNA (*15, 20, 71*) and drastically reduce the superhelicity of the folded *E. coli* chromosome (*46, 47, 72*). Intercalating agents remove negative supercoils and cause curing, that is, a preferential loss of plasmids (*73*). Sublethal doses of novobiocin can similarly cure (*74, 75*), and genetic impairment of gyrase enhances sensitivity to the intercalating agent, acriflavin (*28*). In fact, the discovery of gyrase and its effect on supertwisting has provided the most compelling evidence that supercoils do exist in the cell and are not an artifact of protein loss or ionic changes on isolation of the DNA. Analogously to supercoiling in eukaryotes (discussed below), the bacterial histone-like proteins could make some contribution to supertwisting or help stabilize gyrase-induced supercoils (*76*).

The striking physiological effect of inactivation of gyrase is the complete inhibition of replicative DNA synthesis. This inhibition occurs after treatment with

nalidixic acid or coumermycin A_1 (*77*) and temperature shift upwards of a *gyrA* temperature-sensitive mutant (*29, 30*). The rapidity of the inhibition implies a role in the elongation phase of replication. Gyrase could function as an active version of the replication "swivel" first postulated by Cairns (*78*) to relieve the positive twisting stress generated by the unwinding of the double helix during replication (*79*). Alternatively, or additionally, the maintenance of negative superhelicity by gyrase may be required for the binding of replication proteins. This is exemplified in the early finding that DNA gyrase is required for ϕX174 DNA replication. Supercoiling of ϕX174 replicative form DNA is needed for the initial nicking by *cisA* protein (*80*), and thus DNA synthesis is blocked by nalidixic acid or novobiocin (*81*). In the recent achievement of replication of ϕX174 DNA with the use of only purified proteins, DNA gyrase completed the requisite set of enzymes (*82*). Novobiocin has an interesting selective effect on *B. subtilis* DNA synthesis (*83*). Only a limited segment of the chromosome at the origin is replicated in the presence of the drug and this allowed precise mapping of this region.

Gyrase is required for other metabolic processes involving DNA as substrate or template. In their evolution, such processes may have taken advantage of the supercoiling of DNA for recognition and activity (*22*). This seems to be the case for at least some aspects of DNA transcription, repair, and recombination.

Treatment with gyrase inhibitors or mutational inactivation of gyrase generally results in only a partial inhibition of overall RNA synthesis (*29*); however, nalidixic acid completely blocks phage S13 transcription (*84*). The resolution of this puzzle seems to be that the dependence of transcription on gyrase varies for different operons (*85-90*). The very same gene may be either sensitive or resistant, depending on the promoter it is read from (*87, 88, 90*). An illuminating example is that of phage N4 early transcription, which is obliterated by nalidixic acid, coumermycin A_1, or *gyrA* conditional lethal mutations (*86, 91*). The purified N4 RNA polymerase transcribes only single-stranded N4 DNA in vitro. However, in the cell it is likely that supercoiling of duplex N4 DNA facilitates local denaturation of the duplex and binding of the polymerase to the appropriate strand. A similar explanation may apply to other instances of gyrase effects on transcription, and the assistance needed could vary depending on the ease of helix unwinding at the promoter site. Consistent with this is the report that gyrase inhibitors selectively interfere with the initiation of transcription (*90*). Promoters are generally associated with palindromic DNA sequences and these loop out to form hairpin-like structures when contained in supercoiled DNA generated by gyrase (*22*).

The role of gyrase in λ *int*-promoted recombination has been demonstrated and, indeed, led to the initial discovery of supertwisting activity. Coumermycin A_1 effectively blocks both supercoiling of λ DNA and *int*-promoted recombination in vivo and in vitro, and both effects are absent in a drug-resistant *gyrB* mutant (*71, 92*). Furthermore, the requirement for gyrase in this recombinational process is simply to provide negative superhelicity, since the enzyme is totally dispensable when a supertwisted DNA substrate is provided (*14*). An involvement of gyrase in other recombination pathways is also possible. The quintessence of recombination is transient double-strand breaks that are inherent in the gyrase reaction mechanism, and a logical start point for recombination is the interaction of single-stranded DNA with a homologous supertwisted DNA duplex (*93*). The properties of DNA cleavage by gyrase have also led to a suggested role in the transposition of genetic elements, an example of nonhomologous recombination (*94*).

Whereas early experiments implied that repair of damaged DNA was resistant to gyrase antagonists (*77*), a recent more sensitive study based on the restoration of infectivity of ultraviolet-irradiated phage λ DNA has shown clear inhibition (*95*). It is likely that only certain repair pathways are dependent on gyrase—in fact, nalidixic acid induces the *recA* gene product and an error-prone DNA repair pathway (*96*).

An exciting possibility is that the newly discovered catenation, uncatenation, knotting, and unknotting activities of DNA gyrase (*40, 51*) are also physiologically relevant. Catenanes are present wherever there are DNA rings and have been suggested as intermediates in the termination of replication and in recombination (*48*). Convincing evidence for their resolution into constituent monomers has been lacking (*49*), but the discovery of efficient uncatenation by gyrase provides the first enzymatic mechanism for the process. DNA can be knotted during packaging into phage heads (*97*) and, upon infection, the knots need to be untied, presumably by a topoisomerase such as gyrase.

While a fundamental role of gyrase in various physiological processes is clear, there are several complications to consider in interpreting physiological studies. First, the *gyrA* gene product is not just a part of gyrase but also of topo II′ (*24*). It is thus important to determine the involvement of both gyrase subunits in any process. Second, it is likely that nalidixic acid does not just inactivate its target protein but corrupts it, converting it into a poison (*30*). Phage T7 is exquisitely sensitive to nalidixic acid, but mutational inactivation of either the *gyrA* or *gyrB* gene product has no effect on T7 growth or DNA synthesis and, more importantly, prevents inhibition by nalidixic acid (*30, 98*). Therefore, results with drugs must be cross-checked with mutants. Third, an enzyme in vitro might need superhelical free energy to facilitate binding to DNA, but in the cell some other factor may enhance binding. These three limitations suggest caution but, fortunately, the convergence of different approaches has solidified many aspects of gyrase function.

Comparison of Origin of Supercoiling in Prokaryotes and Eukaryotes

With rare exceptions, circular DNA isolated from all natural sources is negatively supercoiled to about the same degree (*5*). Supercoils in the chromatin of eukaryotes almost surely arise from negative coiling of the DNA around histone octets to form nucleosome units (*99*) followed by enzymatic relaxation of compensatory positive supercoils (*100, 101*). Thus, as seems to be true for gyrase, the ultimate motive force for supercoiling in chromatin is spontaneous wrapping of DNA around a protein core. Even the extent of wrapping seems the same. Gyrase and the histone octet each protect about 140 base pairs of DNA from digestion by staphylococcal nuclease and an estimated one and a quarter supercoils from relaxation (*21, 23, 47, 99*). This similarity is surprising since the 400,000-dalton gyrase is much larger than the 110,000-dalton histone assembly.

The similarities in formation of supercoils by prokaryotes and eukaryotes seem to result not from evolutionary homology but from convergence to perform the same function. A distinguishing feature of histones is their basicity, and both gyrase subunits are acidic (*21, 28*). The topoisomerase and the spool for DNA are separate entities in chromatin but are united in gyrase. Gyrase acts catalytically and requires ATP, whereas supercoiling in chromatin is stoichiometric with the number of nucleosomes. The most fundamental difference is that gy-

rase protects a positive supercoil, but DNA is negatively supercoiled in nucleosomes. The free negative coils produced by gyrase represent a physiologically significant source of energy and are ready to assist in separating the double-helical strands (*102*), whereas the nucleosomal supercoils would have to be freed from the stabilizing histones before the energy of supercoiling could be harnessed.

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