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DNA Gyrase: Structure and Function

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ABSTRACT: DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative supercoiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA. The mechanism by which gyrase is able to influence the topological state of DNA molecules is of inherent interest from an enzymological standpoint. In addition, much attention has been focused on DNA gyrase as the intracellular target of a number of antibacterial agents and as a paradigm for other DNA topoisomerases. In this review we summarize the current knowledge concerning DNA gyrase by addressing a wide range of aspects of the study of this enzyme.

KEY WORDS: DNA gyrase, DNA-protein interactions, energy coupling, DNA topoisomerases, supercoiled DNA.

I. INTRODUCTION

A. General Introduction

The bacterial enzyme DNA gyrase (E.C. 5.99.1.3) was discovered in 1976 and found to possess the unique ability to catalyze the introduction of negative superhelical turns into closed-circuit double-stranded DNA.[1] Since then, this enzyme has been the focus of a great deal of attention concerning its structure, mechanism of action, interaction with antibacterial agents, and physiological role. Apart from the intrinsic interest of gyrase, it also serves as a useful model system for the study of DNA-protein interactions and biological energy coupling.

DNA gyrase belongs to a class of proteins called DNA topoisomerases that share the property of catalyzing interconversions between different topological forms of DNA. All topoisomerases are able to relax negatively supercoiled DNA, but only gyrase can also introduce negative supercoils into DNA. In this review we aim to summarize what is known to date about DNA gyrase, focusing our attention on the more recent developments.

B. DNA Supercoiling

In the well-known double-helical structure of DNA, the two antiparallel strands are coiled around each other.[3] A direct consequence of this intertwining is that, if a double-stranded DNA molecule is circular in form with no discontinuity in the backbone of either strand, the complementary single-stranded rings are linked. The topological state of covalently closed-circular DNA

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can be described by a simple equation:[4-6]

$$Lk = Tw + Wr \quad (1)$$

Lk is the linking number, and specifies the number of times the two strands of a DNA duplex circle are interwound. Lk is an integer and is invariant as long as the two strands remain intact. One way to arrive at the value of Lk is to constrain the duplex circle to a plane and count the excess of right-handed over left-handed crossings of one strand over the other. The value of Lk will generally be positive as DNA is normally a right-handed helix. Tw is the twist, and is determined by the local pitch of the DNA helix; it is equivalent to the number of "Watson-Crick" turns of the double helix. Wr is the writhe and is a measure of the contortion of the helix axis in space; this corresponds to the intuitive concept of supercoiling as a spiraling of the axis of the double helix.

Another formulation of linking number takes into account the fact that the axis of DNA does not necessarily lie in a plane, for example, when wrapped around a protein.[7] Here the linking number of surface-wrapped DNA is expressed as:

$$Lk = SLk + \Phi \quad (2)$$

where SLk is the surface linking number, which takes account of the effects of the surface configuration on twist and write, and Φ is the winding number, which is a function of the helical repeat relative to the surface on which the DNA axis lies. Both SLk and Φ are integers. For more rigorous definitions of these parameters see White et al.[7]

If nicked-circular DNA, i.e., a DNA molecule under no torsional constraint, is closed by a DNA ligase the resulting DNA molecules are said to be relaxed and have linking numbers approximately equal to the number of duplex turns in the nicked DNA (Tw°). Tw° is equal to the number of base pairs of the DNA (N) divided by the number of base pairs per helical repeat, often taken to be 10.5.[8-11]

$$Tw° = N/10.5 \quad (3)$$

In fact, under such conditions, a series of DNA topoisomerases is formed, where the linking number of each topoisomer differs from its neighbors by one.[12,13] These can be visualized as a ladder of bands on an agarose gel.[14] The concentration distribution of the DNA topoisomerases in relaxed DNA is Gaussian with a standard deviation of $\sqrt{(RT/2K)}$ centered at Tw°, where R is the gas constant, T the absolute temperature, and K is a constant roughly inversely proportional to the length of the DNA.[12] In relaxed plasmids, the linking number of the most abundant relaxed topoisomer, Lk° approximates to Tw°.

Naturally occurring closed-circular DNA molecules generally have linking numbers, Lk, which are less than Lk°. The difference between these values, $\Delta Lk = Lk - Lk°$, is the linking difference of a DNA molecule.[15] Values of ΔLk may be either positive or negative depending on whether a DNA molecule is overwound or underwound with respect to its relaxed state. If the linking number of a given molecule exceeds Lk°, ΔLk is positive and the DNA is said to be positively supercoiled. Similarly, a negative value of ΔLk defines negatively supercoiled DNA. To compare the topological properties of DNA molecules of different sizes, the superhelical density, $\Delta Lk/Lk°$ (more accurately called the specific linking difference), is usually quoted and normalizes the linking differences with respect to the length of the DNA circle in question. For small DNA circles (say <500 bp), the approximation Lk° = Tw° will not generally be true, in this case the specific linking difference is more accurately expressed as (Lk − Tw°)/Tw°,[16] this is because the most abundant relaxed topoisomer has a linking number that can often be very different from Tw°.

A nicked DNA molecule is able to change its twist or writhe under conditions affecting the geometry of the DNA helix, for example, in the presence of intercalating agents such as ethidium bromide. However, the only way in which a closed-circular DNA can respond to such conditions is by altering both its writhe and twist in a coordinated fashion according to Equation 1 above. The linking number must remain unchanged.[12,13] The linking number of a closed-circular DNA molecule can only be changed by the breakage and resealing of the phosphodiester backbone. For plasmid DNA molecules, it is

thought that moderate changes in linking number affect writhe more than twist.[10,11]

The supercoiling of DNA has been implicated in many *in vivo* processes, e.g., DNA replication, recombination, and transcription.[29-31] Supercoiling reduces the overall three-dimensional volume of closed-circular DNA, so aiding its packaging into the cell. Negatively supercoiled DNA is more easily unwound, and may allow RNA polymerase to bind more readily to the DNA, since this process involves unwinding of the helix, hence promoting enhanced transcription of certain genes,[32] although the transcription of some genes may be suppressed by increased negative supercoiling of the DNA template.[33,34]

C. DNA Topoisomerases

The topological state of closed-circular DNA molecules can be altered by a class of enzymes called DNA topoisomerases. These enzymes are able to catalyze changes in the tertiary structure of DNA both *in vitro* and *in vivo* (for reviews see References 17 to 25). There are two classes of topoisomerase enzymes (termed type I and type II) that can be distinguished by an operational difference; the type I enzymes catalyze DNA interconversions during which the linking number changes in steps of one, while the type II enzymes perform reactions during which Lk changes in steps of two.

DNA topoisomerases have been isolated from viral, prokaryotic, and eukaryotic sources. The first activity of a topoisomerase to be described was the relaxation of negatively supercoiled closed-circular DNA by prokaryotic topoisomerase I.[26] The only topoisomerase so far shown to be able to introduce negative supercoils into DNA is prokaryotic topoisomerase II, also called DNA gyrase.[1] A "reverse gyrase" that introduces positive superhelical turns into DNA in the presence of ATP has been isolated from the thermophilic bacterium *Sulfolobus*.[27,28]

A series of recent reports suggests that prokaryotic DNA topoisomerases are involved in the relaxation of supercoils during transcription of the DNA template.[31,35,36] Some of the eukaryotic topoisomerases are thought to be the intracellular targets for certain anticancer drugs.[37,38] Immunofluorescence studies of chicken topoisomerase II have suggested the enzyme is part of the mitotic chromosome scaffold.[39,40] Recent experiments demonstrate that topoisomerase II is required for chromosome condensation and that its role is likely to be structural.[41] The possible *in vivo* roles of gyrase are discussed in greater detail below (see Section V).

D. DNA Gyrase

1. Genetic Data

DNA gyrase was first discovered in 1976 by Gellert and co-workers,[1] who were attempting to establish the *Escherichia coli* host factors required for bacteriophage λ site-specific integration. One of these host factors was shown to be an ATP-dependent enzyme capable of introducing negative supercoils into closed-circular duplex DNA, and was named DNA gyrase. The discovery of gyrase had been preceded by work on two classes of DNA synthesis inhibitors, the quinolones (e.g., nalidixic acid, oxolinic acid, and ciprofloxacin; see Figure 5A)[42] and the coumarins (e.g., novobiocin, coumermycin, chlorobiocin; see Figure 5B).[43] The cellular target of each of these antibacterial classes has since been found to be DNA gyrase.[44-47] Subsequently, gyrase has been found to be composed of two separate proteins, coded for by genes previously identified as genetic loci determining resistance to either nalidixic acid or coumermycin (*nal*A and *cou*). With the discovery of gyrase these genes are now referred to as *gyr*A and *gyr*B, and are located at 48 minutes and 83 minutes, respectively, on the standard *E. coli* K-12 chromosome map.[48] Temperature-sensitive and drug-resistant mutations of both the gyrase proteins (to be discussed in greater detail later) have been identified and found to map to the loci indicated here.[49,50] The gyrase enzyme has also been identified in other organisms (see Table 1). In a number of cases, where both the *gyr*A and *gyr*B genes have been identified, they have been found to be contiguous within the genome. For example, the *Bacillus subtilis* gyrase genes are both located near to the origin of replication and are separated

TABLE 1
Characteristics of the DNA Gyrase Genes from Different Organisms

Organism	Gram[a]	Gene	Length of gene (bp)	Distance between genes (bp)	Amino acids in protein	Size of protein (kDa)	Ref.
Escherichia coli	–	*gyr*A	2625	~1.5 10[6]	875	97	51
		*gyr*B	2412		804	90	52
Citrobacter freundii	–	*gyr*A				107	53
		*gyr*B				96	
Pseudomonas aeruginosa	–	*gyr*A				~400[b]	54
		*gyr*B					
P. putida	–	*gyr*A			806	90	234
		*gyr*B	2418				
Klebsiella pneumoniae	–	*gyr*A	2628		876	97	55
		*gyr*B					
Borrelia burgdorferi	–	*gyr*A	2430	14	810	90	
		*gyr*B	1917		639	71	
Mycoplasma pneumoniae	–	*gyr*A	5′ only[d]	1 bp overlap			56
		*gyr*B	1953		650	72	
Haloferax	–	*gyr*A	5′ only[d]	1	640		57
		*gyr*B	1920			71	
Neisseria gonorrhoea	–	*gyr*A					
		*gyr*B	2313		771	86[•]	235
Bacillus subtilis	+	*gyr*A	2463	214	821	92	58
		*gyr*B	1914		638	71	
Micrococcus luteus	+	*gyr*A				115	59
		*gyr*B				97	
Staphylococcus aureus	+	*gyr*A	2667	39	889	100	60[f]
		*gyr*B	3′ only[d]				
Streptomyces sphaeroides	+	*gyr*A		>3000			61
		*gyr*B				79[•]	

[a] –, a Gram-negative organism; +, a Gram-positive organism.
[b] Molecular weight of intact gyrase complex.
[c] Data of W. M. Huang, personal communication.
[d] Only partial sequence information is available.
[•] Resistance gene cloned.
[f] Data of L. M. Fisher, personal communication.

by only 214 base pairs.[58] One exception to this is *E. coli* where the gyrase genes are widely separated (Table 1). The two gyrase subunits can be purified to near homogeneity from *E. coli*,[62] and both the genes have been cloned into plasmids that allow their overproduction.[63-65]

The synthesis of gyrase is itself controlled by the level of DNA supercoiling within the cell.[34] Agents that block DNA gyrase activity, and thus decrease the level of intracellular supercoiling, can increase the *in vivo* rates of synthesis of the A and B subunits up to tenfold.[34] A systematic deletion analysis of the gyrase promoters indicates that a DNA sequence some 20 bp long, that includes the −10 consensus region, the transcription start point, and the first few bases of the gene, are responsible for the property of induction by DNA relaxation.[66]

Both the *gyr*A and *gyr*B genes of *E. coli* have been sequenced[51,52,67] and have been found to encode proteins of 874 (M_r 97,000) and 804 (M_r 90,000) amino acids, respectively. The molecular mass values are in close agreement with those predicted from SDS-polyacrylamide electrophoresis.[68] The A protein seems to have its major role in the breakage and reunion of DNA,[46] while

TABLE 2
Properties of *Escherichia coli* DNA Gyrase

	A Protein	B Protein
Gene	*gyr*A (2625 bp, formerly *nal*A)	*gyr*B (2412 bp, formerly *cou*)
Mol. wt.	96,887 (875 amino acids)	89,893 (804 amino acids)
Measured pI	4.5—5.5	~6
Calculated pI	4.9	5.6
Major role	Breakage and reunion of DNA	ATPase activity
Drug interactions	Likely target of quinolone drugs (e.g., nalidixic acid, ciprofloxacin)	Target of coumarin drugs (e.g., coumermycin A_1, novobiocin)

DNA Gyrase: mol. wt. = 373,560
Subunit structure $-A_2B_2$

the B protein has an ATPase activity.[47,68] Some of the physical properties of the *E. coli* gyrase proteins are listed in Table 2. The *Micrococcus luteus* DNA gyrase appears to resemble the *E. coli* enzyme very closely.[59] From the results of protein cross-linking experiments, the active enzyme from *M. luteus* is thought to be a tetramer of A_2B_2.[69] The tetramer structure has subsequently been confirmed for *E. coli* by small-angle neutron scattering, which yielded an estimated molecular mass of 353 kDa (the A_2B_2 structure has a calculated molecular mass of 374 kDa).[70] In general, both subunits are required for all the reactions of gyrase (see Table 3), although the A subunit does appear to bind DNA in the absence of the B subunit,[71-74] and the B subunit of gyrase is found to have a weak ATPase activity;[75,76] the ATPase activity of the gyrase B protein is only fully stimulated in the presence of the A protein and DNA.[76]

2. Reactions of Gyrase

DNA gyrase performs a number of topological interconversions of DNA molecules. First noted for its negative supercoiling reaction,[1] gyrase is also capable of relaxing negatively supercoiled DNA in the absence of ATP,[45,46] as well as catenating and decatenating two duplex DNA circles[77-80] and resolving a topologically knotted single DNA duplex.[78,81] In the presence of ATP, or the nonhydrolyzable analog 5′-adenylyl-β,γ-imidodiphosphate (ADPNP), gyrase can also relax positively supercoiled DNA (thereby reducing its linking number) in a reaction considered analogous to the introduction of negative supercoils.[82] The characteristics of each of these reactions are outlined in Table 3.

a. Supercoiling and Relaxation

The DNA supercoiling reaction requires, in addition to ATP, a divalent cation, such as Mg^{2+}, and is stimulated in the presence of spermidine.[1] Incubation of gyrase with a single purified DNA topoisomer has indicated that the enzyme alters the linking number in steps of two (characteristic of the type II topoisomerase mechanism).[78,83] This has been interpreted mechanistically in terms of the translocation of a DNA segment through a double-stranded DNA break.

DNA gyrase can supercoil DNA in a processive manner. One molecule has been estimated to produce a linking number difference of about 100/min at 30°C.[62] Since supercoils are introduced in steps of two per reaction, the turnover number for the supercoiling reaction is thus about one per second under these conditions. There is a limit to the degree of negative supercoiling that can be introduced into a DNA mol-

TABLE 3
The Reactions of DNA Gyrase

	Subunits required	ATP required	Inhibited by quinolones	Inhibited by coumarins
Supercoiling	A, B	Yes	Yes	Yes
Relaxation				
Negative supercoils	A, B	No	Yes	No
Positive supercoils	A, B	Yes[a]	Yes	N.D.[b]
Catenation	A, B	Yes	Yes	Yes
Decatenation	A, B	Yes	Yes	N.D.[b]
Unknotting	A, B	Yes	Yes	Yes
DNA cleavage	A, B	No[c]	No[d]	No
ATPase	B[e]	Yes	No[f]	Yes

[a] The nonhydrolyzable ATP analog ADPNP will also support this reaction.
[b] No data available.
[c] ATP can alter the DNA cleavage specificity and efficiency.
[d] The quinolone antibiotics are required to see efficient DNA cleavage by gyrase.
[e] The B subunit has low-level ATPase activity; full ATPase activity is only observed in the presence of the A protein and DNA.
[f] Quinolones do affect the ATPase activity of the B protein in the presence of A and DNA.

ecule. When the plasmid pBR322 is isolated from *E. coli* cells it is found to have a specific linking difference of approximately -0.06, corresponding to a linking number deficit of 25.[84] However, the maximum specific linking difference achievable by gyrase is -0.11,[1,16,85] which corresponds to a linking number deficit of about 46 or 47 for pBR322 DNA. This limit of specific linking difference has been found to be the same for small (150 to 400 bp) and large (4 kb) DNA circles.[16] The *in vivo* level of supercoiling may reflect the antagonistic effects of gyrase and topoisomerase I;[86] recent reports suggest that transcription and other processes may also affect the level of supercoiling *in vitro*[31] (see Section V).

The negative supercoiling of relaxed DNA is ATP-dependent, and is competitively inhibited by ADPNP.[47] ADPNP will, however, support limited negative supercoiling by gyrase. Incubation of relaxed DNA with high levels of gyrase in the presence of ADPNP leads a linking difference (ΔLk) of -0.6 per gyrase tetramer.[47] This may suggest that the binding of nucleotide to gyrase is sufficient to allow a single cycle of supercoiling, but that the hydrolysis of ATP is required to return the enzyme to its starting state for another round of supercoiling. The supercoiling induced by ADPNP is less than stoichiometric to the amount of gyrase added; the fact that ΔLk is not -2 per gyrase could be due either to a proportion of inactive protein, or to some slippage occurring between the binding of nucleotide and DNA strand passage. Attempts to show stoichiometric reactions with negatively supercoiled substrates have indicated that for a negative specific linking difference greater than 0.04, ADPNP is unable to induce any supercoiling,[236] again suggesting that slippage occurs, and that beyond this point nucleotide binding and strand passage are largely uncoupled. It has been found that ADPNP can promote a catalytic relaxation of positive supercoils by gyrase.[87] It is possible, therefore, that ADPNP is able to promote the formation of a complex in which the DNA has been cleaved and the gap is being stabilized by the enzyme. This postulate is given further credence by the observation that ADPNP can induce limited double-stranded cleavage of DNA by gyrase when the complex is disrupted by sodium dodecyl sulfate (SDS).[237] The ATPase activity of gyrase will be discussed in more detail later.

The negative supercoiling reaction performed by gyrase is inhibited by both quinolone and coumarin drugs. The action of drugs on gyrase will be discussed in greater depth later (see Section IV).

In the absence of ATP, gyrase will relax negatively supercoiled DNA.[45,46] The relaxation activity of gyrase is much less efficient than the supercoiling reaction, with about 20 to 40 times as much enzyme required for a comparable rate.[62,88] Gyrase relaxation is inhibited by the quinolone drugs, but not by the coumarins.[45,46] It is possible that DNA relaxation is simply the reverse of the supercoiling reaction, and that ATP hydrolysis is required to drive DNA strand passage in one direction only.

b. Catenation, Decatenation, and Unknotting

Gyrase can catalyze the formation and resolution of DNA catenanes and can unknot knotted DNA. In principle, it would seem that gyrase should also be able to knot double-stranded DNA circles, but this reaction has not been reported. These reactions require ATP and are inhibited by the coumarin and quinolone drugs (see Table 3). Catenation and decatenation are stimulated by spermidine.[77] In principle, catenation and decatenation reactions would be not predicted to require ATP hydrolysis as, under appropriate DNA concentration regimes, these reactions are energetically favorable. It is found, however, that both these reactions require ATP. Indeed it has been shown that the nonhydrolyzable ATP analog ADPNP will not support gyrase-catalyzed decatenation.[238] One possibility is that these reactions proceed via a mechanism akin to DNA supercoiling, where ATP hydrolysis ensures efficient DNA strand passage. If the reactions were to proceed by the ATP-independent relaxation pathway, they might be too slow to detect by conventional methods.

II. STRUCTURE

The topoisomerase enzymes in general, and DNA gyrase in particular, offer intriguing problems to the enzymologist. The manner in which gyrase is apparently able to translocate a section of DNA through at least part of its structure, the nature of the protein-protein interactions within the enzyme complex, and the conformational changes likely to take place during the DNA supercoiling reaction are important problems. However, substantial progress in these areas is likely only with the advent of high-resolution structural information. To date, the three-dimensional structure of a topoisomerase has not been reported. Some success, however, has been achieved with γδ-resolvase, a protein that catalyses site-specific recombination and also possesses type I topoisomerase activity. Crystals of both the intact resolvase protein[89] and a putative catalytic domain[90,91] have been obtained. Recently, the structure of the catalytic domain has been solved at 2.7 Å resolution.[92] Although this part of the protein does not bind independently to DNA, it contains residues involved in catalysis and protein-protein interactions. In the case of gyrase, a number of relatively low-resolution structural techniques have been applied to the enzyme and to the enzyme-DNA complex. More recently, the first reports of crystals of the gyrase proteins have been communicated. These data are discussed in greater detail below.

A. Experimental Evidence for the Gyrase-DNA Complex

1. Biochemical

The A_2B_2 structure of DNA gyrase has been supported by a number of biochemical studies. Mixing different ratios of the gyrase proteins with DNA, followed by separation of the DNA-protein complex from free protein by gel filtration, showed that the gyrase-DNA complex contains equivalent amounts of the A and B proteins.[93] Sedimentation analysis of gyrase complexed with DNA fragments of about 140 bp in length suggested that a particle of molecular mass 470 kDa was formed, suggesting that an A_2B_2 complex is bound to the 140 bp of DNA.[69] This complex was also found to contain roughly equal proportions of the gyrase A and B proteins. Attempts to cross-link *M. luteus* gyrase, using dimethyl suberimidate, yielded a number of protein complexes of molecular masses 420 kDa, 330 kDa, and 230 kDa.[69] These species have been tentatively assigned as the A_2B_2, A_2B, and A_2 complexes, respectively. Cross-linking of the puri-

fied A protein also yielded the 230-kDa species, again suggesting the presence of A_2 dimers, while the purified B protein gave no cross-linked products using dimethyl suberimidate. However, ATPase experiments using the *E. coli* gyrase B protein show a nonlinear dependence of enzyme activity on protein concentration, consistent with oligomerization of this subunit.[75,76] More recently, the molecular mass of *E. coli* gyrase has been calculated from small-angle neutron scattering by measuring the scattering intensity at zero angle[70] (see below). The value obtained, 353 kDa, is again strongly suggestive of an A_2B_2 composition for the active enzyme complex. The apparent disagreement in the molecular weights of the A_2B_2 complexes from *M. luteus* and *E. coli* may be accounted for by the apparent differences in sizes of the A and B subunits from these two species (see Table 1).

The characteristics of the gyrase-DNA complex were initially probed by Liu and Wang.[59] During their studies on the *M. luteus* enzyme, they incubated gyrase with a nicked-circular DNA species. In the absence of ATP, the nicks were sealed with DNA ligase and the DNA was subsequently found to be positively supercoiled. This suggested that the DNA is wrapped around the enzyme with a unique handedness. By calculation from the data of Liu and Wang[59] a positive linking difference of about 0.4 to 0.6 is introduced into the DNA per gyrase molecule present. In a similar experiment, using the highly purified *E. coli* enzyme, a value of 0.8 per gyrase was obtained.[74] It has been assumed that this number is actually 1, corresponding to one complete positive superhelical turn of DNA around the gyrase molecule.[19,59] However, there is no necessity for this number to be an integer. First, the wrapping of DNA around gyrase could indeed be less (or more) than one complete turn, i.e., the DNA does not necessarily enter and exit the complex at the same point; there are currently no data that exclude these possibilities. Second, the wrapped DNA need not lie in a plane; indeed this is not possible if one complete turn is wrapped around gyrase. If the DNA wrap is out of plane, the apparent linking change in the ligation experiment will be affected. In addition, it is possible that there is an alteration of twist in the wrapped segment. Such arguments have been considered in discussions of the wrapping of DNA around the histone octamer in the nucleosome.[94]

Further evidence supporting the notion that DNA is wrapped around the protein in the gyrase-DNA complex has been obtained from the digestion of complexes with nucleases. Digestion of a nicked-circular DNA species with staphylococcal nuclease in the presence of either *E. coli* or *M. luteus* gyrase leads to the formation of DNA fragments of about 140 bp in length.[95] Further digestion of this DNA-protein "core complex" with DNase I leads to the formation of a set of DNA fragments differing in length by approximately 10 nucleotides. The length of DNA resistant to digestion, and the periodicity of the DNase I cleavage pattern are consistent with the wrapping of the DNA around the enzyme. This type of nuclease digestion pattern is reminiscent of that obtained for DNA associated with the eukaryotic nucleosome (reviewed by van Holde, Reference 94), where the DNA is wrapped around the outside of the histone octamer.

The gyrase-DNA complex has also been subjected to analysis by DNase I footprinting techniques.[87,96-99] Gyrase protects approximately 100 to 155 bp of DNA from nuclease attack, with a central region, of some 40 to 50 bp, being most strongly protected. The DNA flanking this region is less well protected, and there is evidence for sites of enhanced sensitivity to DNase I spaced 10 to 11 bp apart; these sensitive sites are staggered by two bases on the complementary DNA strands. Taken together, these data provide evidence for the wrapping of the DNA around the outside of the protein. Gyrase protects approximately 102 bp of DNA from digestion by DNase I in a 172-bp linear DNA fragment;[99] the bound gyrase is also found to hinder access of restriction endonucleases whose recognition sites lie within this 102-bp DNase I-protected region.[100] Given the nuclease protection data, a somewhat surprising result is that little or no protection of the DNA to methylation by dimethyl sulfate is afforded by gyrase,[98] suggesting that the entire length of the DNA in the gyrase-DNA complex is accessible to solvent.

Gyrase-DNA complexes can also be detected by their retention on nitrocellulose filters.[76,98,101-103] The complex, consisting of equal amounts of the gyrase A and B proteins, appears

to be very stable, with a half-life at 23°C of 60 to 70 h and an equilibrium dissociation constant of 0.1 to 0.5 n*M*.[76,103] Based on these data, an association rate constant (k_a) of the order of 10^4 to 10^5 M^{-1} can be calculated; this can be compared to a k_a for the lac repressor of 10^{10} M^{-1} s^{-1},[104] and may suggest that the binding of gyrase to DNA does not proceed via a facilitated mechanism. It was also noted that gyrase binds more strongly to relaxed or linear DNA than to supercoiled DNA by about a factor of ten.[103]

The strength of binding of gyrase to a particular linear DNA is dependent on the length of the fragment used. Morrison et al.[102] found that a more stable complex was formed between gyrase and a 509-bp DNA fragment than with a 176-bp fragment, while no detectable complex was formed with a 76-bp DNA fragment. Maxwell and Gellert[76] noted that gyrase readily forms complexes with fragments of 117 bp or larger, but will only form a complex with a 55-bp fragment at high DNA concentrations. The binding curve for gyrase and the 55-bp fragment was consistent with at least two DNA molecules binding to a single gyrase molecule in a cooperative manner. It is therefore possible that gyrase contains two or more DNA binding sites.[76,105]

Both the gyrase A and B proteins are relatively large (97 kDa and 90 kDa, respectively). Therefore the possibility exists that the proteins are arranged into discrete domains, which may be functional and/or structural entities. The first indication that a domain organization exists within the gyrase proteins came from the observation that purification of the B protein from certain bacterial strains resulted in the isolation of a protein of approximate molecular mass 50 kDa.[82,88] This protein (termed B′ or ν) was found to complement the A protein to generate a complex (topoisomerase II′), which was unable to supercoil DNA, but that had the ability to relax both negative and positive supercoils in the absence of nucleotides. Proteolytic digestion patterns of B′ suggested that it was a fragment of the B protein.[88] When the *gyr*B gene was sequenced, it became apparent that B′ was the C-terminal half of the intact B protein (extending from residue 394 to the C-terminus)[67] and may be produced by proteolysis during cell lysis.[239] It appeared that this fragment had lost either its ATPase activity or the energy transduction process that couples ATP hydrolysis to DNA supercoiling. Hence it was inferred that the ATPase activity of the B protein is located, at least partially, in the N-terminal portion of the B protein, while the C-terminal part is required for interaction with the A protein and DNA.[82] Using genetic manipulation techniques, the N-terminal portion of the B protein (residues 1 to 393) has been produced as a direct gene product.[106] This protein has been found to possess a novobiocin-sensitive ATPase activity and shown to bind novobiocin with a similar affinity to the intact B protein,[240] thus confirming the above inference.

Incubation of *E. coli* gyrase with an ATP analog, pyridoxal 5′-diphospho-5′-adenosine, results in the labeling of two lysine residues of the B protein.[107] These residues (Lys 103 and 110) are both located in the N-terminal region of the gyrase B protein. In addition, a novobiocin-resistant gyrase B protein from *Haloferax* has amino acid changes located in the N-terminal portion of the protein.[57] Therefore, taken with the evidence outlined above, all the amino acids required for the hydrolysis of ATP, and for the interaction with coumarin drugs, are apparently located within the N-terminal fragment. It is likely that the N- and C-terminal portions of the gyrase B subunit represent domains of the protein. Figure 1 shows a diagrammatical representation of the domain organization of both the gyrase A and B proteins.

Treatment of the gyrase A protein with either trypsin or chymotrypsin results in the generation of two large fragments, of approximate molecular masses 64 kDa and 33 kDa, which are relatively stable to further digestion.[74,108] The 64-kDa tryptic fragment (comprising residues 7 to 571 of the intact protein) was found, in the presence of the gyrase B protein, to support a low rate of DNA supercoiling while still able to perform quinolone-directed DNA cleavage as efficiently as the intact A protein. This 64-kDa protein contains both the active-site tyrosine residue (amino acid 122) involved in the covalent attachment of the protein to the DNA[64] and all the sites in the A protein to which quinolone-resistant mutations have been mapped.[109-111] The 33-kDa fragment (residues 572 to 875), however, was unable to support any of the reactions of gyrase, but the

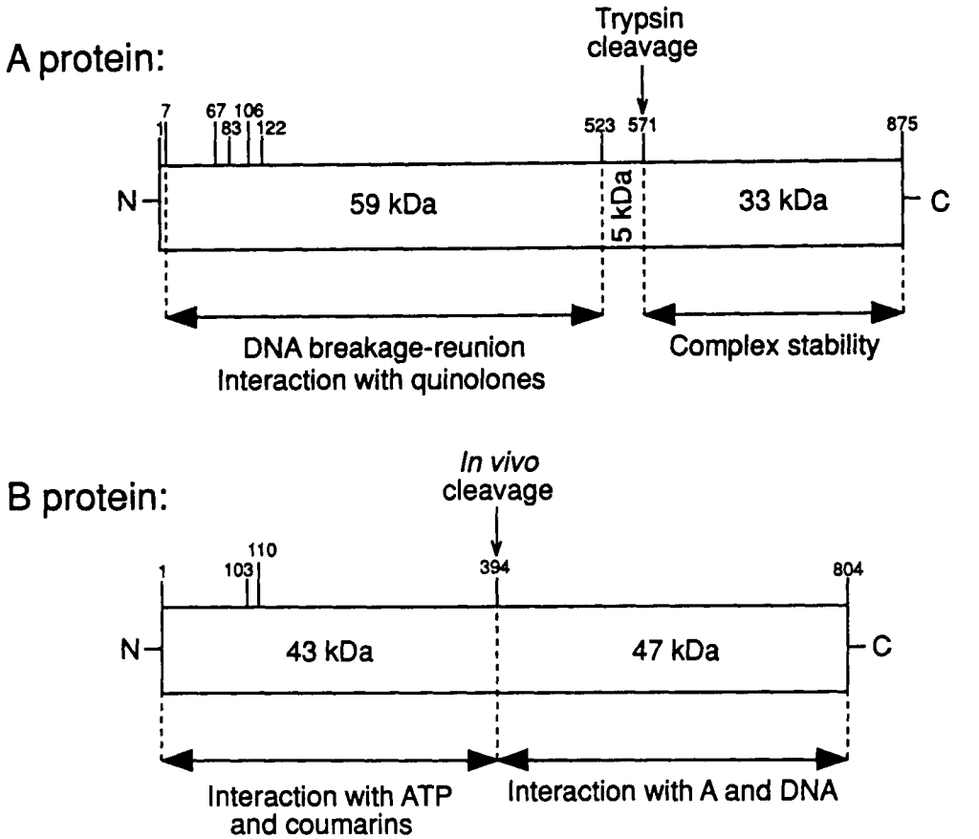

FIGURE 1. Proposed domain organization of *Escherichia coli* DNA gyrase. The gyrase proteins are represented as linear blocks with various amino acid positions indicated. A protein: the site of trypsin cleavage at amino acid 571 is shown.[108] The smallest protein fragment found to be capable of catalyzing DNA breakage and reunion is between residues 7 and 523.[112] Sites of some of the mutations leading to quinolone resistance (67, 83, 106) are indicated (see also Table 5), as is the site of DNA-protein covalent bond formation, Tyr-122.[64] B protein: the site of proposed *in vivo* proteolysis[67] and the position of two lysine residues (103 and 110) proposed to be involved in ATP interaction[107] are shown.

DNA supercoiling reaction catalyzed by the 64-kDa fragment was found to proceed more efficiently in the presence of the 33-kDa fragment.[112] This result has also been noted using the 33-kDa protein overproduced as a direct gene product.[113] It has therefore been suggested that the N-terminal portion of the A protein is principally involved in the breakage and reunion steps of the gyrase reaction, and may in fact form a breakage and reunion domain, while the C-terminal portion of the A protein seems to impart stability to the enzyme-DNA complex.[108] The C-terminal domain of the A protein has recently been shown to bind DNA in a magnesium-independent manner, in contrast to both the binding of A_2B_2 and the A protein alone to DNA.[113] In addition, the 33-kDa protein on its own can be shown to induce positive supercoiling of DNA. It is possible that this portion of the protein provides a nonspecific DNA-binding function that is involved in the wrapping of DNA around DNA gyrase.[113]

The limits of the domain boundaries within the A protein have been defined further by the construction of deletion derivatives of the gyrase A protein.[112] The 64-kDa protein, made as a direct gene product, was found to have the same properties as the trypsin-generated fragment. It has been shown that the N-terminal 6 amino acids of the A protein are completely dispensable for enzymic activity. However, deletion of the N-

terminal 69 amino acids results in the inactivation of the A protein. A number of deletion mutants from the C-terminal end of GyrA have been constructed. The smallest fragment of the protein able to perform quinolone-directed cleavage of DNA was found to be GyrA (7 to 523), i.e., includes amino acid residues 7 to 523 of the intact A protein.[112]

2. Biophysical

Gyrase and complexes between gyrase and DNA can be analyzed by electron microscopy.[72,73,100,114] The active gyrase protein (A_2B_2) appears as a particle some 200 to 250 Å in diameter. It has been reported that the gyrase A protein, in the absence of the B protein, will also form associations with DNA.[72] The A protein alone has also been shown to form a complex with linear DNA fragments using a gel retardation assay.[74,113] Therefore, although both the A and B proteins are required for the catalytic activities of gyrase (see Table 3), the A protein is apparently capable of interacting with DNA on its own.

Moore et al.[72] noted that gyrase was often located at the intersection of two DNA duplexes in electron microscopy images, forming looped structures with closed-circular DNA, suggesting the presence of multiple DNA binding sites on gyrase. However, this phenomenon has not been observed by other groups (e.g., Reference 114) and may be a consequence of differences in the conditions used to form complexes, or in the way they were prepared for electron microscopy. Gyrase and its complexes with DNA have also been studied using high-resolution electron microscopy.[73] These data suggest that the A_2B_2 complex is "heart-shaped" with the A proteins forming the upper, and larger, lobes of the structure. The size of the particle was estimated to be approximately 260 to 280 Å from top to bottom; these values not being corrected for platinum decoration. Estimates of the amount of DNA associated with gyrase in these complexes, by electron microscopy, range from 90 to 115 bp (if the distance between the DNA entry and exit points on the protein particle are measured) or from 155 to 161 bp (measuring only the free DNA),[73,100] the differences being attributed to the length of DNA across the diameter of the gyrase particle. These values are in reasonable agreement with the extent of DNA protected by gyrase from nucleases.

Complexes between gyrase and linear DNA fragments, ranging in size from 127 to 256 bp, have been analyzed by transient electric dichroism.[99] The results, together with complementary nuclease digestion data, support a model whereby the DNA makes a single turn of about 120 bp around the gyrase particle. The entry and exit points of the DNA are thought to be close together, and the average angle between the DNA tails emerging from the protein particle is about 120°. The nonhydrolyzable ATP analog ADPNP, which is known to induce noncatalytic supercoiling by gyrase,[46] was found to induce a dichroism change when added to the gyrase-DNA complex. This change has been interpreted as a consequence of the DNA tails being wrapped onto the protein surface. The quinolone drugs, which inhibit gyrase and promote gyrase-directed double-stranded cleavage of DNA under certain conditions,[45,46] apparently had little effect on the gyrase-DNA complex when monitored by these methods.[99] However, the presence of the quinolone drugs did prevent the presumed conformational change from occurring in the presence of ADPNP. This observation may reflect increased stability and altered properties of gyrase-DNA complexes in the presence of quinolone drugs.[103] The electric dichroism data suggests that the gyrase-DNA complex is a globular particle approximately 170 Å in diameter.[99] A compact globular protein of molecular mass 374 kDa complexed with DNA should have a diameter of about 120 Å. The apparent size of the particle suggests that the gyrase tetramer is not a compact structure. It was therefore postulated that gyrase may contain cavities or channels within its structure. An earlier indication that gyrase may contain cavities was given by the data of Klevan and Wang,[69] who performed sedimentation analysis of gyrase complexed to approximately 140 bp of DNA and obtained a value for the ratio of the frictional coefficient to that of the hydrated equivalent sphere of 1.9, suggesting that there may be extensive bound and entrained solvent associated with the complex.

Gyrase and its complexes with DNA have

been characterized by small-angle neutron scattering and dynamic light scattering.[70] A model consistent with the scattering data comprises an oblate particle with approximate dimensions 175 Å wide by 52 Å thick. The calculated radius of gyration of the particle (64 to 67 Å) is considerably greater than that which would be expected for a 400-kDa compact globular protein (estimated to be 43 Å). This again suggests that gyrase molecules contain channels or cavities, possibly of the order of 15 Å wide.[70] The radius of gyration of the particle was not found to change significantly on the binding of DNA, which may suggest that the DNA is not wrapped around the outside of a protein core as it is in the nucleosome[115] but may be somewhat embedded into the protein.

A potential problem with both the dichroism and scattering experiments is that there may not be a unique interpretation of the data, i.e., the conclusions are highly model dependent. There are also other drawbacks to using these techniques, for example, in electric dichroism experiments of this type, only data from the DNA can be directly obtained; information about the protein must be inferred. During small-angle neutron scattering experiments the molecules are able to take up all possible orientations in solution and therefore the data obtained are spherically averaged. The structures for gyrase obtained by these methods should hence be treated with a degree of caution.

The gyrase proteins have been subjected to analysis by differential scanning microcalorimetry.[241] As the temperature of a protein in solution is raised, it will begin to unfold and denature. At a sufficiently high temperature, the noncovalent interactions, holding the protein's secondary and tertiary structural elements, will break down, and the protein will take up a random coil structure. If a protein consists of two or more domains, it is possible that they will unfold at different temperatures, depending on the relative thermal stability of each domain. The unfolding of a protein domain requires energy, which can be detected in a scanning microcalorimeter as a difference in temperature between a buffer blank and the protein-containing sample. Such an approach has been used successfully to elucidate the domain structure of the λ repressor.[116] Both the gyrase A and B proteins show two thermal unfolding transitions when analyzed in this way. The N-terminal fragments of each of the proteins unfold with a single, almost symmetrical transition. The unfolding temperatures of both the N-terminal fragments can be equated with unfolding transitions occurring in the intact protein. This provides strong supporting evidence for the organization of each of the gyrase proteins into two distinct domains, as suggested by Reece and Maxwell[112] and Jackson et al.,[106] based on biochemical evidence.

B. Crystallography

A number of groups have attempted chrystallographic analysis of the gyrase proteins with little or no success. However, reports have recently appeared suggesting that crystal structures of at least part of the proteins will be possible. Using a lipid monolayer to which novobiocin is covalently attached, Lebeau et al.[117] have succeeded in forming two-dimensional crystals of the B protein under physiological conditions of pH and ionic strength. Electron diffraction data of the two-dimensional crystals at 27 Å resolution have been interpreted in terms of the B protein having a "bean"-like appearance with two asymmetric lobes. These data also suggest that the B_2 dimer is a head-to-tail arrangement of two of the bean-shaped molecules. It is possible, however, that this B_2 structure may have arisen as a result of the packing of the B molecule on the novobiocin-lipid membrane rather than reflecting the true structure.

Reece et al.[118] and Jackson et al.[106] have reported the crystallization and preliminary X-ray analysis of N-terminal fragments of the A and B proteins, respectively (see Figure 1 for the location of these fragments). The N-terminal domain of the A protein can be crystallized from ammonium sulfate solutions, and crystals have been obtained which diffract to 4.5 Å resolution. The N-terminal 393 residues of the B protein, in association with ADPNP, can be crystallized from polyethylene glycol solutions, and crystals have been obtained which diffract to high resolution. Indeed, the structure of the N-terminal portion of the B protein-ADPNP complex has been re-

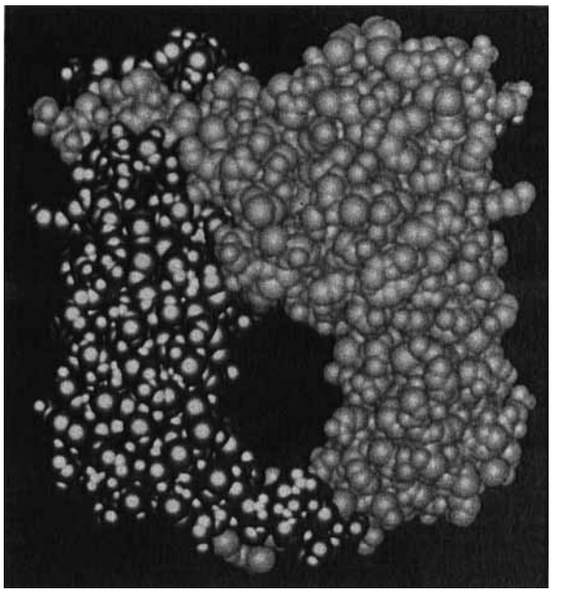

FIGURE 2. Space-filling model of the N-terminal fragment of the gyrase B protein. The two monomers are picked out in different shades of gray. The N-terminal domains are in the upper part of the figure and the C-terminal domains are in the lower part, forming the sides of the hole between the two monomers.

cently solved at 2.5 Å resolution.[119] A space-filling model of this structure is shown in Figure 2. The protein monomer consists of two domains: a N-terminal domain (residues 2 to 220) that contains the bound ADPNP and a C-terminal domain (221 to 392). The protein is proposed to be a dimer with most of the monomer-monomer contacts occurring between the N-terminal domains. This includes a N-terminal arm that protrudes from the surface of the monomer and wraps around the N-terminal domain of the other subunit (Figure 2). The C-terminal domains form the sides of a hole through the protein dimer approximately 20 Å wide. It is possible that this hole forms part of a gateway through which DNA is passed during the supercoiling reaction (see Section III). Again, it is important to stress that the proposed dimer contacts for the N-terminal B fragment could be a consequence of crystal packing.

C. Models for the Gyrase-DNA Complex

The most detailed models of the gyrase-DNA complex have been suggested by Kirchhausen et al.[73] and Krueger et al.,[70] based on interpretations of electron microscopy and scattering data, respectively. Kirchhausen et al.[73] suggested that the gyrase complex is heart shaped and that the DNA wrapped around the protein such that the center of the DNA is located between the heart's upper lobes. Small-angle neutron scattering predicted a model for the gyrase-DNA complex that is similar to the electron microscopy model and suggested that channels or cavities exist within the particle.[70]

Figure 3 shows a proposed model of the gyrase-DNA complex. It is based on those of Kirchhausen et al.[73] and Krueger et al.[70] and can be regarded as an update of those earlier models. This model is intended to emphasize certain features of the gyrase-DNA complex: the wrapping of DNA around the protein, the presence of solvent-filled channels, and the possible domain organization. It can be regarded as a slice through the center of the oblate particle; no significance should necessarily be attached to the shapes of the A and B subunits in this model.

In the gyrase-DNA complex about 120 bp of DNA are wrapped around the protein. The DNA entry and exit points are located close together, and the DNA tails are thought to be at an angle of 120°.[99] A 120-bp segment of B-form DNA should have a length of approximately 410 Å. If we assume that the DNA is smoothly wrapped around gyrase, then the diameter of the resulting circle, at the outside edge of the DNA, will be about 150 Å. The size of the gyrase particle has been estimated to be 175 Å by 52 Å.[70] Therefore, the DNA is likely to be embedded into the protein structure, which will extend beyond the wrapped DNA. The shape of the subunits shown is arbitrary, but the B protein has been drawn as bean shaped, as suggested by Lebeau et al.[117]

The N-terminal two thirds of the A protein has been shown to be involved in the cleavage and reunion of DNA is capable of interacting with the B protein and has the ability to dimerize (our unpublished observations). The C-terminal third of the molecule seems able to contribute to the stability of the DNA-protein complex.[108] The N-terminal half of the B protein possesses an ATPase activity, and is probably able to form dimers,[242] while the C-terminal half of the protein

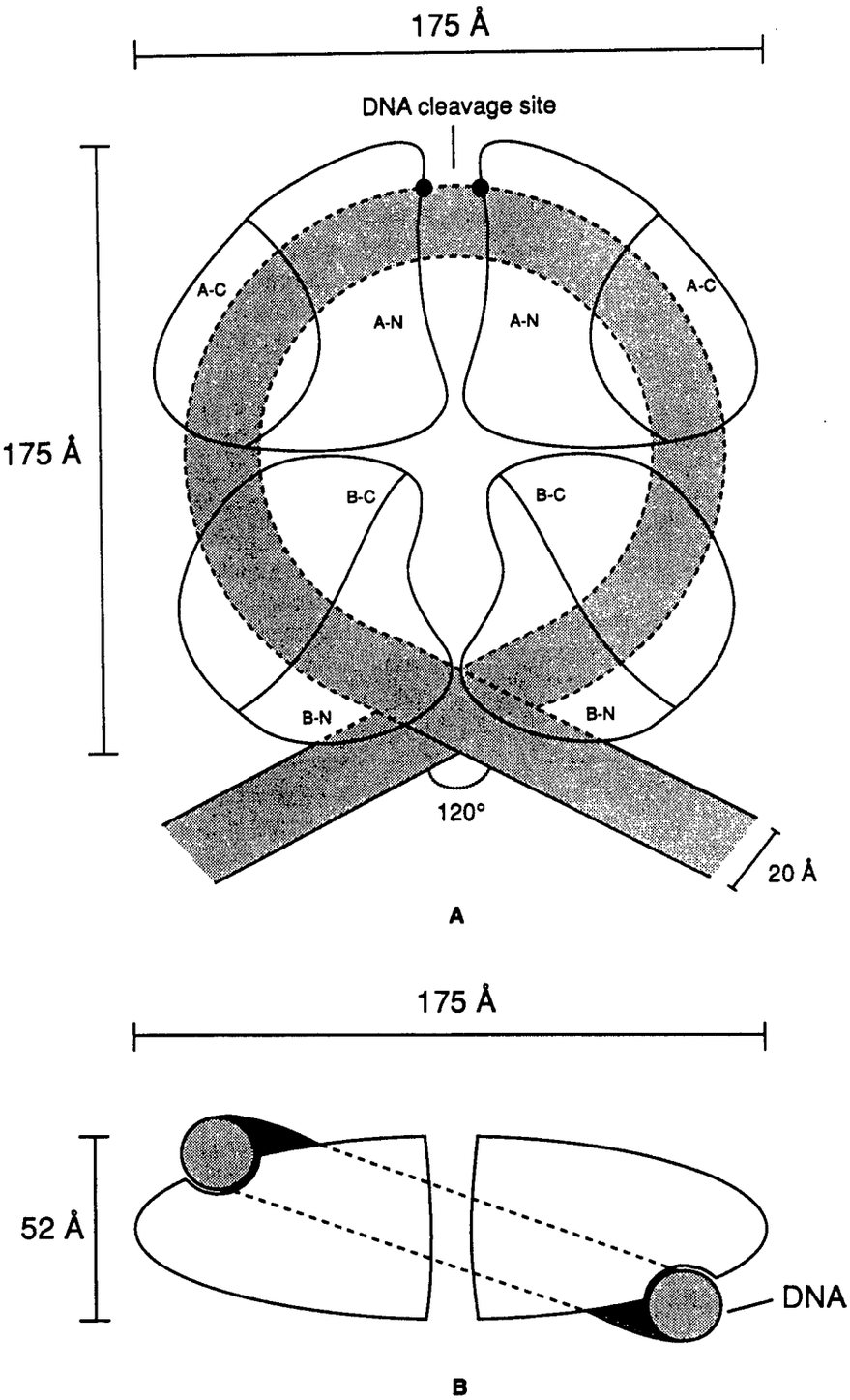

FIGURE 3. Proposed structure of the DNA gyrase-DNA complex. (A) The DNA is shown as a shaded loop wrapped around the A and B subunits. The A proteins are in the upper part of the model and the B proteins in the lower. −N and −C indicate the amino- and carboxy-terminal domains of the proteins. The black dots represent the sites of covalent attachment between the enzyme and the DNA. (B) A transverse section of the model indicating the DNA around the protein complex.

interacts with both the A protein and with DNA.[82,88] Both electric dichroism and small-angle neutron scattering data have suggested that gyrase contains cavities or channels within its structure that could be around 15 Å wide.[70,99] These have been represented in Figure 3 as intersubunit channels. Such structures would provide a route for the translocated DNA to pass through the protein structure.

The sites of DNA cleavage by gyrase mostly occur approximately in the center of the nuclease-protected regions.[96-98] Gyrase cleaves DNA in both strands to yield phosphotyrosine linkages with amino acid 122 of the A protein, with a four-base stagger between the single-stranded breaks.[64,120] The cleavage site is shown in Figure 3 on one side of the DNA only. However, since DNA is helical, with about 10.5 bp per turn in the B form, a difference of four bases will mean that nucleotides in different strands will be on approximately the same side of the helix, and separated by about 15 Å. Therefore the cleavage site could be at the mouth of the channel as shown in Figure 3A.

Figure 3B shows a side-on section of the proposed gyrase-DNA complex. The DNA is wrapped at an angle around the oblate gyrase particle. In this configuration, the DNA should be accessible to nuclease attack, yet the binding of DNA to gyrase would not appreciably alter the size or shape of the particle, in agreement with the neutron-scattering data.[70]

High-resolution X-ray diffraction data will probably be required in order to elucidate the precise structure of the gyrase complex. The fragments of the protein crystallized so far comprise the DNA breakage and reunion, and ATPase activities of the enzyme.[106,118,119] Three-dimensional structural information concerning these domains should give a much clearer indication of how gyrase interacts with both DNA and ATP, but the structure of the whole complex may need to be solved before the exact mechanism of the supercoiling reaction (i.e., ATP-coupled DNA strand passage) can be determined. Perhaps one of the most important potential uses of three-dimensional structural information of the gyrase proteins would be for rational drug design of both quinolones and coumarins, which could lead to important advances in antibiotic development.

III. MECHANISM

A. Introduction

DNA gyrase is unique among the topoisomerase family in being the only enzyme capable of catalyzing the negative supercoiling of DNA. It has been suggested that a negative supercoiling activity exists within *Xenopus* oocytes,[121,122] but these reports have not been substantiated with further evidence. Recently, a factor from the posterior silk gland of *Bombyx mori* has been described that is thought to complement eukaryotic topoisomerase II to produce a supercoiling activity;[123] this factor is required in considerable molar excess over the DNA before the supercoiling reaction can be observed. It is not clear how supercoiling is achieved by this factor, but one proposal is that it may dictate the coiling of DNA around topoisomerase II.[123] It is likely that the observations of these activities in eukaryote cells represent "passive" supercoiling, as distinct from the active supercoiling of DNA gyrase.

All topoisomerase reactions involve the binding of the protein to DNA, DNA cleavage, strand passage, DNA reunion, and in a number of cases ATP hydrolysis, and the enzymes are likely to share a similar mechanism of action to gyrase.[25] Although DNA gyrase conforms to the general topoisomerase mechanism, it must also possess unique mechanistic features that determine its ability to actively supercoil DNA. The observed reactions of DNA gyrase are listed below:

1. ATP-dependent negative supercoiling of closed-circular double-stranded DNA
2. ATP-independent relaxation of negatively supercoiled DNA
3. Nucleotide-dependent relaxation of positively supercoiled DNA
4. Formation and resolution of catenated DNA
5. Resolution of knotted DNA
6. Quinolone or calcium ion-induced double-stranded breakage of DNA
7. DNA-dependent ATP hydrolysis

It is likely that each of the above reactions is an aspect of a single reaction mechanism occurring with different substrates, or under different conditions. Therefore, we shall consider the mech-

anism of the negative supercoiling reaction by gyrase (about which most is known) and attempt to explain the other reactions in terms of this mechanism.

B. DNA Binding, Cleavage, and Reunion

The binding of gyrase to DNA results in the formation of an organized DNA-protein complex in which approximately 120 bp of DNA are wrapped around a protein core. The nature of this complex has already been discussed in some detail (see Section II), and will not be dealt with further here.

In the presence of quinolone drugs (e.g., oxolinic acid, ciprofloxacin), and following subsequent addition of a protein denaturant, such as SDS, *E. coli* DNA gyrase induces double-stranded DNA cleavage with a 4-bp stagger at the cut site.[45,46,87,96,98,120] Following cleavage, the protein is covalently associated with the DNA. Little DNA cleavage by *M. luteus* gyrase is induced by the quinolone/SDS treatment, but the addition of alkali promotes cleavage by this enzyme.[124] Linear, relaxed, and supercoiled DNA are all substrates for cleavage by *E. coli* gyrase,[46] but the cleavage of single-stranded DNA has not been reported. This is consistent with the observation that single-stranded DNA is not bound by gyrase.[25] Cleavage does not require ATP, but ATP or ADPNP can change the efficiency of cleavage and alter the pattern of preference among cleavage sites.[47,96,101] Although DNA is usually broken in both strands, a small proportion of single-stranded breakage can also be detected.[45] If uniformly radiolabeled DNA is used in a cleavage experiment, then, after protein denaturation and digestion of the DNA, the protein can be shown to be labeled. Paper chromatography of the acid-treated labeled protein shows that there is a covalent attachment between gyrase and the cleaved DNA via a phosphotyrosine linkage.[93,124] The labeled protein in such an experiment is the A subunit of gyrase. The 5′-ends of the cleaved DNA are blocked to labeling by T4 polynucleotide kinase, while the 3′-hydroxyl ends of the cut site remain free and able to prime a DNA polymerase reaction.[120] The precise point of attachment between the protein and the 5′-end of the DNA cleavage site has been identified by a series of proteolytic digestions and sequencing of the labeled peptides as tyrosine residue 122 of the A subunit of gyrase.[64]

Efficient DNA cleavage by gyrase can also occur in the absence of quinolone drugs if Ca^{2+} is substituted for Mg^{2+} in reaction mixtures.[108] Cleavage induced by Ca^{2+} occurs at the same loci as that induced by oxolinic acid but with different relative efficiencies.[243]

The site of DNA cleavage by gyrase generally occurs approximately in the center of the region protected from nuclease action.[96-98] Analysis of oxolinic acid/gyrase-induced DNA cleavages sites *in vivo*[125] has suggested the following consensus sequence (also shown is the preferred cleavage site at position 990 in pBR322 DNA):

```
                                    ⇓
consensus seq. 5′-RNNNRNNRTGRYCTYNYNGNY-3′
                              {G}   G   {T}

                                ⇓
pBR322 seq. 5′-GGCTGGATGGCCTTCCCCAT-3′
                          990
```

T and G at the 13th position of the consensus sequence are equally preferred and the G and T in brackets are preferred secondarily to T and G, respectively. DNA cleavage occurs at the site indicated by the arrow. Although there are many degeneracies, this sequence is approximately symmetrical and is consistent with many sites found *in vitro*.[96-98] In the plasmid pBR322 there is a preferred cleavage site at nucleotide position 990 (see above), but a total of 74 quinolone-induced cleavage sites have been mapped *in vivo* using this plasmid.[126]

In vivo treatment of *E. coli* cells with oxolinic acid and subsequent addition of SDS leads to the formation of chromosomal DNA fragments in the size range 25 to 100 kb.[127,128] Snyder and Drlica[127] suggested that the whole *E. coli* genome has 45 to 50 major cleavage sites, corresponding to about one cleavage event per topological domain within the chromosome. A large number of weaker cleavage sites also exist within the genome. A 10-kb region of the *E. coli* chromosome has been examined and found to contain 24 cleavage sites,[129] i.e., the entire *E. coli* chromosome could have in excess of 10,000 cleavage sites, of which

less than 0.5% have been identified as major sites. A possible explanation, assuming that the cleavage sites represent a major fraction of the places where gyrase interacts with DNA, is that a small number of strong interaction sites are used by gyrase to maintain superhelical tension in the chromosome as a whole, and the weaker, dispersed sites allow gyrase to provide local swiveling needed for transcription and replication[130] (see Section V). However, it is possible that there is a limited amount of gyrase distributed among a large number of possible cleavage sites in the *E. coli* chromosome and that only a small proportion of the sites are utilized at any one time.

Recent data support the notion of a limited number of major gyrase binding sites in the *E. coli* chromosome, and suggest that local transcriptional activity may modulate the extent of oxolinic acid-induced cleavage at these sites.[131] One proposal is that major gyrase binding sites (''toposites'') may represent DNA-membrane attachment sites and thus serve as nucleotide domain boundaries[131,132] (see Section V).

There have been several proposals for the existence of specific sites of interaction of DNA gyrase on DNA. In *E. coli* and *Salmonella typhimurium* there exists a family of repetitive extragenic palindromic (REP) sequences. There is evidence that gyrase binds preferentially to these sites and that they may be important sites of gyrase action *in vivo*.[133] In plasmid pSC101, the *par* locus has been proposed as a gyrase binding site[134] (see Section V), and in bacteriophage Mu a centrally located sequence is proposed to be a strong DNA gyrase binding site that is required for efficient replicative transposition.[135]

The major site of quinolone-induced gyrase cleavage in pBR322 (centered at nucleotide 990) has been studied in detail. Mutations at this site can reduce or abolish the cleavage activity.[136] It has also been shown that a 34-bp fragment encompassing the cleavage site is not a substrate for the cleavage reaction, but if this sequence is extended by 85 bp in either direction quinolone-induced cleavage at the site will then occur,[136] i.e., the sequences immediately surrounding the cleavage site are insufficient to support cleavage without flanking DNA. This phenomenon is likely to reflect the length of DNA required by gyrase to form a DNA-protein complex.[76] It is possible that factors other than the DNA sequence, such as the flexibility of the flanking DNA, may influence the cleavage reaction at a particular site.

The gyrase-related enzyme topoisomerase II′ (composed of the A protein and the C-terminal fragment of the B protein) also demonstrates quinolone-dependent DNA cleavage in a similar fashion to the complete enzyme.[82,88]

The mechanism of DNA reunion after cleavage by gyrase is not known. There is no direct experimental evidence available to suggest how this reaction occurs. However, if samples are briefly heated to 80°C before the addition of SDS in a quinolone-directed cleavage reaction, the supercoiled substrate remains intact.[45] This result suggests either that cleavage occurs only after SDS addition, or, more probably, that the broken DNA ends are efficiently resealed prior to protein-DNA dissociation.

C. ATPase Activity

ATP is required for most of the reactions of DNA gyrase. The hydrolysis of ATP to ADP and inorganic phosphate is greatly stimulated by linear, nicked-circular, and relaxed closed-circular double-stranded DNA,[68] but is reported to be less well stimulated by highly negatively supercoiled DNA.[68,137] However, subsequent experiments[236] have suggested that there is no difference between the stimulation of the ATPase reaction by relaxed and highly negatively supercoiled DNA. The number of superhelical turns introduced into an initially relaxed circular DNA has been calculated to be approximately equal to the number of ATP molecules hydrolyzed by gyrase.[137] Therefore it can be suggested that two ATP molecules are hydrolyzed per cycle of reaction by gyrase, leading to the introduction of a linking difference of -2. This seems logical since there are two B subunits in the active enzyme. The energy required to introduce superhelical turns into a closed-circular DNA is dependent on the superhelical density difference between the substrate and the product. It has been calculated that the free energy required to perform the final step in the supercoiling of pBR322 by gyrase is 114 kJ/mol; the maximum specific linking difference achievable by gyrase is -0.11, equivalent to a

linking difference of −46 or −47.[16-85] This is similar to the free energy that can be derived from the hydrolysis of two ATP molecules (−120 kJ/mol),[138] suggesting a straightforward correspondence between the endergonic and exergonic reactions. In studies of the supercoiling of small DNA circles (174 to 427 bp) by gyrase, it was found that a similar limit of specific linking difference was achieved (−0.11).[16] However, calculation of the free energy of supercoiling for such small circles indicates a larger value than that derived from the hydrolysis of two ATP molecules. For example, the calculated free energy change for the gyrase reaction on a 189-bp circle is almost twice that determined for the final supercoiling step in pBR322.[16] One possible explanation for such a result is that more than two ATP molecules are hydrolyzed by gyrase in this case, although it is difficult to envisage how the enzyme can store the energy obtained from multiple rounds of ATP hydrolysis. Other explanations, concerned with the difficulty in deriving the free energy relations for supercoiling of small DNA circles, are also possible.

The gyrase B subunit alone has been found to have a very low level of intrinsic ATPase activity,[76] which is appreciably stimulated when both the A subunit and double-stranded DNA are present. This stimulation is dependent on the length of the DNA used, but generally independent of DNA sequence. DNA molecules of less than about 70 bp in length can only stimulate the ATPase at high concentration. It has been proposed that DNA must bind to at least two sites on the gyrase tetramer before the ATPase reaction can be stimulated.[76,139] Hence, two short DNA molecules must bind to gyrase before ATP can be hydrolyzed, and thus short DNA pieces only activate the ATPase at high concentration. With longer stretches of DNA, however, a single DNA molecule can bind to two or more sites on the enzyme, and thus activate the ATPase at much lower DNA concentrations.[76,105]

The kinetics of ATP hydrolysis by DNA gyrase have been studied by several groups using steady-state methods.[47,75,76,105,137] The B protein alone was found to have a K_M for ATP of 1.7 m*M* and a turnover number of about 1/s.[75] The presence of the A protein and DNA lowers the K_M to 0.3 to 0.5 m*M*,[47,75,76,137] the exact value possibly depending upon the DNA species utilized;[76] the turnover number is apparently unchanged. A variety of nucleotide analogs have been found to be competitive inhibitors of the ATPase reaction,[137] these include ADPNP, ADP, and AMP. Both novobiocin and coumermycin A_1 have also been reported to be competitive inhibitors of ATP hydrolysis,[47,75,137] but there are some reservations about these data (see Section IV).

Maxwell et al.[105] have analyzed the kinetics of ATP hydrolysis by gyrase in the presence of ADP, and found a marked deviation from Michaelis-Menten behavior. These data can be modeled to a scheme involving the binding of two molecules of ATP to each gyrase molecule prior to hydrolysis. Recent experiments addressing the rate of ADPNP binding to gyrase in the presence and absence of ATP suggest positive cooperativity in nucleotide binding to the two B subunits.[244] Such data cast doubt on the steady-state analyses described above and on the kinetic parameters obtained. It would seem perhaps that a better way to analyze the kinetics of ATP hydrolysis by gyrase would be to use a pre-steady-state approach.

D. Mechanistic Models

1. Earlier Models

A number of mechanistic models have been proposed for the supercoiling reaction of DNA gyrase (reviewed by Maxwell and Gellert, Reference 25). Any model must take account of the following features:

1. A DNA segment of about 120 bp is wrapped around the enzyme in a single turn with a positive superhelical sense
2. DNA is cleaved in both strands and the 5′-phosphoryl termini of the DNA are covalently attached to the A protein
3. The linking number of DNA is altered in steps of two
4. The enzyme is able to supercoil, relax, unknot, catenate, and decatenate closed-circular duplex DNA

Two early models of DNA supercoiling by

gyrase proposed the binding of DNA to two parts of the enzyme and the generation of positive and negative supercoils in different regions of the DNA molecule.[59,68,88] The enzyme was then proposed to selectively relax the positive supercoils via the breakage-reunion activity, resulting in the generation of a negatively supercoiled DNA molecule.

Forterre[140] suggested a model for DNA supercoiling by gyrase in which the DNA is wrapped around the protein and a negative writhe is introduced elsewhere in the closed-circular molecule. The strands of the DNA are then cleaved and held tightly by the gyrase A subunits such that no relative movement of the newly formed DNA ends occurs. The A subunits are then proposed to rotate with respect to each other and the DNA is rejoined after a complete rotation, which unwraps the bound DNA. After rejoining, the A subunits rotate again, but in the opposite sense, to renew the wrapping of DNA around the enzyme. However, there is no evidence for the gross conformational changes that must take place in the gyrase molecule to allow the full rotation of the A subunits.

Later models of gyrase action have taken into account the ability of the enzyme to catenate and decatenate DNA, and to resolve knotted DNA. It has been inferred from these reactions that gyrase has the ability to translocate one double-stranded segment of DNA through another. Two models[78,83] assume that the translocated DNA passes through the entire complex (wrapped DNA and protein). One model assumes that the translocated DNA could come from a distant or nearby part of the DNA.[83] The other model proposed that the translocated DNA lies within the wrapped segment;[78] this proposal would ensure the directionality of strand transfer during supercoiling.

Two further models address the question of how the broken DNA ends remain in place during the DNA translocation event, by splitting the translocation process into two parts. The DNA is first translocated through the double-stranded break into the interior of the complex, and then later released through a transient aperture in the protein structure. One of these models[141,142] proposed that the wrapped DNA segment remains in place, with its superhelical sense unchanged, throughout the supercoiling cycle. The DNA segment to be translocated can come from a nearby or remote part of the same molecule or from another DNA molecule. The other model[97,143] postulates a torus-shaped gyrase model with an interior space. The model suggests that the DNA is wrapped around the outside of the protein ring; DNA cleavage then occurs and a DNA duplex is translocated into the center of the protein. The DNA is then proposed to unwrap, at least partially, and the translocated DNA is subsequently released from the center of the complex via the same route by which it entered. However, a model in which the DNA becomes unwrapped and must rebind to gyrase before further turnover of the enzyme can occur is unattractive in the light of the processive nature of the supercoiling reaction performed by gyrase.

2. Summary Model

A possible scheme for the supercoiling reaction of relaxed closed-circular DNA by gyrase based on previous models and recent experimental evidence is shown in Figure 4. Gyrase is represented as an ovoid structure (as suggested by Reference 70), which is able to bind to DNA; 120 bp is wrapped around the protein with a positive superhelical sense. This necessitates the formation of a negative writhe elsewhere in the molecule to relieve the strain generated by the positive writhe. Gyrase then cleaves the wrapped DNA segment in both strands. The 5′-ends of each of the break sites are covalently attached to the A subunits of gyrase via phosphotyrosine residues.[64] The 3′-ends of the broken DNA must be held by noncovalent forces, which may include direct interactions between the enzyme and the free 3′-hydroxyl groups, forces between the wrapped DNA segment and the protein, and the integrity of the DNA double helix itself. Such interactions stabilize the break site and will not allow the DNA to untwist to relieve the strain of the negative writhe.

DNA strand passage then occurs through the break site, and also presumably through at least part of the protein structure. We propose that intersubunit channels within the protein structure facilitate this process (Figure 3) and suggest that the translocated DNA segment is close to, or

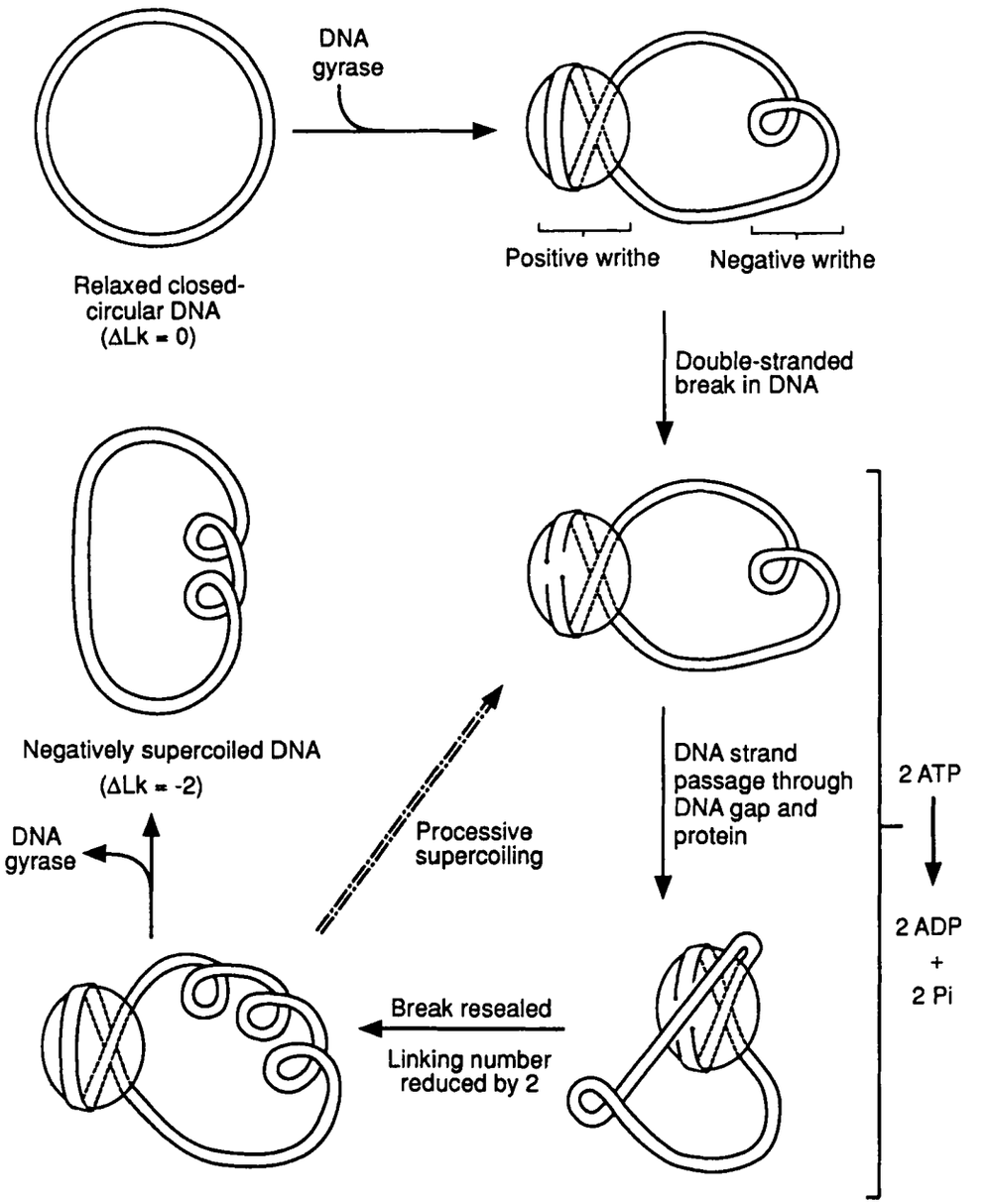

FIGURE 4. The mechanism of DNA supercoiling by DNA gyrase. For an explanation of this figure please refer to the text.

possibly part of, the wrapped DNA (Figure 4). Thus the DNA passes into the interior of the protein complex. It is not clear how the translocated DNA is released. One possibility is that the DNA leaves from the opposite side of the complex to the breakage-reunion site, i.e., close to the entry and exit points of the wrapped DNA (Figure 3). This would entail vectorial passage of the translocated DNA through the gyrase complex and would presumably require large protein conformational changes. Evidence for the existence of a DNA channel between the gyrase B subunits has recently been provided by X-ray crystallographic analysis of the N-terminal portion of the B protein[119] (see Section II). However, this aspect of the gyrase mechanism is poorly understood and requires further investigation.

The passing of a DNA duplex through the

double-stranded break causes the linking number of that DNA to be reduced by 2 (indicated in the diagram as three negative writhes in the DNA and one positive writhe that is a consequence of wrapping of the DNA around gyrase). We suggest that the wrap remains unchanged after this strand translocation step. There are now two possibilities. First, the break in the DNA could be resealed and the gyrase dissociate from the DNA as indicated. This would result in distributive supercoiling since the gyrase would have to bind again in order to carry out further reactions. Second, the break may or may not be resealed and the gyrase remain associated with the DNA to perform other reaction cycles. This would result in the processive supercoiling reaction which is frequently observed for gyrase.[102,108] If the break is not resealed then gyrase will remain covalently attached to the DNA and another DNA strand passage event could occur to further reduce the linking number of the molecule. If the break is resealed after each cycle of reaction, then the DNA must be broken again before further strand passage can occur. At some point in the reaction two ATP molecules are hydrolyzed. However, it is not known how the ATP energy is utilized, or for exactly which part of the reaction it is required. One possibility, based on the experiments involving ADPNP (see Section I), is that nucleotide binding promotes the protein conformational changes that lead to one round of supercoiling but that hydrolysis is required for further catalytic cycles.[101]

The relaxation of positive supercoils by gyrase is likely to occur by the same mechanism as that of negative supercoiling, the only difference being the starting state of the DNA and the fact that the reaction is energetically favorable. Catenation and decatenation can be viewed as the intermolecular counterparts of the supercoiling reaction, the major difference being that the DNA segment to be translocated comes from another DNA molecule. As discussed above (Section I), ATP hydrolysis may merely serve to increase the efficiency of these reactions. Unknotting can proceed via the same type of mechanism. With respect to the relaxation of negative supercoils, it is possible that this reaction proceeds by a mechanism that is essentially the reverse of the supercoiling reaction described in Figure 4, where DNA passes through the enzyme and the break site in the opposite direction. However, it is also possible that this reaction occurs by a distinct mechanism.

TABLE 4
Comparative Effects of Quinolones on Bacterial Growth

Organism	MIC_{90} (μg/ml)[a]		
	NAL	NFX	CFX
Citrobacter spp.	7	0.4	0.1
Escherichia coli	8	0.2	0.08
Klebsiella pneumoniae	20	0.6	0.2
Mycoplasma pneumoniae	N.D.[b]	10	1
Neisseria gonorrhoeae	1	0.05	0.007
Pseudomonas aeruginosa	100	3	0.6
Salmonella spp.	10	0.2	0.09
Serratia marcescens	30	2	0.7
Staphylococcus aureus	100	3	1
Streptococcus pneumoniae	100	3	1

[a] Concentration of drug required to inhibit bacterial growth by 90%. NAL, nalidixic acid; NFX, norfloxacin; CFX, ciprofloxacin.
[b] No data available.

Data from References 151, 153, 158 to 160.

IV. INTERACTION WITH ANTIBIOTICS

The indispensable nature of gyrase in the bacterial cell and the apparent lack of gyrase activity in eukaryotes make DNA gyrase an ideal drug target. Indeed a large number of gyrase specific antibacterial agents have so far been reported. Most of these can be classified into two groups, the quinolones and the coumarins, although there are other compounds that fall outside these two classes. We will discuss here only those drugs that appear to specifically affect gyrase; drugs that bind nonspecifically to DNA and affect the action of other proteins in addition to gyrase (e.g., ethidium bromide) will not be discussed.

A. Quinolone Drugs

There are now more than 5000 antibacterial

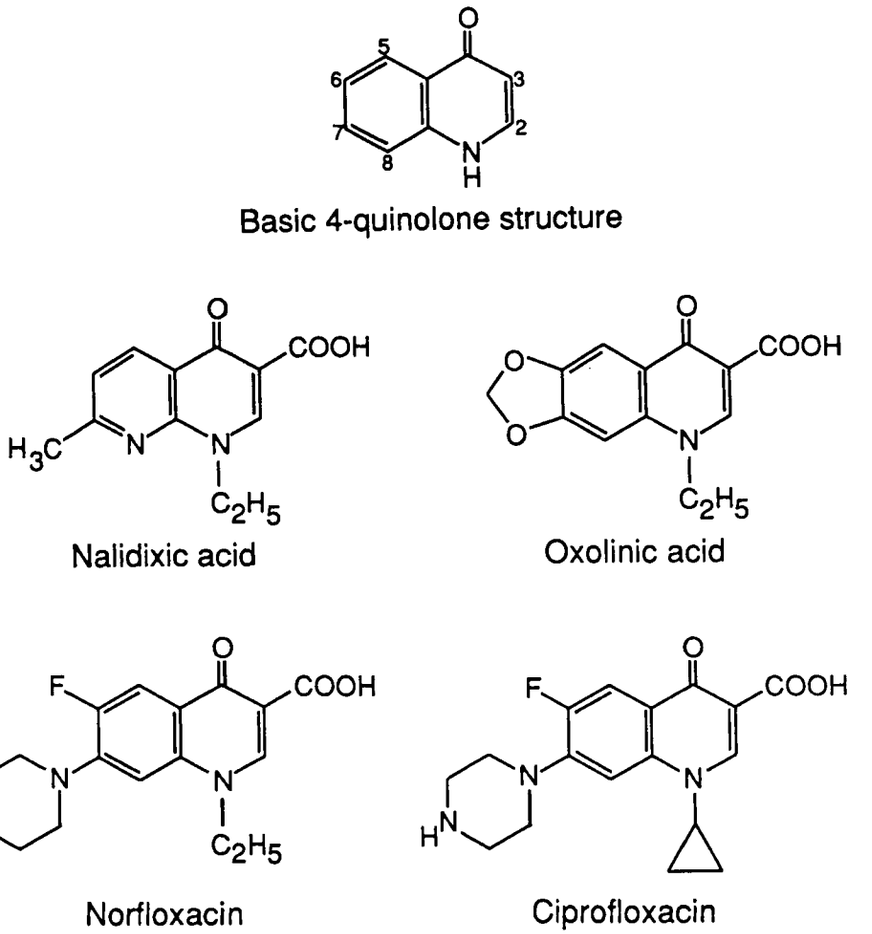

Figure 5A

FIGURE 5 (A). Structures of quinolone antibiotics. (B) Structures of coumarin antibiotics.

compounds based on the 4-oxo-1,4-dihydroquinoline skeleton (Figure 5A), collectively called "4-quinolones"[144] or simply "quinolones". There is an enormous literature concerning these compounds, including a number of reviews. Here we will deal with the aspects of these drugs pertinent to their interaction with DNA gyrase. Other reviews dealing with these aspects include those by Pedrini,[145] Gellert,[19] Smith,[144,146] Drlica and Franco,[147] and Drlica and Coughlin.[130] The reader is also directed to reviews dealing with other aspects of these drugs, such as their chemistry,[144,148-150] pharmacology,[151-153] and bacteriology.[146,154-156]

It is important to note that the quinolone drugs are not natural products but are entirely synthetic. The first of the group to be synthesized was nalidixic acid,[157] which was found to be active against many species of Gram-negative bacteria, but not against eukaryotic cells. However, due to the development of bacterial resistance, the clinical use of this drug has declined. There are now many quinolones with antibacterial activities up to 1000 times that of nalidixic acid (Table 4), in particular those of the fluoroquinolone class. The development of these new drugs has brought many bacteria, which were refractory to the action of the earlier quinolones, within the treatment range of these agents. The considerable success of the new quinolones can be attributed to their broad spectra of activity, minimal toxicity to eukaryotes, ease of penetration into bacterial cells, and

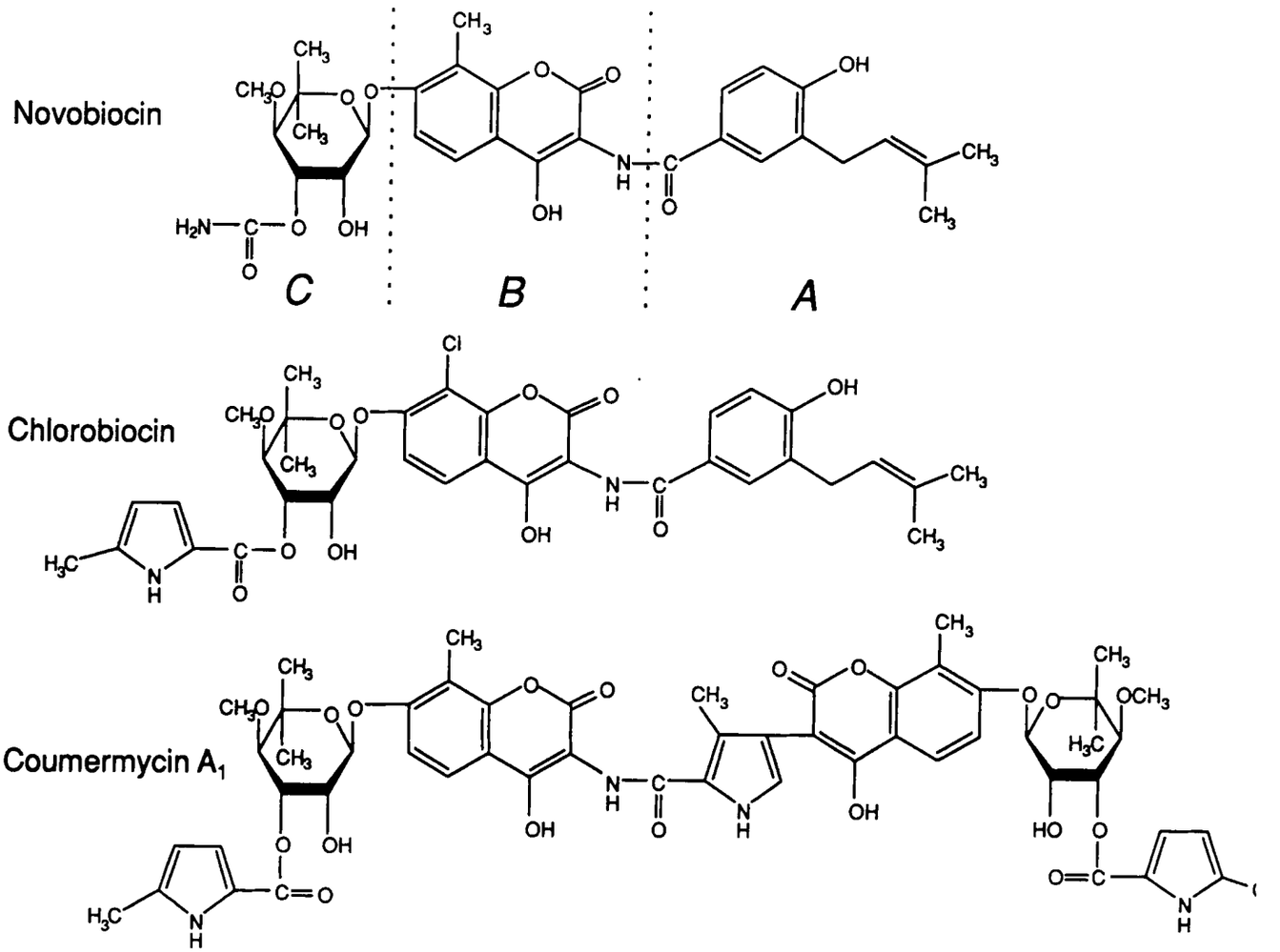

FIGURE 5B

good pharmacokinetic properties.

Systematic substitutions at various positions of the quinolone nucleus have suggested features that contribute to antimicrobial efficacy. For example, the carboxylic acid group at position 3 and the carbonyl group at position 4 appear to be essential to antibacterial activity and may be involved in enzyme-drug or DNA-drug interaction (see below). The substituent at position 1 is also important, and is generally a small aliphatic group such as ethyl (e.g., oxolinic acid and norfloxacin) or cyclopropyl (e.g., ciprofloxacin). The presence of a fluorine atom at position 6 significantly increases bacterial potency[161] and virtually all new analogs include this substituent. Nalidixic acid itself exhibits a relatively narrow spectrum of antibacterial activity, being limited to certain Gram-negative species (Table 4). The newer fluoroquinolones show much broader spectra of activities which include both Gram-positive and Gram-negative bacteria[156] (see Table 4).

Unlike many other types of antibiotics, the quinolones do not appear to be subject to transferable plasmid-mediated resistance. When resistance does occur the mutation is chromosomal and is often found to be in the *gyr*A gene, the structural gene for the DNA gyrase A protein. Several quinolone-resistant mutations in the *E. coli gyr*A gene have now been identified at the DNA sequence level (Table 5). These all fall within a small area of the gyrase A protein, between amino acids 67 to 106, which has been termed the "quinolone resistance-determining re-

gion."[162] Purification of the DNA gyrase A protein from strains bearing such mutations has led to the demonstration that the protein has quinolone-resistant supercoiling activity when complexed with the wild-type B protein *in vitro*.[110,111] Such data would seem to strongly support the notion that the gyrase A protein is the target of the quinolones. However, quinolone-resistant mutations have been found to map elsewhere, including *gyr*B.[164] Indeed two quinolone-resistant mutations in the *E. coli gyr*B gene have been identified at the DNA sequence level (Table 5). In a recent analysis of 25 spontaneous quinolone-resistant mutations of *E. coli* KL16, 13 had *gyr*A mutations and 12 had mutations in *gyr*B.[165] These data suggest that the target of the quinolones may be both subunits of gyrase. However, such data require qualification. Among a group of clinically isolated quinolone-resistant *E. coli* strains five had *gyr*A mutations and only one had a *gyr*B mutation.[165] In a similar study of *Pseudomonas aeruginosa* strains, 33 spontaneous mutants had *gyr*A mutations and none carried mutations in *gyr*B; among clinical isolates 12 had *gyr*A and only 1 had *gyr*B mutations.[165a] The quinolone-resistant *gyr*B mutations so far studied do not exhibit high-level resistance to the drugs,[52,164,165] in contrast with some of the quinolone-resistant *gyr*A mutations that can exhibit much higher levels of resistance[109,110] (Table 5). Indeed one *gyr*B quinoline-resistant allele, *nal*-31 (Lys(447)-Glu), shows resistance to nalidixic acid but hypersensitivity to pipemidic acid, one of the newer quinolones.[52,164]

In addition to the quinolone-resistant mutations mapped to the *E. coli gyr*A genes, Sreedharan and Fisher[166] have determined the amino acid changes leading to quinolone resistance in three *Staphylococcus aureus* mutants. These mutants carry the changes Ser(84)-Leu and/or Ser(85)-Pro. Sequence alignment shows these mutations to be analogous to the *E. coli* changes Ser(83)-Leu and Ala(84)-Pro (Table 5).

One interpretation of these data is that the gyrase A protein is the primary target of the quinolone drugs and that mutations in *gyr*B have secondary effects on quinolone interaction mediated via protein-protein contacts. However, it is certainly a possibility that the drugs interact directly with both subunits. In addition to mutations in the *gyr*A and *gyr*B genes, there have been other resistance mutations mapped elsewhere, many of these appear to confer resistance to cells by alterations in drug permeation. Examples of these include *nor*B, *nor*C, *nfx*B, and *cfx*B,[167-169] which decrease expression of the porin outer-membrane protein OmpF. More detailed discussions of quinolone-resistant mutations mapping at loci distinct from *gyr*A and *gyr*B may be found in other recent reviews.[155,170]

One property of quinolone-resistant mutations of the gyrase genes is that they are recessive to the sensitive alleles. Thus, cells containing nalidixic acid-resistant (Nal^R) and nalidixic acid-sensitive (Nal^S) alleles of the *gyr*A gene are phenotypically drug sensitive.[171] The observation of dominance of quinolone-sensitive over quinolone-resistant gyrase genes hints that the mode of action of the drugs *in vivo* is not simply the inhibition of DNA supercoiling by gyrase (see below).

The addition of a quinolone drug to an *in vitro* reaction containing DNA gyrase, relaxed closed-circular DNA, and ATP leads to the inhibition of DNA supercoiling.[45,46] As discussed above, if such a reaction is terminated by the addition of a protein denaturant, such as SDS, the DNA is found to be cleaved in both strands with the gyrase A protein covalently attached to the newly formed 5′-phosphoryl termini. Other reactions of gyrase that are inhibited by quinolones include DNA relaxation, catenation, and decatenation.[45,77] It appears that these drugs will arrest any gyrase reaction involving double-strand DNA breakage. The DNA-dependent ATPase reaction is not inhibited by quinolones,[78] although there is some evidence of altered kinetic parameters.[245]

The ability of quinolones to induce DNA cleavage by gyrase has been exploited in a number of ways. The gyrase-induced DNA cleavage reaction may be used as a measure of the efficacy of a particular drug as a gyrase inhibitor.[172] This reaction has been taken as evidence for the involvement of a double-stranded cleavage event during the normal DNA supercoiling mechanism. It is proposed that the quinolone drugs block the supercoiling process at the DNA cleavage step. However, it is not clear whether DNA cleavage occurs before or after SDS treatment. Omission

TABLE 5
Quinolone-Resistant Mutations of *Escherichia coli* DNA Gyrase

Subunit	Mutation	MIC (μg/ml)[a] NAL	CFX	Ref.
	Wild type	3.13	0.0125	162
GyrA	Ala(67)-Ser	25	0.05	109
GyrA	Gly(81)-Cys	50	0.1	162
GyrA	Ser(83)-Ala	N.D.[b]	0.06	111
GyrA	Ser(83)-Leu	400	0.39	109, 163
GyrA	Ser(83)-Trp	400	0.39	109, 110, 163
GyrA	Ala(84)-Pro	25	0.1	109
GyrA	Asp(87)-Asn	400	0.2	162
GyrA	Asp(87)-Val	31	0.06	163
GyrA	Gln(106)-Arg	N.D.	0.05	111
GyrA	Gln(106)-His	12.5	0.05	162
GyrB	Asp(426)-Asn	50	N.D.	52
GyrB	Lys(447)-Glu	100	N.D.	52

[a] Minimum inhibitory concentration (MIC) of nalidixic acid (NAL) or ciprofloxacin (CFX) required to inhibit growth of bacteria bearing this mutation.
[b] No data available.

of SDS, or incubation of the gyrase-DNA-quinolone complex at 80°C prior to SDS addition, blocks the DNA cleavage reaction.[45] Thus it is possible that quinolone drugs arrest the supercoiling reaction either prior to, or following, phosphodiester bond hydrolysis. In this respect, it is useful to draw comparisons with eukaryotic type II topoisomerases that are the target of a number of anti-tumor drugs whose mode of action on these enzymes appears similar to that of quinolones on DNA gyrase.[147,172a] Here, it is thought that the drugs inhibit the DNA re-ligation step of the topoisomerase reaction, suggesting that DNA cleavage occurs before the addition of protein denaturant.[172a,172b]

Quinolone-induced cleavage by gyrase occurs at preferred sites on DNA.[87,88,96-98,102,120] It is often assumed that these sites of quinolone-induced cleavage represent sites of action of DNA gyrase on DNA. However, such an assumption is difficult to prove and it has been shown in at least one case that a preferred gyrase binding site (adjudged by filter binding) does not correspond to a site of quinolone-induced cleavage.[98] A large number of quinolone-induced gyrase cleavage sites have been sequenced both *in vitro* and *in vivo* (e.g., References 87, 96, 98, 120, 125, and 126) and a loose consensus cleavage site has been derived (see Section III). The degenerate nature of this cleavage consensus may suggest that factors other than base pair recognition, such as DNA flexibility, may govern cleavage site selection. It is not clear whether the quinolones themselves play a role in determining the cleavage site; the suggestion of direct DNA-quinolone interaction supports this possibility[173] (see below).

Despite the wealth of information about the quinolone drugs, exactly how gyrase-DNA-quinolone interaction leads to cell death is not clear. Comparisons of the concentration of drug required to inhibit supercoiling by gyrase *in vitro* or *in vivo* with that required to inhibit cell growth show that the former exceeds the latter by a factor of between 10 to 100.[45,160,174] Such observations seem paradoxical. One would expect the purified target enzyme to be more (not less) sensitive to the drug. This suggests a mechanism of antibacterial action that does not necessarily involve in-

hibition of DNA supercoiling. One early hypothesis was that the quinolone-gyrase complex forms a DNA lesion[49] that acts as a "poison" and initiates the events that lead to cell death. For example, the gyrase-DNA-quinolone complex could form a barrier to polymerases and thus block the processes of DNA replication and transcription. It is known that quinolones are inhibitors of both these processes *in vivo*.[175,176] Such a poison hypothesis has also been advanced to explain the effects of certain antitumor drugs on eukaryotic DNA topoisomerase III *in vivo*.[177,178] If gyrase-quinolone complexes do form such DNA lesions, then these are likely to be significant when only a small proportion of enzyme molecules are drug bound, i.e., the concentration of drug required to initiate the events leading to cell death would be significantly lower than that required to affect DNA supercoiling, thus accounting for the paradoxical discrepancies between the *in vivo* and *in vitro* effects of quinolones. The poison hypothesis can also explain the observed dominance of quinolone-sensitive over quinolone-resistant *gyr*A alleles in partial diploids.[171] Thus, a cell containing quinolone-susceptible gyrase A protein, even in small amounts, will always be sensitive, irrespective of the presence of quinolone-resistant gyrase A protein.

In addition to the uncertainties surrounding the antibacterial action of quinolones, their mode of action on DNA gyrase is also unclear. The occurrence of point mutations in *gyr*A leading to quinolone resistance suggests that the drugs bind to the gyrase A subunit, but the existence of mutations in *gyr*B that also lead to quinolone-resistance raises the possibility of the involvement of the B subunit (Table 5). Shen and Pernet have investigated norfloxacin binding to gyrase and DNA using equilibrium dialysis and a membrane filtration method.[179] By either technique they found insignificant binding to the gyrase A or B subunit or to the gyrase holoenzyme (A_2B_2), but found evidence of drug binding to DNA. More efficient binding was found to single- compared with double-stranded DNA. Further binding experiments by Shen and co-workers using membrane filtration and a spin-column technique showed that binding of norfloxacin to double-stranded DNA could be stimulated by the presence of DNA gyrase,[173] i.e., the drug can bind to a gyrase-DNA complex. Further characterization of the binding of norfloxacin to DNA showed a preference for guanine-containing homopolymers, and indicted that binding to double-stranded DNA was weak and cooperative.[180] These data have been interpretated as suggesting three different modes of quinolone binding to DNA: weak, noncooperative binding to relaxed double-stranded DNA, noncooperative binding to a single-stranded DNA, and highly cooperative binding to a saturable site in supercoiled DNA. Gyrase seems to enhance the binding of quinolones to double-stranded DNA. These observations have led to the formulation of a model for the interaction of quinolones with the DNA gyrase-DNA complex.[181] The model proposes that gyrase binds to double-stranded DNA, and cleaves the DNA in both strands to reveal a binding site for the quinolone drugs. The drugs are proposed to bind to the exposed single-stranded region at the enzyme's active site. Binding to DNA is proposed to occur via hydrogen bonding between donors on the DNA (in particular guanine residues) and the carbonyl and carboxyl side chains common to all quinolones. Binding of the drug is thought to be cooperative with four or more drug molecules binding in the DNA pocket, interacting with each other by ring stacking. Interaction with gyrase is proposed to occur via the group at C-7 on the quinolone. It is also claimed that binding of the drug to gyrase complexed with relaxed DNA requires the presence of ATP.[181]

This model explains a number of features of gyrase-DNA-quinolone interaction and rationalizes the observations regarding preferential binding of quinolones to single-stranded DNA.[179] Some support for the Shen model has been provided by the data of Tornaletti and Pedrini,[182,183] who showed that nalidixic acid and norfloxacin can cause DNA unwinding in the absence of DNA gyrase. However, there are reports from other laboratories that are in conflict with the data of Shen and co-workers. Bourguignon et al.[184] found no detectable binding of nalidixic acid to calf thymus DNA using absorption spectroscopy, measurement of DNA melting temperatures, and equilibrium dialysis, and Palu et al.[185] failed to detect quinolone-DNA interaction from fluorescence spectroscopy and equilibrium dialysis experiments. Although these data have been criti-

cized,[179,186] they do raise doubts concerning the Shen model.

There are also other comments that can be made concerning the Shen model. It is proposed that the interaction of quinolones with gyrase and relaxed DNA requires ATP.[181] However, it is well documented that quinolone-induced cleavage of relaxed DNA by gyrase can occur in the absence of nucleotide (e.g., Reference 45). The interaction between the drug and the enzyme is proposed to occur between the substituent on the C-7 position of the quinolone and DNA gyrase.[181] However, the C-7 position is variable, having, for example, a methyl group in nalidixic acid, a piperazine ring in norfloxacin, and an alkoxy group in oxolinic acid (Figure 4A). Given that enzyme-ligand interactions are generally highly specific it seems surprising that so much variability would be tolerated. Sequencing of a number of quinolone-resistant mutations in *gyr*A has shown that several of these amino acid changes lead to the removal of hydrogen bond donor or acceptor residues.[109-111] Therefore, it might be expected that enzyme-drug interaction would involve hydrogen bonding. That a pair of essential substituents on the quinolone nucleus (C-3 carboxyl and C-4 carbonyl) have hydrogen-bonding potential suggests that these might be involved in enzyme-drug interaction.[111,149] However, the Shen model proposes that hydrogen bonding occurs between the C-3 and C-4 groups on the quinolone and hydrogen bond donors in DNA.[181] The strongest interactions are proposed to occur between guanine bases in DNA and the carboxyl and carbonyl groups in quinolones. There is some preference for GC over AT in the four base pairs between the cut sites (see Section IV), but only by a factor of 2:1.[125] In addition, there does not seem to be a straightforward correlation between "strong" gyrase-induced cleavage sites and a high content of GC in these four bases.[125] However, factors other than the sequence in the vicinity of the cleavage site are also likely to influence cleavage site preferences.

Despite the reservations regarding the Shen model there have been no other models forthcoming. At the very least the Shen model provides a framework to formulate experiments testing hypotheses of quinolone-gyrase-DNA interactions.

B. Coumarin Drugs

Coumarin drugs are antimicrobial agents originally isolated from *Streptomyces* species. The first of these to be identified was novobiocin,[187,188] which was later followed by coumermycin A_1,[189,190] and chlorobiocin;[191] many synthetic derivatives of these compounds have also been made.[192] In general, the coumarins are found to be more active against Gram-positive than Gram-negative organisms.

The coumarins have been shown to inhibit bacterial DNA, RNA, and protein synthesis, with the primary effect being on the synthesis of DNA.[43] Early experiments mapped coumermycin and chlorobiocin resistance close to the *dna*A gene in *E. coli* (83 min).[43,193] In an attempt to identify the target protein, Ryan employed a novobiocin affinity column and noted that a 37-kDa protein was retained from crude protein extracts prepared from coumermycin-sensitive cells.[43] This protein was not retained when extracts from coumermycin-resistant cells were used. It was concluded that the 37-kDa protein was the likely site of action of the coumorin drugs.[43] With the discovery of DNA gyrase,[1] it was shown conclusively that this enzyme is the target for the coumarin drugs.[44] Later, it was shown that a mutation conferring resistance to chlorobiocin mapped close to *gyr*B (formerly *cou*) the structural gene for the *E. coli* gyrase B protein.[194] One possibility for the identity of the 37-kDa protein identified by Ryan is that it was a N-terminal proteolytic fragment of the gyrase B protein. As discussed in Section II, it is the N-terminal portion of the B protein that binds ATP and coumarin drugs and such a proteolytic fragment would be likely to bind to a novobiocin affinity column.

In vitro, novobiocin has been shown to fully inhibit the supercoiling activity of DNA gyrase,[44] while the ATP-independent relaxation of negatively supercoiled DNA is unaffected.[45,46] This result implies that the coumarin-sensitive gyrase subunit (the B protein) is responsible for the ATP hydrolysis reaction. It was later shown that the B subunit bound ATP, and that this binding could be inhibited by novobiocin.[68] It was further shown that the B subunit alone possessed a novobiocin-sensitive, DNA-independent ATPase activity.[75,76] Thus it is clear that the gyrase B protein

is the site of ATP hydrolysis and that the likely effect of the coumarin drugs is to inhibit this reaction.

The exact mode of inhibition of the gyrase ATPase reaction by coumarins is not clearly established. Steady-state kinetic experiments have suggested that novobiocin and coumermycin are competitive inhibitors of both DNA supercoiling and ATP hydrolysis by gyrase.[47,137] However, examination of the structures of the coumarin drugs (Figure 5B) reveals no striking resemblance to ATP, although there are some common elements (such as a sugar ring). Therefore it seems unlikely that these drugs would behave as ATP analogs and thus competitively inhibit the ATPase reaction. Indeed gyrase is highly specific for ATP, other nucleoside triphosphates are very poor substrates for, and inhibitors of, the ATPase reaction,[1,137] although certain adenosine mono-, di-, and triphosphate derivatives are found to be effective inhibitors. In addition, the kinetics of the DNA supercoiling reaction are difficult to monitor by the gel electrophoretic methods generally used to assay this reaction, and the kinetics of ATP hydrolysis by gyrase have been shown to be distinctly non-Michaelian in other experiments.[105] This latter observation would invalidate the analysis of the ATP hydrolysis by classic Michaelis-Menten steady-state kinetics. Therefore the notion of coumarins as simple competitive inhibitors of the gyrase reaction must be regarded with some caution, and more work in this area is clearly required.

So far there have been no reports of the mapping of coumarin-resistance mutations in the *E. coli* gyrase B protein at the amino acid level. However, Holmes and Dyall-Smith[57] have sequenced the gene of a novobiocin-resistant *gyr*B allele from *Haloferax*. This protein has the amino acids changes Asp(82)-Gly, Ser(122)-Thr, and Arg(137)-His, although it is not clear which of these mutations confers the resistance phenotype. The corresponding amino acids in the *E. coli* B protein are Gly(81), Ser(121), and Arg(136). It is interesting to note that none of these amino acids is directly implicated in the binding of nucleotide in the X-ray structure of the N-terminal fragment of the B protein[119] (see Section II), casting further doubt on the notion of coumarin drugs as competitive inhibitors of the ATPase reaction.

Chemically, novobiocin can be subdivided into three distinct entities (Figure 4B), 3′-isopentenyl-4′-hydroxybenzoic acid (**A**), a coumarin residue (**B**), and the sugar noviose (**C**). It has been found that novenamine (**B** + **C**), novobiocic acid (**A** + **B**), or any of the individual subentities are essentially devoid of any antibacterial activity against whole cells.[195] However, when assayed for their ability to inhibit DNA replication in toluenized *E. coli* cells, or supercoiling by *M. luteus* gyrase *in vitro*, novenamine was found to have activity roughly equivalent to that of novobiocin. This result implies that parts **B** and **C** are the minimum elements required for interaction with gyrase.[195] However, later experiments have shown that both chlorobiocic acid, an analog of novobiocic acid (**A** and **B**), and 3-(carbobenzoxyamino)-4,7-dihydroxy-8-methylcoumarin, an analog of coumarin (**B**), are inhibitors of DNA supercoiling by *M. luteus* gyrase *in vitro*.[196] Taken together, these data point to the importance of the coumarin portion (**B**) in interaction with gyrase, but suggest that appropriate substituents may be required for biological activity.

As stated earlier, novobiocin is produced by *Streptomyces* species. In a study of the novobiocin-producing strain *S. sphaeroides*, evidence for two gyrase B proteins has been found.[61] One of these proteins (molecular mass 79 kDa) is resistant to novobiocin, suggesting that this strain has genes encoding both novobiocin-sensitive and -resistant versions of the gyrase B protein. By expressing the resistant allele at the appropriate time, it is likely that the strain can avoid suicide when producing the antibiotic.[61]

In vitro the gyrase B protein has a high affinity for the coumarin drugs: the B protein and the 43-kDa N-terminal B fragment can be eluted from novobiocin affinity columns at high concentrations of urea.[75,246] In addition, the apparent K_i values for novobiocin and coumermycin A_1 are at least four orders of magnitude lower than the apparent K_M for ATP, measured by ATPase kinetics.[47,137] Despite this, the coumarins have not enjoyed the pharmaceutical success of the quinolones. This is probably a consequence of three major factors. First, the activity of the drugs against bacteria (particularly Gram negatives) is relatively low, probably because of their poor

penetration into bacterial cells. Second, the coumarins have been observed to have effects in eukaryote systems, e.g., inhibition of topoisomerase II,[81] inhibition of DNA polymerase α,[197,198] and inhibition of mitochondrial activity,[199,200] which would tend to compromise their usage as therapeutic agents. Third, the emergence of coumarin-resistant bacterial strains. However, two lines of research may now help to improve the pharmacological prospects of coumarins. First, the systematic dissection of the coumarins' structural elements and assessment of their antibacterial and antigyrase properties independently should help to identify the important parts of the molecule with respect to interaction with gyrase and membrane penetration. Second, high-resolution structural work on the 43-kDa N-terminal domain of the gyrase B protein should lead to identification of the drug-binding site and detailed analysis of drug-protein interaction. Taken together, these two lines of work may permit a rational drug design program to optimize the performance of the coumarin drugs.

C. Other Drugs

There are very few gyrase inhibitors that fall outside the quinolone and coumarin classes. It seems that certain antitumor compounds that inhibit eukaryotic topoisomerase II also show some activity against gyrase. For example, *m*-AMSA (4′-[9-aridinylamino] methane-sulfon-*m* aniside) can weakly stimulate DNA cleavage by gyrase.[239] It is interesting to note that DNA cleavage by T4 topoisomerase II is stimulated by both oxolinic acid and *m*-AMSA.[201,202] Such results suggest that the quinolones and the eukaryotic type II topoisomerase inhibitors may have common mechanisms of action.

Recently, two antibiotics distinct from quinolones and coumarins have been suggested to have DNA gyrase as their intracellular target. Cinodine, which is produced by *Nocardia* species, is an antibiotic of the glycocinnamoylspermidine class. This drug has been shown to bind to DNA[203] and to inhibit DNA supercoiling by *M. luteus* DNA gyrase *in vitro*.[204] Significant inhibition of supercoiling could be detected at 0.1 μg/ml drug. Importantly, neither the restriction enzyme *Bam*HI nor calf thymus topoisomerase I was inhibited at ten times this concentration. Whether cinodine is truly a specific inhibitor of bacterial gyrase is a question requiring further investigation.

Microcin B17 is a glycine-rich peptide (approximate molecular mass 3.2 kDa) that is produced by enterobacteria carrying pMccB17 or related plasmids.[205] These plasmids contain six structural genes for the production of the antibiotic, and a seventh gene that confers immunity to this antibiotic. Microcin B17 is active against many enterobacteria, and has previously been shown to be an inhibitor of DNA replication leading to the rapid arrest of DNA synthesis, induction of the SOS response, DNA degradation, and cell death.[206] Vizán et al. have mapped two independently isolated microcin B17-resistance mutants of *E. coli*, and found them both to contain a single point mutation in the *gyr*B gene that converts Trp(751)-Arg in the B protein.[207] Microcin B17 was also found to be able to induce DNA cleavage, in a similar fashion to the quinolones (i.e., after protein denaturation), when incubated with DNA and cell-free extracts of sensitive, but not resistant, bacterial strains. It has therefore been suggested that the cellular target for the antibiotic is DNA gyrase. Further experimentation is required to elucidate the exact mode of action of this antibiotic.

V. *IN VIVO* ROLE OF GYRASE

A. Introduction

There is a plethora of *in vitro* information about the reactions of DNA gyrase, but its precise *in vivo* function remains somewhat obscure. Originally detected as a host factor required for bacteriophage λ site-specific integration,[1] gyrase has also been implicated in the processes of DNA replication, transcription, the control of overall chromosomal superhelical tension, and a number of other cellular processes. The *in vivo* role of topoisomerases has been reviewed previously,[19,208,209] therefore we shall limit our discussion to issues pertinent to gyrase.

B. DNA Replication

DNA gyrase is essential for DNA replication *in vivo*. Clearly, replication would be expected to be promoted by negative supercoiling given the DNA unwinding at the replication fork. The antibiotics that inhibit gyrase are also inhibitors of DNA replication. In addition, temperature-sensitive mutants of the *gyr*A and *gyr*B genes in *E. coli* block DNA replication at the nonpermissive temperature.[49,50] It appears that *gyr*A and *gyr*B mutants affect different aspects of replication. A *gyr*B mutant was found to inhibit the initiation of replication, but not chain elongation,[50] whereas a *gyr*A mutant led to a rapid arrest of chain elongation.[49] It is not clear why mutations in *gyr*A and *gyr*B have different effects, but only a limited number of mutations have been studied. Nevertheless it seems that gyrase has a dual function in DNA replication and is required for both initiation and elongation. It has been suggested that gyrase acts in elongation at a site close to replication forks facilitating fork movement, possibly by preventing the accumulation of positive supercoils ahead of the advancing fork.[210] The precise role of gyrase at the initiation of replication is unknown. Lother et al.[114] have shown that there is a preferred gyrase binding site at the *E. coli* origin of replication (*oriC*). However, an exact role for a gyrase molecule acting at this site has not been forthcoming. One possibility is that gyrase facilitates the formation of the DNA-protein complex at the initiation site.

Following the replication of chromosomal DNA in *E. coli*, chromosome partition occurs. This process can be divided into two aspects: topological resolution or decatenation of replicated chromosomes, and topological segregation or local separation of daughter chromosomes.[211,212] The ability of gyrase to decatenate DNA has already been mentioned (see Section I), and indeed experimental evidence supports a role for gyrase in the decatenation of daughter chromosomes. For example, nucleoids obtained from cells carrying a *gyr*B temperature-sensitive mutation are found to be doublets when isolated at the nonpermissive temperature.[213] These doublets can be resolved by gyrase *in vitro*. Mutants defective in chromosome partitioning are termed Par mutants, and five loci (named *par*A to E) have so far been identified. Several of these have now been linked in various ways to DNA gyrase. The ParA phenotype has been shown to be attributable to a mutation in *gyr*B, and thus is likely to be a consequence of a defect in decatenation.[214] The ParD phenotype has been shown to be a consequence of two mutations, one of which maps to *gyr*A and is thought to be primarily responsible for the partition defect.[215,216] Again this defect may be attributable to an impaired decatenation reaction.

Recently, the *E. coli par*C and *par*E genes (which map at about 65 min) have been sequenced and found to encode proteins of molecular masses 75 and 67 kDa, respectively.[217] The ParC protein shows homology to the gyrase A subunit, while ParE shows homology with the B subunit. Preliminary experiments indicate that ParC and ParE in combination can relax negatively supercoiled DNA and may represent a new type II topoisomerase in *E. coli*, which has been named topoisomerase IV.[217] It is not clear whether gyrase and topoisomerase IV have distinct functions in the partition process; *in vitro* experiments on purified topoisomerase IV will be required to address this question.

Evidence also exists for a role for DNA gyrase in the partitioning of plasmids in *E. coli*. For example, Wahle and Kornberg have analyzed the *par* locus of plasmid pSC101 and propose that it contains a strongly preferred binding site for gyrase.[134] They suggest, from sequence analysis, that this preference is due, in part, to the intrinsic bendability of the *par* DNA, which favors the wrapping of DNA around the gyrase particle. The role of gyrase in binding to the pSC101 *par* locus is proposed to be structural rather than catalytic.[134]

C. Transcription

Topoisomerases are also involved in the regulation of transcription of a number of prokaryotic genes. Negatively supercoiled DNA can be shown to have enhanced transcriptional ability over relaxed, nicked, or linear DNA and the drugs that inhibit DNA gyrase activity can affect the level of gene expression.[32,33] Transcription can be both activated and inhibited by negative supercoiling. The expression of the gyrase genes

themselves is activated by a reduction in negative supercoiling. This may be part of a homeostatic mechanism of supercoiling control in *E. coli*.[34]

Recently, evidence has been growing for a role for topoisomerases in the transcription process itself. Translocation of an elongating RNA polymerase complex along right-handed double-helical DNA will result in the relative rotation of the RNA polymerase complex around the DNA. During coupled transcription and translation, the rotational resistance of the large RNA polymerase complex (and associated ribosomal proteins) will be great, and twisting of the DNA axis is a more likely result. This process will generate positive supercoiling ahead of the advancing polymerase complex, and negative supercoiling behind it.[31] The transcription of a plasmid containing two genes in opposite orientations (e.g., the *tet* and *bla* genes of pBR322) means these two effects will reinforce each other and positively and negatively supercoiled "domains" will arise in the DNA. It has therefore been suggested that the role of gyrase during the transcription process is to relax the positive supercoils ahead of the transcription complex, while topoisomerase I relaxes the negative supercoils behind it.[31,218]

Two previously observed phenomena can now be readily explained in terms of this "transcriptional buffering". First, Lockshon and Morris[219] noted that after treatment of *E. coli* cells with inhibitors of DNA gyrase (oxolinic acid or novobiocin), positively supercoiled pBR322 DNA could be isolated. This supports the idea that gyrase is involved in the relaxation of the positive supercoils generated as transcription proceeds. Second, pBR322 DNA isolated from *top*A mutants (encoding topoisomerase I) is found to be highly negatively supercoiled.[220,221] This high degree of negative supercoiling was found to be dependent on the transcription of the *tet* gene of pBR322 DNA. If the above model is correct, then in the absence of topoisomerase I only the positive supercoils will be relaxed by gyrase, and this will lead to a net accumulation of negative supercoils. Inversion of the *tet* gene relative to the *bla* gene in pBR322 (so that the two genes are no longer divergent) causes a significant decrease in the degree of negative supercoiling of the plasmid when it is isolated from a *top*A background.[35]

Although the above experiments suggest that the transcription of two genes in opposite orientations (e.g., *tet* and *bla*) is required to generate supercoiling in plasmids, this may also occur as a consequence of the transcription of a single gene whose protein product is anchored to the membrane. Lodge et al.[132] have found that high negative supercoiling of pBR322 in *top*A cells does not require the transcription of the *bla* gene. Indeed it appears that the supercoiling effect can be observed when only the N-terminus of the TetA protein (e.g., 98 amino acids) is produced. Given that TetA is an inner membrane protein, this result can be explained by a model in which, during coupled transcription and translation, the N-terminus of TetA is anchored to the membrane and another barrier to rotation, such as the binding of the replication origin to the membrane, occurs elsewhere. This situation could lead to two domains of supercoiling and consequently to high negative supercoiling of the plasmid in a *top*A strain.[132]

An *in vitro* investigation of the transcriptional buffering model has been performed by Tsao et al.[222] They found that, in the presence of *E. coli* topoisomerase I, transcription from a single promoter resulted in the rapid positive supercoiling of the template DNA. The accumulation of positive supercoils appears to require the presence of nascent RNA chains on the RNA polymerase complex, suggesting that the active polymerase complex is resistant to rotational movement so the DNA becomes supercoiled. The magnitude of the supercoiling introduced into the DNA template during transcription suggests that transcription may be one of the principal factors influencing intracellular DNA supercoiling.[222]

It therefore appears that both topoisomerase I and DNA gyrase play a role in controlling the topology of the DNA template during transcription. Whether DNA gyrase normally functions as a supercoiling enzyme or as an enzyme that relaxes positive supercoils *in vivo* remains at issue. Although the introduction of negative supercoils and the removal of positive supercoils are likely to be mechanistically equivalent, it has been suggested that, under certain conditions, these two processes are separable.[222] Further experimentation is obviously required to resolve these questions.

D. Other Roles for Gyrase

DNA gyrase is a host factor required for phage λ site-specific integration into the *E. coli* chromosome.[1] Gyrase can be dispensed with in this reaction if the λ DNA (carrying *att*P phage attachment site) is already negatively supercoiled.[223] The DNA carrying *att*B (the bacterial attachment site) need not be supercoiled in order for the recombination process to occur. It has been suggested that *att*P wraps around the Int protein (a phage-encoded recombination protein) and that supercoiling favors this interaction in a manner similar to the wrapping of DNA around histones in the nucleosome.[223]

DNA gyrase is also thought to have a role in other types of recombination. Generalized recombination is mediated by the *rec*A gene product, a protein that is known to unwind DNA *in vitro*.[224] Therefore, it is possible that the negative supercoiling generated by gyrase facilitates RecA binding, thereby aiding RecA-dependent recombination. Recombination in *gyr*B mutants is reduced compared with that of wild-type cells.[225]

Illegitimate recombination is a DNA rearrangement between nonhomologous and nonspecific DNA sequences (for a recent review see Reference 226). *In vitro* illegitimate recombination has been demonstrated to occur when pBR322 DNA is present in a λ phage packaging system, with ampicillin-resistant transducing phages arising with a frequency of approximately 10^{-7} per total plaque-forming units.[227] These hybrid plaques are formed by the insertion of the pBR322 *bla* gene into λ DNA, or by the substitution of a λ DNA segment by the plasmid DNA. The addition of quinolone drugs to the packaging system stimulates the recombination process some tenfold; this stimulation is blocked by coumarin drugs. It is therefore suggested that the quinolone-induced recombination is mediated by gyrase. The structure of the recombinants formed in the presence of quinolone drugs between pBR322 and λ have been analyzed.[228,229] It is found that there is no homology greater than 4 bp between the recombination sites. Some of the sites where illegitimate recombination occurs resemble the consensus for gyrase-induced DNA cleavage.[230] It is possible that illegitimate recombination occurs through subunit exchange between different gyrase complexes. An A protein with DNA attached may swap with an A protein from a different gyrase tetramer at another site, resulting in the formation of the recombination product. Illegitimate recombination does occur *in vivo* (reviewed in Reference 226), but the role of gyrase in this process has not been conclusively demonstrated. Gyrase has also been implicated in the spontaneous deletion of DNA that can occur *in vivo*.[231,232]

In *E. coli*, deletion of the *top*A gene (encoding topoisomerase I) is lethal. However, other mutations can occur that compensate for the loss of this enzyme and allow the cells to grow.[233] Some of these secondary mutants have been mapped to the gyrase genes: *gyr*A and *gyr*B. This suggests that excessive supercoiling is lethal to the cell, and that the expression of the topoisomerase genes must be under tight control. The gyrase genes themselves are thought to be under homeostatic regulatory control. Increased supercoiling of the DNA template downregulates the expression of the gyrase genes and a decrease in the superhelix density of the template leads to expression of the genes.[34,66]

The action of quinolone drugs on gyrase, and the subsequent addition of a protein denaturant, can lead to fragmentation of the DNA both *in vitro* and *in vivo*. Approximately 45 to 50 major gyrase cleavage sites have been estimated for *E. coli*.[127] This is roughly equal to the number of supercoiled loops found in the *E. coli* chromosome. This may just be coincidence or gyrase binding may be involved in controlling the overall tertiary structure of the chromosome by separating loops of different superhelical density within the chromosome. This could also involve a structural role for gyrase in anchoring DNA within the cell. A structural role has also been suggested for eukaryotic type II topoisomerases.[39]

VI. CONCLUDING REMARKS

DNA gyrase was first discovered over 15 years ago and, as this review testifies, a great deal is now known about the enzyme. The wide range of scientific journals in which papers about gyrase can now be found attests to the broad spectrum of workers interested in this enzyme;

these include biophysicists, biochemists, microbiologists, medicinal chemists, and clinicians. However, our picture of DNA gyrase is far from complete and it is possible to identify key areas where our knowledge is deficient and where progress clearly needs to be made. First, high-resolution structural information is vital to the study of a number of aspects of DNA gyrase. Some success has been achieved with fragments of the gyrase proteins, but this must be regarded as a stepping-stone en route to the structure of the DNA-protein complex. Second, the mechanism of DNA supercoiling by gyrase has provided a continued source of fascination for workers in this field, but crucially important elements of this reaction remain largely unexplained, e.g., the strand-passage step and the mechanism of energy coupling. These processes can be found in other enzymes, and understanding these elements of the gyrase reaction would have far-reaching implications. Third, many workers have been drawn into the gyrase field through the study of antibacterial agents, particularly those of the quinolone class. Despite the attention that these drugs have received, their mode of action is still largely obscure. Clearly, it is important to understand how these gyrase-specific drugs act in order to potentiate the design of more active derivatives. Fourth, the indispensability of gyrase to the bacterial cell is well known, but its exact *in vivo* role is a contentious issue. The possibility that its primary function may not be the maintenance of *in vivo* supercoiling raises fundamental questions about the organization of the bacterial chromosome.

It is hoped that during the next few years, through the combined efforts of workers from a variety of disciplines, progress in these areas will be made, and our picture of this remarkable enzyme will become more complete.

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