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DNA Supercoiling and Relaxation by ATP-Dependent DNA Topoisomerases [and Discussion]

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DNA supercoiling and relaxation by ATP-dependent DNA topoisomerases

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SUMMARY

Bacterial DNA gyrase and the eukaryotic type II DNA topoisomerases are ATPases that catalyse the introduction or removal of DNA supercoils and the formation and resolution of DNA knots and catenanes. Gyrase is unique in using ATP to drive the energetically unfavourable negative supercoiling of DNA, an example of mechanochemical coupling: in contrast, eukaryotic topoisomerase II relaxes DNA in an ATP-requiring reaction. In each case, the enzyme–DNA complex acts as a 'gate' mediating the passage of a DNA segment through a transient enzyme-bridged double-strand DNA break. We are using a variety of genetic and enzymic approaches to probe the nature of these complexes and their mechanism of action. Recent studies will be described focusing on the role of DNA wrapping on the A_2B_2 gyrase complex, subunit activities uncovered by using ATP analogues and the coumarin and quinolone inhibitors, and the identification and functions of discrete subunit domains. Homology between gyrase subunits and the A_2 homodimer of eukaryotic topo II suggests functional conservation between these proteins. The role of ATP hydrolysis by these topoisomerases will be discussed in regard to other energy coupling systems.

1. INTRODUCTION

Many biological transactions that involve DNA require enzymes that couple ATP hydrolysis to the performance of work. Among these ATP-dependent proteins may be mentioned the helicases which separate the strands of duplex DNA to allow initiation of DNA replication, or movement of a replication fork. Another example is provided by the type I restriction enzymes that bind DNA and induce DNA breakage several thousand base pairs (b.p.) away in a reaction coupled to ATP hydrolysis. However, perhaps the best-characterized DNA-dependent ATPases are the type II DNA topoisomerases that change the topological state of DNA, e.g. by introducing or removing DNA supercoils, and thereby influence many processes including DNA replication, transcription and recombination (Austin & Fisher 1990*a*; Wang 1985). These enzymes act as 'gates' in DNA allowing the passage of one DNA segment through another, and are essential for cellular viability. Moreover, they are the targets for medically important antitumour and antimicrobial agents (Liu 1989). These considerations have led to studies of their enzyme mechanisms. In particular, studies of ATP-dependent DNA supercoiling catalysed by DNA gyrase (Gellert *et al.* 1976*a*) may provide new insights that complement understanding of other mechanochemical coupling systems such as actomyosin.

2. CIRCULAR DNA AND THE ENERGETICS OF DNA SUPERCOILING

The enzymic properties of topoisomerases have been elucidated by using circular DNA substrates, therefore we need first to review topological aspects of DNA. Closed circular DNA occurs widely in biological systems and includes the genomes of bacteria, mitochondria and chloroplasts. Many plasmids and viral DNAs are also circular. When isolated intact from cells, the DNA is usually in a supercoiled state, i.e. superimposed on the intertwining of the complementary DNA strands is a three-dimensional coiling of the DNA helix itself. DNA supercoiling may be understood in the following way. A closed circular DNA may be defined topologically by its linking number, L, the number of times one DNA strand goes around the other (figure 1). The linking number is necessarily an integer, and can only be changed by breaking and rejoining DNA strands. If a circular DNA duplex with an interruption in one strand is sealed such that the DNA lies flat on a plane, then the DNA is said to be relaxed, and its linking number, L_o, will be N/h, where N is the number of base pairs in the molecule and h is the helical pitch. However, if L is less than (or more than) L_o, then the DNA will undergo a three-dimensional writhing in space, which has the physical connotation of DNA supercoiling. The linking difference, $|L-L_o|$ or ΔL, is used to define the degree of

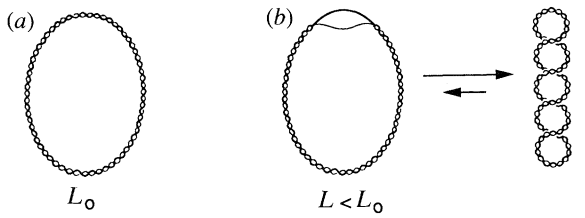

Figure 1. Negative supercoiling arises from a reduction in DNA linking number L. (*a*) A relaxed DNA has a linking number L_o. (*b*) Underwound DNA has $L < L_o$, generating negative supercoils.

supercoiling. This can be defined in a length-independent fashion by using the specific linking difference, $\Delta L/L_o$. Circular DNA isolated from most biological systems is underwound, i.e. $L < L_o$, and the DNA exhibits negative supercoiling. Overwound DNA molecules, i.e. with $L > L_o$, undergo positive DNA supercoiling.

Supercoiled DNA molecules are torsionally stressed relative to their relaxed counterparts. Underwound DNA exerts a persistent torque tending to unwind local regions of the DNA molecule (figure 1). Thus, negative supercoiling can promote formation of DNA cruciforms or left-handed (Z) DNA or facilitate the opening of the DNA helix at promoter sequences or replications origins. In contrast, positive supercoiling exhibited by certain viral DNAs isolated from thermophilic Archaebacteria would tend to force the DNA strands together, and may function to stabilize the DNA helix at high temperature.

The free energy of supercoiling, ΔG_s, varies with ΔL^2 (figure 2) (Horowitz & Wang 1984). This quadratic dependence of ΔG_s on linking number has interesting consequences for enzymic DNA supercoiling.

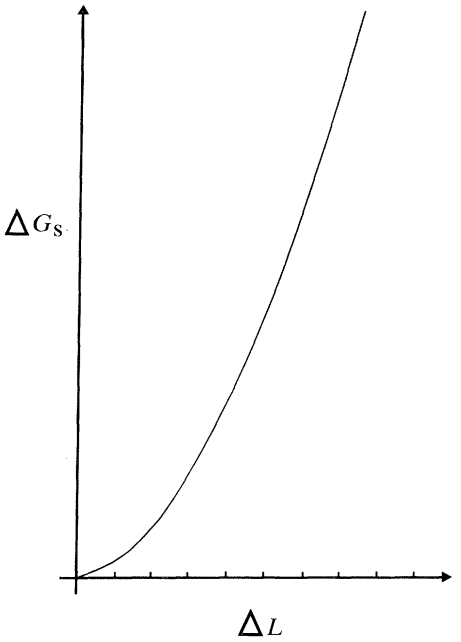

Figure 2. Free energy of supercoiling ΔG_s shows a quadratic dependence on the linking difference ΔL.

3. DNA TOPOISOMERASES CATALYSE DNA BREAKAGE–REUNION REACTIONS

DNA topoisomerases promote the interconversion of topological isomers of DNA, e.g. the introduction or removal of DNA supercoils or the formation or resolution of DNA knots and catenanes. The salient feature of such proteins is that they act by transiently breaking and rejoining the phosphodiester backbone bonds of the substrate DNA (figure 3). This process involves the formation of a covalent enzyme–DNA intermediate that preserves the phosphodiester bond

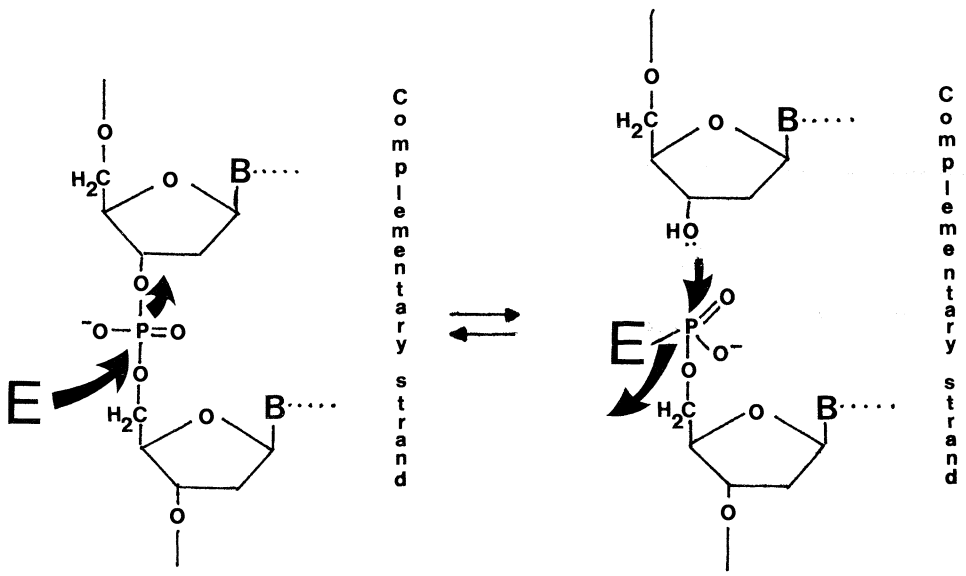

Figure 3. DNA topoisomerases promote transient DNA breakage by forming a covalent enzyme–DNA intermediate. Diagram depicts the formation of a 5′ phosphoenzyme intermediate, e.g. by bacterial topo I. For type II enzymes, two subunits in the protein complex act on complementary DNA strands forming a transient double-strand break.

Table 1. *General properties of type I and type II DNA topoisomerases*

	type I	*type II*	
ΔL	± 1	± 2	
e.g.	ω	gyrase	topo II
ATP	−	+	+
−ve supercoils	↓	↑	↓
Structure	monomer	A_2B_2	A_2
gene	*topA*	*gyrA,B*	*TOP2*

(Note that whereas eukaryotic type I enzymes relax both positive and negative supercoils, bacterial topo I (formerly called ω protein) only removes negative supercoils increasing the linking number.)

energy for later rejoining. For many topoisomerases, a specific tyrosine residue forms a reversible covalent link to a 5′, or less often the 3′, end of the DNA (figure 3). Topoisomerases are classed based on whether they act on one strand (type I) or both strands (type II) of the DNA. The main mechanistic features of each class are briefly summarized in table 1. Type I topoisomerases, whose archaetype is the *E. coli* omega protein, remove supercoils by changing L in unit steps and do not require ATP. (One exception is reverse gyrase, identified in thermophilic Archaebacteria, which utilizes ATP to remove negative supercoils and then introduces positive supercoils (Nakasu & Kikuchi 1985). In contrast, type II enzymes act by passing a duplex DNA segment through a transient double-strand break in DNA (Brown & Cozzarelli 1979; Mizuuchi *et al.* 1980). This reaction requires two subunits acting on complementary DNA strands and changes the linking number in steps of two (table 1). DNA gyrase which catalyses ATP-dependent DNA supercoiling is perhaps the best characterized type II topoisomerase. Eukaryotic type II enzymes are less well understood but utilize ATP to catalyse the energetically favourable removal of both negative and positive supercoils from DNA (figure 2). Gyrase and the eukaryotic type II enzymes also both promote the ATP-dependent formation and resolution of DNA knots and catenanes in DNA, reactions that involve DNA strand passage (Brown & Cozzarelli 1979; Mizuuchi *et al.* 1980 (figure 4).

4. TOPOISOMERASE ACTIVITIES AND CELLULAR FUNCTIONS

All cells examined thus far appear to contain at least one type I and one type II topoisomerase. The functions of these proteins are only now being elucidated. In *E. coli*, DNA gyrase activity is essential for DNA replication and for the segregation of daughter chromosomes (reviewed in Austin & Fisher 1990*a*) (figure 4). Moreover, the competing activities of gyrase and topo I appear to play a role in regulating intracellular DNA supercoiling. Mutations that eliminate *topA* can be tolerated provided the cells acquire compensatory mutations in other loci that redress the intracellular level of DNA supercoiling (figure 5). In two instances, compensatory mutations have been mapped in *gyrA* and *gyrB*, which encode the DNA gyrase A and B subunits and lead to less effective gyrase activity (see McEachern & Fisher 1989). Recent work has identified two other topoisomerases in *E. coli*, topo III and topo IV, which may act in concert with gyrase and topo I.

Other studies have shown that transcription of genes in *E. coli* can itself induce DNA supercoiling.

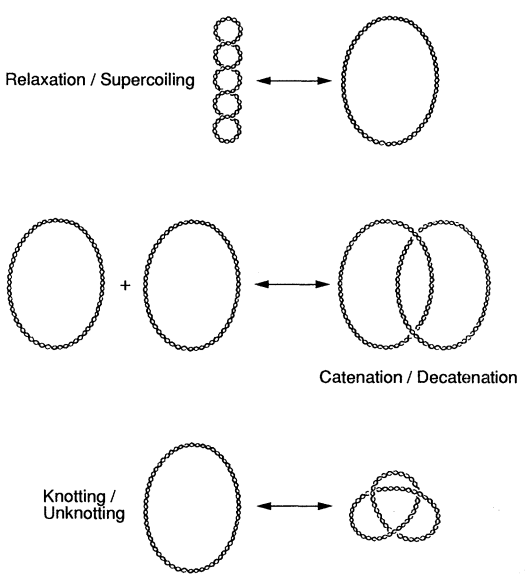

Figure 4. Topological interconversions of DNA catalysed by gyrase and eukaryotic type II topoisomerases. Gyrase alone among type II enzymes promotes ATP-driven DNA supercoiling.

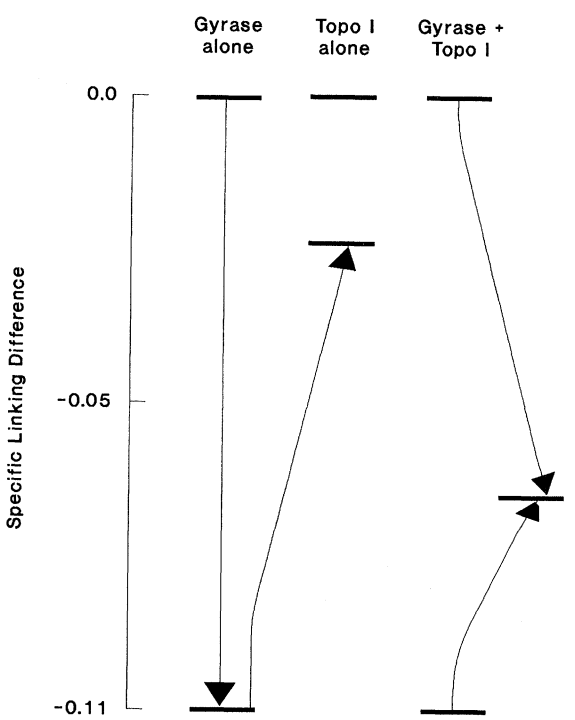

Figure 5. The countervailing activities of gyrase and topo I lead to an intermediate level of bacterial DNA supercoiling. (Other DNA-relaxing enzymes recently discovered in *E. coli*, e.g. topo III and topo IV, could also be involved.)

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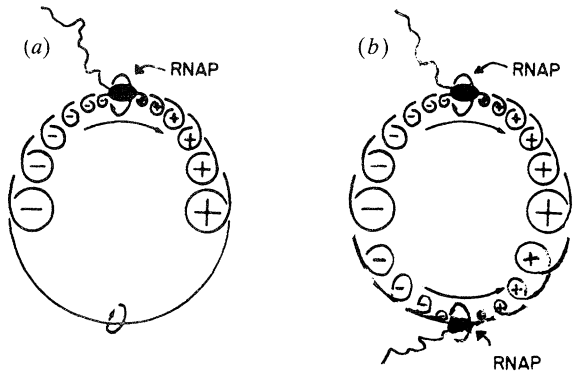

Figure 6. Movement of DNA through the RNA polymerase complex during transcription generates positive supercoils ahead of the enzyme and negative supercoils behind it (modified from Wu *et al.* 1988). (*a*) Rotation of DNA connecting the domains may allow negative and positive supercoils to cancel each other out. (*b*) When DNA rotation is restricted, e.g. by the presence of a second convergently transcribing RNA polymerase complex, the two supercoiled domains may coexist.

According to the twin domain model of Liu & Wang 1987, tracking of the relatively bulky RNA polymerase complex and attendant RNA transcript during transcription will generate domains of positive supercoiling ahead of the complex and negative supercoiling behind (figure 6). If the DNA molecule is constrained elsewhere, e.g. by attachment to a membrane or chromosome scaffold, or by another RNA polymerase complex tracking in the opposite direction, then these supercoiled domains will persist. According to the model, cellular topoisomerases act to relax these transcription-induced supercoils. Recent studies show that, in *E. coli*, topo I acts on negatively supercoiled domains whereas gyrase acts to take out the positive supercoils (Wu *et al.* 1988). Thus the degree of intracellular supercoiling is intimately related to the transcription status of the template.

5. MECHANISM OF DNA SUPERCOILING BY DNA GYRASE

DNA gyrase, a 400 000 M_r A_2B_2 complex, is unique among topoisomerases in catalysing negative supercoiling of DNA driven by ATP hydrolysis. The reaction is a simple type of mechanochemical coupling system in which the free energy of ATP hydrolysis is stored in supercoiled DNA. There is a division of labour in the reaction with the 97 kDa A-subunits (GyrA) mediating DNA breakage–reunion, whereas the 90 kDa B-subunits (GyrB) bind and hydrolyse ATP (Mizuuchi *et al.* 1978). Many of the salient features of DNA supercoiling by gyrase were elucidated in the early 1980s and resulted in a number of mechanistic models (Brown & Cozzarelli 1979; Mizuuchi *et al.* 1980; Wang *et al.* 1980; Morrison & Cozzarelli 1981).

Two attractive and similar models (Mizuuchi *et al.* 1980; Wang *et al.* 1980) envisage that the gyrase complex acts as a directional gate in DNA as shown in figure 7 (reviewed in Fisher 1981). The enzyme

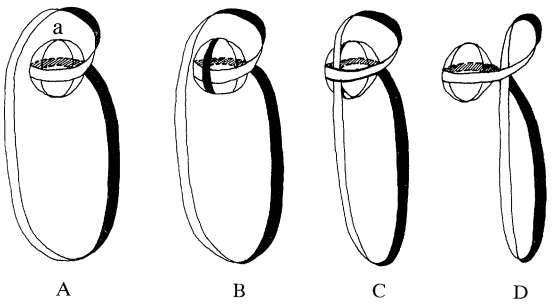

Figure 7. Generalized model for DNA strand passage by DNA gyrase (see text). The gyrase–DNA complex acts as a 'gate' allowing translocation of one duplex DNA segment through another and between the subunit interfaces of the gyrase complex (Mizuuchi *et al.* 1980; Wang *et al.* 1980). In the model of Mizuuchi *et al.* (1980), loop a is wrapped on the enzyme giving directionality to the strand crossing event.

introduces a transient DNA break within a wrapped DNA loop (A,B) and passes another DNA segment through the break and into the enzyme complex. The DNA break is then resealed, resulting in a change of 2 in linking number (C) (Brown & Cozzarelli 1979; Mizuuchi *et al.* 1980). ATP hydrolysis occurs and the passed DNA strand is then released from the complex ready for another cycle of DNA strand passage (D).

DNase I footprinting studies have shown that gyrase binds a 120–150 b.p. loop of DNA which it wraps on its surface in a positive superhelical turn providing evidence for A in figure 7 (Liu & Wang 1978; Fisher *et al.* 1981; Morrison & Cozzarelli 1981). Moreover, nuclease digestion of gyrase bound to DNA allows the isolation of gyrase complexes containing 140 b.p. of DNA termed 'gyrasomes' (Klevan & Wang 1980). Recent work from this group suggests that the determinants for DNA wrapping may be similar to those described for the nucleosome (Hopewell & Fisher unpublished results). The directionality of the reaction to generate a negatively supercoiled product could arise from the positive wrapping of DNA on the complex. Indeed, one version of the model in figure 7 accommodates this idea by proposing that the translocated DNA strand is part of the wrapped DNA segment (Mizuuchi *et al.* 1980). (Interestingly, eukaryotic topo II, which does not have supercoiling activity *in vitro*, binds only a relatively short 25 b.p. DNA region and does not wrap DNA. Moreover, reverse gyrase, which catalyses ATP-driven positive supercoiling, although by a type I mechanism, wraps DNA in the opposite sense to that of gyrase (Jaxel *et al.* 1989).)

Recently, Kirchhausen *et al.* 1985, based on electron microscopy studies, have shown that gyrase is a 'heart shaped' molecule and suggested that the translocated DNA strand has to pass both a 'DNA gate' (B→C) and a 'protein gate' (C→D). They suggested that protein–protein interactions involving GyrB–GyrB subunit contacts hold complex B together. Release of the translocated DNA strand after DNA resealing (C→D) would require opening of the protein gate. The actual molecular details of how strand passage is achieved remain to be elucidated.

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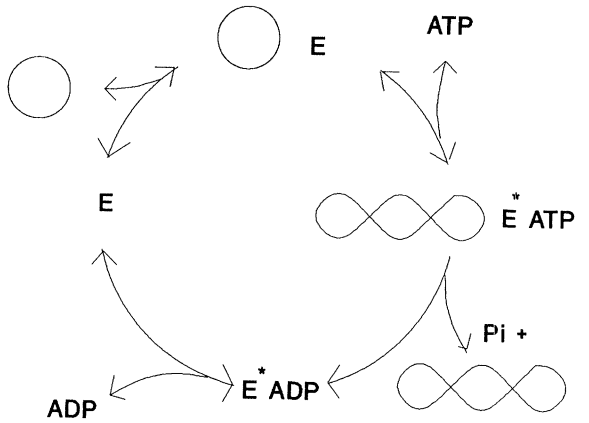

Figure 8. Model for energy transduction during supercoiling by DNA gyrase (after Sugino *et al.* 1978). E and E* are two different conformational states of the protein. After strand passage, the DNA need not be fully released as shown: gyrase acts processively on relaxed substrates and distributively on highly supercoiled DNA.

That ATP hydrolysis occurs after DNA strand passage is suggested by the observation that ADPNP, a non-hydrolysable ATP analogue, supports limited DNA supercoiling stoichiometric with added gyrase (Sugino *et al.* 1978). ADPNP-induced supercoiling changes linking number by 2, consistent with a single cycle of catalysis (Brown & Cozzarelli 1979; Mizuuchi *et al.* 1980). The result suggests that ATP binding may drive a conformation change enabling strand crossing to occur but hydrolysis is necessary to reset the complex for further activity (figure 8). This situation is similar to that described for other energy coupling systems. Although it is difficult to quantitate exactly, the data suggest that two ATP molecules are hydrolysed per strand passage event (Sugino & Cozzarelli 1980).

There are some intriguing energetic aspects of the supercoiling reaction. The limit of the superhelical density of the supercoiled product produced by gyrase is about -0.11. This level of supercoiling in a 4000 b.p. DNA circle corresponds to a linking difference of about -40, or 20 strand-crossing events by gyrase. However, assuming that the quadratic dependence of ΔG_s on linking difference holds up to these high superhelix densities, an ever-increasing free energy input would be required for each subsequent strand passage by gyrase. The observed superhelical limit of the gyrase reaction could involve thermodynamic factors or steric factors (Bates & Maxwell 1989). Alternatively, it may simply reflect the fact that it is more difficult to bind a highly negatively supercoiled DNA to gyrase than a relaxed DNA substrate. Clearly, further work will be required to answer this question.

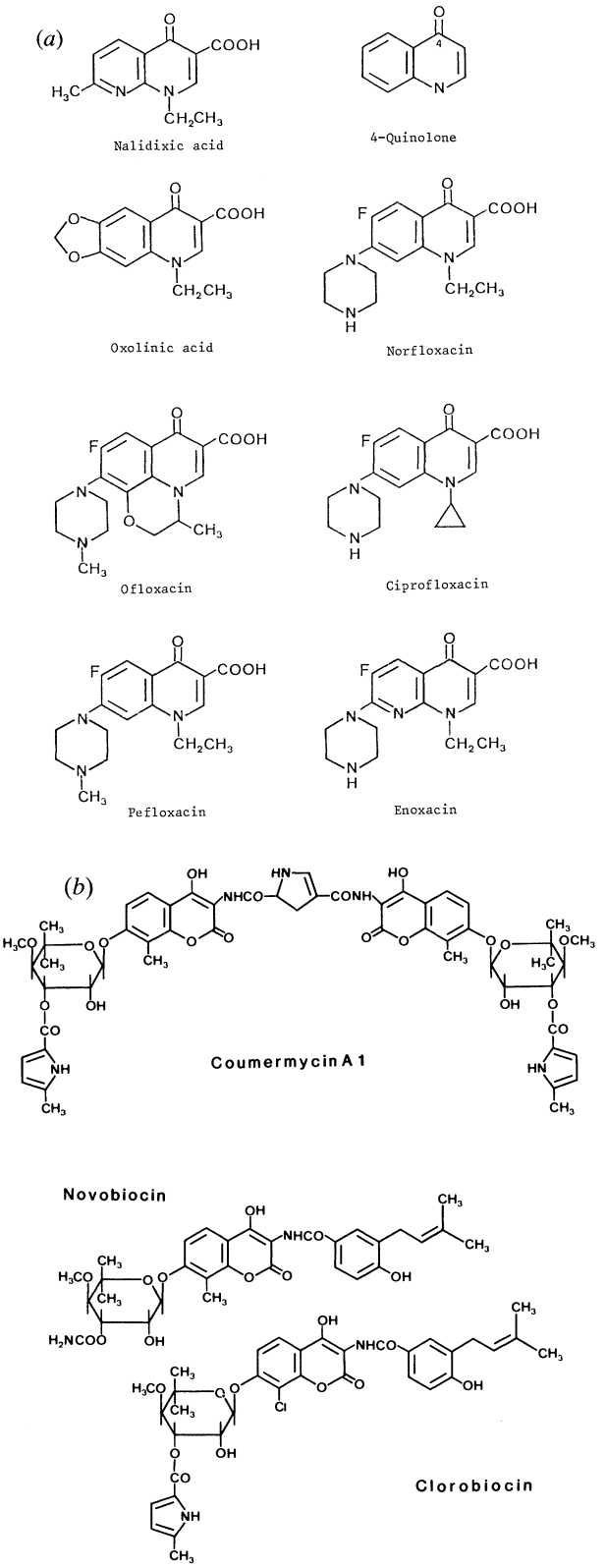

Figure 9. Structures of (*a*) 4-quinolone and (*b*) coumarin inhibitors of DNA gyrase.

6. MOLECULAR ASPECTS OF DNA GYRASE

(*a*) *Gyrase inhibitors*

Two classes of antimicrobial gyrase inhibitors (figure 9) have illuminated different and complementary aspects of the enzyme reaction. The 4-quinolones inhibit the topoisomerase activities of gyrase (e.g. DNA supercoiling and relaxation) by interrupting DNA breakage–reunion by the gyrase complex, and are thought to act to a first approximation at the gyrase A-subunits (Gellert *et al.* 1977). Detergent

treatment of gyrase–DNA complexes formed in the presence of quinolones leads to double-strand breakage at specific sites on DNA and attachment of a GyrA protein to each 5′ end of the break (figure 10). These gyrase cleavage sites are thought to arise from trapping of the covalent enzyme–DNA intermediate and identify potential locations of gyrase action on DNA (see Fisher *et al.* (1986) and references therein). Peptide analysis has shown that the gyrase A-subunits form a 5′phosphotyrosyl bond with DNA and identify Tyr-122 as the catalytic group involved in DNA breakage–reunion (Horowitz & Wang 1987).

Mutations that confer quinolone resistance map either in *gyrA* or *gyrB* genes encoding the gyrase A- and B-subunits. Sequence analysis has shown that such mutations generate amino acid substitutions in a region of GyrA encompassed by Ala-67–Gln-106, i.e. adjacent to the catalytic tyrosine-122 (Cullen *et al.* 1989; Oram & Fisher 1991; Yoshida *et al.* 1990). Interestingly, Ser-83 in *E. coli* GyrA (or its functional equivalent in a range of other bacteria) appears to be a mutational 'hotspot' conferring high-level resistance to the 4-quinolones (Cullen *et al.* 1989; Sreedharan *et al.* 1990; Yoshida *et al.* 1990). Low-level resistance arises from mutation of Asp-426 and Lys-447 of GyrB (Yamagishi *et al.* 1986). Although the precise mechanism of quinolone action is contentious, it is conceivable that quinolones bind at the interface between GyrA and GyrB subunits, and that residues implicated in quinolone resistance may be involved in drug binding (Cullen *et al.* 1989).

Quinolones do not markedly inhibit the ATPase activity of the gyrase complex when assayed with relaxed, linear or supercoiled DNA substrate (Sugino & Cozzarelli 1980). However, the presence of ATP does modulate the relative efficiency of quinolone-promoted gyrase cleavage at its sites on DNA (Fisher *et al.* 1986; Gellert *et al.* 1979; Sugino *et al.* 1978).

In contrast, coumarin inhibitors such as novobiocin and coumermycin (figure 9) inhibit DNA supercoiling (but not DNA relaxation) by blocking ATP hydrolysis at the Gyr B subunits (Gellert *et al.* 1976*b*). The coumarins do not exhibit a close structural similarity to ATP, and they may act by binding to a region of GyrB that overlaps the nucleotide triphosphate site. The K_i for novobiocin measured in the supercoiling reaction of gyrase is some 10^5-fold lower than the K_m for ATP which is about 0.3 mM. It is interesting that coumermycin, a more potent inhibitor, structurally resembles a notional dimer of novobiocin (figure 9*b*). It may be that a molecule of coumermycin can occupy binding sites on both GyrB subunits of the gyrase complex.

(*b*) *Domain structure of gyrase subunits*

Both gyrase A- and B-subunits appear to be domain proteins (figure 10). Evidence for this idea first came from the isolation from *E. coli* extracts of a 50 kDa protein which could complement GyrA to generate the DNA relaxing and cleaving activities of gyrase but which did not promote ATP-dependent DNA supercoiling (Gellert *et al.* 1979). These observations and peptide mapping data suggested that the protein was a GyrA binding fragment of GyrB that was missing the ATP binding site. Subsequent experiments have confirmed these findings and identified the 43 kDa N-terminal region of GyrB as carrying the nucleotide binding site (Tamura & Gellert 1991; Wigley *et al.* 1991). Similarly, tryptic digestion of the GyrA subunit yields a 66 kDa N-terminal DNA breakage–reunion domain and a 33 kDa DNA binding fragment (Reece & Maxwell 1989).

The isolation of discrete functional domains has opened the way for X-ray crystal structure analysis. Indeed, the N-terminal GyrB fragment has been recently crystallized in the presence of ADPNP and its structure determined to 2.5 Å (Wigley *et al.* 1991). The protein crystallizes as a dimer which exhibits some interesting features. First, the N-terminal region of each fragment, notably Tyr-5, reaches over to form part of the ATP binding site of the other protein fragment in the dimer. This arrangement could provide a mechanism for coordinating ATP hydrolysis at the two sites. In the absence of ATP or ADPNP, the N-terminal GyrB fragment is reported to exist as a monomer. Second, the C-terminal regions of the GyrB fragments in the dimer form a hole that is 20 Å in diameter, and which is lined with positively charged residues. The hole could accommodate the DNA duplex that is translocated into the gyrase complex during strand passage. It is interesting to speculate in this regard that the N-terminal regions of the GyrB fragment may constitute the protein gate postulated by Kirchhausen *et al.* 1985 and discussed above.

(*c*) *Mutations affecting gyrase activity*

Site-directed mutagenesis is a powerful tool in delineating the role of particular residues in enzyme

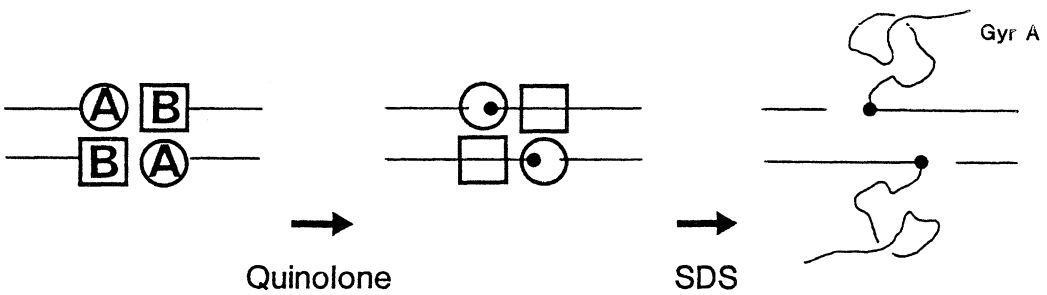

Figure 10. Quinolones trap a 'cleavable complex' of gyrase on DNA. Antitumour inhibitors of eukaryotic topo II may act similarly.

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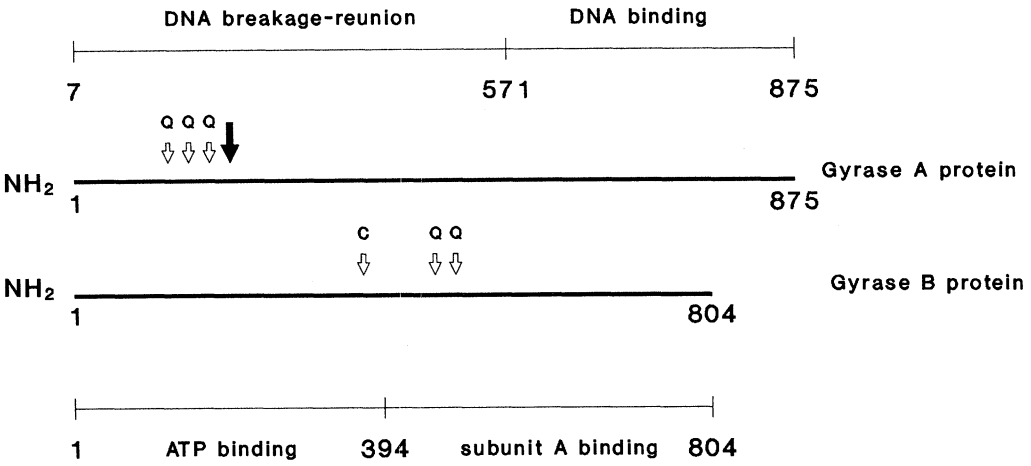

Figure 11. Schematic representation of the gyrase A and B subunits and the locations and functions of known domains. Numbers, amino acid residues; Q and C, locations of quinolone resistance and compensatory mutations, respectively; bold arrow, position in GyrA of catalytic tyrosine 122 involved in DNA breakage–reunion.

action. However, the large size of the gyrase subunits and the absence of detailed structural information has made this approach difficult. An alternative is to study natural mutations in gyrase genes known to make the enzyme less effective in catalysis, e.g. compensatory mutations. We have used the *E. coli* strain DM800 which carries a *gyrB* compensatory mutation that renders GyrB tenfold less effective in promoting DNA supercoiling when complemented with GyrA protein. DNA cloning and sequence analysis of the DM800 *gyrB* gene revealed that it carried an additional Ala–Arg sequence at position 382 in the 804 residue protein, directly following a tandem repeat of the same sequence (McEachern & Fisher 1989). This compensatory mutation lies at the junction between the ATP binding and GyrA binding domains of the GyrB protein (figure 11). It seems possible that the mutation affects the spacing of domains, resulting in suboptimal coupling in the complex and diminished gyrase activity. Such mutations may be useful probes in examining cooperation between gyrase subunit domains in the supercoiling reaction.

7. EUKARYOTIC ATP-DEPENDENT DNA TOPOISOMERASES

DNA sequence analysis of yeast topo II has shown that the A_2 homodimer shares sequence homology with DNA gyrase (Lynn *et al.* 1986). Thus, the N-terminal and C-terminal regions of the polypeptide are homologous with *Bacillus subtilis* GyrB and GyrA proteins. It is known that in *B. subtilis* and *Staphylococcus aureus* the gyrase genes are adjacent on the chromosome and are transcribed in the order *gyrB–gyrA* (Hopewell *et al.* 1990). Therefore each subunit of eukaryotic topo II may have arisen by a gene fusion event and carry functions that are present on separate proteins in the gyrase complex. Nevertheless, eukaryotic topo II functions as an ATP-driven gate much as DNA gyrase, except that the enzyme is unable to supercoil DNA. Rather eukaryotic topo II requires ATP to catalyse the energetically favourable removal of negative or positive supercoils (figure 2).

Unlike gyrase, topo II interacts with a limited DNA region of about 25 b.p. at its binding sites on DNA. Relaxation of DNA by topo II may not need the extensive DNA wrapping observed for gyrase. No supercoiling activity has yet been observed for topo II *in vitro*. It is conceivable, therefore, that the requirement for ATP hydrolysis by topo II has to do with driving the necessary conformational changes of the enzyme complex involved in DNA strand passage. Why human cells contain two forms of topo II, p170 and p180, and how they differ in their catalytic properties, are questions that remain to be answered (Austin & Fisher 1990*b*; Drake *et al.* 1989).

8. CONCLUSIONS

Gyrase and eukaryotic type II topoisomerases are proteins that act as gates in DNA allowing the passage of one DNA duplex through another in a reaction coupled to ATP hydrolysis. In the case of DNA gyrase, ATP-coupled strand crossing occurs vectorially to generate a negatively supercoiled DNA product. Although the models accommodating the known features of strand passage were proposed some time ago, the molecular description of this important process has remained elusive. One difficulty has been the absence of structural information on the gyrase complex. Recent work on the three-dimensional structure of gyrase fragments, interactions with gyrase inhibitors and enzymic properties of gyrase mutants is now beginning to shed light on the problem. An understanding of strand passage would also be aided by studies of trapped translocation intermediates.

Finally, it may be mentioned that passage of DNA through a topo II 'gate' would appear to have some analogy with systems that promote the transport of solutes across membranes. For example, the so-called ABC proteins catalyse the vectorial transport of small molecules against a concentration gradient at the

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expense of ATP (Higgins 1990). These proteins consist of four domains (or subunits). Two membrane-spanning hydrophobic domains form a gate allowing solute transport. Two ATP binding proteins on the cytoplasmic face of the membrane are thought to provide the energetic requirement for transport. The stoichiometry of ATP hydrolysis to uptake has been measured for the transport of maltose by the *E. coli* maltose transport system and of glycine betaine by its transporter (Higgins 1990). In each case, the stoichiometry is two ATP molecules hydrolysed for each molecule transported across the membrane: hydrolysis of two ATP molecules also occurs in each DNA strand passage by gyrase. It remains to be seen whether these analogies reflect similar gating mechanisms used by the two systems.

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REFERENCES

Austin, C.A. & Fisher, L.M. 1990*a* DNA topoisomerases: enzymes that change the shape of DNA. *Sci. Prog.* **74**, 147–162.

Austin, C.A. & Fisher, L.M. 1990*b* Isolation and characterization of a human cDNA clone encoding a novel DNA topoisomerase II homologue from HeLa cells. *FEBS Lett.* **266**, 115–117.

Bates, A.D. & Maxwell, A. 1989 DNA gyrase can supercoil DNA circles as small as 174 basepairs. *EMBO J.* **8**, 1861–1866.

Brown, P.O. & Cozzarelli, N.R. 1979 A sign inversion mechanism for enzymatic supercoiling of DNA. *Science, Wash.* **206**, 6110–6114.

Cullen, M.E., Wyke, A.W., Kuroda, R. & Fisher, L.M. 1989 Cloning and characterization of a DNA gyrase A gene from *Escherichia coli* that confers clinical resistance to 4-quinolones. *Antimicrob. Ag. Chemother.* **33**, 886–894.

Drake, F.H., Hofmann, G.A., Bartus, H.F., Mattern, M.R., Crooke, S.T. & Mirabelli, C.K. 1989 Biochemical and pharmacological properties of p170 and p180 forms of topoisomerase II. *Biochemistry* **28**, 8154–8160.

Fisher, L.M. 1981 DNA supercoiling by DNA gyrase. *Nature, Lond.* **229**, 607–608.

Fisher, L.M., Mizuuchi, K., O'Dea, M.H., Ohmori, H. & Gellert, M. 1981 Site-specific interaction of DNA gyrase with DNA. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4165–4169.

Fisher, L.M., Barot, H.A. & Cullen, M.E. 1986 DNA gyrase complex with DNA: determinants for site-specific DNA breakage. *EMBO J.* **5**, 1411–1418.

Gellert, M., Fisher, L.M. & O'Dea, M.H. 1979 DNA gyrase: purification and catalytic properties of a fragment of gyrase B protein. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6289–6293.

Gellert, M., Mizuuchi, K., O'Dea, M.H., Itoh, T. & Tomizawa, J.-I. 1977 Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4772–4776.

Gellert, M., Mizuuchi, K., O'Dea, M.H. & Nash, H.A. 1976*a* DNA gyrase: an enzyme that introduces superhelical turns into DNA. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3872–3876.

Gellert, M., O'Dea, M.H., Itoh, T. & Tomizawa, J.-I. 1976*b* Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4474–4478.

Higgins, C.F. 1990 Transport systems and their role in drug resistance in bacteria and mammalian cells. In *New antibacterial strategies* (ed. H. C. Neu), pp. 193–212. London: Churchill Livingstone.

Hopewell, R., Oram, M., Briesewitz, R. & Fisher, L.M. 1990 DNA cloning and organization of the *Staphylococcus aureus gyrA* and *gyrB* genes: close homology among gyrase proteins and implications for 4-quinolone action and resistance. *J. Bact.* **172**, 3481–3484.

Horowitz, D.S. & Wang, J.C. 1984 Torsional rigidity of DNA and length dependence of the free energy of DNA supercoiling. *J. molec. Biol.* **173**, 75–91.

Horowitz, D.S. & Wang, J.C. 1987 Mapping the active site tyrosine of *Escherichia coli* DNA gyrase. *J. biol. Chem.* **262**, 5339–5344.

Jaxel, C., Nadal, M., Miranbeau, G., Forterre, P., Takahashi, M. & Duguet, M. 1989 Reverse gyrase binding to DNA alters the double helix structure and produces single strand cleavage in the absence of ATP. *EMBO J.* **8**, 3135–3139.

Kirchhausen, T., Wang, J.C. & Harrison, S.C. 1985 DNA gyrase and its complexes with DNA: direct observation by electron microscopy. *Cell* **41**, 933–943.

Klevan, L. & Wang, J.C. 1980 Deoxyribonucleic acid gyrase–deoxyribonucleic acid complex containing 140 base pairs of DNA and an $\alpha_2\beta_2$ protein core. *Biochemistry* **19**, 5229–5234.

Liu, L.F. 1989 DNA topoisomerase poisons as antitumour agents. *A. Rev. Biochem.* **58**, 351–375.

Liu, L.F. & Wang, J.C. 1978 DNA–DNA gyrase complex: the wrapping of the DNA duplex outside the enzyme. *Cell* **15**, 979–984.

Liu, L.F. & Wang, J.C. 1987 Supercoiling of the DNA template during RNA transcription. *Proc. natn. Acad. Sci. U.S.A.* **84**, 7024–7027.

Lynn, R., Giaever, G., Swanberg, S.L. & Wang, J.C. 1986 Tandem regions of yeast DNA topoisomerase II share homology with different subunits of bacterial gyrase. *Science, Wash.* **233**, 647–649.

McEachern, F. & Fisher, L.M. 1989 Regulation of DNA supercoiling in *Escherichia coli*: genetic basis of a compensatory mutation in DNA gyrase. *FEBS Lett* **253**, 67–70.

Mizuuchi, K., Fisher, L.M., O'Dea, M.H. & Gellert, M. 1980 DNA gyrase action involves the introduction of transient double strand breaks into DNA. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1847–1851.

Mizuuchi, K., O'Dea, M.H. & Gellert, M. 1978 DNA gyrase subunit structure and ATPase activity of the purified enzyme. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5960–5963.

Morrison, A. & Cozzarelli, N.R. 1981 Contacts between DNA gyrase and its binding site on DNA: features of symmetry and asymmetry revealed by protection from nucleases. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1416–1420.

Nakasu, S. & Kikuuchi, A. 1985 Reverse gyrase: ATP-dependent type I topoisomerase from *Sulfolobus*. *EMBO J.* **4**, 2705–2710.

Oram, M. & Fisher, L.M. 1991 4-quinolone resistance mutations in the DNA gyrase of *Escherichia coli* clinical isolates identified by using the polymerase chain reaction. *Antimicrob. Ag. Chemother.* **35**, 387–389.

Reece, R.J. & Maxwell, A. 1989 Tryptic fragments of the *Escherichia coli* DNA gyrase A protein. *J. biol. Chem.* **264**, 19 648–19 653.

Sreedharan, S., Oram, M., Jensen, B., Petersen, L.R. & Fisher, L.M. 1990 DNA gyrase *gyrA* mutations in ciprofloxacin-resistant strains of *Staphylococcus aureus*: close similarity with quinolone resistance mutations in *Escherichia coli*. *J. Bact.* **172**, 7260–7262.

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Sugino, A. & Cozzarelli, N.R. 1980 The intrinsic ATPase of DNA gyrase. *J. biol. Chem.* **255**, 6299–6306.

Sugino, A., Higgins, N.P., Brown, P.O., Peebles, C.L. & Cozzarelli, N.R. 1978 Energy coupling in DNA gyrase and the mechanism of action of novobiocin. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4838–4842.

Tamura, J.K. & Gellert, M. 1991 Characterization of the ATP binding site on *Escherichia coli* DNA gyrase. *J. biol. Chem.* **265**, 21 342–21 349.

Wang, J.C. 1985 DNA topoisomerases. *A. Rev. Biochem.* **54**, 665–697.

Wang, J.C., Gumport, R.I., Javaherian, K.J., Kirkegaard, K., Klevan, L., Kotewicz, M.L. & Tse, R.-C. 1980 DNA topoisomerases. In *Mechanistic studies of DNA replication and genetic recombination* (ICN-UCLA Symp molec. cell. biol. **19**) (ed. B. M. Alberts & C. F. Fox), pp. 769–784. New York: Academic Press.

Wigley, D.B., Davies, G.J., Dodson, E.J., Maxwell, A. & Dodson, G. 1991 Crystal structure of an N-terminal fragment of the DNA gyrase B protein. *Nature, Lond.* **351**, 624–629.

Wu, H.-Y., Shyy, S., Wang, J.C. & Liu, L.F. 1988 Transcription generates positively and negatively supercoiled domains in the template. *Cell* **53**, 433–440.

Yamagishi, J.-I., Yoshida, H., Yamayoshi, M. & Nakamura, S. 1986 Nalidixic acid mutations of the *gyrB* gene of *Escherichia coli*. *Molec. gen. Genet.* **204**, 367–373.

Yoshida, H., Bogaki, M., Nakamura, M. & Nakamura, S. 1990 Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. *Antimicrob. Ag. Chemother.* **34**, 1271–1272.

Discussion

C. Cremo (*Department of Biochemistry, Washington State University, Pullman, U.S.A.*). Could the authors speculate further about the hole in the structure?

L. M. Fisher. It is not for me to talk about the X-ray structure work. In a sense, we have known about the mechanism of gyrase and had a working model for about ten years. The idea that there is a DNA gate and a protein gate has been well known and seemed to be a good working model. The significance of the X-ray work on the ATP-binding fragment is that it provides the first real glimpse of what any part of the gyrase complex looks like. The problem is how this whole system is coupled.

P. L. Dutton (*Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, U.S.A.*). With reference to drugs, is it the case that other anthracycline-type drugs, such as adriamycin or daunomycin, work similarly in the inhibition? There are a broad range of structures.

L. M. Fisher. Yes. Drugs which interfere with topoisomerase II can either be intercalators, such as m-AMSA (amsacrine) and the ones in the question, or they can be non-intercalative, such as etoposide and teniposide. We do not know how any of those drugs cause breakage. Perhaps they work equivalently to the quinolones by interfering in some way with the DNA breaking and rejoining part of the polypeptide chain. That would be quite a useful idea, except we must bear in mind the cautionary note that some etoposide resistance mutations map within the gyrase B-like region of topoisomerase II. They do not map in the gyrase A-like domain. There are also some inhibitors of *E. coli* gyrase which appear to bind to the B subunit and yet also cause cleavage.

J. F. Eccleston (*National Institute for Medical Research, Mill Hill, London, U.K.*). Some of the inhibitors looked as though they might be fluorescent. Would that be of any use to investigate the mechanism of their action?

L. M. Fisher. Yes, it may be useful in the mechanistic work.

J. F. Eccleston. Is the drug incorporated into the protein covalently?

L. M. Fisher. I do not think so. It is actually not known how quinolones work on gyrase. Some people claim they bind to the DNA and to gyrase, some people claim they bind to gyrase, and some people claim they bind just to the DNA. My hypothesis is that there are two binding sites for the quinolone which lie at A and B subunit interfaces, so if there is a tetrameric A_2B_2 complex, this would explain the mutations which lie both within gyrase A and gyrase B protein, giving rise to resistance. It would also explain why at low quinolone concentrations, there is a single-strand break of the DNA and not a double-strand break.

A. J. Crompton (*Avon, U.K.*). Much of this experimental work is on *E. coli* and *Staphylococcus aureus*, yet for the drug therapy it is important to think about replication in human cells. Could the authors tell us the role of the topoisomerase in normal mitosis in mammalian cells?

L. M. Fisher. The short answer is no, but it is known that in yeast, topoisomerase II is required for the segregation of chromosomes during mitosis. That has been established by using topoisomerase II temperature-sensitive mutants. Not much is known about mammalian cells except to say that topoisomerase II is a component of the metaphase chromosome, and is located at the base of chromosome loops. If you have a chromosome and you look at the sister chromatids, and if an antibody which decorates topoisomerase II is used to look at the two sister chromatids during mitosis, they have opposite helicities in terms of their topoisomerase II distribution. I do not know how that is explained.