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Biology of Bacterial Deoxyribonucleic Acid Topoisomerases

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INTRODUCTION

Twenty years ago hydrodynamic studies by Vinograd and co-workers (160) showed that closed, circular DNA molecules are more compact than their nicked or linear counterparts. When examined by electron microscopy, the closed, circular DNA molecules appeared to be twisted (166). The term supercoiling was coined to signify this compaction and twisting, and subsequent studies showed that supercoiling is a ubiquitous property of DNA extracted from natural sources whenever DNA molecules lack ends capable of rotation. Enzymes that regulate supercoiling were discovered by Wang (163) and Gellert et al. (42), and many details are now known about the biochemistry of these enzymes called topoisomerases (reviewed in references 17 and 38). From biochemical characterizations, methods were developed to perturb topoisomerase function in living cells, and it is now clear that DNA supercoiling plays a central role in bacterial chromosome biology. This review focuses on the physiology of supercoiling and the cellular events associated with perturbations of topoisomerase activities.

Before describing topoisomerases and the tools used to inactivate them, it is useful to introduce the concept of supercoiling by restating key biophysical points made previously (2, 38). The topology of closed, circular DNA is described by the relationship

$$W = L - T \quad (1)$$

where W is writhe (the geometric contortion of the helix axis), L is the linking number (the number of times one strand of the double helix passes over the other if the molecule were constrained to lie on a plane), and T is twist (the number of helical turns in the molecule in its native conformation). Supercoiling, which is the intuitive expression for W, arises when $L \neq T$. Currently, W and T cannot be readily measured; consequently, supercoiling is more commonly described by the relationship

$$\tau = L - L_0 \quad (2)$$

where τ, the number of titratable superhelical turns, is the difference between L, the linking number of the topoisomer in question, and L_0, the linking number of a relaxed topoisomer, a closed, circular DNA created by ligation of a nicked circle (for discussion see references 38 and 165). In all natural cases, supercoiling arises when topologically constrained DNA has a deficiency of duplex turns relative to relaxed DNA ($L < L_0$, equation 2). By convention, supercoiling arising from a deficiency of duplex turns is said to be negative. This is the type of supercoiling found in cellular DNA. Positive supercoiling, which arises when constrained DNA has an excess of duplex turns, can be generated under laboratory conditions, but there is no evidence that it plays a major role in living organisms.

For DNA having free ends that can rotate, the number of primary helical turns per unit length (and therefore per DNA molecule) is a function of environmental parameters such as temperature, ionic strength, and concentration of certain DNA-binding ligands. Consequently, relaxed topoisomers formed under different environmental conditions will have different numbers of duplex turns; the value of L_0 in equation 2 will vary with environmental conditions. However, no strand rotation can occur in a closed, circular DNA molecule, so values of L are unaffected by environmental changes. Instead, they are changed by transient strand breakage catalyzed by DNA topoisomerases. Thus, supercoiling can be altered either by topoisomerase activity that changes L or by environmental factors that change L_0. It is important to stress that environmental conditions in vitro may differ considerably from those in vivo; consequently, estimates of supercoiling from hydrodynamic studies may

not accurately reflect the superhelical tension experienced by the DNA in vivo. In the following sections linking differences (τ) inferred from hydrodynamic measurements will be referred to as titratable supercoils to distinguish them from superhelical tension occurring in vivo, a parameter that has not been measured frequently.

Another important point is that supercoiled DNA has a greater free energy than relaxed DNA: supercoiling is spontaneously lost whenever a nick occurs in the DNA. In a sense, supercoiled DNA is under strain and is energetically activated. Processes which unwind DNA (DNA replication and transcription) are expected to be energetically favored in negatively supercoiled DNA molecules relative to linear or relaxed DNA because these processes relieve negative superhelical strain. This type of chromosome activation can be achieved only in DNA molecules that are circular or otherwise topologically constrained.

Four bacterial DNA topoisomerases have been isolated from *Eschericheria coli* extracts: (i) DNA topoisomerase I (omega protein or swivelase; 163); (ii) gyrase (DNA topoisomerase II; 42); (iii) DNA topoisomerase II′ (5, 39); and (iv) DNA topoisomerase III (19). In addition, topoisomerase activities are encoded by bacteriophage T4 (product of phage genes 39, 52, and 60 [for review see reference 76), bacteriophage λ (product of the *int* gene [for review see reference 110), and transposon Tn*3* (resolvase [19]). Topoisomerases alter DNA linking numbers by facilitating the passage of DNA strands through a transient single- or double-strand break (for reviews see references 17 and 38). The reaction can be intra- or intermolecular. Intramolecular strand passage can have three effects. First, in the case of a negatively supercoiled molecule, titratable supercoils decrease if an intramolecular strand passage increases the linking number, making it closer to that of a comparable relaxed DNA and lowering the linking difference (τ) (see equation 2). In vitro all topoisomerases exhibit this activity, which is called relaxation. Second, titratable supercoils increase if intramolecular strand passage decreases the linking number. Only gyrase exhibits this activity in vitro. Third, knots can be introduced or removed. Intermolecular strand passage leads to catenation (interlinking) and decatenation of separate circular DNAs.

The biological function of DNA topoisomerases and superhelical tension has been investigated by examining the physiological consequences of inactivating topoisomerases. Of the *E. coli* enzymes, mutations are available only for gyrase and topoisomerase I; thus, almost all of the inactivation studies have focused on these two enzymes. Gyrase is composed of two types of subunit, the *gyrA* and the *gyrB* gene products, and each type of subunit can be selectively inhibited with temperature-sensitive mutations (31, 32, 39, 75, 100, 115) or by treating cells with specific antibiotics. Drugs that affect the *gyrA* protein are nalidixic acid, oxolinic acid, and a number of related compounds (41, 151, 174). Novobiocin, coumermycin A_1, and chlorobiocin inhibit the *gyrB* protein (29, 43, 115). No specific inhibitors have been found for topoisomerase I, but this activity is eliminated by mutations in the *topA* gene (108, 148, 157). A third activity, topoisomerase II′, is composed of the *gyrA* protein and a fragment of the *gyrB* protein; it is sensitive to inhibitors of the *gyrA* but not the *gyrB* protein (39). Since the possibility exists that topoisomerase II′ is a proteolytic artifact of isolation, it has received little attention. No mutations or inhibitors are known that affect topoisomerase III (19).

A popular working hypothesis is that many of the effects due to inactivation of topoisomerases arise from changes in DNA superhelical tension. This hypothesis is based on the following line of reasoning. The physiological changes roughly correlate with changes in titratable supercoiling in extracted DNA. In turn, changes in titratable supercoiling reflect changes in linking number occurring inside cells, for linking number is not expected to change when DNA is extracted (providing that topoisomerases do not act during the extraction process). These linking changes reflect changes in superhelical tension in vivo if it is assumed that the particular perturbation of topoisomerase activity has no significant effect on environmental parameters affecting helical pitch in vivo. Although this important assumption has not been extensively tested, Sinden et al. (138) have provided experimental evidence that bacterial DNA is under superhelical tension in vivo. These measurements are discussed in the next section, which focuses on how superhelical tension is controlled.

DNA SUPERCOILING AND TOPOISOMERASES

The only direct measurements of superhelical tension in vivo come from psoralen-binding studies (138, 140). Like other intercalating dyes that unwind DNA, psoralen preferentially binds to negatively supercoiled DNA. Binding of psoralen to bacterial DNA in vivo shows a preference for intact DNA relative to nicked DNA. This preference is the same as that observed in vitro for supercoiled DNA relative to nicked DNA. The preference is small (70% increase in binding), but consistent differences are seen when several treatments expected to remove superhelical tension are used. For example, treatment of cells with coumermycin A_1, which inhibits gyrase and results in a decrease in titratable supercoils in nucleoids isolated from treated cells (see below), lowers psoralen binding to DNA by the same amount as do nicks introduced into the DNA by gamma irradiation. In addition, psoralen binding to chromosomal DNA decreases as the DNA becomes nicked after infection of *E. coli* by bacteriophage T4 (140). Thus, bacterial DNA is under superhelical tension. This conclusion rests on the reasonable assumption that the primary effect of nicking is on DNA linking number, not on as yet unknown environmental factors that change helical pitch.

The psoralen-binding studies do not indicate what fraction of the DNA is under superhelical tension: parts of the chromosome might be constrained into nucleosome-like structures not under tension and thus might exhibit the same lack of preference for psoralen binding as observed with eucaryotic cells (138). A DNA nicking study suggests that this might be the case (121). DNA in *E. coli* cells harboring an F-factor was nicked with gamma irradiation (>96% of the plasmids were nicked). After a brief period for DNA repair was allowed, during which time coumermycin A_1 was present to block gyrase activity, plasmid DNA was isolated. This DNA contained about half as many titratable supercoils as unirradiated DNA. If the coumermycin A_1 treatment effectively blocked gyrase activity, which is difficult to establish rigorously, these results mean that some of the supercoils detected in vitro are restrained in vivo so that DNA nicks do not cause relaxation.

None of these studies indicates whether superhelical tension is a general property of the entire chromosome, whether it is restricted to specific regions, or whether levels of tension vary slightly from one region to another. To address these questions, nucleotide sequence-specific probes must be developed that are sensitive to changes in superhelical tension.

Soon after the discovery of gyrase (42), it was realized that the subunits of this enzyme are the targets of well-known antibiotics (41, 43, 151). Cells treated with inhibitors of gyrase exhibit two effects on supercoiling. First, specific inactivation of either subunit of gyrase blocks the introdution of titratable supercoils into relaxed, circular bacteriophage lambda DNA during superinfection of a lysogen (41, 43). Second, inhibition of either subunit leads to a loss of titratable supercoils from the bacterial chromosome (25, 88) and from plasmid DNA molecules (65, 84, 121). In some of these antibiotic studies gyrase was shown to be the target of the drugs: identical experiments on mutants with drug-resistant gyrase genes show no change in titratable supercoiling. Subsequently, temperature-sensitive gyrase mutants became available. As expected, incubation of either *gyrA* or *gyrB* mutants at restrictive temperature blocks the introduction of titratable supercoils into small, circular DNA molecules (36, 40, 62) and leads to a loss of titratable supercoils from chromosomes (147, 161) and plasmids (84, 100). Thus, gyrase both introduces and maintains DNA supercoiling in bacterial cells.

If supercoiling activates chromosomes for processes such as transcription, then supercoiling must be maintained throughout the cell cycle; gyrase cannot simply act to relieve the topological problems generated by DNA replication. In this context, it is important to note that gyrase maintains supercoiling even in the absence of DNA or protein synthesis (25). How the level of supercoiling is established is discussed below.

Since *E. coli* extracts contain DNA-relaxing activities as well as supercoiling activities, the hypothesis arose that in vivo the two types of activities compete to produce the proper level of superhelical tension. Indeed, closed, circular DNAs treated with gyrase can become more highly supercoiled than DNAs extracted directly from cells (42). When mutations became available in *topA* (108, 148), the gene encoding topoisomerase I (148, 156), it became possible to test this hypothesis. DNA from *E. coli* cells containing point mutations in *topA* and *Salmonella typhimurium* cells containing deletion or nonsense mutations in *topA* have higher-than-normal levels of titratable supercoiling (124; G. Pruss, unpublished data). Titratable supercoiling returns to normal when a wild-type *topA* gene is introduced into a mutant cell line. Thus, it appears that topoisomerase I is involved in modulating the level of bacterial DNA superhelical tension by opposing gyrase.

Not all *topA* mutants fit the pattern described above (Fig. 1). In *E. coli*, a *topA* deletion mutant exhibits a 25% reduction in titratable supercoiling relative to wild-type cells, a result opposite to that described in the preceding paragraph (124) (step 3, Fig. 1). Introduction of a wild-type *topA* gene into this strain failed to raise titratable supercoil-

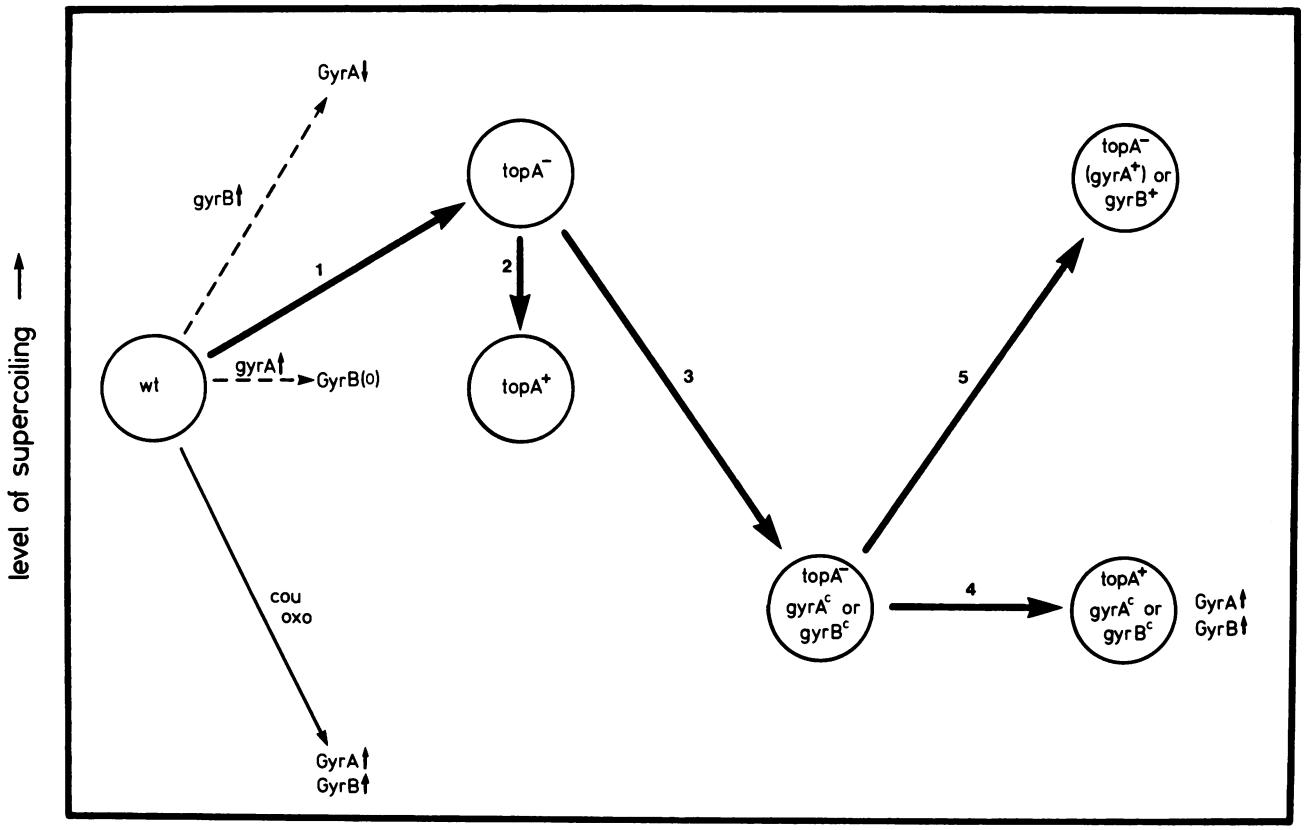

FIG. 1. Relationship between topoisomerase gene expression and DNA supercoiling. Wild-type *E. coli* cells have been manipulated in various ways, and levels of titratable supercoiling in chromosomal DNA have been measured. Thick arrows (numbered 1 to 5) indicate steady-state changes generated by the construction of the indicated strains by transduction. Thin arrow indicates a shift in supercoiling. Dashed arrows indicate predicted shifts in supercoiling after overproduction of *gyrA* or *gyrB* protein from cloned genes. Designations $gyrA^c$ and $gyrB^c$ represent mutations in gyrase that compensate for the absence of *topA*. The result arising from arrow 5 has been tested for $gyrB^+$ but not for $gyrA^+$. Relative rates of gyrase gene expression: increase (↑), decrease (↓), no change (0). The figure is a composite derived form data in references 25, 40, 88, 98, and 124.

ing back to normal levels, suggesting that a suppressor mutation had occurred (step 4, Fig. 1). The presence of suppressor mutations, some of which map in *gyrA* and some in *gyrB* (22, 124), reduces the level of titratable supercoiling (124). The suppressor mutations appear to compensate for the loss of topoisomerase I activity, as judged by effects on cell growth. For example, deletions in *topA* are easily transduced into strains containing a suppressor mutation by selecting for flanking markers. However, similar transductions into wild-type strains produce slow-growing Δ*topA* transductants colonies. Upon subculturing, these Δ*topA* transductants begin to grow rapidly, presumably because members of the clones have acquired the appropriate suppressor mutation (22).

In the studies described above, DNA linking numbers were perturbed (L, equation 2); the conclusion that topoisomerases affect DNA superhelical tension rests on the assumption that no significant changes occurred in the pitch of the DNA helix (L_0, equation 2). Recently, we have taken a different approach and have treated cells in ways expected to affect the pitch of the DNA helix to see whether the level of superhelical tension is tightly controlled. In vitro, temperature changes alter helical twist and supercoiling in a well-defined manner (20, 126). In terms of the equations presented above, lowering the growth temperature should tighten the DNA helix, increasing L_0 (equation 2). At the lower temperature τ (equation 2) should become more negative; i.e., supercoiling should increase. However, if superhelical tension is tightly regulated by the action of topoisomerases, then DNA linking numbers should be higher at the lower temperature to offset the effect of temperature on helical pitch. We find this to be the case when plasmids are extracted from cells grown at different temperatures and differences in linking numbers are measured (43b). The magnitude and direction of the linking change are consistent with the idea that topoisomerases maintain a constant level of superhelical tension in DNA. When cells are shifted from 17 to 37°C, linking numbers equilibrate in 10 to 30 min, presumably through increased gyrase activity. In a downshift, equilibration occurs in 1 to 2 h, presumably as topoisomerase I (or perhaps topoisomerase III) corrects for the change.

To summarize, the simplest interpretation of data obtained with bacterial systems is that gyrase increases DNA superhelical tenison by lowering DNA linking numbers and topoisomerase I carries out the opposite reaction. The two activities are balanced to produce an optimal level of superhelical tension. The topoisomerases maintain a tight distribution of topoisomers (about ±10% in plasmids [124]). Moreover, there is little deviation in supercoiling from one wild-type strain to another. We have found that the average ethidium concentration required to titrate the supercoils in isolated nucleoids from eight strains of *E. coli* K-12 has a standard deviation of <2% (unpublished data). A mutation in *topA* that partially inactivates topoisomerase I activity allows gyrase to introduce higher-than-normal levels of superhelical tension. In the case of deletion of *topA* in *E. coli*, the level of superhelical tension may become unacceptably high, halting cell growth. Compensatory mutations then arise in *gyrA* and *gyrB* that lower the level of superhelical tension and allow cell growth.

Whereas there is little doubt that superhelical tension is important to cell physiology, few details are known about precisely how topoisomerases control superhelical tension. We sense that gyrase and topoisomerase I compete, but we are left with the nagging question of where topoisomerase III fits into the scheme.

CONTROL OF TOPOISOMERASE GENE EXPRESSION

The relationships between topoisomerase mutations and titratable supercoiling in DNA described above raise the question of how superhelical tension is controlled. Since part of the answer may lie in the regulation of topoisomerase gene expression, Gellert and his colleagues have focused on the regulation of gyrase. Both in vitro and in vivo studies suggest that DNA supercoiling itself is important in controlling gyrase expression: lowering levels of supercoiling leads to an increase in gyrase production. When plasmids carrying either *gyrA* or *gyrB* are added to a coupled transcription-translation system in vitro, more gyrase is synthesized from relaxed DNA than from supercoiled DNA (98). In vivo studies (summarized in Fig. 1) show that inhibition of gyrase by coumermycin A_1 or by incubation of a temperature-sensitive *gyrB* mutation at restrictive temperature leads to an increase in both *gyrA* and *gyrB* proteins (98). Both of these treatments lower titratable supercoiling in chromosomal DNA (25, 147, 161). A smaller increase in gyrase expression is observed when gyrase is inactivated by nalidixic acid (98). Oxolinic acid, and probably nalidixic acid, also relaxes chromosomal DNA (88) at the drug concentrations used by Menzel and Gellert (98). It is not clear whether relaxation associated with DNA cleavage occurs in vivo under the conditions used by Menzel and Gellert (98); moderate concentrations of oxolinic acid, which is a more potent drug than nalidixic acid, do not result in DNA cleavage unless nucleoids are exposed to protein-denaturing agents (see next section; 88, 145).

Since reduced levels of supercoiling lead to increased gyrase expression, it has been proposed that a homeostatic relationship exists between the two (98). If this is the case, then cells having higher-than-normal levels of supercoiling should contain lower-than-normal amounts of gyrase. This possibility can now be explored with cells having mutations in *topA*.

The effect of *gyrA* expression on *gyrB* expression and vice versa has also been examined (40, 98). Isotope uptake into *gyrA* and *gyrB* proteins was measured in cells harboring a multicopy plasmid into which either *gyrA* or *gyrB* had been cloned. Under these conditions either the *gyrA* or the *gyrB* protein was overproduced. An asymmetric result was observed: overproduction of *gyrB* reduces *gyrA* expression by about threefold, but overproduction of *gyrA* has no effect on *gyrB* expression. The first result can be explained if overproduction of *gyrB* leads to increased levels of supercoiling which in turn decrease the expression of gyrase. The second result can be explained if overproduction of the *gyrA* protein has no effect on supercoiling. Such a situation could occur if the *gyrA* protein is normally in excess relative to the *gyrB* protein, a supposition consistent with data from enzyme purification studies (55, 146). Although measurements of chromosomal DNA supercoiling have not been made under these conditions, we may find that the concentration of *gyrB* protein, not *gyrA* protein, is involved in controlling the level of supercoiling. Another possibility to be tested is that excess *gyrB* protein decreases the stability of the *gyrA* protein.

Topoisomerase I also affects gyrase gene expression, but it is not clear that the effect is mediated by changes in DNA supercoiling. Gellert et al. (39) compared the synthesis of *gyrA* and *gyrB* proteins in Δ*topA* and *topA*$^+$ strains (both

contained a compensatory mutation in *gyrB*). Introduction of *topA*$^+$ into the *topA* deletion mutant by transduction increased both *gyrA* and *gyrB* expression by about threefold (step 4, Fig. 1). In addition, supercoiling activity in a cell extract from the *topA*$^+$ strain was found to be 5 to 10 times higher than that from the Δ*topA* strain, and in vivo gyrase activity increased due to introduction of *topA*$^+$ when the formation of titratable supercoils in superinfecting bacteriophage lambda was measured. Thus, it appears that gyrase gene expression and the resulting supercoiling activity are enhanced by the presence of topoisomerase I. Increased gyrase and increased topoisomerase I activities seem to counterbalance each other, for a similar pair of Δ*topA*-*topA*$^+$ strains exhibit no difference in average chromosomal titratable supercoiling (124). Thus, a homeostatic relationship between gyrase expression and DNA supercoiling is not sufficient to explain all of the data (Table 1). An interesting speculation is that topoisomerase I directly affects gyrase gene expression.

Gyrase expression is also affected by the *himA* gene product, one of the components of a protein complex important for bacteriophage lambda integrative recombination. In a *himA* deletion mutant, *gyrA* expression is reduced about fourfold whereas *gyrB* expression is unchanged (40). It is interesting to note that supercoiling activity in the *himA* deletion mutant, as measured by introduction of titratable supercoils into superinfecting bacteriophage lambda, is the same as that in wild-type strains (35, 40). This finding supports the idea discussed above that the concentration of the *gyrB* protein is more important than the concentration of the *gyrA* protein in controlling supercoiling. The *himA* deletion, presumably through its effect on lowering production of *gyrA* protein, does have an effect on gyrase activity if placed in a strain having a temperature-sensitive *gyrB* mutation: supercoiling activity, measured with superinfecting bacteriophage lambda in cells incubated at restrictive temperature, is lower than that in cells having only the *gyrB* mutation (35, 40).

TABLE 1. Interactions among gyrase, topoisomerase I, and DNA supercoiling[a]

Expt manipulation	Cellular response[b]			
	gyrA synthesis	*gyrB* synthesis	Gyrase activity[c]	Chromosomal supercoiling[d]
Inhibition of gyrase	+	+	−	−
topA point mutation	ND	ND	ND	+
Overproduction of *gyrA*		0	ND	ND
Overproduction of *gyrB*	−		ND	ND
Underproduction of *gyrB*	+		ND	ND
Δ*topA* replaced[e] by *topA*$^+$	+	+	+	0
Δ*himA*	−	0	0	ND
Δ*himA*, inhibition of *gyrB*	ND	ND	—[f]	ND

[a] References 25, 40, 41, 43, 88, 98, and 124.
[b] +, Increase; 0, no change; −, decrease; ND, not determined.
[c] Measured by introduction of titratable supercoils into bacteriophage lambda superinfecting a lysogen.
[d] Titratable supercoils in bacterial nucleoids.
[e] By P1-mediated transduction.
[f] Relative to inhibition of *gyrB* protein alone.

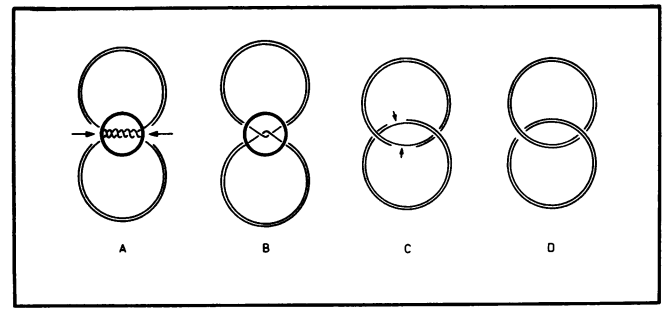

FIG. 2. Formation of catenated DNA molecules. Catenated daughter chromosomes will form if replication stops before all helical overlaps are removed. (A) Bidirectional replication of a circular DNA near the end of a round. Arrows indicate the direction of replication fork movement and central enlargement shows helical overlaps in parental DNA. (B) Replication forks halt while two single-strand overlaps remain (enlargement). (C) Free-floating catenane arises, with gaps (arrows) remaining in each daughter chromosome. (D) Gaps are filled, producing covalently closed, circular catenane. If replication forks stop before (B), catenanes will be multiply intertwined (see reference 152).

Another aspect of the *himA*-*gyrA* relationship involves the SOS response to DNA-damaging agents (for review of the SOS response, see reference 81). The *himA* gene appears to be under *lexA* control and becomes induced when cells are treated with UV light (99). The data discussed in the previous paragraph lead us to expect *gyrA* protein production also to increase during the SOS response. Surprisingly, Menzel and Gellert (98) found that the absence of the *recA* gene, which is needed for induction of SOS-controlled genes, has no effect on induction of *gyrA* or *gyrB* by nalidixic acid or UV irradiation. Perhaps the *himA* protein is at saturating concentration with respect to gyrase expression in wild-type cells before induction of the SOS response.

ROLE OF TOPOISOMERASES AND SUPERCOILING IN DNA REPLICATION

Three aspects of DNA topology are important when considering DNA replication. First, a swivel problem arises during movement of a replication fork. DNA unwinding and strand separation are expected to generate torsional tension ahead of replication forks in topologically constrained DNA molecules, and without a topoisomerase acting as a swivel, the torsional strain will eventually become so great that fork movement will stop. Second, some proteins involved in initiation of DNA replication may require a supercoiled substrate for binding. Third, the persistence of helical overlaps after completion of a round of replication (Fig. 2) will leave daughter chromosomes catenated (interlinked). Proper chromosome segregation may require a decatenating activity. The following paragraphs review evidence that topoisomerases play roles in all three of these aspects of DNA replication.

Elongation Phase of Replication

Participation of gyrase in replication fork movement is more a logical construct than a phenomenon well documented by in vitro studies. One line of support for the concept that negative supercoils facilitate fork movement is found in an observation by Liu and Wang (83). When supercoiled bacteriophage PM2 DNA is partially denatured and then renatured in the presence of small, denatured PM2 DNA fragments, DNA molecules are obtained which have

D-loops where the fragments have annealed to intact strands. The annealed fragments act as primers for *E. coli* DNA polymerase I. When DNA synthesis occurs, the primers are extended and the D-loops become larger. A limit D-loop size is reached at the point where all of the negative superhelical turns in the DNA have been removed by replication fork movement. Another line of support was provided by Itoh and Tomizawa (64). They found that the size of the DNA products is larger if gyrase is included in a DNA-synthesizing system utilizing a circular DNA substrate. Thus, one function of gyrase might be to maintain negative superhelical tension in DNA which would in turn eliminate topological barriers to fork movement; in effect, the negative superhelical turns would pull the replication fork along. Such a role would not require direct interaction of gyrase with the replication apparatus.

Although it is conceptually pleasing to think that negative supercoiling facilitates a number of strand separation processes including replication fork movement, it need not be the solution to the swivel problem. Any topoisomerase that relaxes positive superhelical tension will suffice. Of these, topoisomerase I is the least likely candidate, for whereas it can relax positive supercoils in vitro (78), *topA* deletion mutants reproduce at rates similar to those found in wild-type bacteria (148). The swivel problem could also be solved by winding the newly replicated duplexes around each other (10); double-strand passage by topoisomerases behind replication forks would eliminate this winding.

Several physiological studies, in which gyrase has been inactivated in vivo, are most easily interpreted as gyrase or supercoiling or both participating in the elongation phase of replication. In two cases, temperature-sensitive gyrase mutations are thought to inhibit elongation because inhibition of DNA synthesis at restrictive temperature occurs more rapidly than expected from an initiation mutant (*gyrA43* [75]; *gyrB402* [32a]). It was further reasoned that if a gyrase mutation affects elongation, mutant cells should show reduced residual DNA synthesis compared with wild-type cells if (i) both were shifted to temperatures nonpermissive for the mutant and (ii) initiation of replication were blocked in both by treatment with rifampin or chloramphenicol. Such a response occurs in the *gyrB402* mutant (32a) but not in two other *gyrB* mutants (32, 32a, 115). It is not clear how the *gyrA43* and *gyrB402* mutations differ functionally from the other mutations, since all temperature-sensitive mutants examined have reduced levels of titratable supercoiling after incubation at restrictive temperatures (147). Perhaps cells having gyrase mutations other than *gyrA43* and *gyrB402* retain enough residual gyrase activity to allow replication fork movement but not enough activity to allow other aspects of replication to occur.

Other physiological studies involve antibiotic inhibitors of gyrase. The two classes of inhibitors (represented by coumermycin A_1/novobiocin and nalidixic/oxolinic acid) both lower DNA synthesis rates rapidly. However, each class has unique features. Coumermycin A_1, for example, causes a loss of titratable supercoils from the bacterial chromosome (25), and in so doing the drug also decreases rates of DNA synthesis (25). The gradual loss of superhelical tension associated with coumermycin A_1 treatment may eliminate part of the driving force needed for fork movement. In contrast, low concentrations of oxolinic acid decrease DNA synthesis rates with little or no loss of titratable supercoils (145). With this drug even partial inhibition of DNA synthesis is very rapid, as if inactivation of gyrase by oxolinic acid blocks replication fork/DNA movement (24, 145).

Interpretation of the action of oxolinic and nalidixic acids relies heavily on how these drugs interact with gyrase in vitro. The two drugs appear to trap a gyrase-DNA reaction intermediate in which gyrase has cleaved its DNA substrate and the *gyrA* protein has become covalently bound to the DNA (reviewed in reference 120). A similar reaction probably occurs in vivo since cleaved DNA can be isolated from permeabilized (122) and intact (56, 145) cells treated with the drugs. In vivo a saturable number of gyrase-DNA complexes exist, as determined by measurement of DNA fragment lengths, and the number of cleavage events correlates with the extent of inhibition of DNA synthesis (145). At low but saturating concentrations of oxolinic acid, little or no loss of supercoiling occurs (145). Thus, formation of drug-gyrase-DNA complexes appears responsible for inhibition of DNA replication.

Quantitatively, inhibition of DNA synthesis by oxolinic acid does not fit simple models. If one views DNA as moving through a stationary replication apparatus, formation of a single complex anywhere on the chromosome might rapidly block DNA movement and therefore replication, much in the way ^{32}P decay-generated strand scission is thought to block replication from a distance (8). In the case of oxolinic and nalidixic acids this explanation seems unlikely since there are on average about 50 gyrase-DNA targets for oxolinic acid scattered over the genome, and the degree of inhibition of DNA synthesis is proportional to the number of complexes formed per genome, not the number of genomes with a single drug-gyrase-DNA complex (145). Alternatively, one could imagine that the replication apparatus moves along the DNA; movement of replication forks could be interrupted whenever a fork encounters a drug-gyrase-DNA complex. This explanation must take into account the observation that inhibition is rapid even at very low drug concentrations, concentrations at which only partial inhibition occurs. These concentrations are so low that complexes, if randomly distributed among 50 targets, are not expected to be near enough to forks to cause rapid, but partial, inhibition. A third speculation is that gyrase preferentially binds near replication forks in addition to being distributed over the entire genome; complex formation near forks would quickly block DNA replication. Since gyrase preferentially binds to relaxed DNA (54) and DNA near replication forks is expected to be relaxed due to discontinuous synthesis and DNA unwinding, it is reasonable for gyrase to be concentrated near forks. Consistent with this notion are the observations that pulse-labeled DNA is cleaved by oxolinic acid into fragments smaller than those from long-term-labeled DNA (24) and inhibition of DNA synthesis exhibits biphasic kinetics (24, 28).

Since gyrase preferentially binds to linear DNA, it is not surprising that linear DNA phages such as T7 are sensitive to nalidixic acid (1); formation of nalidixic acid-gyrase-DNA complexes should block phage DNA replication. What fraction of this binding of gyrase to phage DNA is fortuitous and what fraction is required for phage replication have not been determined. Certainly high levels of gyrase are not required because bacteriophage T7 replication occurs in a temperature-sensitive host gyrase mutant (*gyrA43*; 75) at restrictive temperature. Since inhibition of the GyrB protein by coumermycin A_1 blocks T7 replication (21, 63), we examined phage production in a temperature-sensitive *gyrB* mutant. We found that the phage burst size is markedly reduced at restrictive temperature (T. R. Steck and K. Drlica, unpublished data). Thus, at least the *gyrB* protein appears to be required for the growth of this bacteriophage.

It is still not understood why restrictive temperatures relieve the inhibitory effect of nalidixic acid on T7 growth in the *gyrA43* mutant (75). The effect is not explained by the hypothesis that the mutant *gyrA* protein is unable to form drug-gyrase-DNA complexes at restrictive temperatures because chromosomal replication remains sensitive to the drug (75).

Two notes of caution need mentioning when discussing the action of gyrase inhibitors. First, the possibility exists that the drugs bind to gyrase before the enzyme becomes bound to DNA. If so, the location of complexes might not reflect normal gyrase function. Since the presence of oxolinic acid does not change the location of gyrase binding to DNA in vitro, as determined by nuclease protection studies (33, 105), this possibility has not received much attention. Second, drug-DNA complexes involving the *gyrA* protein do not necessarily reflect gyrase-DNA interactions, for this peptide functions in vitro as a component of topoisomerase II′ as well as gyrase (5, 39). Topoisomerase II′ has a relaxing activity, acting on both positive and negative supercoils (5, 39). Thus, it is potentially capable of relieving the positive superhelical tension that might arise ahead of replication forks due to DNA unwinding. However, there is no experimental support for topoisomerase II′ behaving in this way in vivo, and since this activity may result from a proteolytic breakdown product of purification, it too has received little attention.

Initiation of Replication

The clearest example that DNA supercoiling is involved in initiation of DNA replication is found in the replication of bacteriophage φX174 replicative intermediates. Replication in vitro is initiated by a site-specific cleavage of the replicative intermediates by the phage gene *A* protein; this cleavage event requires that the replicative intermediate DNA be supercoiled (89).

Supercoiling is probably also involved in initiation of bacteriophage T4 replication, but in this case the molecular details are poorly understood. Conditional mutations in any of the three genes encoding structural proteins for the T4 type II topoisomerase (phage genes 39, 52, and 60 [82, 149]) cause a delay in acceleration of the T4 DNA synthesis (107). Since these mutations reduce the number of replication forks but not the rate of fork movement (93), it appears that the T4 topoisomerase plays a role in initiation of phage replication. So far supercoiling activity has not been found to be associated with the T4 topoisomerase (76), but the host cell gyrase will partially compensate for the loss of activity in vivo: inactivation of gyrase by coumermycin A_1 blocks nearly all phage DNA synthesis in T4 topoisomerase mutants (92). Gyrase, however, is not required for wild-type phage replication, for with these phages replication is affected little by coumermycin A_1 (92).

Our understanding of how supercoiling affects initiation of bacterial chromosome replication may soon be clarified. An in vitro system for studying initiation of replication has been developed in which plasmid replication requires the chromosomal origin of replication, the *dnaA* protein, and a number of other factors (37). Three points have emerged that are germaine to the present discussion. First, gyrase is required for replication. Second, topoisomerase I appears to act to maintain the specificity of the system for the *dnaA* protein and the *oriC* sequence (23; R. S. Fuller, L. C. Bertsch, N. E. Dixon, J. E. Flynn, Jr., J. M. Kaguni, R. L. Low, T. Ogawa, and A. Kornberg, *in* N. Cozzarelli [ed.], *Mechanisms of DNA Replication and Recombination*: *UCLA Symposia on Molecular and Cellular Biology*, vol. 10, in press). Third, HU protein, a bacterial histone-like protein, stimulates the system (23). Since topoisomerases appear to play a role in chromatin formation in eucaryotic cells (43a, 134a), it is reasonable to postulate that a similar phenomenon occurs in bacteria which provides specificity and stimulation for *dnaA* protein action.

Physiological studies support the general concept that gyrase and supercoiling are involved in initiation of replication. In one type of investigation temperature-sensitive gyrase mutants of *E. coli*, incubated at restrictive temperature, exhibit reduced rates of DNA synthesis without showing a decrease in elongation rates (32, 115). Thus, it appears that initiation is blocked under these conditions. Antibiotic studies with *Bacillus subtilis* are also consistent with the hypothesis. Germinating spores from a thymine-requiring strain develop the potential to initiate replication, but no replication occurs until thymine is supplied. Treatment with coumermycin A_1, but not nalidixic acid, before addition of thymine leads to a loss of initiation potential (111). In this case the conclusion that DNA supercoiling is involved in this phenomenon relies on titratable supercoiling measurements made with *E. coli*: coumermycin A_1 causes DNA relaxation (25), whereas oxolinic acid, at moderate concentrations, does not (145).

Several intriguing genetic observations with a temperature-sensitive *dnaA* initiation mutant have also been suggested as evidence for involvement of gyrase or supercoiling or both in the initiation reaction. First, the *dnaA46* mutant, growing at permissive temperatures, is three to four times more sensitive to inhibitors of gyrase than is the appropriate wild-type strain (31). Second, spontaneous novobiocin- or nalidixic acid-resistant mutations arise in the *dnaA46* strain at a frequency 20 to 30 times lower than that observed in the wild-type strain (31). Third, when the *dnaA46* mutation is suppressed by an RNA polymerase mutation (*rpoB*), this double mutant exhibits a three- to fourfold-lower sensitivity to novobiocin than does the strain having only the *dnaA46* mutation (32). Since the *dnaA46* mutation affects the thermal stability of the gene product itself (155), it is unlikely that these three observations arise from a promoter mutation making *dnaA* particularly sensitive to DNA superhelical tension. Perhaps RNA polymerase and the *dnaA* protein interact in a way that is sensitive to DNA superhelical tension.

Termination of Replication

Evidence for gyrase participation in daughter chromosome resolution also comes from inactivation of gyrase (146a). The sedimentation coefficients of nucleoids isolated from a temperature-sensitive gyrase mutant increase after cells are shifted to the nonpermissive temperature (146a, 147). Microscopic examination of nucleoids isolated from the mutant revealed that almost 90% of them become dumbbell-shaped doublets (in the comparable wild-type strain the amount is <20%) (146a). These doublet nucleoids probably correspond to daughter chromosomes that have failed to segregate in the absence of gyrase activity. While the block in chromosome segregation could be due to the inability either to complete a round of replication or to decatenate interlinked chromosomes, incubation of isolated doublet nucleoids with purified gyrase causes many of the doublets to separate into singlets; gyrase activity appears to be sufficient to separate the daughter chromosomes in vitro under conditions in which no DNA synthesis should occur.

The simplest interpretation of these results is that the decatenating activity of gyrase is required for proper chromosome segregation.

Plasmid Replication In Vivo

Relationships between plasmid replication and topoisomerase activity have also been explored by inhibiting gyrase. Two differences have been found between plasmids and the host chromosome. First, bacterial strains can be cured of some types of plasmids by treatment with sublethal concentrations of inhibitors of the *gyrB* protein (9, 58, 96, 153, 170, 171) or by incubation of temperature-sensitive *gyrB* mutants at intermediate temperatures (36, 170). Among the plasmids reported to be sensitive to these treatments are ColA, ColV, pBR322, R28K, R1*drd-19* (in some host strains), F*lac*, and pMG110. Second, higher concentrations of oxolinic acid are required to inhibit ColE1 replication than chromosomal replication (Pruss, unpublished data). So far no unifying theme has emerged which explains these differences between plasmid and chromosome replication.

ROLE OF TOPOISOMERASES AND SUPERCOILING IN GENE EXPRESSION

An understanding of the relationships between supercoiling and transcription developed primarily from in vitro studies. Hayashi and Hayashi (52) first noticed that the initial rate of RNA synthesis is about three times higher from a supercoiled bacteriophage template than from a relaxed one. Subsequently, DNA topoisomers having different superhelical densities were used to show a proportionality between the level of DNA supercoiling and transcriptional activity (4, 132, 164). Two lines of evidence indicate that superhelical tension facilitates initiation of transcription. First, chain initiation, measured by incorporation of γ-^{32}P labeled ATP and GTP, is greater with a supercoiled template (132, 133). Second, the maximum number of RNA polymerase molecules that can form heparin-resistant complexes with DNA, complexes capable of initiating RNA synthesis, is greater with supercoiled DNA (133).

In vitro studies also showed that superhelical tension does not affect all genes equally. In the case of bacteriophage lambda DNA, increasing supercoiling raises the amount of RNA synthesis and lowers the relative amount of early gene transcription (4) by increasing transcription from promoters not used on linear DNA (3). In another approach, specific inhibitors were used to assess the effect of gyrase on coupled transcription and translation in a DNA-directed, cell-free system (175). Two types of DNA were examined. In the first case, relaxed plasmid DNA was added to the reaction mixture. Novobiocin blocked an endogenous supercoiling activity and reduced the relative synthesis of some proteins (colicin E1, tetracycline resistance protein) but not others (β-lactamase). In the second case, DNA from lambda transducing phages was used as a template. Expression from the *lacZ* and *rrnB* promoters, but not from the *trp* promoter, was reduced by novobiocin. In both of these cases gyrase was shown to be the target of novobiocin because little drug effect was observed when the cell-free system was prepared from mutants containing novobiocin-resistant gyrase. However, the conformation of the lambda DNA templates was not determined; these DNAs may have been linear. Since linear plasmid DNA also shows selective inhibition of gene expression by novobiocin, the interesting possibility exists that gyrase introduces localized superhelical tension into linear DNA molecules, a phenomenon that may be relevant to transcription of linear bacteriophage DNA in vivo (21, 30).

Three types of physiological studies support the concept that transcription rates are higher from DNA under superhelical tension and that individual genes respond differently to changes in superhelical tension. The first study used phage mutants to compare transcription from supercoiled and nicked forms of phage replicative intermediates. During normal infection with a phage such as ϕX174 or S13, phage DNA enters the cells as single-stranded circles. Double-stranded, circular replicative intermediates are synthesized, supercoiled by gyrase, and then nicked by the product of the phage *A* gene to initiate replication (reviewed in reference 73). A pool of nicked and supercoiled DNAs then serves as templates for phage mRNA synthesis. In gene *A* mutants, the replicative forms fail to become nicked and thus remain supercoiled. Puga and Tessman (125) found that phage DNA in these mutants has a template efficiency four to five times that found in cells infected with wild-type phages. Thus, it appears that the effect of superhelical tension on transcription in vivo is quantitatively similar to that observed in vitro.

The second type of study linked gyrase to transcription. Antibiotics (29, 46, 134) and temperature-sensitive mutations (32a, 75, 100, 114, 115) affecting gyrase activity reduce levels of RNA synthesis in vivo. The antibiotics are selective in their inhibitory action: cells treated with inhibitors exhibit reduced expression of some genes, increased expression of others, and unchanged expression of still others (Table 2). Since coumermycin A_1 and temperature-sensitive gyrase mutations cause a loss of titratable supercoils from bacterial nucleoids (25, 147, 161) and plasmids (65, 84, 100, 121), it is likely that these two treatments affect transcription by relaxing DNA superhelical tension. Indeed, there is a rough correlation between the change in total RNA synthesis rate and the change in titratable supercoils when cells are treated with coumermycin A_1 (88).

Inhibition of gyrase by oxolinic and nalidixic acids presents a more complicated situation, for the cellular response is very dependent on drug dose. At low drug concentrations, DNA synthesis is drastically inhibited (45, 46), DNA cleavage sites become saturated with drug molecules (145), a small fraction of total RNA synthesis is inhibited (88), and little change in average DNA supercoiling occurs (88, 145). At high drug concentrations, chromosome supercoiling decreases (88), total RNA synthesis is substantially reduced (46), and the expression of some genes is inhibited (135, 137, 142). Moreover, the SOS response (reviewed in reference 81) is induced (see next section). Whereas control experiments with drug-resistant mutants support the contention that inhibition of gyrase is responsible for selective inhibition of certain genes by oxolinic and nalidixic acids, the relationship to superhelical tension is not clear: we have observed a counterexample in which oxolinic acid causes an increase in titratable supercoiling while at the same time causing a decrease in total RNA synthesis (88). Perhaps the oxolinic acid-*gyrA* protein-DNA complexes discussed above block RNA synthesis as well as DNA synthesis.

The third physiological approach has focused on topoisomerase mutations that alter steady-state levels of titratable supercoiling (Table 3). The *supX* mutations, the first group discovered (108), were identified in *S. typhimurium* by their ability to suppress the *leu-500* promoter mutation. In this system, the suppressor effect arises from a loss of a gene mapping near *cys* and *trp*, almost 50 min away from the leucine operon. When *topA* was mapped to this same region (148, 156), it became apparent that *supX* action resulted from the loss of topoisomerase I activity (157). It is likely that *supX* acts to change supercoiling: *supX* mutants have higher-

than-normal levels of titratable supercoiling (Pruss, unpublished data). In *E. coli*, *topA* mutations also exist (148) that appear to exhibit elevated levels of β-galactosidase induction under certain conditions (148); these cells also have higher-than-normal levels of titratable supercoiling (124). Another example of altered supercoiling affecting gene expression is associated with compensatory mutations arising in gyrase in response to *topA* deletions (22, 124). As mentioned in a previous section, these mutations result in lower-than-normal levels of titratable supercoiling (124). In these gyrase mutants the *bgl* operon, which is not expressed in wild-type strains, becomes inducible (22).

Whereas it is clear that levels of supercoiling or topoisomerase activity affect the expression of a few genes (Tables 2 and 3), in most cases it is not known whether the effect is direct or mediated by altered expression of other genes. Nor is the general scope of the effect known. However, the availability of topoisomerase mutants in which titratable supercoiling is either higher or lower than normal now makes it possible to survey the relative abundance of a large number of proteins to determine whether the level of supercoiling found in wild-type cells is optimal for expression of most genes.

TABLE 2. Effect of supercoiling on gene expression: inactivation of gyrase by antibiotics

Gene, promoter, operon, or gene product[a]	Drug tested[b]	Reference(s)
Expression reduced		
Alkaline phophase[c]	N, C	159
Colicin[d]	c	175
cys JIH[e]	N, C	116
gal	N	135
Histidase[c]	C	159
lacZ	N, n, c	135, 137, 175
malE malF	N	135
malK lamB	N, C	135, 137
malP malQ	N	135, 137
$P_L(\phi 80)^f$	N, C	142
$P_L(\lambda^f)^d$	N, C	77
$P_R(\lambda^f)^d$	N, C	77
$P_{RM}(\lambda^f)$	N	51
$P_E(\lambda^f)^d$	N, C	77
rRNA	N, C, n, c	113, 114, 162
rrnB	c	175
T7 late genes[f]	C	21
Tetracycline resistance[d]	N, C, c	44, 175
trp[d,f,g]	N, C	75, 77
tna	N	135
Expression stimulated		
gyrA	N, C, c	98
gyrB	N, C, c	98
lacI^Q	N, C	135
lacZ-UV5	N	135
Expression unaffected		
Amylase[c]	N, C	159
bla[d]	N, C, c	44, 175
cysE[e]	N, C	116
Glucose 6-phosphodehydrogenase	N	137
Histidase[c]	N	159
Homoserine dehydrogenase	N	137
thrA	N, C	135
trp[g]	N, C, c	135, 175

[a] All listings are for *E. coli* unless indicated otherwise.
[b] Abbreviations: N, nalidixic acid or oxolinic acid (in vivo measurement); C, coumermycin A_1 or novobiocin (in vivo measurement); n, nalidixic acid or oxolinic acid (in vitro measurement); c, coumermycin A_1 or novobiocin (in vitro measurement).
[c] *B. subtilis*.
[d] Plasmid.
[e] *S. typhimurium*.
[f] Bacteriophage.
[g] In some cases, *trp* expression is inhibited by drugs affecting gyrase, whereas in other cases it is not.

CELLULAR RESPONSES TO NALIDIXIC ACID

Inhibitors of gyrase are bacteriocidal (45, 134, 144); in retrospect, this is not surprising since gyrase and supercoiling play a central role in bacterial chromosome structure and function. However, the bacteriocidal effect, especially in the cases of oxolinic and nalidixic acids, arises from more than a simple absence of gyrase activity. As mentioned above, these drugs form complexes in which the *gyrA* protein and the drug are bound to DNA (41, 101, 105, 106, 145, 150, 151, 158), and it is likely that these complexes act as barriers to replication fork movement: DNA synthesis is rapidly inhibited when cells are treated with nalidixic or oxolinic acid (46). Blockage of DNA replication, which is readily reversed by removal of the drug (50, 66), leads to induction of the SOS regulatory pathway (for review, see reference 81). As a result, cell division stops and some proteins thought to participate in DNA repair increase in abundance. It is not known how nalidixic acid-induced lesions are repaired. The SOS response is probably involved since mutations in some of the genes of that pathway increase the bacteriocidal effect of nalidixic acid (Table 4). A simplified version of the SOS response, as it pertains to nalidixic acid, is illustrated in Fig. 3.

The *recA* gene is the most extensively studied component of the SOS regulatory pathway, and induction of *recA* is commonly used as an assay for the response. In the case of nalidixic acid, inhibition of DNA synthesis is one factor in the induction of *recA*, for cells undergoing little or no DNA replication (temperature-sensitive *dnaA* mutants incubated at restrictive temperature) show little induction of *recA* after drug treatment (50, 80). Factors other than inhibition of DNA synthesis are probably also involved in *recA* induction, for the same amount of inhibition of DNA synthesis by thymine starvation induces less *recA* protein than does inhibition by nalidixic acid (50). Moreover, higher nalidixic acid concentrations induce more *recA* protein than lower ones even when the extent of inhibition of DNA synthesis is roughly the same (50). One testable hypothesis is that the decrease in titratable supercoiling associated with high concentrations of the drug (88) enhance expression of *recA*.

Generating the signal for induction of *recA* by nalidixic acid requires the presence of wild-type alleles of *recB* and *recC* (49, 79). Since the *recB* and *recC* gene products exhibit DNA exonuclease V activity in vitro (for review, see 154), the idea developed that exonuclease V degrades DNA (34, 143) when DNA synthesis is rapidly blocked. In this model, DNA degradation would create an oligonucleotide signal that would in turn convert the *recA* protein into a catalyst of proteolytic cleavage of the *lex* protein, the repressor of *recA* (Fig. 3; see reference 81 for review of *lex* and *recA* interactions). Since DNA degradation is potentially harmful and probably contributes to the cytotoxicity of nalidixic acid (15, 16, 34, 49, 66, 130), the presence of wild-type *recB* and *recC* alleles might not always be favorable for survival. Such a case has been reported; wild-type cells are more sensitive than *recB* and *recC* mutants to inhibitors of gyrase if the cells

TABLE 3. Effect of topoisomerase mutations and supercoiling on gene expression

Gene, promoter, operon, or gene product[a]	Mutation	Effect	Reference
bgl	*gyrB*[b]	Becomes inducible	22
ilv	*himA gyrB him*(Ts)	Becomes auxotrophic at 42°C	35
leu-500[c]	*top*[d]	Promoter mutation suppressed	108
lacL1[e]	*top*[d]	Promoter mutation suppressed	27
lacL8[e]	*top*[d]	Promoter mutation suppressed	26
lacL29[e]	*top*[d]	Promoter mutation suppressed	26
Alkaline phosphatase[c]	*top*[d]	Expression increased	27
Histidinol phosphate[c]	*top*[d]	Expression increased	47
Isocitrate lyase[c]	*top*[d]	Expression increased	47
soc[f]	T4 (gene 39$^-$) *gyrB*(Ts)	Expression increased at 42°C	87
recBC nuclease	*top*[d]	Expression increased	17
xyl	*himAgyrB him*(Ts)	Becomes auxotrophic at 42°C	35

[a] All listings are for *E. coli* unless indicated otherwise.
[b] Mutation causes lower-than-normal levels of titratable supercoiling.
[c] *S. typhimurium*.
[d] Many different point and deletion mutations were used in these experiments; supercoiling was measured in only three (Pruss, unpublished data), and in all three, levels were higher than normal.
[e] *E. coli* mutation on plasmid in *S. typhimurium*.
[f] Bacteriophage T4.

are pretreated with UV irradiation (128). This UV effect is exacerbated by a mutation in *recA* (127), perhaps because the *recA* protein blocks exonuclease V-dependent DNA degradation (14, 90, 136, 167, 168). Such action by the *recA* protein might also explain why high concentrations of nalidixic acid, which lead to high levels of *recA* induction, exhibit less DNA degradation (34) and less cell death (18, 169) than lower concentrations of the drug.

One of the consequences of the SOS response to nalidixic acid is that cell division is blocked. The effect appears to be mediated by the *lex* protein, which normally acts as a repressor of the *sfiA* gene (Fig. 3). The target of the *sfiA* protein appears to be the *sfiB* (*ftsZ*) protein (86), and the latter is probably required for cell division. Thus, nalidixic acid indirectly leads to induction of *sfiA*, inactivation of the *sfiB* protein, and cessation of cell division. In a *sfiB* mutant, the *sfiB* protein might not be readily inactivated by the *sfiA* protein, explaining the observation that a *sfiB* mutation delays inhibition of cell division by nalidixic acid (6).

Recovery of normal cell division after removal of nalidixic acid may involve the *lon* protein. Mutations in *lon* lead to nalidixic acid-induced lethal filmentation (66), a situation in which cells grow lengthwise but do not divide. The *lon* protein exhibits protease activity in vitro (11, 12), and one of its functions may be to degrade the *sfiA* protein after DNA damage has been repaired.

The *recF* pathway is also involved in cell survival during treatment with nalidixic acid; *recF* mutants are hypersensitive to the drug and *recBCF* triple mutants are more sensitive to nalidixic acid than are either *recBC* or *recF* mutants (95). Little can be said about the role of *recF*. The wild-type allele is not required for induction of *recA* by nalidixic acid (67, 97, 141), and under some circumstances it even lowers the level of induction (67). In contrast, induction of *recA* by coumermycin A_1 requires the presence of a wild-type *recF* allele (141).

TOPOISOMERASES, RECOMBINATION, AND REPAIR

Generalized Recombination

Generalized recombination is mediated by the *recA* gene product, and this protein is known to unwind DNA in vitro (112, 173). Thus, we expect negative superhelical tension to facilitate recombination through its effect on DNA unwinding. Indeed, *recA*-dependent recombination in a spheroplast transfection system occurs between single-stranded fragments of φX174 DNA and circular φX174 replicative intermediate at higher frequencies if the replicative form DNA is supercoiled (57).

Two observations suggest that gyrase activity may affect recombination. First, recombination involving UV-irradiated, superinfecting bacteriophage lambda DNA (53) or transformation in *Streptococcus sanguis* (129) is reduced in vivo by inhibitors of gyrase. These inhibitors also lower intramolecular recombination in plasmids in an in vitro, *recA*-dependent system (72). Second, recombination after conjugation is reduced in *gyrB* mutants (161). However, generalized recombination is not very sensitive to small changes in DNA supercoiling: topoisomerase mutants having either 25% higher- or 25% lower-than-normal levels of titratable supercoiling (124) have similar recombination frequencies when measured by P1-mediated transduction (148).

Site-Specific Recombination

Integration and excision of bacteriophage lambda and resolution of cointegrates by transposons involve recombi-

TABLE 4. Mutations that increase sensitivity to nalidixic acid

Gene	Product	Reference(s)
polI	DNA polymerase I	94, 169
lon	ATP-dependent protease	48, 66
recA	RecA protein	48, 95
recF	RecF protein	95
recB[a]	135-kilodalton subunit of DNA exonuclease V	48, 95
recC[a]	125-kilodalton subunit of DNA exonuclease V	48, 95
uvrB[b]	84-kilodalton subunit of pyrimidine dimer DNA endonuclease	95
uvrD(*recL*)	DNA helicase II	95
xsc	DNA exonuclease VII	13

[a] *sbcA* and *sbcB* mutations do not markedly suppress increased sensitivity to nalidixic acid in *recB recC* mutants (95).
[b] *uvrA* mutants do not show increased sensitivity to nalidixic acid (48).

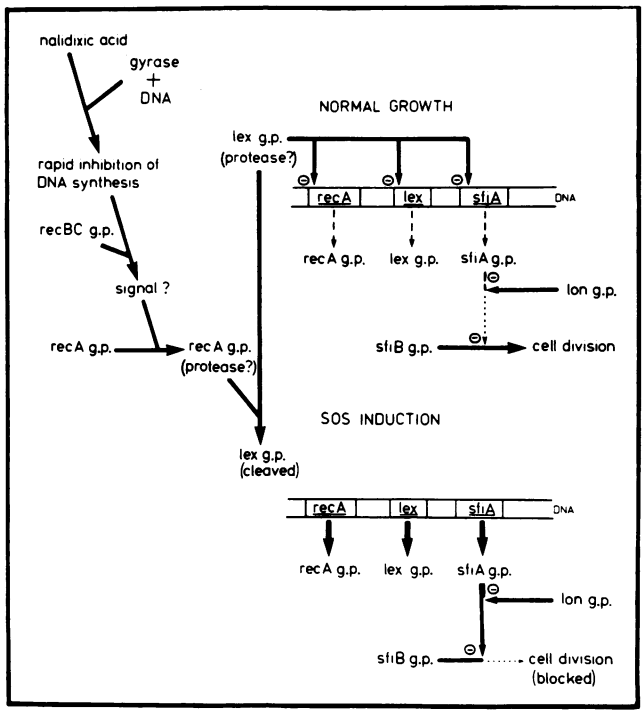

FIG. 3. Nalidixic acid and the SOS response. During normal growth the *lex* gene product (g.p.) represses a number of genes among which are *recA*, *lex*, and *sfiA*. Thus, the concentration of the *sfiA* g.p. is kept low and has little affect on the *sfiB* g.p., a protein required for cell division. In this scheme it is assumed that the *lon* g.p., which has proteolytic activity in vitro, contributes to maintaining concentrations of the *sfiA* g.p. low. Nalidixic acid blocks DNA replication, and an undefined *recB recC*-mediated event occurs that activates the *recA* g.p. Activated *recA* g.p. either acts directly as a protease to cleave the *lexA* g.p. or stimulates an autoproteolytic activity of the *lexA* g.p. to cleave the *lexA* g.p. Cleavage of the *lexA* g.p. leads to induction of the SOS genes. The concentration of the *sfiA* g.p. becomes high enough to inactivate the *sfiB* g.p. and cell division halts. DNA synthesis begins shortly after removal of nalidixic acid, the inducing signal disappears, and the *lex* g.p. restores repression. The *lon* g.p. removes excess *sfiA* g.p., and cell division occurs. The figure is adapted from references 81 and 79a.

nation events in which two short, homologous DNA sequences pair, break, and rejoin. The process is mediated by proteins, some of which exhibit topoisomerase activity in vitro, and the process normally requires a supercoiled DNA substrate (102, 131; for a review see reference 110).

The development of an in vitro integrative recombination system for lambda uncovered the need for supercoiled DNA (104) and led to the discovery of gyrase (42). It was then found that inhibitors of gyrase (69) or mutations in *gyrB* (*himB*; 36, 40) restrict lambda integration, indicating that the requirement for supercoiling also holds in vivo. Integrative recombination can be carried out with in vitro reaction mixtures containing purified lambda *int* gene product (Int), purified integration host factor (the product of *E. coli* genes *himA* and *hip*), and DNA containing two specialized nucleotide sequences, *attP* from the phage and *attB* from the bacterium (110, 123).

Nash and his colleagues (70, 110, 123) suggest that the following events occur during integrative recombination. Int recognizes *attB*, and Int plus integration host factor recognize *attP*; interaction of the recombination proteins leads to synapsis of the homologous nucleotide sequences via a four-stranded helix. The topoisomerase activity of Int (69, 70) leads to cleavage of one DNA strand from each parent followed by swiveling of the parental helices around each uncut strand and ligation of realigned strands. This Holliday junction is then resolved by a second round of Int-dependent cleavage, swiveling, and ligation of the remaining parental strands.

It is not clear which steps are accelerated by supercoiling. An important symmetry consideration arises from the observation that supercoiling of *attP*-containing, but not *attB*-containing, DNA stimulates recombination (103, 123). An attractive idea is that *attP* wraps around Int and that supercoiling favors this event much in the way it favors wrapping of DNA around histones during formation of nucleosomes (123).

Resolvase from transposon Tn*3* also displays topoisomerase activity. This protein resolves a supercoiled cointegrate into two catenated, supercoiled rings (19, 74). Recombination mediated by resolvase is strongly favored in supercoiled DNA molecules (131). During the reaction, a small amount of relaxation occurs in both the parental DNA and the recombinant catenanes (74). Both relaxation and recombination by resolvase require the same substrate, two *res* sites oriented as direct repeats. Neither relaxation nor recombination occurs with DNAs having zero, one, or even two *res* sites if oriented head to head (19, 74). Thus, resolvase is the only topoisomerase which shows strict nucleotide sequence specificity. Moreover, resolvase relaxes and recombines DNA only if the two *res* sites are on the same DNA molecule (74).

Gyrase-Mediated Illegitimate Recombination

Ikeda and co-workers (60, 61, 109) observed that illegitimate recombination occurs in vitro when an ampicillin-resistant plasmid (pBR322) is present in a bacteriophage lambda packaging system: Amp^r transducing phages arise at a frequency of about 10^{-7}. Surprisingly, addition of oxolinic acid to the packaging system stimulates the recombination event by about 10-fold. Coumermycin A_1 blocks the stimulatory effect of oxolinic acid. Thus, it has been suggested that gyrase participates in illegitimate recombination, perhaps through a mechanism involving gyrase subunit exchange. During gyrase-DNA interactions, two *gyrA* proteins and two *gyrB* proteins are thought to form a complex with DNA that results in transient double-strand cleavage (for reviews, see references 17 and 38). Exchange of *gyrA* protein-DNA fragments at this stage would lead to recombination. Oxolinic acid interacts with the *gyrA* protein to trap a reaction intermediate in which the phosphodiester bonds in DNA are broken; by lengthening the lifetime of the reaction intermediate, oxolinic acid could stimulate subunit exchange. Since gyrase does not exhibit strict nucleotide sequence specificity, recombination events could occur at a wide variety of locations on both pBR322 and lambda. Heteroduplex and nucleotide sequence analyses of recombinants show this to be the case. If a cascade of subunit exchange occurs, deletions are expected to arise in the recombinants (Fig. 4); this too has been observed. As predicted by the subunit exchange model, mutations in genes involved in recombination have no effect on the stimulation of illegitimate recombination by oxolinic acid.

Some illegitimate recombination events occurring in vivo are also most easily interpreted by using the gyrase subunit exchange model (71, 91). The reaction mechanism of gyrase is probably similar in vivo and in vitro since oxolinic acid, in

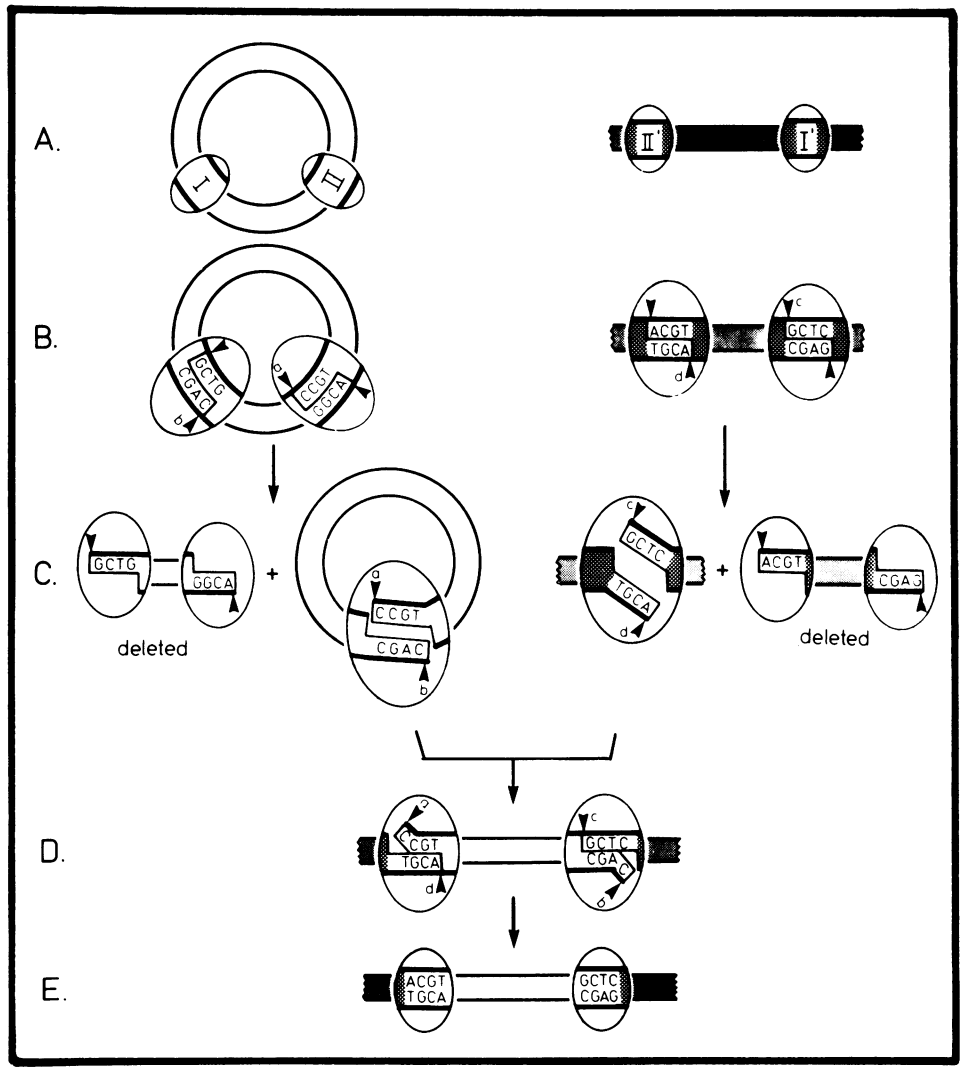

FIG. 4. Illegitimate recombination mediated by gyrase. Illegitimate recombination between plasmid pBR322 and bacteriophage lambda has been observed in *recA* bacteria. Nucleotide sequence analysis suggests that gyrase may be involved in the recombinational event. (A) Plasmid DNA (left) and phage DNA (right, shaded) are shown in which each contains two gyrase cleavage sites (I, I', II, II'). (B) Gyrase binds to each DNA, producing DNA cleavage as a normal intermediate in the supercoiling reaction. Arrowheads depict 5' end of DNA in the cleavage site where a *gyrA* protein is thought to be bound. Lowercase letters identify specific 5' termini. (C) Gyrase subunit exchange occurs between sites I and II, leading to deletion of intervening DNA and formation of unstable intermediates. Intermediates are unstable because there is little base pairing complementarity. (D) A second gyrase subunit exchange occurs in which plasmid and phage DNAs pair. Base pairing complementarity is imperfect. (E) Resealing by gyrase and mismatch repair produce a stable recombinant. The figure is adapted from a model presented by Marvo et al. (91).

combination with protein-denaturing agents, produces double-strand DNA cleavage (145). Nucleotide sequences determined from a recombinant formed in vivo (91) have been included in Fig. 4 to illustrate a possible mechanism.

DNA Repair

Relationships between DNA repair and supercoiling have been investigated by using mutations that alter levels of titratable supercoiling, but so far no simple correlation has emerged. Both *gyrB* mutations and inhibitors of gyrase reduce supercoiling (25, 147, 161); in one case the mutation lowers sensitivity to UV irradiation (161), whereas in others the inhibitor reduces repair of UV-induced damage (53). Mutations in *topA*, some of which raise levels of supercoiling (124; Pruss, unpublished data), raise sensitivity to UV irradiation (118, 148). But UV sensitivity may not arise from supercoiling, for sensitivity is also observed in a *topA* mutant (DM800 [148]) having lower-than-normal levels of supercoiling (124), levels which arise from an additional mutation in *gyrB* (22). It is now necessary to determine which of these effects are due to altered expression of genes involved in repair and which arise from alterations in the DNA that acts as a substrate for repair enzymes.

Another approach is to ask how DNA damage affects topoisomerase activity. In the case of topoisomerase I, supercoiled DNA containing small amounts of UV-induced damage is not efficiently relaxed by topoisomerase I in vitro (119); does this phenomenon allow cells to strongly favor gyrase activity over topoisomerase I activity during recovery from photodamage to DNA?

CONCLUDING REMARKS

Biochemical and physiological studies of topoisomerases have led to the following general view of bacterial chromosome structure and function. Bacterial chromosomal DNA is circular (7), so topoisomerases are able to activate the DNA by maintaining superhelical tension in it. Gyrase introduces tension by lowering DNA linking numbers (42) and topoisomerase I modulates the level (124) by increasing them (163). The level of tension is tightly controlled, and even small alterations induced by changes in temperature seem to be corrected by topoisomerase action (43b). One of the reasons for such tight control of superhelical tension appears to involve gene expression, for expression of certain genes is sensitive to changes in supercoiling or topoisomerase activity or both (Tables 2 and 3). Since nicks or gaps in DNA would lead to relaxation and would adversely affect gene expression, the chromosome is divided into about 50 topologically independent domains (139, 172) to confine relaxation to small (100-kilobase pair) regions. Gyrase is distributed along the DNA at 100-kilobase pair intervals (145), which correspond to about one gyrase per domain. This distribution of gyrase does not diminish when DNA, RNA, or protein synthesis is inhibited (unpublished data), so gyrase is able to maintain superhelical tension under a variety of conditions. As a consequence of domains and gyrase, relaxation associated with discontinuous DNA synthesis is restricted to only 2% of the chromosome at any given time. Thus a new round of replication, which may require supercoiled DNA for initiation, can begin before the previous round finishes. By examining the events at the end of a round of replication, we see one of the costs of chromosome circularity: the need to unlink daughter chromosomes. This event appears to require gyrase activity (Steck and Drlica, in press).

Although we are beginning to understand topoisomerases and supercoiling, a number of questions about their roles in chromosome structure and function remain unanswered. For example, we do not know how topological domains are established, whether specific nucleotide sequences are involved, or if superhelical tension is the same in each. Nor do we know whether cells respond to unfavorable environmental conditions by changing superhelical tension so certain genes can be induced. Finally, we would like to know what role histone-like proteins and nucleosomes play in bacterial DNA topology so we can relate bacterial studies to eucaryotic systems in which nucleosomes tend to dominate our thinking.

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LITERATURE CITED

1. **Baird, J. P., G. J. Bourguignon, and R. Sternlganz.** 1972. Effect of nalidixic acid on the growth of deoxyribonucleic acid bacteriophages. J. Virol. **9**:17–21.
2. **Bauer, W. R.** 1978. Structure and reactions of closed duplex DNA. Annu. Rev. Biophys. Bioeng. **7**:287–313.
3. **Botchan, P.** 1976. An electron microscopic comparison of transcription on linear and superhelical DNA. J. Mol. Biol. **105**:161–176.
4. **Botchan, P., J. C. Wang, and H. Echols.** 1973. Effect of circularity and superhelicity on transcription from bacteriophage λ DNA. Proc. Natl. Acad. Sci. U.S.A. **70**:3077–3081.
5. **Brown, P. O., C. L. Peebles, and N. R. Cozzarelli.** 1979. A topoisomerase from *Escherichia coli* related to DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **76**:6110–6114.
6. **Burton, P., and I. B. Holland.** 1983. Two pathways of division inhibition in UV-irradiated *E. coli*. Mol. Gen. Genet. **190**:309–314.
7. **Cairns, J.** 1963. The chromosome of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **28**:43–46.
8. **Cairns, J., and C. I. Davern.** 1966. Effect of ^{32}P decay upon DNA synthesis by a radiation-sensitive strain of *Escherichia coli*. J. Mol. Biol. **17**:418–427.
9. **Cejka, K., I. Holubova, and J. Hubacek.** 1982. Curing effect of chlorobiocin on *Escherichia coli* plasmids. Mol. Gen. Genet. **186**:153–155.
10. **Champoux, J. J., and M. D. Been.** 1980. Topoisomerases and the swivel problem, p. 809–815. *In* B. Alberts (ed.), Mechanistic studies of DNA replication and genetic recombination. Academic Press, Inc., New York.
11. **Charette, M. F., G. W. Henderson, F. J. Kezdy, and A. Markovitz.** 1982. Molecular mechanism for dominance of a mutant allele of an ATP-dependent protease. J. Mol. Biol. **162**:503–510.
12. **Charette, M. F., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon* (*capR*) protein of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **78**:4728–4732.
13. **Chase, J. W., and C. C. Richardson.** 1977. *Escherichia coli* mutants deficient in exonuclease VII. J. Bacteriol. **129**:934–947.
14. **Clark, A. J., M. Chamberlin, R. P. Boyce, and P. Howard-Flanders.** 1966. Abnormal metabolic response to ultraviolet light of a recombination deficient mutant of *Escherichia coli* K-12. J. Mol. Biol. **19**:442–454.
15. **Cook, T., K. Brown, J. Boyle, and W. Goss.** 1966. Bactericidal action of nalidixic acid on *Bacillus subtilis*. J. Bacteriol. **92**:1510–1514.
16. **Cook, T., W. Deitz, and W. Goss.** 1966. Mechanisms of action of nalidixic acid on *Escherichia coli*. IV. Effects on the stability of cellular constituents. J. Bacteriol. **91**:774–779.
17. **Cozzarelli, N. R.** 1980. DNA gyrase and the supercoiling of DNA. Science **207**:953–960.
18. **Crumplin, G. C., and J. T. Smith.** 1975. Nalidixic acid: an antibacterial paradox. Antimicrob. Agents Chemother. **8**:251–261.
19. **Dean, F., M. Krasnow, R. Otter, M. Matzuk, S. Spengler, M. Pastorcio, and N. Cozzarelli.** 1983. *Escherichia coli* Type I topoisomerases: identification, mechanism and role in recombination. Cold Spring Harbor Symp. Quant. Biol. **47**:769–777.
20. **Depew, R. E., and J. C. Wang.** 1975. Conformational fluctuations of DNA helix. Proc. Natl. Acad. Sci. U.S.A. **72**:4275–4279.
21. **DeWyngaert, M., and D. C. Hinkle.** 1979. Involvement of DNA gyrase in replication and transcription of bacteriophage T7 DNA. J. Virol. **29**:529–535.
22. **DiNardo, S., K. A. Voelkel, R. Sternglanz, A. E. Reynolds, and A. Wright.** 1982. *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. Cell **31**:43–51.
23. **Dixon, N. E., and A. Kornberg.** 1984. Protein HU in the enzymatic replication of the chromosomal origin of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **81**:424–428.
24. **Drlica, K., E. C. Engle, and S. H. Manes.** 1980. DNA gyrase on the bacterial chromosome: possibility of two levels of action. Proc. Natl. Acad. Sci. U.S.A. **77**:6879–6883.
25. **Drlica, K., and M. Snyder.** 1978. Superhelical *Escherichia coli* DNA: relaxation by coumermycin. J. Mol. Biol. **120**:145–154.
26. **Dubnau, E., A. B. Lenny, and P. Margolin.** 1973. Nonsense mutations of the *supX* locus: further characterization of the

supX mutant phenotype. Mol. Gen. Genet. **126**:191–200.
27. **Dubnau, E., and P. Margolin.** 1972. Suppression of promoter mutations by the pleiotropic SupX mutations. Mol. Gen. Genet. **117**:91–112.
28. **Engle, E. C., S. H. Manes, and K. Drlica.** 1982. Differential effect of antibiotics inhibiting gyrase. J. Bacteriol. **149**:92–98.
29. **Fairweather, N. F., E. Orr, and I. B. Hoalland.** 1980. Inhibition of deoxyribonucleic acid gyrase: effects on nucleic acid synthesis and cell division in *Escherichia coli* K-12. J. Bacteriol. **142**:153–161.
30. **Falco, S. C., R. Zivin, and L. B. Rothman-Denes.** 1978. Novel template requirements of N4 virion RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **75**:3220–3224.
31. **Filutowicz, M.** 1980. Requirement of DNA gyrase for the initiation of chromosome replication in *Escherichia coli* K-12. Mol. Gen. Genet. **177**:301–309.
32. **Filutowicz, M., and P. Jonczyk.** 1981. Essential role of *gyrB* gene product in the transcriptional event coupled to *dnaA*-dependent initiation of *Escherichia coli* chromosome replication. Mol. Gen. Genet. **183**:134–138.
32a. **Filutowicz, M., and P. Jonczyk.** 1983. The *gyrB* gene product functions in both initiation and chain polymerization of *Escherichia coli* chromosome replication: suppression of the initiation deficiency in *gyrB*-ts mutants by a class of the *rpoB* mutations. Mol. Gen. Genet. **191**:282–287.
33. **Fisher, L. M., K. Mizuuchi, M. H. O'Dea, H. Ohmori, and M. Gellert.** 1981. Site-specific interaction of DNA gyrase with DNA. Proc. Natl. Acad. Sci. U.S.A. **78**:4165–4169.
34. **Fradkin, G. E., O. A. Aizenberg, and I. I. Samoilenko.** 1977. Stabilization and repair of metabolic breaks in DNA chains resulting from an imbalance between protein and DNA syntheses. Mol. Biol. (Moscow) **11**:498–506.
35. **Friedman, D. I., E. J. Olson, D. Carver, and M. Gellert.** 1984. Synergistic effect of *himA* and *gyrB* mutations: evidence that Him functions control expression of *ilv* and *xyl* genes. J. Bacteriol. **157**:484–489.
36. **Friedman, D. I., L. C. Pantefaber, E. J. Olson, D. Carver, M. H. O'Dea, and M. Gellert.** 1984. Mutations in the DNA *gyrB* gene that are temperature sensitive for lambda site-specific recombination, Mu growth, and plasmid maintenance. J. Bacteriol. **157**:490–497.
37. **Fuller, R. S., J. M. Kaguni, and A. Kornberg.** 1981. Enzymatic replication of the origin of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. U.S.A. **78**:7370–7374.
38. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
39. **Gellert, M., L. M. Fisher, and M. H. O'Dea.** 1979. DNA gyrase: purification and catalytic properties of a fragment of gyrase B protein. Proc. Natl. Acad. Sci. U.S.A. **76**:6289–6293.
40. **Gellert, M., R. Menzel, K. Mizuuchi, M. H. O'Dea, and D. Friedman.** 1983. Regulation of DNA Supercoiling in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **47**:763–767.
41. **Gellert, M., K. Mizuuchi, M. H. O'Dea, T. Itoh, and J.-I. Tomizawa.** 1977. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc. Natl. Acad. Sci. U.S.A. **74**:4772–4776.
42. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3872–3876.
43. **Gellert, M., M. H. O'Dea, T. Itoh, and J.-I. Tomizawa.** 1976. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **73**:4474–4478.
43a. **Glikin, G., I. Rubenti, and A. Worcel.** 1984. Chromatin assembly in *Xenopus* oocytes: *in vitro* studies. Cell **37**:33–41.
43b. **Goldstein, E., and K. Drlica.** 1984. Regulation of bacterial DNA supercoiling: plasmid linking numbers vary with growth temperature. Proc. Natl. Acad. Sci. U.S.A. **81**:4046–4050.
44. **Gomez-Eichelmann, M. C.** 1981. Effect of nalidixic acid and novobiocin on pBR322 genetic expression in *Escherichia coli* minicells. J. Bacteriol. **148**:745–752.
45. **Goss, W. A., W. H. Deitz, and T. M. Cook.** 1964. Mechanism of action of nalidixic acid on *Escherichia coli*. J. Bacteriol. **88**:1112–1118.
46. **Goss, W. A., W. H. Deitz, and T. M. Cook.** 1965. Mechanism of action of nalidixic acid on *Escherichia coli*. II. Inhibition of deoxyribonucleic acid synthesis. J. Bacteriol. **89**:1068–1074.
47. **Graf, L. H., and R. O. Burns.** 1973. The *supX*/*leu500* mutations and expression of the leucine operon. Mol. Gen. Genet. **126**:291–301.
48. **Green, M., J. Donch, and J. Greenburg.** 1970. Effect of inhibitors of DNA synthesis on UV-sensitive derivatives of *Escherichia coli* strain K-12. Mutat. Res. **9**:149–154.
49. **Grigg, G. W.** 1970. Stability of DNA in dark-repair mutants of *Escherichia coli* B treated with nalidixic acid. J. Gen. Microbiol. **61**:21–25.
50. **Gudas, L. J., and A. B. Pardee.** 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459–477.
51. **Hawley, D. K., and W. R. McClure.** 1982. Mechanism of activation of transcription initiation from the P_{RM} promoter. J. Mol. Biol. **157**:493–525.
52. **Hayashi, Y., and M. Hayashi.** 1971. Template activities of the $\phi\times174$ replicative allomorphic deoxyribonucleic acids. Biochemistry **10**:4212–4218.
53. **Hays, J. B., and S. Boehmer.** 1978. Antagonists of DNA gyrase inhibit repair and recombination of UV-irradiated phase λ. Proc. Natl. Acad. Sci. U.S.A. **75**:4125–4129.
54. **Higgins, N. P., and N. R. Cozzarelli.** 1982. The binding of gyrase to DNA: analysis by retention by nitrocellulose filters. Nucleic Acids Res. **10**:6833–6847.
55. **Higgins, N. P., C. L. Peebles, A. Sugino, and N. R. Cozzarelli.** 1978. Purification of subunits of *Escherichia coli*, DNA gyrase and reconstitution of enzymatic activity. Proc. Natl. Acad. Sci. U.S.A. **75**:1773–1777.
56. **Hill, W. E., and W. L. Fangman.** 1973. Single-strand breaks in deoxyribonucleic acid and viability loss during deoxyribonucleic acid synthesis inhibition in *Escherichia coli*. J. Bacteriol. **116**:1329–1335.
57. **Holloman, W. K., and C. M. Radding.** 1976. Recombination promoted by superhelical DNA and the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **73**:3910–3914.
58. **Hooper, D. C., J. S. Wolfson, G. L. McHugh, M. D. Swartz, C. Tung, and M. N. Swartz.** 1984. Elimination of plasmid pMG110 from *Escherichia coli* by novobiocin and other inhibitors of DNA gyrase. Antimicrob. Agents Chemother. **25**:586–590.
59. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**:1137–1150.
60. **Ikeda, H., K. Aoki, and A. Naito.** 1982. Illegitimate recombination mediated *in vitro* by DNA gyrase of *Escherichia coli*: structure of recombinant DNA molecules. Proc. Natl. Acad. Sci. U.S.A. **79**:3724–3728.
61. **Ikeda, H., K. Moriya, and T. Matsumoto.** 1981. *In vitro* study of illegitimate recombination: involvement of DNA gyrase. Cold Spring Harbor Symp. Quant. Biol. **45**:399–408.
62. **Isberg, R. R., and M. Syvanen.** 1982. DNA gyrase is a host factor required for transposition of Tn5. Cell **30**:9–18.
63. **Itoh, T., and J.-I. Tomizawa.** 1977. Involvement of DNA gyrase in bacteriophage T7 DNA replication. Nature (London) **270**:78–80.
64. **Itoh, T., and J.-I. Tomizawa.** 1979. Initiation of replication of plasmid ColE1 DNA by RNA polymerase, ribonuclease H, and DNA polymerase I. Cold Spring Harbor Symp. Quant. Biol. **43**:409–417.
65. **Kano, Y., T. Miyashita, H. Nakamura, K. Kuroki, A. Nagata, and F. Imamoto.** 1981. *In vivo* correlation between DNA supercoiling and transcription. Gene **13**:173–184.
66. **Kantor, G. J., and R. A. Deering.** 1968. Effect of nalidixic acid and hydroxyurea on division ability of *Escherichia coli fil*$^+$ and *lon*$^-$ strains. J. Bacteriol. **95**:520–530.
67. **Karu, A. E., and E. D. Belk.** 1982. Induction of *E. coli* recA protein via recBC and alternate pathways: quantitation by enzyme-linked immunosorbent assay (ELISA). Mol. Gen. Genet. **185**:275–282.
68. **Kikuchi, Y., and H. A. Nash.** 1978. The bacteriophage λ *int* gene

product. J. Biol. Chem. **253**:7149–7157.
69. **Kikuchi, Y., and H. A. Nash.** 1979. Integrative recombination of bacteriophage λ: requirement for supertwisted DNA in vivo and characterization of Int. Cold Spring Harbor Symp. Quant. Biol. **43**:1099–1109.
70. **Kikuchi, Y., and H. Nash.** 1979. Nicking-closing activity associated with bacteriophage λ *int* gene product. Proc. Natl. Acad. Sci. U.S.A. **76**:3760–3764.
71. **King, S. R., M. A. Krolewski, A. L. Marvo, P. J. Lipson, K. L. Pogue-Geile, J. H. Chung, and S. R. Jaskunas.** 1982. Nucleotide sequence analysis of in vivo recombinants between bacteriophage λ DNA and pBR322. Mol. Gen. Genet. **186**:548–557.
72. **Kolodner, R.** 1980. Genetic recombination of bacterial plasmid DNA: electron microscopic analysis of *in vitro* intramolecular recombination. Proc. Natl. Acad. Sci. U.S.A. **77**:4847–4851.
73. **Kornberg, A.** 1980. DNA replication. W. H. Freeman & Co., San Francisco.
74. **Krasnow, M. A., and N. R. Cozzarelli.** 1983. Site-specific relaxation and recombination by Tn3 resolvase: recognition of the DNA path between oriented *res* sites. Cell **32**:1313–1324.
75. **Kreuzer, K. N., and N. R. Cozzarelli.** 1979. *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. J. Bacteriol. **140**:424–435.
76. **Kreuzer, K. N., and W. M. Huang.** 1983. T4 DNA topoisomerase, p. 90–96. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget (ed.), Bacteriophage T4. American Society for Microbiology, Washington, D.C.
77. **Kubo, M., Y. Kano, H. Nakamura, A. Nagata, A., and F. Imamoto.** 1979. *In vivo* enhancement of general and specific transcription in *Escherichia coli* by DNA gyrase activity. Gene **7**:153–171.
78. **Kung, V. T., and J. C. Wang.** 1977. Purification and characterization of a ω protein from *Micrococcus luteus*. J. Biol. Chem. **252**:5398–5402.
79. **Little, J. W.** 1983. Variations in the *in vivo* stability of lexA repressor during the SOS regulatory cycle, p. 369–378. *In* Cellular responses to DNA damage. Alan R. Liss, Inc., New York.
79a. **Little, J. W.** 1984. Autodigestion of lexA and phage λ repressors. Proc. Natl. Acad. Sci. U.S.A. **81**:1375–1379.
80. **Little, J. W., and P. C. Hanawalt.** 1977. Induction of protein X in *Escherichia coli*. Mol. Gen. Genet. **150**:237–248.
81. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of Escherichia coli. Cell **29**:11–22.
82. **Liu, L. F., C. C. Liu, and B. M. Alberts.** 1979. T4 DNA topoisomerase: a new ATP-dependent enzyme essential for initiation of T4 bacteriophage DNA replication. Nature (London) **281**:456–461.
83. **Liu, L. F., and J. C. Wang.** 1975. *In vitro* DNA synthesis on primed covalently double-stranded templates. I. Studies with *Escherichia coli* DNA polymerase I, p. 38–63. *In* M. Goulian, E. C. Friedberg, and B. A. Bridges (ed.), DNA synthesis and its regulation. W. A. Benjamin, Menlo Park, Calif.
84. **Lockshon, D., and D. R. Morris.** 1983. Positively supercoiled plasmid DNA is produced by treatment of *Escherichia coli* with DNA gyrase inhibitors. Nucleic Acids Res. **11**:2999–3017.
85. **Lother, H., R. Lurz, and E. Orr.** 1984. DNA binding and antigenic specifications of DNA gyrase. Nucleic Acids Res. **12**:901–914.
86. **Lutkenhaus, J. F.** 1983. Coupling of DNA replication and cell division: *sulB* is an allele of *ftsZ*. J. Bacteriol. **154**:1339–1346.
87. **MacDonald, P. M., E. Kutter, and G. Mosig.** 1984. Regulation of a bacteriophage T4 late gene, soc, which maps in an early region. Genetics **106**:17–27.
88. **Manes, S. H., G. J. Pruss, and K. Drlica.** 1983. Inhibition of RNA synthesis by oxolinic acid is unrelated to average DNA supercoiling. J. Bacteriol. **155**:420–423.
89. **Marians, K. J., J.-E. Ikeda, S. Schlagman, and J. Hurwitz.** 1977. Role of DNA gyrase in ϕx replicative-form replication *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74**:1965–1968.
90. **Marsden, H. S., E. C. Pollard, W. Ginoza, and E. P. Randall.** 1974. Involvement of *recA* and *exr* genes in the in vivo inhibition of the *recBC* nuclease. J. Bacteriol. **118**:465–470.
91. **Marvo, S. L., S. R. King, and S. R. Jaskunas.** 1983. Role of short regions of homology in intermolecular illegitimate recombination events. Proc. Natl. Acad. Sci. U.S.A. **80**:2452–2456.
92. **McCarthy, D.** 1979. Gyrase-dependent initiation of bacteriophage T4 DNA replication: interactions of *Escherichia coli* gyrase with novobiocin, coumermycin and phage DNA-delay gene. J. Mol. Biol. **127**:265–283.
93. **McCarthy, D., C. Minner, H. Bernstein, and C. Bernstein.** 1976. DNA elongation rates and growing point distribution of wild type T4 and DNA delay amber mutant. J. Mol. Biol. **106**:963–981.
94. **McCoy, E. C., L. A. Petrullo, and H. S. Rosenkranz.** 1980. Non-mutagenic genotoxicants: novobiocin and nalidixic acid, two inhibitors of DNA gyrase. Mutat. Res. **79**:33–43.
95. **McDaniel, L. S., L. H. Rogers, and W. E. Hill.** 1978. Survival of recombination-deficient mutants of *Escherichia coli* during incubation with nalidixic acid. J. Bacteriol. **134**:1195–1198.
96. **McHugh, G. L., and M. N. Swartz.** 1977. Elimination of plasmids from several bacterial species by novobiocin. Antimicrob. Agents Chemother. **12**:423–426.
97. **McPartland, A., L. Green, and H. Echols.** 1980. Control of *recA* gene RNA in *E. coli*: regulating and signal genes. Cell **20**:731–737.
98. **Menzel, R., and M. Gellert.** 1983. Regulation of the genes for *E. coli* DNA gyrase: homeostatic control of DNA supercoiling. Cell **34**:105–113.
99. **Miller, H. I., M. Kirk, and H. Echols.** 1981. SOS induction and autoregulation of the *himA* gene for site-specific recombination in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:6754–6758.
100. **Mirkin, S. M., and Z. Schmerling.** 1982. DNA replication and transcription in a temperature-sensitive mutant of E. coli with a defective DNA gyrase subunit. Mol. Gen. Genet. **188**:89–95.
101. **Mizuuchi, K., L. M. Fisher, M. O'Dea, and M. Gellert.** 1980. DNA gyrase B action involves the introduction of transient double-strand breaks into DNA. Proc. Natl. Acad. Sci. U.S.A. **77**:1847–1851.
102. **Mizuuchi, K., M. Gellert, and H. A. Nash.** 1978. Involvement of supertwisted DNA in integrative recombination of bacteriophage lambda. J. Mol. Biol. **212**:375–392.
103. **Mizuuchi, K., and M. Mizuuchi.** 1979. Integrative recombination of bacteriophage λ: *in vitro* study of the intermolecular reaction. Cold Spring Harbor Symp. Quant. Biol. **43**:1111–1114.
104. **Mizuuchi, K., and H. A. Nash.** 1976. Restriction assay for integrative recombination of bacteriophage λ DNA in vitro: requirement for closed circular DNA substrate. Proc. Natl. Acad. Sci. U.S.A. **73**:3524–3528.
105. **Morrison, A., and N. R. Cozzarelli.** 1979. Site-specific cleavage of DNA by *E. coli* DNA gyrase. Cell **17**:175–184.
106. **Morrison, A., N. P. Higgins, and N. R. Cozzarelli.** 1980. Interaction between DNA gyrase and its cleavage site on DNA. J. Biol. Chem. **255**:2211–2219.
107. **Mufti, S., and H. Berstein.** 1974. The DNA-delay mutants of bacteriophage T4. J. Virol. **14**:860–871.
108. **Mukai, F. H., and P. Margolin.** 1963. Analysis of unlinked suppressors of an 0° mutation in Salmonella. Proc. Natl. Acad. Sci. U.S.A. **50**:140–148.
109. **Naito, A., S. Naito, and H. Ikeda.** 1984. Homology is not required for recombination mediated by DNA gyrase in *Escherichia coli*. Mol. Gen. Genet. **193**:238–243.
110. **Nash, H. A.** 1981. Integration and excision of bacteriophage λ: the mechanism of conservative site specific recombination. Annu. Rev. Genet. **15**:143–167.
111. **Ogasawara, N., M. Seiki, and H. Yoshikawa.** 1981. Initiation of DNA replication in *Bactillus subtilis*. V. Role of DNA gyrase and superhelical structure in initiation. Mol. Gen. Genet. **181**:332–337.
112. **Ohtani, T., T. Shibata, M. Iwabuchi, H. Watabe, T. Iino, and T. Ando.** 1982. ATP-dependent unwinding of double helix in closed circular DNA by *RecA* protein of *E. coli*. Nature (London) **299**:86–89.

113. **Oostra, B., A. Geert, and M. Gruber.** 1980. Involvement of DNA gyrase in the transcription of ribosomal RNA. Nucleic Acids Res. **8**:4235–4246.
114. **Oostra, B., A. VanVliet, A. B. Geert, and M. Gruber.** 1981. Enhancement of ribosomal ribonucleic acid synthesis by deoxyribonucleic acid gyrase activity in *Escherichia coli*. J. Bacteriol. **148**:782–787.
115. **Orr, E., N. F. Fairweather, I. B. Holland, and R. H. Pritchard.** 1979. Isolation and characterization of a strain carrying a conditional lethal mutation in the *cou* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **177**:103–112.
116. **Ostrowski, J., and D. Hulanicka.** 1981. Effect of DNA gyrase inhibitors on gene expression of the cysteine region. Mol. Gen. Genet. **181**:363–366.
117. **Overbye, K. M., S. K. Basu, and P. Margolin.** 1983. Loss of DNA topoisomerase I activity alters many cellular functions in *Salmonella typhimurium*. Cold Spring Harbor Symp. Quant. Biol. **47**:785–791.
118. **Overbye, K. M., and P. Margolin.** 1981. Role of the *supX* gene in ultraviolet light-induced mutagenesis in *Salmonella typhimurium*. J. Bacteriol. **146**:170–178.
119. **Pedrini, A. M., and G. Ciarrocchi.** 1982. Inhibition of *micrococcus luteus* DNA topoisomerase I by UV photoproducts. Proc. Natl. Acad. Sci. U.S.A. **80**:1787–1791.
120. **Peebles, C. L., N. P. Higgins, K. N. Kreuzer, A. Morrison, P. O. Brown, A. Sugino, and N. R. Cozzarelli.** 1979. Structure and activities of *Escherichia coli* DNA gyrase. Cold Spring Harbor Symp. Quant. Biol. **43**:41–52.
121. **Pettijohn, D. E., and O. Pfenninger.** 1980. Supercoils in prokaryotic DNA restrained *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **77**:1331–1335.
122. **Pisetsky, D., I. Berkower, P. Wickner, and J. Hurwitz.** 1972. Role of ATP in DNA synthesis in *Escherichia coli*. J. Mol. Biol. **71**:557–571.
123. **Pollock, T. J., and H. A. Nash.** 1983. Studies on the role of DNA supercoiling in lambda integrative recombination, p. 41–57. *In* K. F. Chater, C. A. Callis, D. A. Hopwood, A. W. B. Johnston, and H. W. Woolhouse (ed.), Genetic rearrangement. Sinauer Associates, Sunderland, Mass.
124. **Pruss, G. J., S. H. Manes, and K. Drlica.** 1982. *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. Cell **31**:35–42.
125. **Puga, A., and I. Tessman.** 1973. Mechanism of transcription of bacteriophage S13. I. Dependence of messenger RNA synthesis on amount and configuration of DNA. J. Mol. Biol. **75**:83–97.
126. **Pullyblank, D. E., M. Shure, D. Tang, J. Vinograd, and H.-P. Vosberg.** 1975. Action of nicking-cloning enzyme on supercoiled and nonsupercoiled closed circular DNA: formation of a Boltzmann distribution of topological isomers. Proc. Natl. Acad. Sci. U.S.A. **72**:4280–4284.
127. **Purdy, M., and K. L. Yielding.** 1976. Nalidixic acid inhibition of post-ultraviolet recovery in *Escherichia coli* K-12: requirement for *recBC* function. Antimicrob. Agents Chemother. **10**:182–184.
128. **Purdy, M., and K. C. Yielding.** 1979. Enhancement of post-ultraviolet killing in *Escherichia coli* K-12 through the action of gyrase inhibitors: evidence for associated gyrase-*recBC* deoxyribonuclease function. Antimicrob. Agents Chemother. **16**:247–251.
129. **Raina, J. L., and A. W. Ravin.** 1979. Superhelical DNA in *Streptococcus sanguis*: role in recombination *in vivo*. Mol. Gen. Genet. **176**:171–181.
130. **Ramareddy, G., and A. Reiter.** 1969. Specific loss of newly replicated deoxyribonucleic acid in nalidixic acid-treated *Bacillus subtillis* 168. J. Bacteriol. **100**:724–729.
131. **Reed, R.** 1981. Transposon-mediated site-specific recombination: a defined *in vitro* system. Cell **25**:713–719.
132. **Richardson, J. P.** 1974. Effects of supercoiling on transcription from bacteriophage PM2 deoxyribonucleic acid. Biochemistry **13**:3164–3169.
133. **Richardson, J. P.** 1975. Initiation of transcription by *Escherichia coli* RNA polymerase from supercoiled and nonsupercoiled bacteriophage PM2 DNA. J. Mol. Biol. **91**:477–487.
134. **Ryan, M. J.** 1976. Coumermycin A_1: a preferential inhibitor of replicative DNA synthesis in *Escherichia coli*. I. *In vivo* characterization. Biochemistry **15**:3769–3777.
134a. **Ryoji, M., and A. Worcel.** 1984. Chromatin assembly in *Xenopus* oocytes: *in vivo* studies. Cell **37**:21–32.
135. **Sanzey, B.** 1979. Modulation of gene expression by drugs affecting deoxyribonucleic acid gyrase. J. Bacteriol. **138**:40–47.
136. **Satta, G., L. J. Gudas, and A. B. Pardee.** 1979. Degradation of *Escherichia coli* DNA: evidence for limitation *in vivo* by protein X, the recA gene product. Mol. Gen. Genet. **168**:69–80.
137. **Shuman, H., and M. Schwarz.** 1975. The effect of nalidixic acid on the expression of some genes in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **64**:204–209.
138. **Sinden, R. R., J. Carlson, and D. E. Pettijohn.** 1980. Torsional tension in the DNA double helix measured with trimethylpsoralen in living *E. coli* cells. Cell **21**:773–783.
139. **Sinden, R. R., and D. E. Pettijohn.** 1981. Chromosomes in living *Escherichia coli* cells are segregated into domains of supercoiling. Proc. Natl. Acad. Sci. U.S.A. **78**:224–228.
140. **Sinden, R. R., and D. E. Pettijohn.** 1982. Torsional tension in intracellular bacteriophage T4 DNA. Evidence that a linear DNA duplex can be supercoiled *in vivo*. J. Mol. Biol. **162**:659–677.
141. **Smith, C. L.** 1983. recF-dependent induction of recA synthesis by coumermycin, a specific inhibitor of the B subunit of DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **80**:2510–2513.
142. **Smith, C. L., M. Kubo, and F. Imamoto.** 1978. Promoter-specific inhibition of transcription by antibiotics which act on DNA gyrase. Nature (London) **275**:420–423.
143. **Smith, C. L., and M. Oishi.** 1978. Early events and mechanisms in the induction of bacterial SDS functions. Analysis of the phage repressor inactivation process *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **75**:1657–1661.
144. **Smith, D. H., and B. D. Davis.** 1965. Inhibition of nucleic acid synthesis by novobiocin. Biochem. Biophys. Res. Commun. **18**:796–800.
145. **Snyder, M., and K. Drlica.** 1979. DNA gyrase on the bacterial chromosome: DNA cleavage induced by oxolinic acid. J. Mol. Biol. **131**:287–302.
146. **Staudenbauer, W. C., and E. Orr.** 1981. DNA gyrase: affinity chromatography on novobiocin-sepharose and catalytic properties. Nucleic Acids Res. **9**:3589–3603.
146a. **Steck, T. R., and K. Drlica.** 1984. Bacterial chromosome segregation: evidence for DNA gyrase involvement in decatenation. Cell **36**:1081–1088.
147. **Steck, T. R., G. J. Pruss, S. H. Manes, L. Burg, and K. Drlica.** 1984. DNA supercoiling in gyrase mutants. J. Bacteriol. **158**:397–403.
148. **Sternglanz, R., S. DiNardo, K. A. Voelkel, Y. Nishimura, Y. Hirota, K. Becherer, L. Zumstein, and J. C. Wang.** 1981. Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affecting transcription and transposition. Proc. Natl. Acad. Sci. U.S.A. **78**:2747–2751.
149. **Stetler, G. L., G. J. King, and W. M. Huang.** 1979. T4 DNA-delay proteins, required for specific DNA replication, form a complex that has ATP-dependent DNA topoisomerase activity. Proc. Natl. Acad. Sci. U.S.A. **76**:3737–3741.
150. **Sugino, A., N. P. Higgins, and N. R. Cozzarelli.** 1980. DNA gyrase subunit stiochiometry and the covalent attachment of subunit A to DNA during cleavage. Nucleic Acids Res. **8**:3865–3874.
151. **Sugino, A., C. Peebles, K. Kruezer, and N. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *Escherichia coli nalA* gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S.A. **74**:4767–4771.
152. **Sundin, O., and A. Varshavsky.** 1980. Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers. Cell **21**:103–114.
153. **Taylor, D. E., and J. G. Levine.** 1979. Characterization of a

plasmid mutation affecting maintenance, transfer, and elimination by novobiocin. Mol. Gen. Genet. **174**:127–133.

154. **Telander-Muskovitch, K. M., and S. Linn.** 1981. RecBC-like enzymes: exonuclease deoxyribonuclease, p. 233–250. *In* P. D. Boyer (ed.), The enzymes, vol. 14. Academic Press, Inc., New York.
155. **Tippe-Schindler, R., G. Zahn, and W. Messer.** 1979. Control of the initiation of DNA replication in *Escherichia coli*. I. Negative control of initiation. Mol. Gen. Genet. **168**:185–195.
156. **Trucksis, M., and R. DePew.** 1981. Identification and localization of a gene that specifies production of *Escherichia coli* DNA topoisomerase I. Proc. Natl. Acad. Sci. U.S.A. **78**:2164–2168.
157. **Trucksis, M., E. Golub, D. Zabel, and R. DePew.** 1981. *Escherichia coli* and *Salmonella typhimurium supX* genes specify deoxyribonucleic acid topoisomerase I. J. Bacteriol. **147**:679–681.
158. **Tse, Y.-C., K. Kirkegaard, and J. C. Wang.** 1980. Covalent bonds between protein and DNA. J. Biol. Chem. **255**:5560–5565.
159. **Vazquez-Ramos, J. M., and J. Mandelstam.** 1981. Inhibition of sporulation by DNA gyase inhibitors. J. Gen. Microbiol. **127**:11–17.
160. **Vinograd, J., J. Lebowtiz, R. Radloff, R. Waton, and P. Laipis.** 1965. The twisted circular form of polyoma viral DNA. Proc. Natl. Acad. Sci. U.S.A. **53**:1104–1111.
161. **von Wright, A., and B. Bridges.** 1981. Effect of *gyrB*-mediated changes in chromosome structure on killing of *Escherichia coli* by ultraviolet light: experiments with strain differing in deoxyribonucleic acid repair capacity. J. Bacteriol. **146**:18–23.
162. **Wahle, E., and K. Mueller.** 1980. Involvement of DNA gyrase in rRNA synthesis *in vivo*. Mol. Gen. Genet. **179**:661–667.
163. **Wang, J. C.** 1971. Interaction between DNA and an *Escherichia coli* protein. J. Mol. Biol. **55**:523–533.
164. **Wang, J. C.** 1974. Interactions between twisted DNAs and enzymes: the effects of superhelical turns. J. Mol. Biol. **87**:797–816.
165. **Wang, J. C., L. J. Peck, and K. Becherer.** 1983. DNA supercoiling and its effects on DNA structure and function. Cold Spring Harbor Symp. Quant. Biol. **47**:85–91.
166. **Weil, R., and J. Vinograd.** 1963. The cyclic helix and cyclic coil forms of polyoma viral DNA. Proc. Natl. Acad. Sci. U.S.A. **50**:730–738.
167. **Willetts, N. S., and A. J. Clark.** 1969. Characteristics of some multiply recombination-deficient stains of *Escherichia* coli. J. Bacteriol. **100**:231–239.
168. **Williams, J. G. K., T. Shibata, and C. M. Radding.** 1981. *Escherichia coli* recA protein protects single-stranded DNA or gapped duplex DNA from degradation by recBC DNase. J. Biol. Chem. **256**:7573–7582.
169. **Winshell, E. B., and H. S. Rosenkranz.** 1970. Nalidixic acid and the metabolism of *Escherichia coli*. J. Bacteriol. **104**:1168–1175.
170. **Wolfson, J. S., D. C. Hooper, M. N. Swartz, and G. L. McHugh.** 1982. Antagonism of the B subunit of DNA gyrase eliminates plasmid pBR322 and pMG110 from *Escherichia coli*. J. Bacteriol. **152**:338–344.
171. **Wolfson, J. S., D. C. Hooper, M. N. Swartz, M. D. Swartz, and G. L. McHugh.** 1982. Novobiocin-induced elimination of F′*lac* and mini-F plasmids from *Escherichia coli*. J. Bacteriol. **156**:1165–1170.
172. **Worcel, A., and E. Burgi.** 1972. On the structure of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **71**:127–147.
173. **Wu, A. M., M. Bianchi, C. DasGupta, and C. M. Radding.** 1983. Unwinding associated with synapsis of DNA molecules by *recA* protein. Proc. Natl. Acad. Sci. U.S.A. **80**:1256–1260.
174. **Yamagishi, J., Y. Furutani, T. Ohue, S. Inoue, S. Nakamura, and M. Shimizu.** 1981. New nalidixic acid resistance mutations related to deoxyribonucleic acid gyrase activity. J. Bacteriol. **148**:450–458.
175. **Yang, H.-L., K. Heller, M. Gellert, and G. Zubay.** 1979. Differential sensitivity of gene expression in vitro to inhibitors of DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **76**:3304–3308.