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Inhibitors of DNA Topoisomerases†

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DNA topoisomerases alter linking relationships within or between DNA molecules, and in so doing they introduce or remove DNA superhelical tension, tie or untie DNA knots, and catenate or decatenate circular DNA molecules. Both prokaryotic and eukaryotic cells contain two major types of topoisomerase. The type I enzymes alter DNA topology by a single-stranded DNA passage mechanism, and the best-studied bacterial and eukaryotic enzymes of this type are called topoisomerase I (bacteria also contain a type I enzyme called topoisomerase III which has received little attention). The type II enzymes DNA gyrase, bacteriophage T4 topoisomerase II, and eukaryotic topoisomerase II hydrolyze ATP and alter topology by a double-stranded DNA passage mechanism. All of these enzymes remove negative supercoils from DNA; as a purified protein only gyrase has been shown to introduce supercoils. The availability of specific inhibitors of DNA topoisomerases and mutations in the genes encoding topoisomerases have made it possible to study how these enzymes influence the activities of DNA. Below we discuss selected aspects of DNA topoisomerase activity, focusing on the physiology of their inhibition.

Inhibitors of DNA topoisomerases are listed in Table I. The best known agents block the type II enzymes. Drugs such as coumermycin A_1, novobiocin, and chlorobiocin, collectively called coumarins, inhibit gyrase (Gellert et al., 1976) and eukaryotic topoisomerase II (Hsieh & Brutlag, 1980). They probably act by competing with ATP for binding to the enzyme (Sugino et al., 1978). The coumarins also inhibit reverse gyrase (Nakasu & Kikuchi, 1985) and the vaccinia virus type I topoisomerase (Foglesong & Bauer, 1984). Other antibiotics act by trapping a topoisomerase–DNA reaction intermediate; subsequent treatment with a protein denaturant reveals DNA cleavage. The quinolones nalidixic acid, oxolinic acid, and related compounds act this way with bacterial gyrase (Gellert et al., 1977; Sugino et al., 1977), and a variety of antitumor drugs, including *m*-AMSA[1] and the epipodophyllotoxins, behave in a similar way with eukaryotic topoisomerase II (Tewey et al., 1984a; Chen et al., 1984). Both the quinolones and the antitumor drugs interact with T4 topoisomerase II (Kreuzer & Alberts, 1984; Rowe et al., 1984). Camptothecin, an inhibitor of eukaryotic topoisomerase I, also appears to act by trapping a DNA–enzyme reaction intermediate (Hsiang et al., 1985).

Table I: Inhibitors of DNA Topoisomerases

class of drug	examples	topoisomerases inhibited
coumarins	novobiocin	bacterial gyrase (B subunit)
	coumermycin A_1	eukaryotic topoisomerase II
	chlorobiocin	reverse gyrase
		vaccinia type I topoisomerase
quinolones	nalidixic acid	bacterial gyrase (A subunit)
	oxolinic acid	phage T4 topoisomerase
	norfloxacin	
acridines	*m*-AMSA	eukaryotic topoisomerase II
		phage T4 topoisomerase
anthracyclines	5-iminodaunorubicin	eukaryotic topoisomerase II
ellipticines	2-Me-9-OH-E+	eukaryotic topoisomerase II
epipodophyllo-toxins	VP-16	eukaryotic topoisomerase II
	VM-26	
alkaloids	camptothecin	eukaryotic topoisomerase I

Since the major classes of inhibitor differ in their physiological effects, they are discussed separately. Within this framework we take a comparative approach to facilitate the application of information derived from prokaryotic studies to eukaryotic ones and vice versa. Readers interested in the biochemical properties of topoisomerases are referred to reviews by Gellert (1981) and Wang (1985).

The Coumarins

Drug Targets. In vitro the coumarins inhibit the enzymatic activities of bacterial gyrase and eukaryotic topoisomerase II. Drug-resistant mutations were obtained in bacteria, and they made it possible to show that novobiocin and coumermycin block the ATPase activity of the B subunit of gyrase and that gyrase is the primary target of the coumarins in vivo (Gellert

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[1] Abbreviations: *m*-AMSA, *N*-[4-(acridinylamino)-3-methoxyphenyl]methanesulfonamide; kbp, kilobase pair(s); EDTA, ethylenediaminetetraacetic acid.

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et al., 1976). In eukaryotic systems no drug-resistant mutation is available to clearly establish topoisomerase II as the physiological target of the coumarins. Consequently, drug concentration and incubation time become important considerations when trying to distinguish between inhibition of topoisomerase II and interference with a variety of other enzymatic activities and protein-mediated processes such as DNA polymerase α (Edenberg, 1980), aminoacyl-tRNA synthetases (Wright et al., 1981), mitochondrial oxidative phosphorylation (Gallagher et al., 1986), and histone–DNA interactions (Sealy et al., 1986).

Effects on DNA Supercoiling. The most striking feature of the coumarins is that they cause the relaxation of negative DNA supercoils. In bacterial systems purified gyrase, which normally introduces supercoils, removes them in the presence of the coumarins (Gellert et al., 1976). In vivo the coumarins block the introduction of supercoils into relaxed bacteriophage λ DNA during superinfection of a lysogen (Gellert et al., 1976) and cause the bacterial chromosome to relax (Drlica & Snyder, 1978). The two most likely sources of the relaxation are gyrase and topoisomerase III. Topoisomerase I probably contributes little since coumermycin-induced relaxation of chromosomal DNA occurs equally rapidly in *top*A$^-$ and *top*A$^+$ cells (Pruss et al., 1986).

Although coumermycin at high concentration (>20 μg/mL) causes DNA relaxation bacteria, at low concentration (1–2 μg/mL) it causes an *increase* in negative supercoiling (Manes et al., 1983). The increase correlates with increased gyrase expression (Franco and Drlica, unpublished observations). Apparently the induction of gyrase expression by the coumarins (Menzel & Gellert, 1983) produces more than enough enzyme to titrate the drug when present at low concentration, and the excess gyrase leads to the increase in supercoiling.

The coumarins can even generate high levels of positive supercoiling in the plasmid pBR322 (Lockshon & Morris, 1983). This observation may be related to the ability of *top*A mutations to create very high levels of negative supercoiling in pBR322 (Pruss, 1985). In the latter case the high level of supercoiling is eliminated by deletion of an actively transcribed gene from the plasmid (Pruss & Drlica, 1986) or by treatment of plasmid-containing cells with rifampicin, an inhibitor of RNA synthesis (Drlica, unpublished observations). These phenomena have been explained in the following way (Liu & Wang, 1987). On the plasmid two genes transcribing in opposite directions would effectively divide the DNA molecule into two domains, providing that the transcription–translation apparatus does not freely rotate around the DNA. As transcription proceeds, one of the domains will tend to become positively supercoiled and the other negatively supercoiled. Normally the positive supercoils would be removed by gyrase and the negative ones by topoisomerase I. Thus treatment of wild-type cells with coumermycin removes the ability of gyrase to eliminate positive supercoils, and the absence of topoisomerase I in *top*A mutants allows the high levels of negative supercoils to be generated. Similar effects probably occur in the chromosome, but they appear to be masked by the large amount of DNA. For example, in one Δ*top*A *gyr*B mutant average chromosomal supercoiling is below normal levels (Pruss et al., 1982) while pBR322 supercoiling is higher than normal (Pruss, 1985).

The comparisons of supercoiling described above involve dye titration of extracted plasmids or nucleoids. In bacteria about half of these supercoils appear to be constrained by proteins while the other half probably exist as torsional stress (Sinden et al., 1980; Pettijohn & Pfenninger, 1980; Bliska & Cozzarelli, 1987). Manipulations that alter torsional stress [coumermycin (Sinden et al., 1980) and *top*A mutations (Bliska & Cozzarelli, 1987)] cause comparable changes in supercoiling of extracted DNA (Drlica & Snyder, 1978; Pruss et al., 1982). Thus, it is likely that the supercoiling changes observed in extracted DNA reflect changes in intracellular stress.

The situation may be different in eukaryotic cells. Here the bulk of the DNA does not appear to be torsionally strained (Sinden et al., 1980), yet the coumarins cause relaxation when DNA is analyzed by dye titration methods (Mattern & Painter, 1979; Ryoji & Worcel, 1984). Such relaxation may be related to chromatin assembly. Wrapping DNA into nucleosomes by histones is equivalent to introducing constrained negative supercoils. Compensating positive supercoils would be generated elsewhere in the DNA molecule, and these might be relaxed by topoisomerase II as a part of chromatin assembly. Subsequent removal of the histones would then produce a DNA molecule having unrestrained negative supercoils (Germond et al., 1975). According to this idea inhibition of topoisomerase II by novobiocin would prevent assembly. In the absence of chromatin assembly, relaxed DNA might accumulate as products of DNA replication and of topoisomerase I activity on torsionally strained regions of chromatin. Such a scheme does not require that eukaryotic cells contain a gyrase, an activity that has been elusive.

Support for the scheme described above comes from studies of active chromatin assembly (Ryoji & Worcel, 1984; Glikin et al., 1984; Kmiec & Worcel, 1985; Kmiec et al., 1986). Novobiocin, at the same concentration required to inhibit the activity of topoisomerase II, blocks assembly and relaxes DNA. A source of the relaxation may be topoisomerase I, since addition of this enzyme to assembled chromatin leads to DNA relaxation (Kmiec et al., 1986). At higher concentrations and much longer incubation times novobiocin also interferes with a passive chromatin assembly system that lacks topoisomerase II (Sealy et al., 1986). This raises the possibility that under certain conditions novobiocin might also affect assembly by interferring with histone–DNA interactions.

Inhibition of DNA Replication. A second consequence of coumarin treatment of cells is inhibition of DNA synthesis (Smith & Davis, 1967). In bacteria, inhibition of DNA synthesis parallels DNA relaxation (Drlica & Snyder, 1978), and it now appears that initiation of replication (Ogasawara et al., 1981; van der Ende et al., 1985), elongation (Itoh & Tomizawa, 1979; Filutowicz & Jonczyk, 1983), and decatenation (Steck & Drlica, 1984; Bliska & Cozzarelli, 1987) all require negative supercoiling and/or gyrase activity.

DNA replication in eukaryotic cells also requires topoisomerase activity. In yeast, mutations are available in the genes encoding topoisomerase I and topoisomerase II. DNA synthesis appears to proceed normally in single mutants, but it is blocked in double mutants (Brill et al., 1987). Thus either topoisomerase I or topoisomerase II can satisfy the topological requirements of DNA replication, and it is not obvious why the coumarins inhibit DNA synthesis in higher eukaryotes (Edenberg, 1980; Mattern & Scudiero, 1981; Lavin, 1981). Indeed, coumermycin inhibits SV40 viral and human lymphoblastoid cellular DNA synthesis without causing measurable DNA relaxation (Edenberg, 1980; Lavin, 1981). These observations, plus the finding that the coumarins inhibit DNA polymerase α (Edenberg, 1980), limit the utility of the coumarins for the study of topoisomerase II involvement in DNA replication.

Effects on Gene Expression. The relaxing activities of the coumarins provide a way to relate changes in supercoiling with

intracellular expression of particular genes. In general, these results correlate well with in vitro studies in which supercoiling and gene expression have been examined [for a list, see Drlica (1984)]. Decreasing supercoiling below normal levels with coumermycin decreases expression from some promoters, increases it from others, and causes no change from still others. The drug also reverses the effects of abnormally high levels of supercoiling. This is seen in a strain of *Salmonella typhimurium* in which a *top*A mutation suppresses the *leu*500 promoter mutation (Mukai & Margolin, 1963), probably by raising DNA supercoiling above normal levels. Low concentrations of coumermycin lower supercoiling to normal levels and reverse the suppression of *leu*500 (Pruss & Drlica, 1985).

Recently, genes whose expression is increased by the relaxing activity of coumermycin have been attracting attention. They appear to be common, since about 70% of random gene fusions exhibit a drug-induced increase in expression (Jovanovich & Lebowitz, 1987). With some promoters there is clearly an optimal level of supercoiling for expression in vitro (Borowiec & Gralla, 1985, 1987; Brahms et al., 1985), and it may be that in vivo many promoters are optimally expressed at levels of supercoiling that are below normal. DNA relaxation may also disrupt "silencing elements" of the type found associated with a chloroplast promoter. In this system novobiocin increases expression of the promoter in *Chlamydomonas* and in *Escherichia coli*, and upstream deletions remove both the silencing element(s) and the stimulatory effect of novobiocin on expression (Thompson & Mosig, 1987). Since the supercoiling-sensitive upstream region contains sequences that behave as "bent" DNA, as well as sequences similar to binding sites for integration host factor, the "silencer" may be a protein–DNA complex in which the DNA is wrapped into a supercoiling-dependent, nucleosome-like structure [for review of bacterial DNA wrapping, see Drlica and Rouviere-Yaniv (1987)].

The coumarins also affect gene expression in eukaryotic systems where most of the experimental emphasis has been placed on understanding chromatin structure. The most extensively studied system is transcriptional activation of the 5S ribosomal RNA gene. Activation in vitro parallels the introduction of supercoils during chromatin assembly; novobiocin, at the same concentration that inhibits purified topoisomerase II, quickly relaxes the DNA and blocks transcription (Ryoji & Worcel, 1984; Kmiec et al., 1986). But superhelical tension per se is not required for activation of the 5S rRNA gene since relaxation induced by topoisomerase I has no effect on transcription (Kmiec et al., 1986). Understanding the inhibitory activity of novobiocin on the 5S rRNA system still requires additional knowledge about protein transcription factors and their interactions with the drug. For example, it is not known why factor TFIIIA is required for novobiocin-induced DNA relaxation following assembly of nucleosomes (Kmiec & Worcel, 1985) or whether the blockage of some transcription factor interactions by novobiocin plays a role in DNA relaxation (Gottesfeld, 1986; Felts et al., 1987).

Two other examples have been described in which novobiocin prevents or reverses changes in chromatin structure associated with gene expression. One occurs during induction of the major heat shock genes in *Drosophila* tissue culture cells. Nuclei isolated from *Drosophila* cells exhibit a characteristic pattern of nuclease-sensitive sites in the heat shock genes; heat shock changes this pattern, and novobiocin blocks the alteration and heat shock gene expression (Han et al., 1985). In the other case the DNase I sensitivity characteristic of activatable β-globin genes is lost following novobiocin treatment (Villeponteau et al., 1984). In neither of these cases have the effects of novobiocin been correlated with changes in DNA topology or directly attributed to inhibition of topoisomerase II.

DNA Lesion-Forming Drugs Affecting Type II Topoisomerases

Lesions in Bacterial DNA. Soon after the discovery of bacterial gyrase both Gellert and Cozzarelli (Gellert et al., 1977; Sugino et al., 1977) found that the enzyme is inhibited by nalidixic acid and related antibiotics. Addition of protein denaturants such as sodium dodecyl sulfate to reaction mixtures containing gyrase, DNA, and nalidixic acid leads to DNA cleavage with the *gyr*A gene product remaining covalently bound to the 5′ end of each DNA strand [for reviews, see Peebles et al. (1979) and Wang (1985)]. DNA cleavage does not occur in a known *gyr*A Nal^r mutant, establishing gyrase as the target of the drug in vivo (Snyder & Drlica, 1979). Since protein denaturation is required to reveal the discontinuity in DNA, it is unlikely that the drugs generate free DNA ends inside bacterial cells. This prediction is borne out experimentally: intact nucleoids containing negatively supercoiled DNA can be isolated from oxolinic acid treated bacteria if sodium dodecyl sulfate is omitted from cell lysis procedures (Snyder & Drlica, 1979; Manes et al., 1983). Subsequent treatment of the isolated nucleoids with sodium dodecyl sulfate then leads to DNA fragmentation.

In vitro the quinolones bind preferentially to single-stranded DNA (Shen & Pernet, 1985), and it has been suggested (Shen, personal communication) that the drugs bind to a single-stranded pocket of DNA formed during the strand passage reaction catalyzed by gyrase. Drug binding presumably traps a gyrase–DNA reaction intermediate. Consequently, drug-induced DNA cleavage should mark where at least some of the gyrase molecules act on DNA, and this idea is supported by the following observations: (1) the sites on DNA where gyrase induces cleavage are identical whether or not oxolinic acid is present (Fisher et al., 1981, 1986), (2) DNA cleavage induced by gyrase produces DNA fragments whose length is unaffected by the presence of oxolinic acid [K. Kreuzer as cited in Lockshon and Morris (1985)], and (3) norfloxacin, one of the most potent quinolones, has no effect on the DNase I protection pattern generated by binding of gyrase to DNA (Rau et al., 1987). Oxolinic acid does not by itself cleave DNA (Sugino et al., 1977), nor does it appear that cleavage arises from secondary effects such as the activation of nucleases because the saturation of sites by oxolinic acid occurs rapidly and protein denaturation is required to reveal cleavage (Snyder & Drlica, 1979).

The sites of DNA cleavage have been studied with plasmids treated with gyrase and oxolinic acid in vitro (Morrison et al., 1980; Morrison & Cozzarelli, 1979; Fisher et al., 1981; Kirkegaard & Wang, 1981) and with plasmids extracted from drug-treated cells (O'Connor & Malamy, 1985; Lockshon & Morris, 1985). pBR322 contains more than 70 such sites scattered around the plasmid (O'Connor & Malamy, 1985). These sites are unevenly spaced but show no obvious pattern relative to the genes in the plasmid (O'Connor & Malamy, 1985; Lockshon & Morris, 1985). Comparison of the nucleotide sequences at the cleavage sites reveals that cleavage is not highly dependent on nucleotide sequence in the simple way found for type II restriction endonucleases; however, a loose consensus sequence has been derived (Morrison & Cozzarelli, 1979; Fisher et al., 1981; Kirkegaard & Wang, 1981; Lockshon & Morris, 1985) . The major cleavage site in pBR322, which matches the consensus almost exactly, is identical in vitro and in vivo, and some point mutations in the

cleavage site that change the nucleotide sequence from the consensus reduce cleavage frequency. Mutations that maintain the consensus do not alter cleavage. Thus nucleotide sequence appears to dictate where cleavage occurs.

Not all of the cleavage sites identified on a given DNA molecule are cleaved when oxolinic acid is added to cells. On individual chromosomes the distribution of cleaved sites can be estimated from size measurements of DNA fragments generated by the drug. From sedimentation studies we estimated that those fragments are about 100 kbp, indicating that the drug cleaves chromosomal DNA into about 50 fragments (Snyder & Drlica, 1979). On average, this corresponds to about one cleavage event per topological domain (Worcel & Burgi, 1972; Sinden & Pettijohn, 1981). Recently it has been found that cleavage is especially frequent at a small number of specific sites on the chromosome (G. Condemine and C. Smith, personal communication). A large number of weaker sites also exist. We examined a small, 10-kbp region of the chromosome by indirect end labeling and found 24 sites (Franco & Drlica, 1988). Extrapolation of this cleavage site density to the entire chromosome indicates that there might be in excess of 10 000 sites where cleavable complexes form. Thus fewer than 0.5% of the sites appear to be cleaved at a given time. One speculation, assuming that the cleavage sites represent a major fraction of places where gyrase interacts with DNA, is that a small number of strong interaction sites are used by gyrase to maintain superhelical tension in the chromosome as a whole and that the weak, widely dispersed sites allow gyrase to provide local swiveling needed for transcription and replication (replication is discussed in more detail below).

Lesions in Eukaryotic DNA. Eukaryotic topoisomerase II is relatively insensitive to nalidixic acid and its older derivatives (Hussy et al., 1986); however, a variety of antitumor drugs stimulate formation of cleavage complexes with topoisomerase II that are analogous to those formed with bacterial gyrase. Four categories of antitumor drug, the acridines (*m*-AMSA), the anthracyclines (5-iminodaunorubicin), the ellipticines (2-Me-9-OH-E+), and the epipodophyllotoxins (VP-16 and VM-26), stimulate formation of cleavable complexes with topoisomerase II in vitro and induce protein-linked DNA breaks in vitro and in vivo (Tewey et al., 1984a; Ross et al., 1984). As in bacterial systems, protein denaturation is required to reveal the DNA cleavage, a reaction that can be reversed by treatment with NaCl or EDTA prior to protein denaturation (Tewey et al., 1984a; Udvardy et al., 1986). Although well-characterized drug-resistant mutants are unavailable to define the drug's target genetically, five types of evidence support the idea that drug-induced cleavages mark the sites of topoisomerase II–DNA interactions: (1) cleavage occurs at many of the same sites in the presence or absence of the drugs (Udvardy et al., 1985, 1986), (2) cleavage sites are similar in intracellular DNA and in DNA treated in vitro with purified enzyme and drug (Yang et al., 1985b), (3) novobiocin blocks cleavage in vivo and in vitro (Marshall et al., 1983; Yang et al., 1985a), (4) the protein-linked DNA is specifically precipitated by antisera against topoisomerase II (Yang et al., 1985b), and (5) the cleavage activity copurifies with topoisomerase II (Yang et al., 1985a; Rowe et al., 1986a). However, still unexplained is why different anticancer agents produce different cleavage patterns (Tewey et al., 1984a,b).

As pointed out in the section on coumarins, eukaryotic topoisomerase II is thought to be involved in gene expression. Thus there has been interest in mapping topoisomerase II mediated DNA cleavage sites near genes before and after activation. SV40 virus has provided one system for this type of study (Yang et al., 1985b). As infection proceeds, the relative cleavage frequency at different sites changes, and late in infection a single, strong cleavage site is observed in the enhancer region around nucleotide 270. DNase I hypersensitivity is also found in this region at the same time, and accessibility to both enzymes appears to correlate temporally with late gene expression. The site around nucleotide 270 is cleaved weakly in vitro by topoisomerase II, suggesting that chromatin structure is related to the location of topoisomerase II–DNA interaction sites. A second system examined in this way is a *Drosophila* heat shock gene (Rowe et al., 1986b; Udvardy et al., 1986). Heat shock induces the gene and also produces changes in the pattern of DNA cleavage induced by VM26 in and around the gene. Thus correlations between gene expression and changes in DNA cleavage patterns support the idea that topoisomerase II participates in gene expression.

Effects on DNA Supercoiling. Lesion-forming drugs such as oxolinic acid are not potent relaxing agents. Even at saturating concentrations with respect to DNA cleavage, oxolinic acid fails to relax bacterial DNA (Snyder & Drlica, 1979). This is a surprising result since these wild-type cells contain topoisomerase I, an important source relaxing activity (Pruss et al., 1982; DiNardo et al., 1982; Richardson et al., 1984). At very high concentrations oxolinic acid will cause partial relaxation of the bacterial chromosome (Manes et al., 1983), even in *top*A mutants having little topoisomerase I activity (Pruss et al., 1986). Thus in the presence of oxolinic acid topoisomerase I is not a major relaxing activity. The possibility exists that in vivo topoisomerase I is somewhat sensitive to the quinolones; slight sensitivity has been reported for purified topoisomerase I (Sugino et al., 1977; Gellert et al., 1977; Burrington & Morgan, 1978).

In certain genetic backgrounds oxolinic acid can cause chromosomal supercoiling to *increase*. This occurs when a Δ*top*A *gyr*A(Nal^R)*gyr*B225 mutant, partially resistant to oxolinic acid, is treated with the drug (Manes et al., 1983; Pruss et al., 1986). Oxolinic acid stimulates gyrase expression by about 2-fold (Franco and Drlica, unpublished observations), and with the Δ*top*A mutation this appears to be sufficient to overcome the small inhibitory effect of the drug on gyrase.

Lesion-Forming Drugs as Poisons. The lesion-forming drugs appear to exert their physiological effects through complex formation with topoisomerases and DNA rather than through simple elimination of topoisomerase activity. In this sense they can be considered poisons. Two observations with bacteriophages support this idea. First, growth of some bacteriophages is very sensitive to nalidixic acid but not to the elimination of gyrase activity through temperature-sensitive mutations (Kreuzer & Cozzarelli, 1979). Second, *m*-AMSA reduces yields of wild-type T4 bacteriophage by 10-fold whereas its target, T4 topoisomerase II, is a nonessential enzyme. Indeed, the drug has no effect if an amber mutation in phage gene 52 eliminates topoisomerase II [K. Kreuzer as cited in Rowe et al. (1984)]. These considerations, plus the weak relaxing ability of the quinolones (see above), make the lesion-forming drugs poor agents for examining relationships between the loss of topoisomerase activity and chromosomal activities such as gene expression.

Inhibition of DNA Synthesis. Trapping topoisomerases on DNA has several consequences, the most striking of which is rapid inhibition of DNA replication (Goss et al., 1964). In bacteria, oxolinic acid–gyrase–DNA complexes form quickly, and within a few minutes DNA synthesis rates drop below 5% of that observed in untreated controls. Inhibition is rapid even at low drug concentrations where inhibition is only partial and

few cleavable complexes form (Snyder & Drlica, 1979). Under these conditions no DNA relaxation was observed (Snyder & Drlica, 1979), and it was not obvious how rapid inhibition could arise from a few drug–gyrase interactions that on average would be far from replication forks. Subsequent studies showed that newly replicated DNA is cleaved into smaller fragments by oxolinic acid than is the bulk of the chromosomal DNA (Drlica et al., 1980), suggesting that gyrase is clustered around replication forks as well as being distributed at 100-kbp intervals on the chromosome. Formation of a drug complex with fork-associated gyrase would lead to rapid inhibition while a slower onset of residual inhibition, which is also observed, would arise from forks proceeding until they encounter one of the widely spaced complexes (Drlica et al., 1980; Engle et al., 1982).

Although neither eukaryotic topoisomerase I nor topoisomerase II is required for DNA synthesis, they appear to be involved since each can substitute for the other in yeast mutants (Brill et al., 1987). If so, the poisoning effect of lesion-forming drugs should block replication; this is the case with an inhibitor of topoisomerase I (see below). Treatment of cells with *m*-AMSA generates topoisomerase II–DNA complexes concentrated on newly replicated DNA (Nelson et al., 1986), analogous to the situation reported earlier with oxolinic acid and bacterial DNA (Drlica et al., 1980).

Camptothecin, an Inhibitor of Topoisomerase I

Camptothecin appears to trap eukaryotic topoisomerase I on DNA in a way similar to that observed with the lesion-forming drugs that interact with the type II topoisomerases. The drug blocks the catalytic activity of calf thymus topoisomerase I, and detergent treatment of drug–enzyme–DNA mixtures reveals protein bound covalently to the 3′ end of the DNA (Hsiang et al., 1985). Camptothecin blocks replication of SV40 DNA, and analysis of the resulting replication intermediates suggests that topoisomerase I acts near replication forks (Snapka, 1986).

Topoisomerase I is also associated with some active genes. High-affinity binding sites for topoisomerase I are located in nontranscribed spacers near ribosomal RNA genes (Bonven et al., 1985), and immunological and photo-cross-linking methods show that the enzyme is preferentially associated with transcriptionally active regions of *Drosophila* polytene chromosomes (Fleischmann et al., 1984; Gilmour et al., 1986). Following induction of a *Drosophila* heat shock gene, camptothecin causes preferential DNA cleavage in the transcribed region of the gene, particularly at its 3′ end (Gilmour & Elgin, 1987; Rowe, Couto, and Kroll, unpublished observations). In vitro the drug inhibits rRNA synthesis when the DNA template is topologically closed (Garg et al., 1987), consistent with the idea that the enzyme plays a role in relieving torsional strain generated by transcription.

Unresolved Issues

Our understanding of many aspects of topoisomerase biology is still incomplete, and important questions such as whether eukaryotic cells contain a gyrase and precisely how topoisomerases influence gene expression remain unanswered. Below we elaborate on three points.

One concerns the regulation of gyrase expression in bacteria. Both the coumarins and the quinolones cause a decrease in chromosomal supercoiling (Drlica & Snyder, 1978; Manes et al., 1983) and an increase in gyrase expression (Menzel & Gellert, 1983, 1987). These observations led to the idea that supercoiling and topoisomerase expression are homeostatically regulated (Menzel & Gellert, 1983). The homeostatic hypothesis is supported by the observations that topoisomerase I expression is lowered by a treatment of cells that relaxes DNA (Tse-Dinh, 1985) and raised by a treatment that increases supercoiling (Y.-T. Tse-Dinh, personal communication). But the model does not explain why gyrase expression increases when cells are treated in ways that increase supercoiling (treatment of wild-type cells with low concentrations of coumermycin or treatment of partially resistant mutants with oxolinic acid) (Manes et al., 1983; Pruss et al., 1986; Franco and Drlica, unpublished observations).

Another involves interactions of topoisomerases with DNA. Studies with the lesion-forming drugs show that there are many sites where topoisomerases interact with DNA, and correlations have been found between changes in cleavage patterns and levels of gene expression. But we do not know what increased cleavage frequency at a particular site means in terms of DNA structure. For example, would a plasmid having a large number of very strong sites have an abnormally high level of supercoiling? Nor has it been established that cleavage sites mark all of the positions where gyrase acts on DNA; thus we must remain cautious about equating cleavage sites with topoisomerase–DNA interaction sites.

The third issue focuses on the mechanism of cell killing by the lesion-forming drugs. The formation of protein–drug complexes on DNA appears to rapidly block DNA replication, but complex formation itself is not cytocidal. Drug action is generally rapidly reversible (Khachatourians, 1981), and at present there is no evidence that free DNA ends are generated. Protein synthesis must occur for cells to be killed (Winshell & Rosenkranz, 1970; Crumplin & Smith, 1975); thus it appears that killing is an active process. In bacteria, one of the active processes induced by the quinolones, and to a lesser extent by the coumarins (Smith, 1983), is expression from a series of genes that collectively comprise the SOS response [for review, see Little and Mount (1982)]. The products of many of these genes are involved in DNA repair, and mutations in some of the key genes, such as *recA*, make cells more sensitive to the quinolones. But could it be that other members of the SOS pathway cause misrepair of the lesions and thus kill the cell?

Conclusions

The anti-topoisomerase drugs, combined with mutations in the genes encoding the topoisomerases, reveal at a gross level how topoisomerases influence chromosome structure and function. In bacteria, gyrase introduces superhelical tension (Gellert et al., 1976) and decatenates replicated daughter chromosomes (Steck & Drlica, 1984; Bliska & Cozzarelli, 1987); topoisomerase I modulates the effect of gyrase by providing a relaxing function (Pruss et al., 1982; DiNardo et al., 1982). Supercoiling itself appears to influence expression of the genes encoding gyrase and topoisomerase I in a way that would result in the homeostatic regulation of supercoiling (Menzel & Gellert, 1983; Tse-Dinh, 1985). Even the small changes in superhelical tension expected to arise from changes in temperature or intercalating dyes appear to be corrected by corresponding changes in linking difference (Goldstein & Drlica, 1984; Esposito & Sinden, 1987).

In eukaryotic cells temperature-sensitive topoisomerase I and II mutants of yeast behave as if both enzymes relax DNA in vivo (Saavedra & Huberman, 1986), and the requirement of continuous topoisomerase activity for DNA replication can be met by either topoisomerase (Brill et al., 1987; Yang et al., 1987). That both enzymes relax DNA could explain why eukaryotic chromosomes contain little unconstrained superhelical tension (Sinden et al., 1980). In eukaryotic DNA the

supercoils appear to be due to the assembly of chromatin, a process that probably involves topoisomerase II. How chromatin structure and topoisomerase activities affect gene expression has not yet been clearly defined. Topoisomerase II in particular may act as a swivel to relieve strain introduced by transcription (Kmiec et al., 1986), an activity that may also be carried out by topoisomerase I in the case of rRNA genes (Bonven et al., 1985; Brill et al., 1987). Decatenation of daughter chromosomes appears to be carried out by topoisomerase II (DiNardo et al., 1984; Snapka, 1986; Yang et al., 1987), and this protein appears to be so abundant on metaphase chromosomes that it may even act as a structural component (Earnshaw et al., 1985; Gasser et al., 1986).

The lesion-forming drugs, although poor choices for studying the physiology of topoisomerase inactivation because the complexes they form act as poisons, are emerging as powerful probes for locating sites of topoisomerase–DNA interactions. Strong interaction sites have been identified in both prokaryotic and eukaryotic systems, and by placing these near promoters in plasmids or in viruses it may be possible to examine how topoisomerases influence transcription. Locating cleavage complexes may also help us better understand how chromosomal activities such as transcription affect topoisomerase–DNA interactions and supercoiling.

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