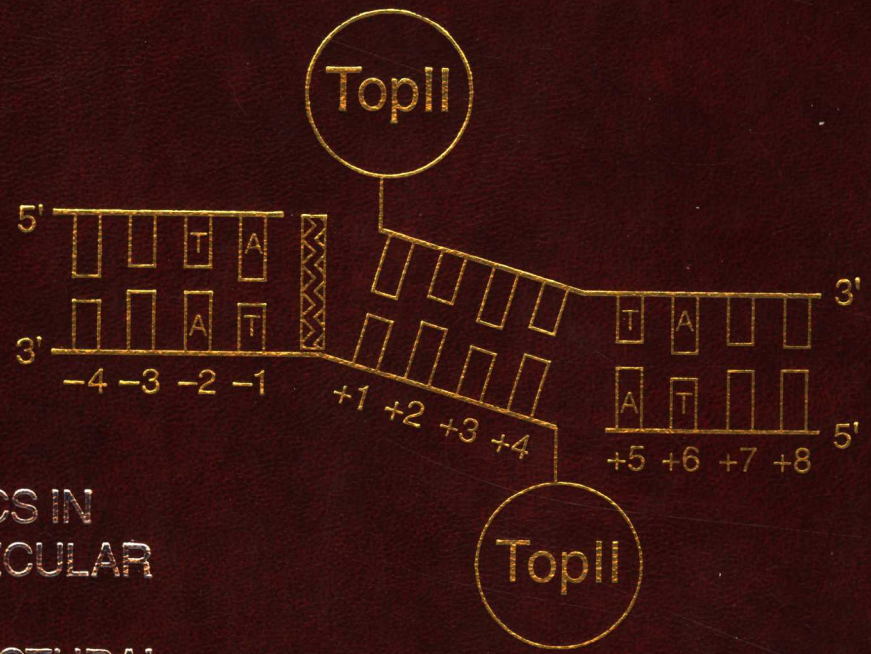
Molecular Aspects of Anticancer Drug–DNA Interactions

VOLUME 2

EDITED BY
Stephen Neidle
Michael Waring



TOPICS IN MOLECULAR AND STRUCTURAL BIOLOGY

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MOLECULAR ASPECTS OF ANTICANCER DRUG–DNA INTERACTIONS
Volume 2

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Preface

DNA has long been a key target for cancer chemotherapy. Indeed, the first agents to be employed clinically in the treatment of human cancer (the nitrogen mustards) are DNA cross-linking agents. Spectacular advances have occurred during recent years in the treatment of childhood leukaemia and testicular cancer, largely as a result of the development of better DNA-interactive agents. Even though the majority of solid tumours remain resistant to chemotherapy, there is real promise that a new, third-generation of platinum compounds will prove successful in the treatment of ovarian cancer. Clinical advances in such key areas are the ultimate objective of much current research in cancer chemotherapy and biology. Future progress must surely result from wise application of the large body of fundamental knowledge being accumulated from studies in a whole range of disciplines. No one doubts that clinical success will increasingly depend upon the exploitation of such knowledge and on the interplay between it and more applied disciplines. This is especially important as the molecular and cellular bases of malignant cell growth become better understood. So the study of drug–DNA interactions has moved on from the position of a dozen years ago, when our understanding of the molecular basis of drug action was relatively poor, as were the prospects for rational design of new drugs, to a much more positive position with new horizons.

These two volumes survey our current knowledge about the mode of action of the major classes of DNA-interactive antitumour agents, and in so doing provide pointers for the discovery of new therapeutic substances. The reader will notice that certain related topics have been grouped together; indeed in one instance (that of topoisomerase inhibitors), what were originally planned as two separate chapters by different authors have been amalgamated into one (by mutual consent!) so as to produce a more balanced and co-ordinated treatment. Elsewhere the

relationships between topics may be less obvious, but we hope that our choices will stimulate cross-fertilization of ideas.

An enterprise involving many authors such as this requires the cooperation of all the contributors if it is to succeed. We are grateful to everyone for their efforts in ensuring delivery of their manuscripts promptly and for making our task as editors such a pleasurable one. Both of us are indebted to the Cancer Research Campaign for supporting work on drug–DNA interactions in our own laboratories over a number of years. To the hard-working staff of the Campaign, as well as to those who devote their lives to the alleviation of cancer at the bedside and in the laboratory, we dedicate this pair of volumes.

Sutton and Cambridge, 1993

S. N.
M. W.

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DNA Topoisomerases

Raymond K. Ralph, Warren Judd, Yves Pommier and Kurt W. Kohn

1 Introduction

The simple elegance of the antiparallel, plectonemic DNA double helix revealed by Watson, Crick and Wilkins gave substance to the concept of DNA as a repository of genetic information and a rationale for its replication (Watson and Crick, 1953). Subsequently the complexity of chromosomes has been slowly revealed (Gasser *et al.*, 1989; Filipski *et al.*, 1990), bringing with it the realization that simple models for DNA replication must be modified to explain the replication and resolution of knotted DNA, concatemeric DNA, radial looped DNA, nucleosome-coiled DNA or other phenomena such as DNA replication from multiple origins in eukaryotic cells (Hamlin *et al.*, 1991). In addition, the progression of RNA or DNA polymerase produces topological effects on DNA templates which, if left unresolved, inhibit RNA and DNA synthesis (Brill *et al.*, 1987; Uemura *et al.*, 1987; Yamagishi and Nomura, 1988). The discovery of topoisomerases, enzymes that can resolve topological constraints in DNA, gave a clue to the mechanism(s) used by cells to overcome some of the problems resulting from DNA twisting (Vosberg, 1985; Wang, 1985). As their functions are revealed, these apparently magical enzymes can be seen as central to most of the events involving DNA, gene expression and growth of cells. Consequently, they are good potential targets for anticancer drugs.

Two main types of topoisomerases exist in eukaryotic cells and both types bind to DNA. They also recognize and bind to cross-overs in DNA, which are more abundant in supercoiled DNA (Zechiedrich and Osheroff, 1990). Type I DNA topoisomerases transiently nick and reseal one strand of double-stranded DNA. Passing the intact DNA strand through the nick in the other permits relaxation of a circularly constrained double-helical supercoiled DNA (Vosberg, 1985; Wang, 1985). Other activities of type I

topoisomerases have also been suggested involving DNA strand rearrangements (Halligan *et al.*, 1982). Type II DNA topoisomerases nick and reseal both strands of double-stranded supercoiled DNA and they relax the DNA by passing one double-stranded section of the DNA through the transient break to reduce supercoiling. Eukaryotic type II topoisomerases require ATP to function, although some organisms (e.g. trypanosomes) may have type II topoisomerases that do not need ATP (Douc Rasy *et al.*, 1986). Bacterial DNA gyrase, a type II topoisomerase, has the additional feature that it will introduce negative supercoils into DNA when provided with ATP (Vosberg, 1985). No equivalent activity has been reported in eukaryotes to date. Preliminary evidence for additional topoisomerases or topoisomerase variants exists, but these are not yet well characterized (Fink, 1989).

DNA topoisomerases are targets for a number of anticancer drugs. The molecular mechanisms of anticancer drug action upon topoisomerases and DNA were previously reviewed by Marshall and Ralph (1985), Wang (1985), Maxwell and Gellert (1986), Kohn *et al.* (1987), Ralph and Schneider (1987), Lock and Ross (1987), D'Arpa and Liu (1989), Liu (1989) and Schneider *et al.* (1990).

It is our intention to focus upon more recent studies of topoisomerases that are pertinent to the action of anticancer drugs on cells. However, this inevitably requires some appreciation of the mechanism of topoisomerase cleavage of DNA which proceeds via transient protein–DNA complexes (PDCs) in which the 3′ ends (top I) or 5′ ends (top II) of nicked DNA strands are temporarily covalently linked via phosphate ester bonds to tyrosine hydroxyl residues in the respective topoisomerases. These open protein–DNA complexes are the targets for anticancer drugs that act upon topoisomerases and inhibit the resealing of DNA, sometimes contributing to successful chemotherapy.

Precisely why or how topoisomerase inhibitors cause cancer cell death has still to be defined, although various drugs that inhibit topoisomerases can cause DNA scission, affect RNA or DNA synthesis and produce sister-chromatid exchange, chromosomal rearrangements, recombination and other chromosome aberrations which undoubtedly disrupt many normal cellular processes. Some of the recent information related to topoisomerases has come from studies with yeasts, where genetic modification is possible, or from other organisms such as *Drosophila* or *Xenopus*, where the advantages of such systems can be exploited. In general, it is believed that the information obtained from these systems reflects events in other eukaryotic cells.

2 Topoisomerase I (top I)

General Properties and Overview

The principal type I topoisomerase (top I) in human cells is a monomeric protein of M_r 90 649 Da calculated from the sequence of the cloned gene (D'Arpa *et al.*, 1988) or M_r 100 000 Da in SDS–polyacrylamide gels (Liu and Miller, 1981).

The major source of the enzyme in cells is the nucleus, where it is associated particularly with actively transcribed regions of DNA in different types of cells (Fleischmann *et al.*, 1984; Bonven *et al.*, 1985; Gilmour and Elgin, 1987; Stewart and Schütz, 1987). However, mitochondria also contain an ATP-independent type I topoisomerase with a molecular weight of 63–64 kDa estimated in polyacrylamide gels (Castora and Lazarus, 1984; Castora *et al.*, 1985; Lazarus *et al.*, 1987). The mitochondrial enzyme from platelets is inhibited by camptothecin (Kosovsky and Soslau, 1991). Recently a M_r 165 kDa tissue-specific top I variant in *Xenopus* oocytes which disappeared during maturation was described, which, it was conjectured, plays a role in ribosomal DNA excision and amplification (Richard and Bogenhagen, 1991).

The gene coding for top I is a single-copy gene located on human chromosome 20q12-13.2 (Juan *et al.*, 1988; Kunze *et al.*, 1989). The gene is composed of 21 exons spread over 85 kilobase pairs (kb) of DNA, and with SP1, cAMP and octamer motifs in its promoter region (Kunze *et al.*, 1991). In addition, there are at least two truncated, processed and hypomethylated pseudogenes on chromosomes 1 and 22, respectively, in different human cell lines (Kunze *et al.*, 1989; Zhou *et al.*, 1989; Yang *et al.*, 1990; Hsieh, 1990).

Top I–DNA complexes are targets for the alkaloid camptothecin and certain analogues, which bind reversibly to top I–DNA complexes but not to isolated DNA or top I alone (Hsiang *et al.*, 1985; Hsiang and Liu, 1988; Hsiang *et al.*, 1989a,b; Bjornsti *et al.*, 1989). The bound drug reversibly traps and stabilizes the top I–DNA intermediate involved in DNA unwinding, ultimately causing DNA damage and cell death (Hertzberg *et al.*, 1989a). There is good evidence that camptothecin prevents the resealing of transient top I-induced single-strand breaks in DNA, since an increase in open or 'cleavable' complexes can be demonstrated with protein denaturants in cells or nuclei treated with the drug (Hsiang *et al.*, 1985; Hsiang and Liu, 1988; Covey *et al.*, 1989). Studies with yeast topoisomerase mutants have shown that the effects of camptothecin on cells are due to its action on top I (Nitiss and Wang, 1988; Eng *et al.*, 1988; Bjornsti *et al.*, 1989). Moreover, top I purified from camptothecin-resistant leukaemia and other cells is resistant to camptothecin (Andoh *et al.*, 1987; Kjeldsen *et al.*, 1988a; Gupta *et al.*, 1988).

Camptothecin and related drugs that inhibit the action of top I can have strong antitumour activity against a variety of experimental tumours (Gallo *et al.*, 1971; Neil and Homan, 1973; Tsuruo *et al.*, 1988; Giovanella *et al.*, 1989). However, problems with non-specific cytotoxicity seem to have precluded their general use as anticancer agents, although extracts of *Camptotheca accuminata* have been used to treat some solid tumours and leukaemias in China (Gottlieb and Luce, 1972; Moertel *et al.*, 1972; Muggia *et al.*, 1972). New derivatives of camptothecin that might be exploited as anticancer drugs have recently led to renewed interest in top I inhibitors, while a novel new drug, saintopin, inhibits both types I and II topoisomerases, causing DNA cleavage (Hsiang *et al.*, 1989b; Yamashita *et al.*, 1991). Acidic phospholipids have also been shown to inhibit top I by blocking binding of the enzyme to DNA (Tamura *et al.*, 1990). Whether this observation can be exploited to produce a new class of drugs active against the enzyme remains to be established, as does the significance of the observation *in vivo*.

Reaction Mechanisms

The enzymatic function of top I is to change the number of twists of one DNA strand about the other. This is accomplished in four steps: (1) binding of top I to DNA; (2) cleavage of one DNA strand and covalent linkage of top I to the 3′ terminus of the strand break; (3) rotation of the free strand segment around the intact strand; and (4) resealing of the strand break.

The binding of top I is only mildly constrained by DNA sequence and therefore can occur in most regions of the genome, although regions of bent or supercoiled DNA are preferred (Muller *et al.*, 1985; Camilloni *et al.*, 1989; Caserta *et al.*, 1989, 1990; Krogh *et al.*, 1991). Binding appears to occur over a span of at least 20 base pairs of duplex DNA and to be tighter for the DNA segment upstream from the cleavage site than for the downstream segment, as indicated by DNA footprinting (Stevnsner *et al.*, 1989), base-sequence specificity (Been *et al.*, 1984; Jaxel *et al.*, 1991a,b; Porter and Champoux, 1989b) and oligonucleotide cleavage experiments (Jaxel *et al.*, 1991a; Svejstrup *et al.*, 1990). Top I does not bind to single-stranded DNA (unless the strand can form a duplex region due to diadic symmetry of sequence) (Been and Champoux, 1984; Jaxel *et al.*, 1991a). Top I can bind to duplex DNA at the site of a single-strand break (McCoubrey and Champoux, 1986) and can cleave the intact strand to produce a double-strand cut which may promote DNA recombination.

Strand scission occurs through a transesterification in which a tyrosyl hydroxy group (Tyr 723 in human top I) links to the 3′ oxygen of a phosphodiester bond, displacing the 5′ phosphate to generate a DNA

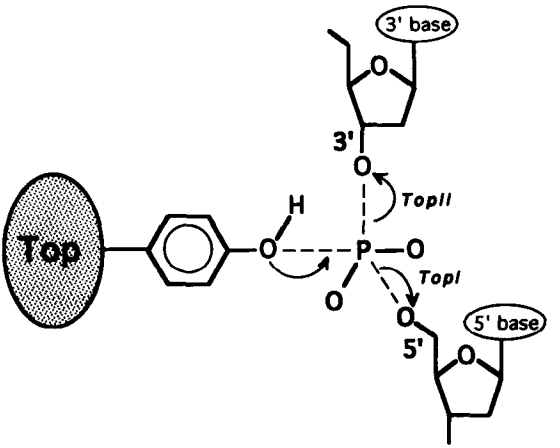

Figure 1.1 Reaction between topoisomerase tyrosyl and DNA phosphodiester bond. The tyrosyl OH group attacks the phosphodiester bond; a curved arrow represents an unshared electron pair moving from the tyrosyl O atom towards the P atom during covalent bond formation. As this new O–P bond forms, a P–O bond of one or other phosphodiester bond must cleave so as to leave the P atom linked to 4 oxygens. In the case of top I, the P–O bond to the 5′ DNA strand terminus is cleaved; a curved arrow shows an electron pair being sucked into the 5′ oxygen. The tyrosyl residue then remains bound to the 3′ strand terminus. Conversely, in the case of top II, the P–O to the 3′ terminus is cleaved and the enzyme tyrosyl residue becomes bound to the 5′ terminus. The reactions are reversible

strand break (Figure 1.1) (Champoux, 1981; Lynn *et al.*, 1989; Tse *et al.*, 1980; Wang, 1987). The easy reversibility of this reaction evoked the term 'cleavable complex' to designate a topoisomerase–DNA complex which readily converts between a state in which the DNA is intact and a state in which a strand is cleaved (Wang, 1985, 1987). The sites of cleavable complex formation have a strong preference for T at the 3′ terminus (Been *et al.*, 1984; Porter and Champoux, 1989b; Jaxel *et al.*, 1991a,b); it is interesting to note that this preferred T could remain in close proximity to the incoming tyrosyl residue.

A change in the number of twists of one strand about the other ('linking number') could occur either by free rotation of the 3′ side of the cleaved strand around the intact strand or by passing the intact strand through a gap in the cleaved strand (Champoux, 1990). In the latter model, the two termini of the cleaved strand would remain fixed to the enzyme, while the intact strand passes through the gap. There is, however, evidence against strong binding of the 3′ side of the cleaved strand, in that this segment can be exchanged for other single-strand fragments bearing 5′-OH termini (Halligan *et al.*, 1982; McCoubrey and Champoux, 1986). This strand exchange process may reflect the ability of top I to promote genetic recombination.

Following change in the DNA linking number, the cleaved strand can readily reseal, owing to the intrinsic reversibility of the transesterification reaction (Figure 1.1). The rate of resealing is affected by the local DNA sequence, and sites that reseal slowly are more effectively inhibited by camptothecin (Porter and Champoux, 1989a,b).

Mechanisms of Drug Actions

Top I has become an important target for cancer chemotherapy since the discovery of its specific inhibition by camptothecin (Hsiang *et al.*, 1985). Inhibitors might have been expected to act by blocking top I functions, such as relaxation of the DNA supercoiling stress generated during transcription or replication. Some recently identified top I inhibitors may act in this way. However, the major action of camptothecin is to form stabilized cleavable complexes. Two actions of top I inhibitors that are likely sources of cytotoxicity are (1) inhibition of RNA synthesis due to the accumulation of supercoiling stress in transcribed regions of the genome; and (2) interaction with top I–DNA so as to cause stabilization of cleavable complexes which, when encountered by a polymerase, particularly a replication fork, may become an unrepairable DNA defect. These processes are discussed further in later sections.

Clues to the manner of interaction of camptothecin with the top I–DNA complex were obtained from structure–activity studies. The single chiral position in camptothecin (position 20, Figure 1.2) must be in the *S* configuration, both for action on top I and for antitumour activity. The alternative isomer, *R*-camptothecin, is totally inactive. Studies of derivatives bearing substituents on the A ring gave further indications of the steric specificity of the camptothecin binding site (Wani *et al.*, 1987; Jaxel *et al.*, 1989; Kingsbury *et al.*, 1991; Pommier *et al.*, 1991c). Substituents at position 12, and to a lesser degree bulky groups at position 11, abolish activity, suggesting that this region is in close proximity to the binding site on the enzyme–DNA complex. Similar substituents at the 9 or 10 position, on the other hand, generally increase activity (Hsiang *et al.*, 1989b; Jaxel *et al.*, 1989; Pommier *et al.*, 1991c).

The close parallel between the activities of camptothecin derivatives against top I and against murine tumours strongly supports top I as an effective antitumour target (Jaxel *et al.*, 1989; Pommier *et al.*, 1991c).

Although camptothecin binds neither to DNA (Fukada, 1985; Hsiang *et al.*, 1985; Kuwahara *et al.*, 1986) nor to top I alone, radiolabelled drug has been found to bind to complexes of DNA plus top I (Hertzberg *et al.*, 1989a). A camptothecin derivative bearing an alkylating group at the 9 position was found to bind covalently to the top I but not to the DNA component of the complex (Hertzberg *et al.*, 1990), indicating that a

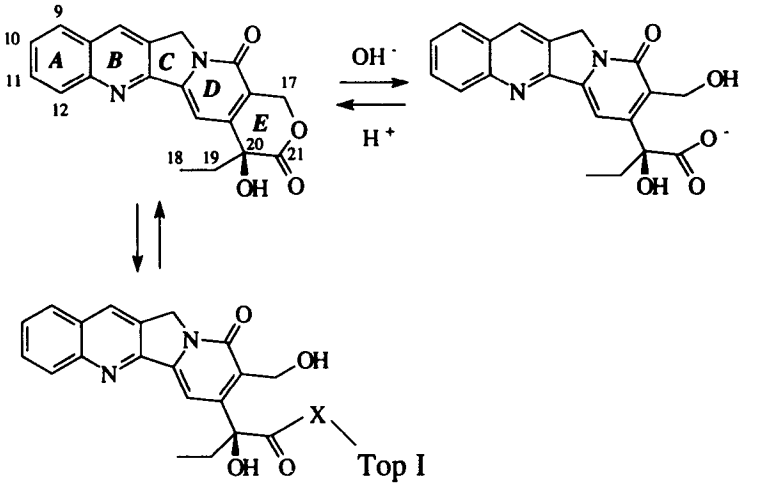

Figure 1.2 Chemistry of camptothecin. The sole chiral position, C20, has the *S* configuration in the active form of the drug. The base-catalysed opening of the lactone ring (ring E) is reversible and facilitated by the OH group at position 20. The kinetics of ring opening and closing depend on pH (half-times are of the order of an hour under physiologic conditions). Lactone ring opening may occur by way of attack by hydroxide ion at position 21. The attack may also come from a suitably placed nucleophile, such as $-SH$ or $-NH_2$ on the top I protein, leading to reversible covalent bonding between drug and enzyme

nucleophilic group of top I is within reach of position 9 of bound camptothecin.

The lactone ring of camptothecin is critical to activity. If the O in the lactone ring is replaced by an N to make a lactam, all activity against top I as well as against tumours is lost (Hertzberg *et al.*, 1989b; Jaxel *et al.*, 1989; Pommier *et al.*, 1991c). The lactone ring opens spontaneously (more rapidly at higher pH) to form a carboxylate salt which is itself inactive. The salt form can, however, convert spontaneously (more rapidly at lower pH) to the active lactone. The opening and closing of the lactone ring is facilitated by the OH group at position 20. This interconversion chemistry suggests the possibility that camptothecin may bind covalently and reversibly with a sulfhydryl, amino or other reactive group on the enzyme (Figure 1.2). Evidence for reversible covalent binding of camptothecin to top I has been reported by Hertzberg *et al.* (1990).

There is good evidence that bound camptothecin inhibits the resealing of the cleaved form of top I–DNA complexes (Hsiang *et al.*, 1985; Hsiang and Liu, 1988; Champoux and Aronoff, 1989; Covey *et al.*, 1989; Porter and Champoux, 1989a; Svejstrup *et al.*, 1990).