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DNA Topoisomerase II, Genotoxicity, and Cancer

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Abstract

Type II topoisomerases are ubiquitous enzymes that play essential roles in a number of fundamental DNA processes. They regulate DNA under- and overwinding, and resolve knots and tangles in the genetic material by passing an intact double helix through a transient double-stranded break that they generate in a separate segment of DNA. Because type II topoisomerases generate DNA strand breaks as a requisite intermediate in their catalytic cycle, they have the potential to fragment the genome every time they function. Thus, while these enzymes are essential to the survival of proliferating cells, they also have significant genotoxic effects. This latter aspect of type II topoisomerase has been exploited for the development of several classes of anticancer drugs that are widely employed for the clinical treatment of human malignancies. However, considerable evidence indicates that these enzymes also trigger specific leukemic chromosomal translocations. In light of the impact, both positive and negative, of type II topoisomerases on human cells, it is important to understand how these enzymes function and how their actions can destablize the genome. This article discusses both aspects of human type II topoisomerases.

1. Introduction

1.1 DNA topology

Perhaps the most striking feature of DNA is the intertwining of the two complementary strands of the double helix [1]. Discovery of this characteristic led to the immediate recognition that biological processes such as replication would be severely affected by the topological state of the genetic material [2].

DNA is globally underwound (*i.e.*, negatively supercoiled) in all species ranging from eubacteria to humans [3–6]. This underwinding makes it easier to separate complementary DNA strands from one another and greatly facilitates initiation and elongation of replication and transcription. Once the replication or transcription machinery begins to travel along the DNA template, however, deleterious effects of topology manifest themselves. Since helicases separate, but do not unwind the two strands of the double helix, fork movement results in acute overwinding (*i.e.*, positive supercoiling) of the DNA ahead of the tracking systems [3,5–7]. In contrast to underwinding, overwinding dramatically increases the difficulty of separating

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duplex DNA into individual strands. Therefore, accumulation of positive supercoils presents a formidable block to replication, transcription, and other essential DNA processes [5,7–10].

In addition to issues related to DNA under/overwinding, nuclear processes such as recombination and replication generate knots and tangles in the genetic material. If knots accumulate in the genome, DNA tracking systems are unable to separate the two strands of the double helix [3,5–7,11]. Moreover, if tangled (*i.e.*, catenated) daughter chromosomes are not resolved prior to cell division, cells will die of mitotic failure [7,12–16].

1.2 DNA topoisomerases

The topological state of DNA in the cell is modulated by enzymes known as topoisomerases [5,7,12–19]. These ubiquitous enzymes regulate DNA over- and underwinding, and remove knots and tangles from the genetic material by creating transient breaks in the sugar-phosphate backbone of the double-helix [5,7,12–19]. Topoisomerases maintain genomic integrity during this process by forming covalent attachments between active site tyrosyl residues and the terminal DNA phosphates that are generated during the cleavage reaction [5,12,14–20]. This covalent linkage is the hallmark characteristic of all DNA topoisomerases.

Cells encode two classes of topoisomerases that are distinguished by their catalytic mechanisms. Type I topoisomerases act by generating a transient single-stranded break in the double helix, followed by either a single-stranded DNA passage event or controlled rotation about the break [5,12,14,18,21,22]. As a result, these enzymes are able to alleviate torsional stress (*i.e.*, remove superhelical twists) in duplex DNA. Type I topoisomerases are involved in all DNA processes that involve tracking systems and play important roles in maintaining genomic integrity [5,7,13,14,18,21,22].

Type II topoisomerases act by generating a transient double-stranded DNA break, followed by a double-stranded DNA passage event [14–16,19]. Consequently, these enzymes are able to remove superhelical twists from DNA and resolve knotted or tangled duplex molecules. Type II topoisomerases function in numerous DNA processes and are required for recombination, the separation of daughter chromosomes, and proper chromosome structure, condensation, and decondensation [5–7,12–16].

2. Topoisomerase II

2.1 Topoisomerase II isoforms

Whereas lower eukaryotes such as yeast and *Drosophila* encode only a single type II topoisomerase [23,24], vertebrate species express two discrete forms of the enzyme, topoisomerase IIα and IIβ [14,19,25,26]. These enzymes display a high degree of amino acid sequence identity (~70%), however, they differ in their protomer molecular masses (170 *vs.* 180 kDa, respectively) and are encoded by separate genes [7,14–16,19,25–30]. Topoisomerase IIα and IIβ can complement the loss of topoisomerase II in yeast [31–33], and with the exception of their abilities to discern DNA geometry (discussed later in this article), display similar enzymological characteristics [15,16,19,25,26,30]. Despite their similarities, the two enzymes have distinct patterns of expression and physiological functions in vertebrate cells [7,13,15, 16,19,30].

Topoisomerase IIα is essential for the survival of actively growing cells. Enzyme concentrations are upregulated dramatically during periods of cell proliferation [34–36]. Furthermore, topoisomerase IIα levels increase over the cell cycle and peak in G2/M [36–38]. Topoisomerase IIα is found at replication forks and remains tightly associated with chromosomes during mitosis [13,39]. Thus, topoisomerase IIα is believed to be the isoform that functions in growth-dependent processes, such as DNA replication and chromosome

segregation [7,13]. In contrast, expression of the β isoform is independent of proliferative status and the enzyme dissociates from chromosomes during mitosis [13,30,36,40]. Topoisomerase IIβ cannot compensate for the loss of topoisomerase IIα in mammalian cells [30,41,42] and its physiological functions have yet to be defined. Although topoisomerase IIβ appears to be dispensable at the cellular level, it is required for proper neural development in mice [43].

2.2 Topoisomerase II domain structure

The primary structures of topoisomerase IIα and IIβ are very similar and can be divided into three domains based on sequence homology with the bacterial type II enzyme, DNA gyrase (Fig. 1) [5,13,15,19,30,44]. The N-terminal domain (first ~670 amino acids) of topoisomerase II is homologous to the B-subunit of DNA gyrase (GyrB). This portion of the enzyme contains the site of ATP binding and hydrolysis [14,19,45]. Crystal structures of this domain recently were solved for yeast topoisomerase II (Fig. 1) [46] and human topoisomerase IIα [47].

The central domain (amino acids ~671–1200) of topoisomerase II is homologous to the A-subunit of DNA gyrase (GyrA) [14,19,48]. This portion of the enzyme contains the active site tyrosine (amino acid 805 for topoisomerase IIα and 821 for topoisomerase IIβ) required for DNA cleavage and ligation. A crystal structure for this domain in the absence of a DNA substrate was solved for yeast topoisomerase II (Fig. 1) [44].

The C-terminal domain (amino acids ~1201–1521 for topoisomerase IIα and ~1201–1621 for topoisomerase IIβ) is highly variable among species and between the two human isoforms. While it is dispensable for catalytic activity *in vitro*, this domain contains nuclear localization sequences [49–55] and sites of phosphorylation [49,56–58]. For many years, the C-terminal domain was thought to contribute little to the enzymatic activity of any type II topoisomerase. However, several recent studies suggest that this portion of the protein plays an intriguing and important role in the recognition of DNA geometry [59–65]. As such, it may impart unique attributes, such as the ability to supercoil DNA [59–61] or act in front of replication forks [62,64], to specific type II enzymes. Unfortunately, no structural information is available for the C-terminal domain of any eukaryotic type II enzyme at the present time.

2.3 Topoisomerase II catalytic cycle

Human topoisomerase IIα and IIβ function as homodimers and interconvert different topological forms of DNA by a "double-stranded DNA passage reaction" [15,19,44]. Briefly, these enzymes bind two separate segments of DNA, create a double-stranded break in one of the segments, translocate the second DNA segment through the cleaved nucleic acid "gate," rejoin (*i.e.*, ligate) the cleaved DNA, release the translocated segment through a gate in the protein, close the protein gate, and regain the ability to start a new round of catalysis [15,19, 20,44,66–70]. The scissile bonds on the two strands of the double helix that are cut by topoisomerase II are staggered and are located across the major groove from one another. Thus, the enzyme generates cleaved DNA molecules that contain 4-base single-stranded ends at their 5′-termini. [71,72] During its cleavage event, topoisomerase II covalently attaches to these newly generated 5′-termini [48,71–73]. This covalent enzyme-cleaved DNA complex is known as the "cleavage complex."

Topoisomerase II requires two cofactors in order to carry out its catalytic double-stranded DNA passage reaction. First, it needs a divalent cation for all steps beyond enzyme-DNA binding [67,71,72,74]. Magnesium(II) appears to be the divalent cation that the enzyme uses *in vivo*. Second, topoisomerase II uses the energy of adenosine triphosphate (ATP) to drive the overall DNA strand passage reaction [66,69,75,76]. While ATP is not required for either DNA cleavage or ligation, the binding of this nucleoside triphosphate triggers the translocation of DNA through the double-stranded nucleic acid gate [75,77]. ATP hydrolysis is necessary for

enzyme recycling [66]. Normally, topoisomerase II binds two molecules of ATP [76]. Although ATP hydrolysis is not a prerequisite for the strand passage event, it appears that this step proceeds more rapidly if it is preceded by hydrolysis of one of the bound ATP molecules [77].

3. Topoisomerase II as a genotoxic enzyme

Topoisomerase II-DNA cleavage complexes are transient in nature and their cellular concentration is tightly regulated (Fig. 2). Cleavage complex formation is essential for topoisomerase II to perform is cellular functions [5,14–16,19]. If the level of topoisomerase II-DNA cleavage complexes falls too low (*i.e.*, enzyme activity is lowered), cells are unable to undergo chromosome segregation and ultimately die of mitotic failure [7,12–16].

Although the cleavage complex is a requisite intermediate in the catalytic cycle of topoisomerase II, it also is potentially deleterious to the cell (Fig. 2). When a nucleic acid tracking system, such as a replication or transcription complex, attempts to traverse the cleavage complex, it converts this transient enzyme-DNA interaction to a permanent double-stranded break in the genetic material [15,16,78–81]. The resulting strand breaks, as well as the inhibition of essential DNA processes, initiate multiple recombination/repair pathways [15,16,80,82–84]. Accumulation of DNA breaks can lead to chromosome translocations and other DNA aberrations [82,84,85]. If the accumulation of breaks is overwhelming, they trigger apoptotic pathways and kill the cell [83]. If these DNA strand breaks do not result in cell death, chromosomal translocations may be present in surviving populations [84].

Increased levels of topoisomerase II-mediated DNA cleavage in humans is associated with translocations that involve the *MLL* (mixed lineage leukemia) gene at chromosome band 11q23 (Fig. 2) [84,86–94]. As discussed later in this article, these translocations often are linked to the initiation of specific types of acute leukemias. The mechanistic basis for the initiation of these leukemias has not been definitively elucidated, but it appears to be related to the function of the protein product of the *MLL* gene. MLL, which is the human homolog of the *Drosophila* trithorax and yeast Set1 proteins, is a histone methyltransferase that is involved in transcriptional regulation in hematopoietic cells [94–98]. Accumulating evidence suggests that the fusion of the MLL protein with other cellular partner proteins alters enzyme function and affects the differentiation of pluripotent hematopoietic stem cells or committed myeloid or lymphoid stem cells by deregulating the expression of the HOX gene [94–96,98,99].

4. Topoisomerase II poisons as anticancer agents and cellular toxins

Agents that increase levels of topoisomerase II-DNA cleavage complexes are known as “topoisomerase II poisons” because they convert this essential enzyme to a potent cellular toxin. Topoisomerase II poisons increase levels of enzyme-DNA cleavage complexes by two non-mutually exclusive mechanisms [15,16,79–81]. Some poisons act by inhibiting the ability of topoisomerase II to ligate the cleaved substrate [15,16,81,100]. These agents not only increase the level of cleavage complexes, but also increase the lifetime of these complexes. Other poisons have little effect on the rate of enzyme-mediated ligation and are believed to act primarily by enhancing the forward rate of cleavage complex formation [15,16,81,101]. The exact mechanism by which this second group of drugs increases levels of DNA cleavage is unknown. They may specifically act to enhance the forward rate of DNA scission. Alternatively, they may have some effect on the DNA binding/dissociation equilibrium, as the level of topoisomerase II-mediated DNA cleavage is proportional to the amount of enzyme bound.

Topoisomerase II poisons can be categorized into three broad classes: The first increases levels of enzyme-DNA cleavage complexes by interacting with topoisomerase II at the protein-DNA

interface in a non-covalent manner; the second acts by covalently modifying the enzyme; and the third acts by covalently altering the structure of DNA. These will be discussed individually below.

4.1 Non-covalent topoisomerase II poisons

Topoisomerase IIα and IIβ are the targets of a diverse group of natural and synthetic compounds [15,16,19,79–81,102–104], some of which are depicted in Fig. 3. Although the compounds shown vary in their ring structures, all are potent topoisomerase II poisons *in vitro* and in human cells. Many of these agents are in wide clinical use as anticancer agents and represent some of the most successful chemotherapeutic drugs currently used for the treatment of human malignancies. On the basis of genetic and mutagenesis studies in yeast and cultured human cells, topoisomerase II is believed to be the cytotoxic target of drugs such as those shown in Fig. 3 [15,16,19,79–81,104]. Some of these drugs appear to favor either topoisomerase IIα or IIβ, however, no truly "isoform-specific" agents have been identified. The relative contribution of the two enzyme isoforms to the chemotherapeutic effects of drugs has yet to be resolved.

Half of all chemotherapy regimens include topoisomerase II-targeted drugs, and six such agents are approved for use in the USA [15,16,19,79–81,102–104]. Drugs such as etoposide and doxorubicin are front-line therapy for a variety of systemic cancers and solid tumors.

Bioflavonoids, such as genistein, are polyphenolic compounds that are constituents of many fruits, vegetables, legumes, and plant leaves [105–107]. They are an integral component of the human diet and are believed to provide a number of health benefits to adults, including chemoprevention leaves [105–112]. Bioflavonoids have a variety of effects on human cells. They represent the most abundant natural source of antioxidants, potently inhibit tyrosine kinases, and exhibit anti-proliferative and pro-apoptotic affects leaves [105–109,112–116]. However, they also display cytotoxic and genotoxic properties and many are potent topoisomerase II poisons [117–120]. Although the physiological actions of bioflavonoids are complex, the sensitivity of cells to genistein-induced toxicity has been correlated to the activity of the type II enzyme [119,121].

Quinolones, such as CP-115,953, are the only drugs that show high activity against eukaryotic and prokaryotic type II enzymes [101,122–125]. While this last drug class has not yet been exploited to treat cancer, quinolones such as ciprofloxacin and levofloxacin that target bacterial type II topoisomerases are the most active and broad-spectrum oral antibacterials in clinical use [124,126,127].

Non-covalent topoisomerase II poisons vary dramatically in their DNA binding properties. For example, etoposide is a non-intercalative compound that displays weak, if any, interaction with DNA in the absence of topoisomerase II [81,128]. Similarly, genistein and quinolones are also non-intercalative [101,123–125,129]. In contrast, amsacrine, doxorubicin, and mitoxantrone are all intercalative in nature, and the latter two compounds bind DNA with very high affinities [130–132]. It was originally thought that topoisomerase II-targeted drugs acted through interactions with DNA, "highjacking" the enzyme to sites of drug binding. We now believe that this is incorrect. All available evidence indicates that non-covalent topoisomerase II poisons act within the active site of the enzyme at the interface between the protein and DNA substrate [15,16,133–137]. Furthermore, mechanistic studies suggest that it is actually the interactions between topoisomerase II and these compounds that serve as the point of entry into the enzyme-DNA complex [135–138]. Although the non-intercalative/intercalative nature of compounds appears to have little effect on the action of drugs within the ternary topoisomerase II-DNA-drug complex, it has the potential to modulate the efficacy of these agents in a physiological setting. This issue is discussed in greater detail later in this article.

Despite the importance of topoisomerase II as a target for cancer chemotherapy, considerable evidence suggests that the enzyme also initiates chromosomal translocations that lead to specific types of leukemia [84,86,94,139–141]. For example, ~2–3% of patients treated with regimens that include etoposide ultimately develop acute myelocytic leukemia [84,86,94, 139,140]. Recently, correlations between the rising use of mitoxantrone to treat breast cancer and the development of secondary leukemias have been reported [142]. Over 50% of these leukemias display translocations within an 8.3 kb breakpoint cluster region in the *MLL* gene at chromosomal band 11q23 [84,86,94,139,140,143]. DNA breakpoints found in these secondary leukemias are in close proximity to topoisomerase II cleavage sites [144–146]. It is notable that therapy-related leukemias with 11q23 rearrangements are unique to chemotherapeutic regimens that contain topoisomerase II-targeted drugs [84,86,94,139].

A high percentage of infant leukemias also display translocations involving chromosomal band 11q23 [147]. Even though genistein and other bioflavonoids appear to be chemopreventative in adults, the maternal consumption (during pregnancy) of foods that are naturally high in these topoisomerase II poisons increases the risk of developing these infant leukemias more than 3-fold [148]. Once again, chromosomal breakpoints in these leukemias are proximal to topoisomerase II cleavage sites [149].

Although the involvement of topoisomerase II-mediated DNA cleavage in the development of leukemias with *MLL* translocations is widely accepted, the mechanism by which these translocations arise is controversial and poorly understood. The evidence discussed above suggests that breaks induced by topoisomerase II within the *MLL* gene play a direct role in the translocation process [94,147,150]. However, it also has been proposed that the breaks induced by topoisomerase II play an indirect role in the process and that breaks induced by apoptotic nucleases ultimately lead to the translocations [151–153] In support of this later theory, a major site of apoptotic cleavage is located in the breakpoint cluster region of the *MLL* gene [151–153]. Ultimately, the pathway(s) that converts topoisomerase II-mediated DNA cleavage into 11q23 chromosomal translocations is likely to be highly complex and may contain elements of both processes.

4.2 Covalent topoisomerase II poisons

Several recent studies indicate that selected sulfhydryl-reactive chemicals, especially quinones, are potent topoisomerase II poisons [154–157]. These compounds appear to act by covalently adducting to the enzyme. The best-characterized covalent topoisomerase II poisons are quinones (Fig. 3). Consequently, discussion will be confined to this class of compounds.

Quinones display the unusual property of acting as topoisomerase II poisons when incubated with the enzyme-DNA complex, but as inhibitors of enzyme activity when incubated with topoisomerase II in the absence of its nucleic acid substrate [155–161]. It has been proposed that this attribute is related to the ability of quinones to cross-link the N-terminal gate of the enzyme [157] [157]. This cross-linking traps DNA within the active site topoisomerase II, but blocks entry of nucleic acid molecules that are not already engaged with the enzyme.

Studies with quinones have focused on human topoisomerase IIα [155–161]. The few experiments carried out with topoisomerase IIβ suggest that these compounds are somewhat less reactive towards this isoform [156]. Quinones such as 1,4-benzoquinone (a major benzene metabolite), *N*–acetylbenzoquinone amine (the major toxic metabolite of acetaminophen), and polychlorinated biphenyl (PCB) quinones (PCB metabolites such as 4′Cl-2,5pQ) increase levels of DNA cleavage mediated by topoisomerase IIα in cultured human cells [155–157, 160]. However, since quinones are reactive towards a number of proteins, it is not clear what role (if any) topoisomerase II plays in the cytotoxic or genotoxic effects of these compounds.

Benzene is carcinogenic in humans and causes primarily hematopoietic malignancies [162–164]. Several lines of evidence link benzene-induced leukemias to topoisomerase II. Occupational exposure to benzene has been associated with t(8;21), which that also are seen in patients that have been treated with topoisomerase II-targeted drugs [94,165–168]. In addition, there is also a case report of leukemia with t(4;11)(q21;q23) following exposure to benzol [169]. Finally, cigarette smoke is an environmental source of benzene, smoking during pregnancy is associated with chromosomal instability in fetal amniocytes, and the genomic region most affected by tobacco is 11q23 [170].

The mechanism by which benzene induces leukemias has not been fully elucidated. However, it is believed that benzene acts through a series of reactive phenolic and quinone-based metabolites [171]. The metabolite that is believed to be central to benzene-induced leukemias is 1,4-benzoquinone [171]. This compound is generated in high concentrations in the bone marrow by oxidation of hydroquinone by endogenous myeloperoxidase [166,171]. 1,4-Benzoquinone is reduced back to the less reactive hydroquinone by NAD(P)H:quinone oxidoreductase 1 (NQO1) [166,167,172]. It is notable that individuals who are heterozygous or homozygous for the *C609T* polymorphism of the *NQO1* gene, which encodes an inactive form of NQO1, display an increased risk for leukemias with 11q23 chromosomal translocations [166,167,172]. The finding that 1,4-benzoquinone is a potent topoisomerase II poison *in vitro* and in cultured human cells [156,190] provides a provocative biochemical link between the type II enzyme and these benzene-induced leukemias.

4.3 DNA lesions as topoisomerase II poisons

The remarkable ability of anticancer drugs to convert topoisomerase II to a toxic enzyme suggests that these agents take advantage of preexisting pathways and argues for the existence of endogenous topoisomerase II poisons. In this regard, a number of naturally occurring DNA lesions that result from endogenous or environmental stress increase levels of topoisomerase II-DNA cleavage complexes [173–179]. Both the α and β isoforms of the enzyme are affected similarly *in vitro* [177,179]. Furthermore, DNA damaging agents stimulate DNA cleavage mediated by topoisomerase IIα in cultured human cells (the cellular effects of DNA damage on topoisomerase IIβ have not been reported) [179].

The ability to distort the double helix appears to be a common feature among lesions that act as topoisomerase II poisons [174,176–179]. Abasic sites, which are the most common lesions in the cell, are among the strongest of poisons [173,174,176,177,179]. Abasic sites are generated by a variety of methods, including spontaneous hydrolysis, DNA reactive chemicals, ionizing radiation, and base excision repair pathways [180–182]. It is estimated that some human cells may contain in excess of 100,000 abasic sites per genome, even in the absence of environmental stress [182]. The presence of ~4 abasic sites randomly generated in a plasmid ~4,400 bp in size increases enzyme-mediated DNA cleavage by either topoisomerase IIα or IIβ to approximately the same extent as the presence of ~10,000 molecules of etoposide [177]. In addition to abasic sites, exocyclic DNA adducts, such as ethano-bases are strong topoisomerase II poisons [177,179]. These adducts are generated following peroxidation of cellular lipids or by exposure of cells to industrial chemicals such as vinyl chloride [183, 184].

The position of the DNA damage relative to the scissile bond cleaved by the type II enzyme is critical to the actions of lesions as topoisomerase II poisons [173–179]. As discussed earlier, the two scissile bonds on the opposite strands of the double helix are separated by 4 base pairs. Lesions that occur within these 4 base pairs often stimulate topoisomerase II-mediated DNA cleavage. Conversely, lesions located immediately outside of the scissile bonds generally inhibit, or have little effect, on DNA cleavage.

The cellular consequences of interactions between type II topoisomerases and DNA lesions are not well understood. It may be that these interactions help to trigger the excision of chromosomal loops by topoisomerase II during the late stages of apoptosis or help to kill cells following oxidation by macrophages [185,186]. The ability of DNA lesions to poison topoisomerase II provides a fascinating teleological link between the actions of anticancer drugs and programmed cell death pathways.

5. Effects of DNA supercoiling on topoisomerase II-mediated DNA cleavage and the actions of anticancer drugs

As discussed above, topoisomerase II-DNA cleavage complexes are transient in nature and are converted to permanent DNA strand breaks in the cell when replication or transcription complexes (or other nucleic acid tracking systems) collide with the covalently attached enzyme [15,16,78,79]. It is these permanent DNA strand breaks that ultimately initiate the cytotoxic effects of topoisomerase II poisons [83].

Globally, DNA in all eukaryotic cells is underwound (*i.e.*, negatively supercoiled) [3–6]. This puts energy into the genetic material and makes it easier to separate the two strands of the double helix. In contrast to global underwinding, the movement of DNA tracking systems through the genetic material results in an acute overwinding (*i.e.*, positive supercoiling) immediately ahead of replication or transcription enzymes (Fig. 4) [3,5–7]. Therefore, collisions with tracking systems, which are critical for the conversion of topoisomerase-DNA cleavage complexes to permanent strand breaks, most likely occur in overwound rather than underwound regions of the genome.

Because of the universal nature of underwound DNA, negatively supercoiled substrates have been used for most previous studies that characterized the catalytic actions of type II topoisomerases and anticancer drugs that target these enzymes. Other studies have utilized linear nucleic acid substrates, especially for mapping sites of drug-induced DNA scission. Recent reports have begun to describe interactions between type II topoisomerases and positively supercoiled substrates [61,62,64,65,187,188]. Several unexpected findings have emerged from these experiments.

First, human topoisomerase IIα removes (*i.e.*, relaxes) positive DNA supercoils >10–fold faster than it does negative supercoils [62]. Thus, the α isoform, which is involved in replicative processes, displays preferential activity with the DNA substrate that accumulates ahead of replication forks. In contrast, topoisomerase IIβ, which is not believed to play a role in DNA replication, relaxes positive and negative superhelical twists at similar rates [62]. On the basis of amino acid sequence comparisons between the two human topoisomerase II isoforms, as well as studies on bacterial and viral type II enzymes [59–61,65], it has been proposed that the ability to discern the geometry of DNA supercoils during relaxation resides in the C-terminal domain of human topoisomerase IIα.

Second, both topoisomerase IIα and IIβ maintain lower levels (~2– to 4–fold) of DNA cleavage complexes with positively supercoiled substrates [62,64]. This attribute decreases the probability that a collision with a replication fork (or other DNA tracking system) will result in the formation of a topoisomerase II-associated double-stranded chromosomal breaks and thus makes these enzymes “safer” participants in DNA processes. However, it also potentially renders them less lethal targets for anticancer drugs.

Third, the geometry of DNA supercoils differentially affects the efficacy of non-intercalative and intercalative topoisomerase II-targeted drugs [64]. In the case of non-intercalative drugs, such as etoposide, the supercoil geometry has relatively little effect on drug action. The relative

increase in DNA cleavage mediated by topoisomerase IIα or IIβ is similar with positively or negatively supercoiled substrates [62,64]. However, overall levels of DNA scission are reduced in accordance with the lower baseline (*i.e.*, no drug) levels of cleavage observed with overwound DNA [64]. The effects of supercoil geometry are considerably different with intercalative drugs such as amsacrine. Intercalative drugs produce the typical "bell-shaped" curve for the induction of topoisomerase II-mediated DNA cleavage with negatively supercoiled substrates, with scission dropping (sometimes precipitously) at high drug concentrations [64]. In contrast, levels of cleavage rise and remain high with positively supercoiled molecules [64].

The differential consequences of DNA geometry on the actions of intercalative drug probably result from the effects of intercalation on nucleic acid structure and drug binding. Since intercalative compounds locally underwind DNA, they induce compensatory unconstrained positive superhelical twists in distal regions of covalently closed circular molecules [130, 189]. Thus, as the concentration of an intercalating agent increases, DNA that is topologically negatively supercoiled would appear to contain positive superhelical twists. Since baseline levels of DNA cleavage mediated by human type II topoisomerases are lower with positively supercoiled substrates [64], the apparent intercalation-induced change in DNA topology could diminish the ability of a compound to enhance cleavage with underwound substrates. In contrast, the apparent geometry of a positively supercoiled plasmid (which already is overwound) would not change substantially upon addition of an intercalative drug.

Although topoisomerase II-targeted anticancer drugs act at the enzyme-DNA interface [15, 16,104,133–137], the accumulation of drugs in the double helix has the potential to inhibit enzyme binding or activity. Because the generation of positive superhelical twists by DNA intercalation induces torsional stress in the double helix [130,189], the ability of these molecules to absorb intercalative compounds is limited. Since overwound DNA is under positive torsional stress even in the absence of drugs, it cannot bind as many intercalative molecules as underwound DNA. Therefore, enzyme activity on positively supercoiled substrates is less likely to be inhibited by the accumulation of bound drug.

The differential influence of DNA geometry on the actions of intercalative anticancer drugs that target topoisomerase II allows these agents to maintain their efficacy ahead of tracking systems over a broad drug concentration range [64]. This aspect of drug activity may contribute to the clinical success of intercalative agents such as doxorubicin, mitoxantrone, and amsacrine.

6. Conclusions

Type II topoisomerases are ubiquitous enzymes that play essential roles in a number of critical DNA processes. In addition, they are the cytotoxic targets for a number of highly successful anticancer drugs. Despite the significance of topoisomerase IIα and IIβ to the survival of human cells and the efficacy of cancer chemotherapy, considerable evidence indicates that these enzymes have significant genotoxic effects and can trigger specific leukemic chromosomal translocations. In light of the profound impact, both good and bad, of type II topoisomerases on human cells, it is important to continue research on these fascinating enzymes. For example, recent work with positively supercoiled DNA has helped to elucidate why some topoisomerase II-targeted drugs may display unexpected activity in physiological settings. Future studies hopefully will generate even greater revelations.

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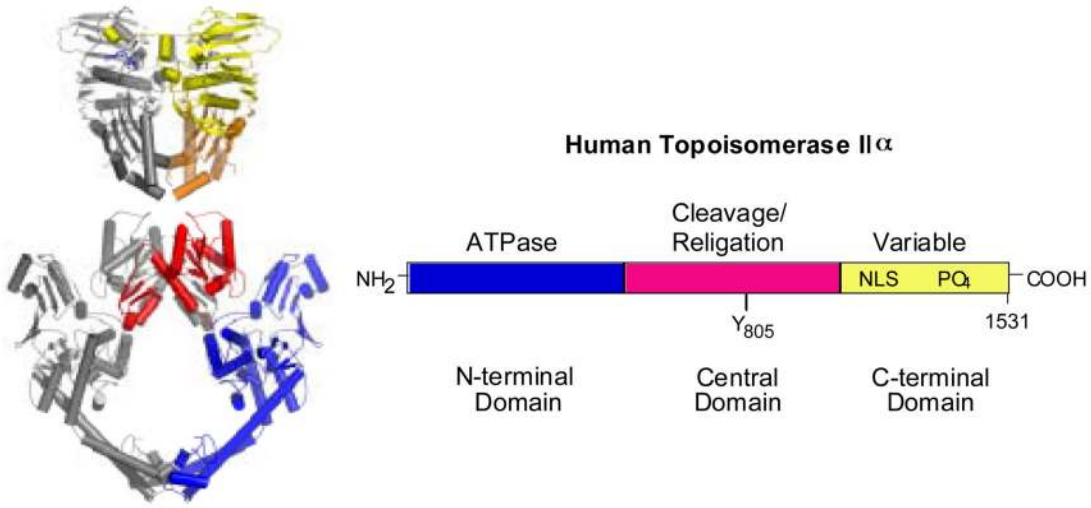

Fig. 1.

Structure of topoisomerase II. A ribbon diagram representing the crystal structure of a homodimer of yeast topoisomerase II is shown at left. The N-terminal domain is on the top (yellow and orange) and the central domain is on the bottom red and blue. At the present time, there is no structural information available for the C-terminal domain of any eukaryotic type II topoisomerase. The domain structure of human topoisomerase IIα is shown at right. The N-terminal domain is homologous to the B-subunit of DNA gyrase (GyrB) and contains the site of ATP binding and hydrolysis. The central domain is homologous to the A-subunit of DNA gyrase (GyrA) and contains the active site tyrosine (Y805) required for DNA cleavage and ligation. The C-terminal domain is highly variable among species and contains nuclear localization sequences (NLS) and sites of phosphorylation (PO4). Although the C-terminal domain was thought to contribute little to the enzymological activity of any type II topoisomerase, several recent studies suggest that this portion of the protein plays an important role in the recognition of DNA geometry.

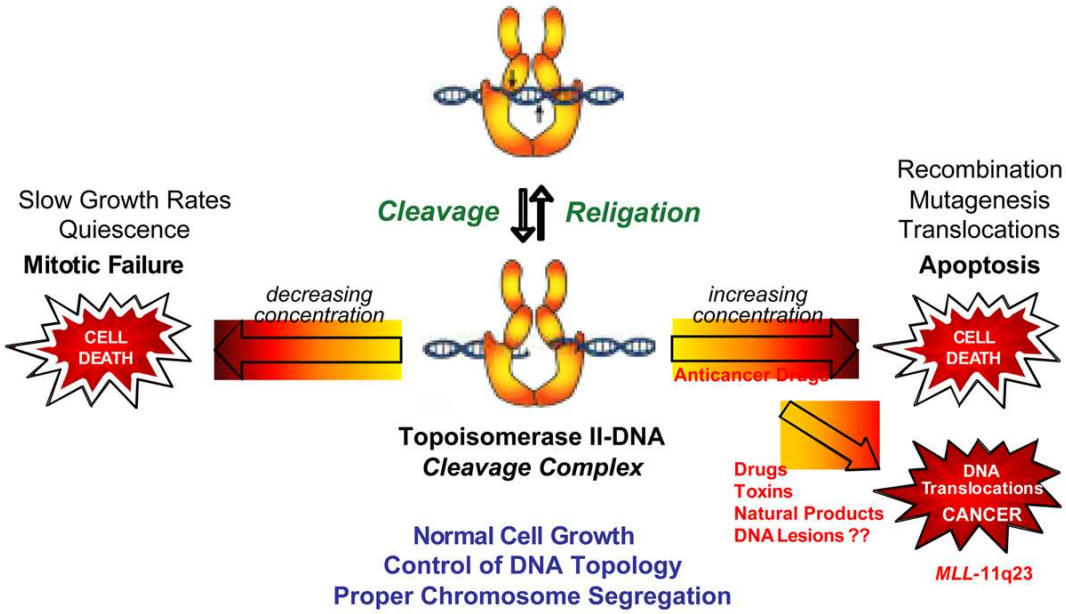

Fig. 2.

Topoisomerase II is an essential, but genotoxic enzyme. The formation of topoisomerase II-DNA cleavage complexes is required for the enzyme to perform its essential cellular functions. If the level of cleavage complexes falls too low (left arrow), cells are unable to undergo chromosome segregation and ultimately die of mitotic failure. If the level of cleavage complexes becomes too high (right arrow) the actions of DNA tracking systems can convert these transient complexes to permanent double-stranded breaks in the genetic material. The resulting strand breaks, as well as the inhibition of essential DNA processes, initiate multiple recombination/repair pathways and generate chromosome translocations and other DNA aberrations. If the DNA strand breaks overwhelm the cell, they trigger apoptotic pathways. This is the basis for the actions of several widely prescribed anticancer drugs. If the concentration of topoisomerase II-mediated DNA strand breaks is too low to overwhelm the cell, chromosomal translocations may be present in surviving populations and trigger the formation of leukemias that involve the *MLL* (mixed lineage leukemia) gene at chromosome band 11q23.

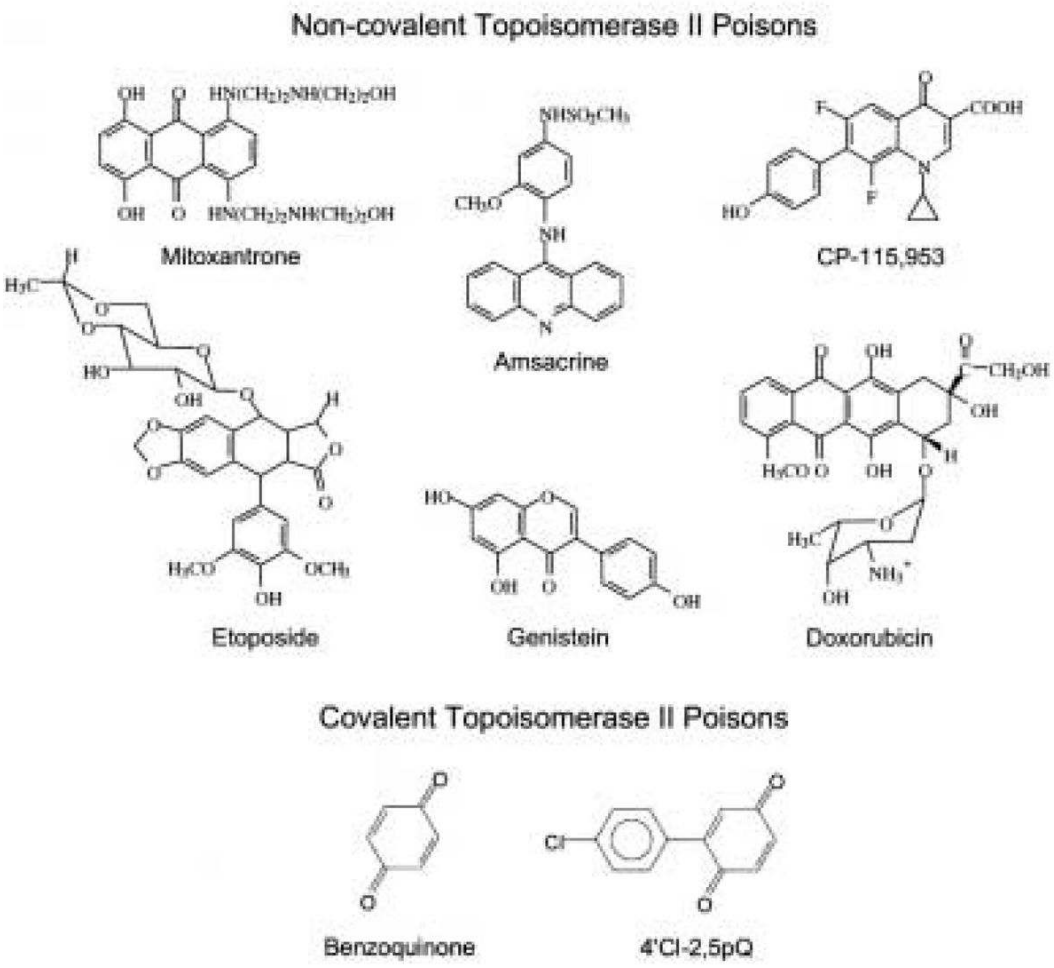

Fig. 3.

Structures of selected topoisomerase II poisons. Agents that act in a non-covalent fashion at the topoisomerase II-DNA interface are shown at the top. Quinones that act by covalently adducting the type II enzyme are shown at the bottom.

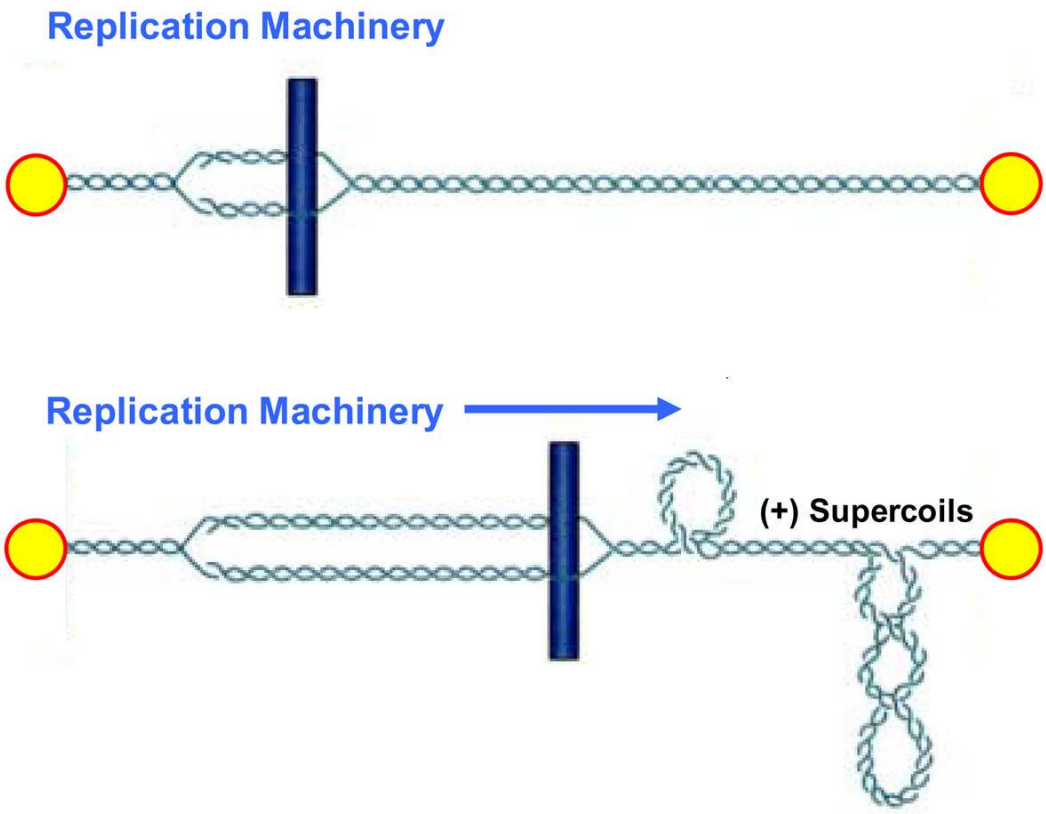

Fig. 4.

Generation of positive DNA supercoils (+SC) ahead of DNA tracking systems. The replication machinery is represented by a rod moving through the double helix. DNA ends are anchored to hypothetical immobile structures existing in the nucleus. Upon initiation of DNA replication, the two strands of duplex DNA are separated and the replication fork is formed (top). Movement of the replication machinery through the immobilized DNA template strands induces acute overwinding (*i.e.*, positive supercoiling) ahead of the fork (bottom). Since collisions with DNA tracking systems (such as replication forks) are critical for the conversion of topoisomerase II-DNA cleavage complexes to permanent cytotoxic strand breaks, these collisions most likely occur in overwound regions of the genome.