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Functional and Regulatory Characteristics of Eukaryotic Type II DNA Topoisomerase

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ABSTRACT: DNA topoisomerases are ubiquitous nuclear enzymes that govern the topological interconversions of DNA by transiently breaking/rejoining the phosphodiester backbone of one (type I) or both (type II) strands of the double helix. Consistent with these functions, topoisomerases play key roles in many aspects of DNA metabolism. Type II DNA topoisomerase (topo II) is vital for various nuclear processes, including DNA replication, chromosome segregation, and maintenance of chromosome structure. Topo II expression is regulated at multiple stages, including transcriptional, posttranscriptional, and posttranslational levels, by a multitude of signaling factors. Topo II is also the cellular target for a variety of clinically relevant anti-tumor drugs. Despite significant progress in our understanding of the role of topo II in diverse nuclear processes, several important aspects of topo II function, expression, and regulation are poorly understood. We have focused this review specifically on eukaryotic DNA topoisomerase II, with an emphasis on functional and regulatory characteristics.

KEY WORDS: DNA supercoiling, topoisomerases, chromosome segregation, genome integrity, cancer, anti-tumor agents.

I. INTRODUCTION

Over 3 decades ago, J. Vinograd and his colleagues discovered that polyoma virus chromosome was a circular, double-stranded, supercoiled DNA (Weil and Vinograd, 1963; Vinograd et al., 1965). This observation implied that the enzymatic machinery involved in duplication of DNA faces a formidable problem in ensuring faithful replication of genetic material. The biological solutions to such topological problems were revealed by the discovery of DNA topoisomerase I, originally denoted as omega protein (Wang, 1971), followed by DNA gyrase (Gellert et al., 1976) from *Escherichia coli*. It is now established that *Escherichia coli* encodes four distinct topoisomerases. These include topoisomerase I (*topA*) (Wang, 1971), topoisomerase II (*gyrA* and *gyrB*) (Gellert et al., 1976), topoisomerase III (*topB*) (DiGate and Marians, 1988), and topoisomerase IV (*parC* and *parE*) (Kato et al., 1990, 1992). These enzymes perform various nonoverlapping functions *in vivo* (reviewed in Roca, 1995). Additionally, a "reverse gyrase", capable of introducing positive supercoils into DNA, in

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an ATP-dependent manner, has been characterized from hyperthermophiles (Kikuchi and Asai, 1984; Kozyavkin et al., 1994).

Before discussing functional and regulatory characteristics of topo II, it is useful to define certain concepts of DNA topology. The superhelical density of a covalently closed circular duplex molecule can be defined using three parameters: the linking number (Lk), the twist (Tw), and the writhe (Wr). The linking number (Lk) represents the number of times one strand of the duplex crosses over the other when viewed on a planar surface. The linking number can be partitioned into twist and writhe (Lk = Tw + Wr). The twist (Tw) represents the local twist of the helix and writhe (Wr) is the measure of the local contortion of the helix axis in space. Lk is necessarily integral, while Tw and Wr are not. For completely relaxed DNA ($Lk = Lk_0$), $Lk_0 = Tw_0 + Wr_0$, where Wr_0 may have a finite value based on the environment, DNA isolated from most natural sources is underwound (i.e., $Lk < Lk_0$). To maximize base pairing, such a molecule adopts a negative writhe and is considered negatively supercoiled. Similarly, a molecule with a positive writhe (i.e., overwound) is considered positively supercoiled. Negative supercoiling is physiologically important as underwound DNA facilitates cellular processes that require strand separation, such as transcription, recombination, and replication. It must be noted that the linking number of a DNA molecule is a topological invariant. A change in linking number involves the breaking of one or both strands of the duplex. Cellular processes requiring topological changes therefore are dependent on the components that catalyze such modifications *viz.* DNA topoisomerases (reviewed in Wang, 1985, 1987; Maxwell and Gellert, 1986).

A fundamental feature of all topoisomerases is their innate ability to transiently cleave and reseal the phosphodiester backbone of DNA via a transesterification reaction. This cleavage gives rise to a covalent enzyme-DNA intermediate with the tyrosine hydroxyl group of the topo-isomerase linked to the 5' or 3' phosphate. The ability of these enzymes to generate either a single- or a double-strand break in the DNA has led to their classification as type I or type II enzymes. Strand passage by type I enzymes leads to changes in linking number in steps of 1 and is independent of the binding or hydrolysis of ATP (except reverse gyrase). Strand passage by type II enzymes is coupled to ATP hydrolysis and leads to changes in linking number in steps of 2. The ability to create a double-strand break confers, on type II enzymes, the capacity to catalyze a variety of topological interconversions, including relaxation of negatively supercoiled DNA and the decatenation of interlinked duplex molecules. The latter reaction is specific to topoisomerase II and underlies its role in other cellular processes, such as chromosome segregation and sister chromatid separation.

II. TYPE II DNA TOPOISOMERASES IN EUKARYOTES

The discovery of topoisomerase I (Wang, 1971) was followed by the identification of a similar activity in cell-free extracts of cultured mouse cells (Champoux and Dulbecco, 1972). Utilizing the assays developed for the identification and isolation of prokaryotic topoisomerases, a number of type I and type II enzymes have been identified, their genes cloned, and characterized from a variety of eukaryotic organisms (reviewed in Wang, 1996; Watt and Hickson, 1994). One significant feature that has emerged from these studies is that, unlike *E. coli* DNA gyrase, none of the known eukaryotic type II enzymes are endowed with supercoiling activity. The literature associated with the studies on eu-

karyotic topoisomerases is exhaustive and has been reviewed extensively (Watt and Hickson, 1994; Wang, 1996). This review emphasizes the progress made in understanding cellular regulation and the roles of eukaryotic topoisomerase II in multiple aspects of DNA metabolism.

A. Topoisomerase II from Yeasts

Because of the simplicity and extensive genetic understanding of yeasts, much of our current knowledge of the mechanistic aspects of eukaryotic type II DNA topoisomerase has been derived from studies on yeast topo II. Topo II has been purified from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The enzyme is a single polypeptide of 150 kDa (Goto et al., 1984) and 165 kDa (Shiozaki and Yanagida, 1991), respectively. Gel filtration and sedimentation analyses revealed that topo II exists as a homodimer in solution (Goto et al., 1984). Isolation and sequence characterization of *TOP2* genes from both these organisms (Goto and Wang, 1984; Uemura et al., 1986) revealed extensive structural homology between yeast topo II and *E. coli* DNA gyrase. The N-terminal and C-terminal domains of yeast topo II are extensively homologous to the GyrB and GyrA subunits of *E. coli* gyrase, respectively (Giaever et al., 1986; Lynn et al., 1986; Uemura et al., 1986). These results suggest that eukaryotic topo II is likely to have evolved from the fusion of *gyrA* and *gyrB* genes.

B. Topoisomerase II from *Drosophila*

Topo II from *Drosophila melanogaster* has been extensively utilized for the elucidation of the mechanistic aspects of the reaction promoted by eukaryotic topo II. The enzyme was purified from embryo extracts and was shown to reversibly catenate DNA in an ATP-dependent manner (Hsieh and Brutlag, 1980; Shelton et al., 1983). *Drosophila* topo II is a 166-kDa polypeptide and exists as a dimer in solution (Shelton et al., 1983). *TOP2* gene from *Drosophila* has been isolated and characterized. It is a single copy gene located near the centromere of chromosome 2 at position 37D (Nolan et al., 1986). A comparison of nucleotide sequences indicates significant homology among the *TOP2* of *Drosophila*, *S. cerevisiae*, and *S. pombe*, and DNA gyrase of *Bacillus subtilis* (Nolan et al., 1986; Wyckoff et al., 1989).

C. Topoisomerase II in Vertebrates

Studies on topoisomerase II from vertebrates, including human (Drake et al., 1987, 1989), mouse (Adachi et al., 1992), rat (Tsutsui et al., 1993; Park et al., 1993), and hamster (Dereuddre et al., 1995) have revealed the existence of two isoforms of topo II: topo IIα and topo IIβ. They are highly similar but genetically distinct and display different expression patterns. *In vivo* studies have revealed that topo IIα is expressed in developing tissues such as thymus and testis, while topo IIβ is expressed in somatic tissues (Holden et al., 1990; Capranico et al., 1992; Juenke and Holden, 1993; Galande and Muniyappa, 1996). The genes for human topo IIα and topo IIβ have been isolated and characterized extensively (Tsai-Pflugfelder et al., 1988; Tan et al., 1992; Lang et al., 1998); these encode polypeptides of 170 and 180 kDa, respectively. Topo IIα gene has been mapped to chromosome 17q21-22 (Tsai-Pflugfelder et al., 1988), while topo IIβ gene resides on chromosome 3p24 (Tan et

al., 1992). Interestingly, human cells produce a variant form of topo IIβ that is five amino acid residues longer and is generated by alternative splicing (Davies et al., 1993). Human topo IIα and topo IIβ have been purified and biochemically characterized (Drake et al., 1989; Chung et al., 1989; Austin et al., 1995). They differ in various enzymatic parameters *in vitro*, including reaction processivity, sensitivity to ionic strength, thermal stability, and drug sensitivity (Drake et al., 1989). These isoforms normally exist as homodimers. However, a small proportion of αβ heterodimers has been detected in cultured cells (Biersack et al., 1996). These are biochemically distinct from homodimers (Gromova et al., 1998), but their physiological significance remains obscure. There is evidence to suggest that human topo IIα and topo IIβ might perform nonoverlapping functions *in vivo* (Meyer et al., 1997; Grue et al., 1998). In this regard, we note that homozygous topo IIβ mutant mice failed to connect motor axons to skeletal muscles, and sensory axons failed to enter the spinal cord. These defects led to breathing impairment and neonatal death (Yang et al., 2000).

D. Topoisomerase II from Parasites

Parasitic diseases are among the leading causes of morbidity and mortality worldwide. The importance of topo II is underscored by the fact that topo II is the cellular target for therapeutic intervention and control of these diseases (Wang, 1996). In recent years, there has been an increasing interest in topo II from parasites with the aim of developing potent antiparasitic chemotherapies (Nenortas et al., 1998). Biochemical studies led to the identification and characterization of topo II in trypanosomes (Douc-Rasy et al., 1986), *Leishmania donovani* (Chakraborty and Majumder, 1987, 1991), *Crithidia fasciculata* (Shlomai et al., 1984; Melendy and Ray, 1989), *Plasmodium* species (Riou et al., 1986; Chavalitshewinkoon et al., 1994), and *Giardia lamblia* (Bell et al., 1993). Subsequently, genes that encode topo II have been isolated and characterized from *Trypanosoma brucei* (Strauss and Wang, 1990), *C. fasciculata* (Pasion et al., 1992), and *Plasmodium falciparum* (Cheesman et al., 1994). The molecular masses of topo II from these organisms are in the range of 137 to 160 kDa. Sequence comparisons suggest that the parasite topo II is related to human enzyme and contains conserved DNA binding, dimerization, and ATPase domains (Nenortas et al., 1998). The genome sequences of all the parasites examined so far indicate the presence of a single copy of topo II gene. However, data from immunolocalization (Melendy et al., 1988) and inhibitor studies (Shapiro and Englund, 1990) indicate that topo II in the nucleus and mitochondria may be biochemically distinct.

E. Topoisomerase II in Plants

Data obtained from plants have helped establish the universality of topo II in eukaryotes. Two groups have reported the isolation and characterization of topo II homologues from *Arabidopsis thaliana* (Xie and Lam; 1994a; 1994b) and pea (Reddy et al., 1999). Others have identified and characterized topo II activity in nuclear extracts of *Zea mays* (Carballo et al., 1991). Computer analysis of structural and evolutionary relationships show that topo II from *Arabidopsis* and pea display significant homology with human, *Drosophila* and yeast topo II (Xie and Lam; 1994a; 1994b; Reddy et

al., 1999). There has been an increasing interest in elucidating the role of topo II in plant growth and development. In this regard, the connection between topo II expression and growth regulatory signals has been strengthened by the demonstration that topo II expression in actively dividing tissues is modulated by light and phytohormones (Carballo et al., 1991; Xie and Lam,1994b; Reddy et al., 1999).

III. MECHANISTIC ASPECTS OF EUKARYOTIC TYPE II DNA TOPOISOMERASE

In recent years, the molecular mechanism of the topo II promoted reaction has been the focus of intense investigation. While the general mechanistic aspects of the catalytic cycle have been elucidated, the molecular details are still obscure. The canonical catalytic mechanism employed by this dyadic enzyme involves the following steps: Topo II binds the first DNA segment (referred to as the G segment), inflicts a double-strand break, and remains covalently linked to the DNA. ATP binding to the N-terminal domain results in a structural change in topo II, which is believed to facilitate capturing of another segment (the T segment). This structural change also leads to the enlargement of the gate in the G segment to permit strand passage. Concomitantly, the T segment is guided through the protein-DNA gate, resulting in a change in the topology of the substrate. ATP hydrolysis results in turnover of the enzyme, thereby initiating the cycling of the molecular machine.

A. Substrate Recognition and Binding

A variety of assays have been used to examine the binding of topo II to DNA. These include glass-fiber and nitrocellulose filter-binding (Sander et al., 1987; Roca and Wang, 1992), electrophoretic mobility shift (Osheroff, 1986; Bechert et al., 1994), nuclease protection (Spitzner and Muller, 1988; Lee et al., 1989), and electron microscopy (Zecheidrich and Osheroff, 1990; Howard et al., 1991). These methods, along with cleavage studies (Udvardy and Schedl, 1991), indicate that the structure, rather than the sequence of DNA, is crucial for topo II binding. In addition, nuclease protection assays have revealed that topo II protects 20 to 30 bp around its recognition site (Lee et al., 1989; Thomsen et al., 1990).

A number of observations suggest that eukaryotic topo II binds a variety of DNA substrates, including scaffold-associated regions (Adachi et al., 1989; Käs and Laemmli, 1992), Z-DNA (Glikin et al., 1991; Arndt-Jovin et al., 1993; Choi et al., 1995), bent DNA (Howard et al., 1991), hairpins (Froelich-Ammon et al., 1994), tetraplexes (Chung et al., 1992), telomeric DNA (Yoon et al., 1998) and intra- or intermolecular crossovers (Zecheidrich and Osheroff, 1990; Roca et al., 1993). Although binding to crossovers is consistent with its inherent property of strand passage, quantitative analysis has revealed that binding to crossovers is not essential for gating (Roca et al., 1993). It must be noted that despite extensive investigations of this particular aspect, the molecular mechanism of DNA substrate recognition by topo II is obscure.

B. DNA Cleavage and Religation

After binding to its substrate, in the presence of Mg^{2+}, topo II inflicts a 4 bp-staggered break in the DNA, forming a 5'-phosphotyrosine enzyme-DNA intermediate with one strand covalently linked to a monomer of topo II (Liu et al., 1983; Sander and

Hsieh, 1983). The reaction is isoenergetic, does not require a nucleotide cofactor, and exists in a cleavage-religation equilibrium. The latter step is an important focal point for various clinically important anti-tumor drugs (see below), which act by uncoupling the cleavage/religation reaction (Tewey et al., 1984; Chen et al., 1984). By stabilizing the "cleavable" intermediate, these drugs have facilitated the analysis of various aspects of the reaction, including sequence preference, kinetics, and the nature of various intermediates (Zecheidrich et al., 1989). These studies have revealed a broad correlation between cleavage sites *in vitro* and *in vivo* (Udvardy et al., 1986; Sander et al., 1987; Udvardy and Schedl, 1993); however, chromatin organization might regulate site accessibility *in vivo* (Udvardy et al., 1986; Capranico et al., 1990; Galande and Muniyappa, 1997). Attempts at generating a 'consensus' cleavage site led to the finding that eukaryotic topo II from a wide variety of sources preferentially cleaves at purine•pyrimidine (R•Y) repeats (Spitzner et al., 1990). However, the stringency of recognition is moderate, and there exist species-specific cleavage sites that bear low sequence homology to the consensus site (Sander and Hsieh, 1985; Spitzner and Muller, 1988; Burden and Osheroff, 1999). It was also demonstrated that binding of a second segment of DNA to the dimer stimulates the cleavage reaction (Corbett et al., 1992a).

C. ATP Binding and Hydrolysis

The role of ATP hydrolysis in the catalytic cycle has been the subject of extensive investigation. Topo II binds ATP cooperatively and this binding triggers an interdomain conformational change in the dimer (Lindsley and Wang, 1991; Lindsley and Wang, 1993a). The conformational transition is believed to play a role in trapping the T segment and enlarging the G segment gate to allow passage of a second segment (Berger and Wang, 1996; Wigley, 1996). ATPase activity of *Drosophila* topo II (Osheroff et al., 1983), yeast topo II (Lindsley and Wang, 1993b), and human topo IIα (Hammonds and Maxwell, 1997) is enhanced after DNA binding. However, topo II promotes one round of strand passage in the presence of a nonhydrolyzable analog of ATP (Osheroff et al., 1983), suggesting that ATP hydrolysis is dispensable for catalysis per se. Steady-state kinetic analyses under ATP-limiting conditions revealed that two ATP molecules are hydrolyzed per DNA transport event. Studies utilizing AMPPNP have indicated that ATP hydrolysis, and release of products thereof, is required for topo II turnover at the end of the catalytic cycle (Osheroff et al., 1983).

Recent studies using rapid quench techniques and pre-steady-state analysis have contradicted this view. These results suggest that topo II hydrolyzes the two bound ATP molecules sequentially (Harkins and Lindsley, 1998; Harkins et al., 1998). The first ATP is hydrolyzed very rapidly; this hydrolysis occurs prior to DNA transport and accelerates strand passage (Baird et al., 1999). Hydrolysis of the second ATP molecule is linked to the opening of the top "clamp" and enzyme turnover. The significance of these findings in the context of the topo II reaction cycle remains to be elucidated.

D. Strand Passage

Two alternate models have been proposed for the passage of the T segment through the topo II-G segment complex. The "one-gate" model posits that the T segment enters the

dimer through one gate (formed by the N-terminal ATPase domains of the monomers), traverses the double-strand break in the G segment, and exits through the same gate. This model requires the release, or a major conformational change, of the G segment prior to T segment release. The "two-gate" model hypothesizes that the T segment enters and exits the topo II dimer using gates located at opposite ends, leaving the topo II-G segment association unchanged (Figure 1). Data from experiments using various forms of catenated DNA substrates and nonhydrolyzable analogs of ATP (Roca and Wang, 1992; Roca and Wang, 1994), or an intradimerically crosslinked topo II (Roca et al., 1996) favor the two-gate model. In contrast, Lindsley (1996) demonstrated that a topo II dimer consisting of two covalently crosslinked monomers was capable of catalysis. However, it is possible that the covalent linkage at the end of the C-terminal domain may allow enough space for the DNA to rearrange itself for another cycle of strand passage. Taken together, the results discussed above indicate that the two-gate model accurately defines the mechanism of topo II-mediated strand passage.

E. Insights into Topo II Reaction Mechanism Through Structural Biology

The overall structural organization of yeast topo II shows striking similarities to the *E. coli* GyrA and GyrB, suggesting that these subunits may have been joined to form eukaryotic topo II. Alignment of yeast topo II amino acid sequence with those of GyrA (harboring the breakage/rejoining core) and GyrB (harboring the ATPase domain) subunits reveals a high degree of sequence homology (Lynn et al., 1986; Uemura et al., 1986; Wyckoff et al., 1989). It has been shown that the N-terminal portion of human topo II is sufficient for the display of DNA-dependent ATPase activity (Gardiner et al., 1998). The C-terminal half of eukayotic topo II harboring both the active site tyrosine and dimerization domain is related to the GyrA subunit, while the N-terminal half is homologous to the GyrB subunit. In addition, eukaryotic type II topoisomerases have a species-specific C-terminal tail consisting of about 250 amino acids beyond the dimerization domain (Uemura et al., 1986; Lynn et al., 1986; Wyckoff et al., 1989). There are a number of functions, both *in vitro* and *in vivo*, attributed to the C-terminal domain. These include nuclear localization (Shiozaki and Yanagida, 1992; Jensen et al., 1996; Mirski et al., 1997), multimerization (Vassetzky et al., 1994), protein-protein interaction (Kroll, 1997), modulation of DNA binding (Crenshaw and Hsieh, 1993) and regulation of catalytic activity (Cardenas and Gasser, 1992; Cardenas et al., 1993). In addition, genetic studies implicate a role for this domain in maintaining topo II function *in vivo* (Thomas et al., 1991; Caron et al., 1994; Yoon et al., 1996). However, it must be noted that certain portions of the C-terminal domain are dispensable for catalytic activity *in vitro* (Shiozaki and Yanagida, 1991; Crenshaw and Hsieh, 1993).

The molecular architecture of eukaryotic topo II has emerged from a combination of electron microscopy and X-ray crystallographic studies. Analysis of EM images of both human and yeast topo II show a tripartite structure. A large globular core comprising the C-terminal part of the dimeric enzyme is connected by linkers to two smaller N-terminal ATPase domains (Benedetti et al., 1996; Schultz et al., 1997). The binding of nonhydrolyzable ATP analogue resulted in the formation of an internal tunnel for the passage of a segment of DNA, as proposed earlier using DNA binding and *in vitro* enzymatic assays (Schultz et al., 1997; Roca and

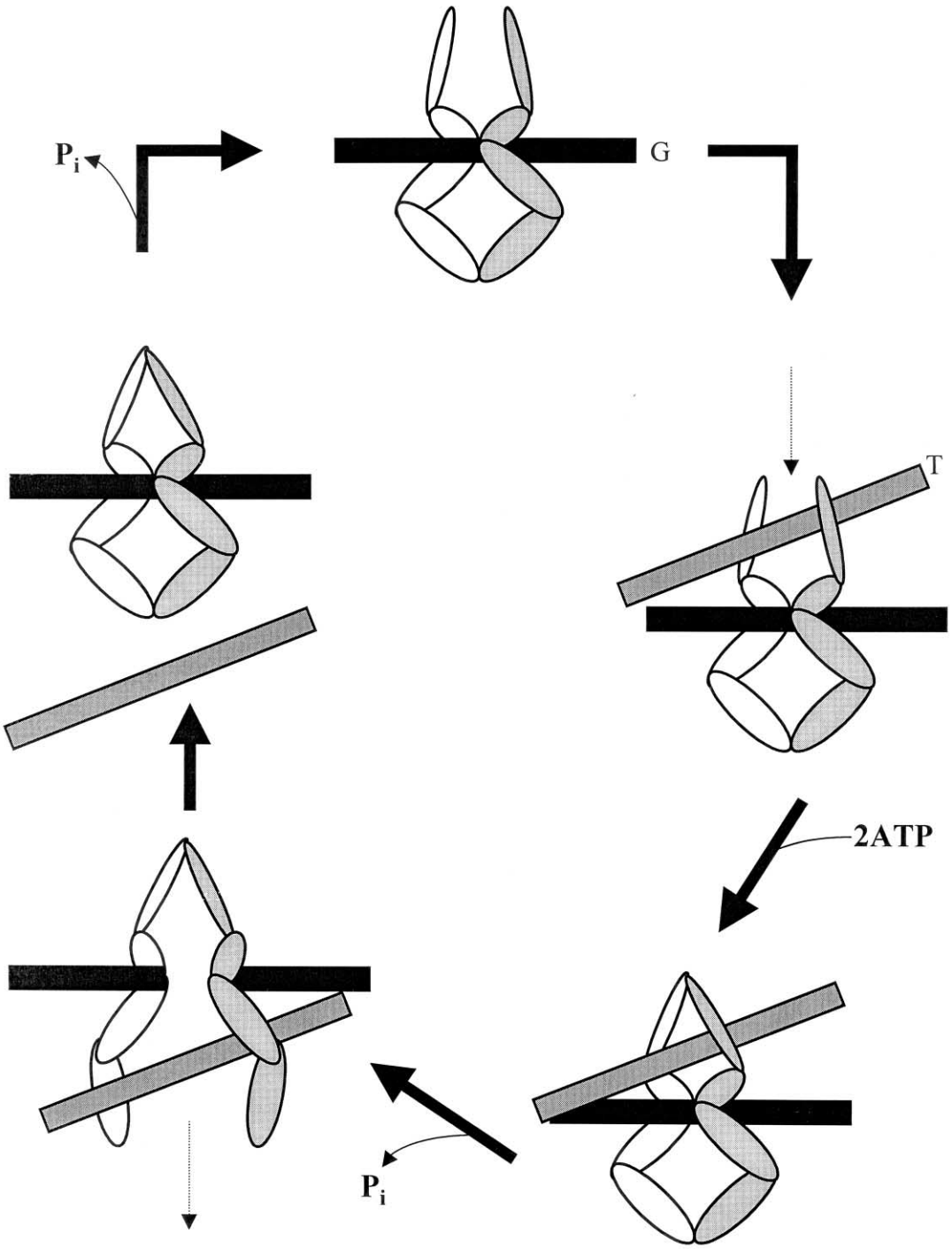


FIGURE 1. Cartoon depicting the two-gate model for topo II reaction mechanism. In the model, topo II dimer, shown as two crescent-shaped monomers, binds to DNA designated as G segment (for gated) and interacts with segment T (for transported) as a protein clamp in an "open" configuration. Binding of two ATP molecules by topo II leads to the conformational change in the N-termini of monomers, resulting in the trapping of the T segment. Hydrolysis of one ATP precedes and accelerates strand passage leading to the eviction of T segment through the lower gate. This process generates topo II-G segment complex in a "closed" conformation. Subsequently, hydrolysis of the second ATP molecule resets the upper clamp in an "open" conformation. Topo II-DNA complex then is ready for the second cycle of catalysis (see text for further details).

Wang, 1992; 1994). X-ray crystal structure of the 92-kDa *S. cerevisiae* topo II spanning amino acid residues 410 to 1202, which lacks the ATP-binding domain but contains the remaining portions of a functional enzyme, has been determined (Berger et al., 1996). An interesting feature of the three-dimensional structure is that it shows topo II as a dyadic enzyme composed of two crescent-shaped monomers. Dimer contacts are established at the tips of crescent near the C- and N-termini of the fragment. These are the two principal regions of contact in the dimer that are stabilized by hydrophobic interactions at the interface. This arrangement of contacts in the dimer results in the formation of a central cavity, which was predicted earlier by DNA binding assays in the presence of AMPPNP (Roca and Wang, 1992). As one might imagine, the shape that the dimer adopts has been likened to a "wasp's" head (Wigley, 1996). However, the structure that helped to understand the organization of topo II by defining the interactions between the monomers has failed to provide a satisfactory explanation regarding the positioning and cleavage of G segment and the passage of T segment through the central cavity. This issue was resolved in the X-ray structure of 59-kDa fragment of *E. coli* gyrase harboring the breakage-reunion domain (Morais Cabral et al., 1997). The gyrase structure reveals new dimer contacts, a grooved surface for binding the G segment, and a cluster of conserved charged residues surrounding the active site tyrosine. Based on the location of the amino acid residues, this structure suggests an enzymatic mechanism of cleavage of G segment and a pathway for the passage of the T segment through the second gate of the dyadic enzyme. However, the biochemical or structural details of how the passage of T segment is achieved through the gate are still poorly understood.

Some of the potential biological implications of conserved and nonconserved regions in topo II have been most clearly revealed in *S. cerevisiae*. Site-directed mutagenesis of functionally important regions has indicated that the catalytic activity of human and *S. cerevisiae* topo II is tolerant to modifications at the inter-domain spacing. Collectively, it appears that the conserved domains are essential for sustained mitotic growth of *S. cerevisiae* and for enzymatic activity *in vitro* (Jensen et al., 1996). A recent study has implicated that in human topo II, the region between ATPase and cleavage/religation domain is essential for effective inter-domain communication consequent to ATP binding (Bjergbaek et al., 2000).

F. Inhibition of Topo II Action

Topo II plays a vital role in maintaining cell viability; consequently, it is an important target for antiproliferative intervention. The cellular target for a large number of antitumor drugs is topo II. These include DNA intercalating agents such as ellipticine (Chu and Hsu, 1992) and amiloride (Besterman et al., 1987), and nonintercalators such as etoposide and teniposide (Chen et al., 1984). Both of these function directly by interfering with the cleavage/religation step of the catalytic cycle, by either enhancing the cleavage reaction (e.g., ellipticine; Froelich-Ammon et al., 1995a), or inhibiting religation (e.g., etoposide; Robinson and Osheroff, 1991; Burden et al., 1996). This results in stabilization of the "cleavable complex", thereby leading to the accumulation of potentially lethal double-strand breaks. Although the cleavage step is the main target of these drugs, they also inhibit other aspects of the

catalytic cycle such as ATP hydrolysis (Robinson et al., 1993) and strand passage (Chen et al., 1984). Certain classes of drugs inhibit topo II activity by interfering with the catalytic cycle at steps other than DNA cleavage/religation equilibrium. For example, aclarubicin inhibits noncovalent DNA binding (Sørensen et al., 1992), *bis*-dioxopiperazines lock the enzyme in the "closed clamp" form (Roca et al., 1994), while staurosporine inhibits the transfer of phosphodiester bonds from DNA to the active site tyrosine (Lassota et al., 1996).

Antitumor agents exert their effects via the formation of a topo II-DNA-drug ternary complex, wherein the drug may interact with both the enzyme and the DNA (Froelich-Ammon et al., 1995a). Accordingly, the efficacy of these agents is assessed by a variety of assays and phenotypes. Investigations have shown that resistance to these drugs arise from changes in the cellular levels of topo II (Pommier et al., 1986; Ganapathi et al., 1993) or alteration of its subcellular localization (Boege et al., 1993). Additional evidence indicates that nucleotide sequence and incorporation of substrate DNA into nucleosomes differentially affect the extent of inhibition by topo II-directed drugs (Sørensen et al., 1990; Spitzner et al., 1995; Galande and Muniyappa, 1997; Larsen and Skladanowski, 1998). There is good evidence that cells that have become resistant to antitumor drugs accumulate point mutations (Froelich-Ammon et al., 1995b; Hsiung et al., 1995) and deletions (Campain et al., 1994) in the coding portion of topo II gene. In addition, altered phosphorylation of topo II has also been noted in the resistant cells (DeVore et al., 1992; Ritke et al., 1994).

Various endogenous DNA lesions are also capable of poisoning topo II activity. UV-radiation-induced cyclobutane pyrimidine dimers inhibit topo II activity by interfering with strand passage (Corbett et al., 1991). The presence of abasic sites in the DNA affects the cleavage step of the topoisomerization reaction. When these sites are located within the four-base overhang generated by DNA incision, they enhance the rate of topo II-mediated scission (Kingma et al., 1995). Apurinic sites are more potent than apyrimidinic sites, and enhance cleavage by 10- to 20-fold (Kingma and Osheroff, 1997a). Interestingly, abasic sites just outside the site of cleavage inhibit strand scission (Kingma et al., 1995). Base mismatches (Bigioni et al., 1996) and cytosine deamination (Kingma and Osheroff, 1997b) also affect DNA strand scission, and do so with a position specificity identical to that observed for abasic sites. Cytosine methylation has a negative effect on topo II-mediated cleavage and may affect the site-specificity of antitumor drugs (Leteurtre et al., 1994). It is believed that all these endogenous lesions affect topo II activity by inducing structural perturbations in the DNA (reviewed in Kingma and Osheroff, 1998).

Although anti-topo II agents have been valuable in gaining insights into the mechanistic aspects of topo II, the basis of drug-induced cell death is not well understood. Accumulation of "cleavable complexes" leads to inhibition of replication (Del Bino et al., 1991), enhancement of sister chromatid-exchange (Pommier et al., 1985), chromosomal translocation (Charron and Hancock, 1991), and arrest of $p34^{cdc2}$ kinase induction (Roberge et al., 1990). While the ultimate result of these events is apoptotic cell death, the molecular links in the signaling pathway are obscure (reviewed in Kaufman, 1998).

IV. CELLULAR FUNCTIONS OF TOPOISOMERASE II

Due to its ability to promote topological interconversions of DNA, topo II plays a

vital role in multiple cellular processes such as chromosome segregation, chromosome condensation, transcription, replication, maintenance of chromosome structure, recombination, and maintenance of genomic integrity. The role(s) of topo II in these processes is reviewed below.

A. Mitotic and Meiotic Chromosome Segregation

Sundin and Varshavsky (1981) first reported that topo II is essential for proper segregation of chromosomes. Their studies on SV40 demonstrated the accumulation of catenated dimers after the arrest of segregation of newly replicated genomes. Similar results were obtained using topo II-directed inhibitors in intact cells (Yang et al., 1987; Snapka et al., 1988). Cytogenetic studies provided firm evidence for the involvement of topo II in chromosome segregation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. *S. pombe* cells bearing a *top2*^{ts} mutation displayed aberrant mitosis when grown at a nonpermissive temperature (Uemura and Yanagida, 1986; Uemura et al., 1987), leading to chromosome breakage and cell death. Similarly, growth of *S. cerevisiae top2*^{ts} cells at a nonpermissive temperature led to lethality when the cells were allowed to progress through mitosis (Holm et al., 1985). In addition, replication of 2 μ plasmid in *top2*^{ts} cells at nonpermissive temperature led to the accumulation of multiply intertwined catenated dimers, indicating that topo II activity was required for segregation of circular DNA molecules (DiNardo et al., 1984). Cytological studies showed a higher frequency of chromosome nondisjunction, at nonpermissive temperatures, in *top2*^{ts} cells, suggesting that aneuploidy may be responsible for lethality (Holm et al., 1989). Subsequently, Spell and Holm (1994) detected chromosome fragmentation in *S. cerevisiae top2*^{ts} cells after cell division at a nonpermissive temperature. Studies in various mammalian cell lines (Downes et al., 1991; Charron and Hancock, 1991; Ishida et al., 1994) and *Xenopus laevis* oocyte extracts (Shamu and Murray, 1992) revealed that topo II inhibitors blocked chromosome segregation at the metaphase/anaphase transition. Injection of teniposide or anti-topo II antibodies into *Drosophila* embryos (Buchenau et al., 1993) yielded similar results *in vivo*. Consistent with the above findings, Downes et al. (1994) identified a catenation-sensitive G2 checkpoint in mammalian cells.

Cytogenetic studies on *top2* mutant strains of *S. cerevisiae* revealed that topo II activity was also required for segregation of recombined homologs during meiosis (Rose et al., 1990; Spell and Holm, 1994). *S. cerevisiae top2*^{cs} mutants failed to finish the first meiotic division when grown at nonpermissive temperature. This defect was overcome by the presence of a mutation that impaired recombination, confirming that topo II activity was necessary for resolving recombined chromosomes (Rose et al., 1990). Cytological analysis of *S. cerevisiae top2* strains identified an important cell cycle checkpoint at late pachytene stage prior to the formation of the meiotic spindle (Spell and Holm, 1994). Together these results suggest that topo II activity is essential for resolving inter- and intrachromosomal tangles generated during meiotic recombination.

B. Transcription and DNA Replication

There is considerable evidence to suggest that unwinding of duplex DNA and helix tracking during transcription, DNA

replication, and recombination results in marked changes in DNA supercoiling. Consequently, topoisomerase activity might be required to annul these changes and allow successful completion of ongoing processes (Liu and Wang, 1987; Dröge, 1994, and references therein). Consistent with this notion, genetic studies using *top1 top2* strains of *S. cerevisiae* have demonstrated the requirement for topo II as a "swivelase" during transcription and DNA replication (Brill et al., 1987; Brill and Sternglanz, 1988; Kim and Wang, 1989a; Ishimi et al., 1992). Several studies using *in vitro*-reconstituted systems, immunodepletion, or use of specific inhibitors also suggested the requirement of topo II in the initiation (Halmer et al., 1998) and late elongation phase of SV40 minichromosome replication (Yang et al., 1987; Snapka et al., 1988; Ishimi et al., 1992). However, it must be noted that topoisomerase I could substitute for the "swivelase" function of topo II in all of the cases mentioned above.

C. Maintenance of Chromosome Structure

A number of observations indicate that topo II is an abundant chromosomal protein in eukaryotes. One possible explanation for the differences in the abundance of topo II family of proteins between prokaryotes and eukaryotes might have to do with the linear structure and complex organization of eukaryotic chromosomes. Accordingly, substantial experimental evidence indicates that topo II plays a key role in chromosomal scaffolding. Biochemical characterization of SC1, an abundant, nonhistone chromosomal protein, revealed that it is identical to topo II (Earnshaw et al., 1985; Gasser et al., 1986). Immunolocalization methods showed that topo II localizes along the central axial region that traversed the length of each chromatid, including the kinetochore (Earnshaw and Heck, 1985; Gasser et al., 1986; Taagepaara et al., 1993). A similar pattern of distribution was also seen in meiotic chromosomes of rooster (Moens and Earnshaw, 1989) and yeast (Klein et al., 1992). The presence of topo II was shown to be essential for the formation of a proper kinetochore structure in a variety of species (Rattner et al., 1996). The preferential binding of topo II to AT-rich scaffold-associated regions (SARs) *in vitro* (Adachi et al., 1989), and the positioning of long range topo II cleavage sites *in vivo* (Gromova et al., 1995), led to the suggestion that topo II may be localized at the base of chromosomal domains. Together these results argue that topo II plays a vital role in the maintenance of mitotic (and meiotic) chromosome structure. However, studies in *Xenopus* (Hirano and Mitchison, 1993) and *Drosophila* (Whalen et al., 1991; Swedlow et al., 1993) indicated that topo II was localized uniformly all along the chromosome and that its continuous association was not necessary for the maintenance of chromosome structure. These discrepancies might arise due to the method of sample preparation and fixation, inherent species-specific differences in the chromosomal localization of topo II, and different epitope-specificities of the anti-topo II antibodies used. Also, the distribution of topo II in any given organism may itself change as cells progress through the cell cycle (reviewed by Warburton and Earnshaw, 1997).

D. Chromosome Condensation

In contrast to the situation discussed above, an essential role for topo II in chromosome condensation has been demonstrated unambiguously in a variety of

model organisms. Genetic analysis in the budding and fission yeasts showed that topo II is necessary for chromosome condensation (Holm et al., 1985; Uemura et al., 1987). Biochemical studies using topo II inhibitors and cell-free extracts from *Xenopus* (Newport, 1987; Newport and Spann, 1987; Adachi et al., 1991; Hirano and Mitchison, 1993), chicken and mammals (Wood and Earnshaw, 1990) are consistent with the above notion. These results were confirmed using entire *Drosophila* embryos (Buchenau et al., 1993) and mammalian cells (Roberge et al., 1990; Ishida et al., 1994; Downes et al., 1994; Bojanowski et al., 1998). Interestingly, recent studies using catalytically inactive topo II (Bojanowski et al., 1998; Mo and Beck, 1999) demonstrated that the enzymatic activity of topo II was not required for chromosome condensation. These results suggest that the formation of higher order chromatin structure may be mediated solely through DNA-binding interactions of topo II. There is considerable evidence to suggest that topo IIα is the primary mediator of chromosome condensation (Taagepaara et al., 1993; Kimura et al., 1996b; Meyer et al., 1997; Grue et al., 1998).

E. Recombination and Genomic Integrity

Topo II (together with topo I) is involved in suppressing mitotic recombination in the rDNA cluster of *S. cerevisiae* (Christman et al., 1988). In this regard, Kim and Wang (1989b) demonstrated that in a *top1 top2-4* mutant, one-half of total rDNA existed as extrachromosomal rings. Expression of either *TOP1* or *TOP2* led to integration of these rings into the chromosome. It has been suggested that the movement of transcription machinery across repetitive DNA sequences creates a topological situation favoring recombination and the subsequent excision of these sequences. Topoisomerases are required to suppress this phenomenon and maintain genomic integrity.

The isolation and characterization of a type II DNA topoisomerase from the archaebacterium *Sulfolobus shibatae* (Bergerat et al., 1997) led to interesting insights into the involvement of topoisomerase-like proteins in meiotic recombination. This enzyme bears homology to *S. cerevisiae* Spo11, which is involved in meiotic recombination. Mutagenesis studies revealed that Spo11p utilizes a tyrosine residue to inflict a double-strand break in DNA, analogous to the mechanism used by type II DNA topoisomerases (Bergerat et al., 1997). It is speculated that Spo11 is a divergent form of topo II that has lost the ability to reseal the double-strand break (Wang, 1997; Haber, 1997). Nonetheless, these observations imply that topoisomerase II-like components might play an important role in meiotic recombination.

The mechanism of topoisomerization reaction promoted by topo II involves the formation of a covalent protein–DNA intermediate. This raises the possibility that the enzyme might mediate illegitimate recombination through subunit exchange. This premise is based on the observation that *E. coli* DNA gyrase (Ikeda et al., 1982) and phage T4 topoisomerase II (Ikeda, 1986a, 1986b) catalyzed illegitimate recombination *in vitro*. Subsequent studies demonstrated that eukaryotic enzymes such as calf thymus DNA topo II (Bae et al., 1988) and *Drosophila* topo II (Gale and Osheroff, 1990, 1992) also mediate the formation of heteroduplex DNA *in vitro*. In this regard, Lamhasni et al. (1995) reported that the yeast topo II dimer had a dissociation constant (K_d) of the order of 10^6 M^{-1}, implying that the intermonomer interaction is rela-

tively weak. On the other hand, Tennyson and Lindsley (1997) demonstrated that the yeast enzyme existed as a stable dimer with a dissociation constant (K_d) of the order of $10^{11} M^{-1}$. It was also shown that dissociation of human topo IIα and topo IIβ dimers was undetectable *in vitro* and *in vivo* (Biersack et al., 1996; Gromova et al., 1998).

In mammalian cells, topo II-mediated rearrangements are observed in cells that are treated with anti-topo II drugs (reviewed in Felix, 1998). While crossovers at topo II cleavage sites led to the formation of hybrid DNA *in vivo* (Zhou et al., 1997), an overwhelming majority of recombinants demonstrated processing of DNA prior to hybrid formation. These results suggest that topo II-mediated illegitimate recombination *in vivo* may be a consequence of the physical disruption of the trapped DNA- topo II complex, rather than subunit exchange.

V. REGULATION OF TOPO II EXPRESSION

A. Transcriptional and Post-transcriptional Regulation of topo II

1. Promoter Structure

Topo IIα gene promoters from human, mouse, hamster, and rat have been isolated and characterized. The human promoter sequence is devoid of a TATA-box, but is GC rich, with a number of putative regulatory motifs embedded in the first 650 base pairs upstream of the translation initiation site (Hochhauser et al., 1992). These motifs include a consensus half-site for the binding of Myc/Max factors, two Sp1 elements, and a proximal Myb-binding site. The Myb site regulates topo IIα expression in human leukemic cells and HeLa cells (Brandt et al., 1997). The functional relevance of the remaining motifs is unclear. In addition, the topo IIα promoter also contains five inverted CCAAT boxes (ICBs), a cell cycle-dependent element (CDE) between ICB2 and ICB3, and an ATF-binding site between ICB3 and ICB4. The region up to 617 base pairs upstream of the translation initiation site contains all of the above-mentioned motifs and directs maximal expression of a linked reporter gene in HeLa cells (Hochhauser et al., 1992). Molecular analysis has revealed that ICB1 governs repression of topo IIα transcription during G_0/G_1 stage (Falck et al., 1999); activation of topo IIα expression following heat-shock (Furakawa et al., 1998) involves alleviation of ICB1-mediated repression. ICB2 is involved in the repression of topo IIα transcription following confluence-mediated arrest of cell division (Isaacs et al., 1996). Interestingly, p53 down-regulates human topo IIa transcription from the minimal (100 bp) promoter. The mechanism by which it does so is unclear (Sandri et al., 1996; Wang et al., 1997).

The hamster topo IIα promoter displays a very high degree of homology to its human homologue, but there is evidence to suggest that the ICBs may be functionally different (Ng et al., 1995). Similarly, the mouse topo IIα promoter shows a high degree of homology to the human and hamster promoters except that it contains two additional CCAAT boxes in the sense orientation (Adachi et al., 1997).

Molecular analysis of the rat topo IIα promoter has revealed significant similarity with the human topo IIα promoter. The first 250-bp sequence upstream of the translation initiation site of rat topo IIα bears a 70% identity with the corresponding human sequence. Although the sequence further up-

stream shows less similarity, the spatial organization of the *cis*-acting elements is preserved. The rat topo IIα promoter also contains an AP1-binding site that is not found in the human, hamster, or mouse promoters. In addition, DNase I footprinting analysis with HeLa cell nuclear extracts has provided evidence for regulation of the rat topo IIα promoter by binding of factors to the Sp1 motifs (Park et al., 1995). Mutational analysis has revealed that ICB4 is required for basal transcription, while Sp1 controls up-regulation during cell-proliferation (Yoon et al., 1999).

The regulation and promoter structure of topo IIβ has not been studied extensively. The 5' flanking region of human topo IIβ gene has been characterized. Although the promoter is devoid of a TATA box and contains GC-rich sequence, it has no obvious homology to the topo IIa promoter. Interestingly, sequences within the first intron, which interact with AP2 and Sp1 proteins, contribute to promoter activity (Ng et al., 1997).

2. Cell Cycle Stage-Specific Regulation

Analysis of whole tissues of rat have shown that expression of topo IIα specific-mRNA is highest in proliferating tissues, while topo IIβ mRNA levels do not correlate with proliferation state (Tsutsui et al., 1993). In HeLa cells, topo IIα mRNA levels rise 10-fold in late S-phase compared with the levels in G1-phase and then decline rapidly following mitosis. In contrast, topo IIβ mRNA levels show a twofold increase during progression through the cell cycle (Isaacs et al., 1998). Run-on transcription assays indicated a twofold difference in the rate of transcription initiation of topo IIa during cell cycle progression in HeLa cells (Goswami et al., 1996). These results suggest that control of mRNA expression might be occurring at the level of mRNA stability. This is supported by the studies of Goswami et al. (1996), who showed that the half-life of topo IIα mRNA was 4 h during S-phase but 30 min in G1 phase. The 3′ UTR was found to be necessary and sufficient for cell cycle stage-specific control of topo IIα mRNA levels (Goswami et al., 2000). This control was mediated via the interaction of redox-sensitive protein complexes with the UTR (Goswami et al., 2000; see Figure 2). In contrast, studies on mouse topo IIα gene expression demonstrated that the mRNA stability was not governed by the 3′ UTR sequence (Kobayashi et al., 1998).

B. Posttranslational Regulation

1. Regulation of Topo II Activity by ADP-Ribosylation

ADP-ribosylation of proteins by poly (ADP)-ribose polymerase plays an important role in the regulation of many cellular processes. Topo II undergoes ADP-ribosylation *in vitro* with concomitant decrease in catalytic activity (Darby et al., 1985). Scovassi et al. (1993) have demonstrated that HeLa cell topo IIα is ADP ribosylated in intact cells. However, the treatment of cells with mutagens failed to enhance ADP ribosylation of topo II. The physiological significance of these results is unclear.

2. Regulation of Topo II by Phosphorylation

Regulation of protein function by phosphorylation-dephosphorylation is a mechanism widely used in biological systems to

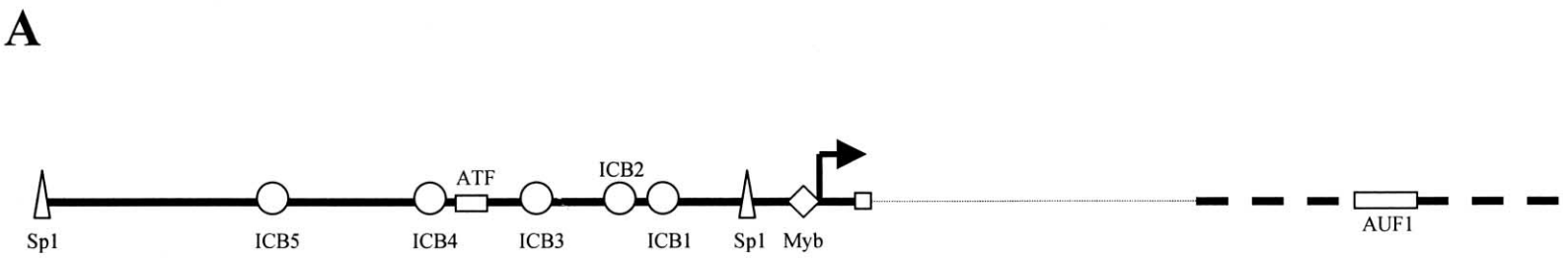
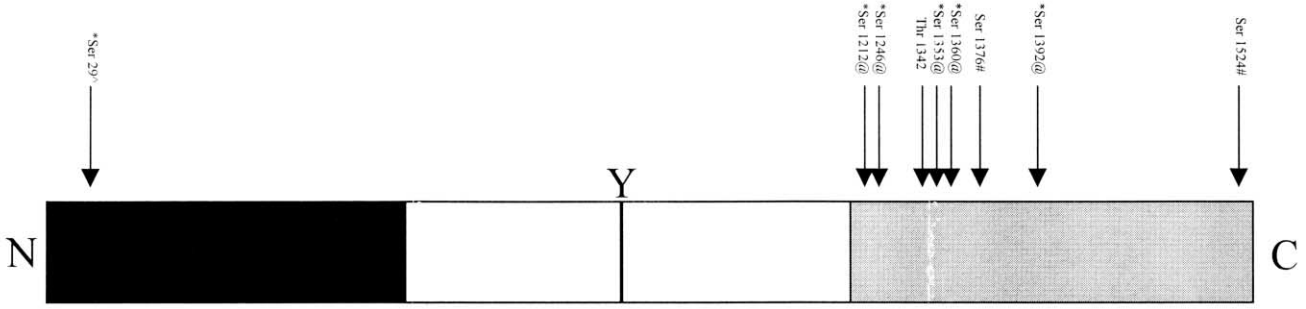
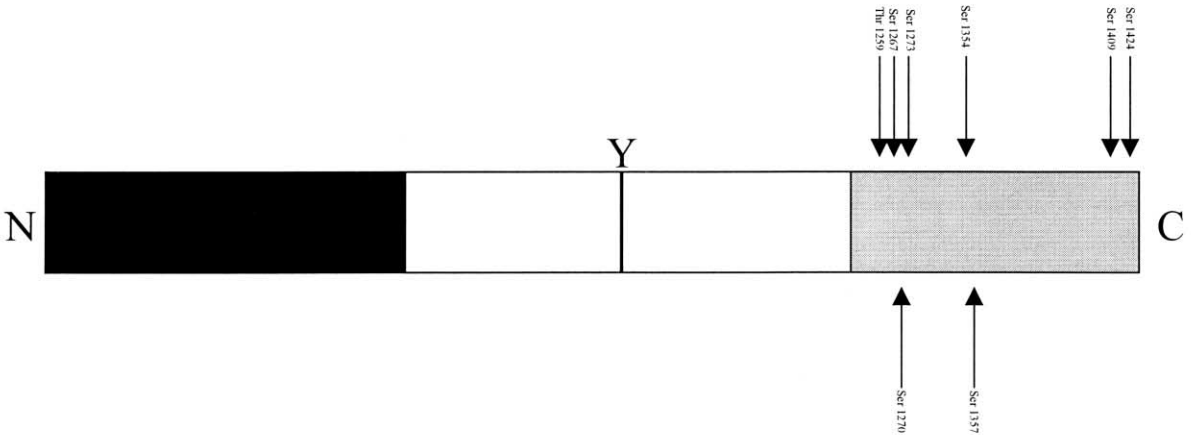


FIGURE 2. Structural organization of human topo IIα gene and protein. (A) Schematic representation of topo IIα gene depicting the promoter (solid line), structural gene (broken line), and the 3' UTR (broken solid line). Topo IIα promoter possesses various *cis*-acting regulatory elements including five Inverted CCAAT Boxes (ICBs), a Myb-binding site, two Sp1-binding sites and an ATF site. Arrow represents the transcription start site of the topo IIa gene. Activation of topo IIα gene expression by serum growth factors and heat shock involves derepression of ICB2 and ICB1, respectively. AUF1 (together with undefined redox-sensitive factors) regulates the half-life of topo IIα-specific mRNA by binding to the 3' UTR in a cell cycle stage-specific manner. The 3′ UTR bears an AUUUUUA motif (AUF1) believed to regulate mRNA degradation. The binding sites of factors that modulate topo IIα gene expression positively (Myb or heat shock) or negatively (AUF1 or redox-sensitive factors) are shown. In addition, p53 downregulates topo IIα transcription, but the molecular mechanism is unclear. **(B)** *(above)* Schematic representation of human topoisomerase IIα showing the amino-terminal ATPase domain (closed box), breakage/reunion domain (open box), carboxy-terminal domain (grey), and active site tyrosine (Y). The arrows represent sites of phosphorylation *in vivo*. Asterisks denote sites of mitosis-specific phosphorylation. A large number of kinases, including PKC (^), CKII (#) and proline-directed MAP kinases (@) phosphorylate topo IIα *in vivo* at the sites indicated. **(B)** *(below)* Schematic representation of *S. cerevisiae* topo II showing sites of phosphorylation by CKII *in vivo*. While phosphorylation affects the biological activity of *S. cerevisiae* topo II, its effect on human topo IIα is obscure. N and C represent amino- and carboxy-terminal ends, respectively.

B



Human topoisomerase IIα (1530 a.a.)



S. cerevisiae topoisomerase II (1429 a.a.)

modulate metabolic pathways (Hunter, 1987). *In vivo*, topo II exists as a phosphoprotein, including yeasts (Cardenas et al., 1992; Shiozaki and Yanagida, 1992), *Drosophila* (Sander et al., 1984; Ackerman et al., 1988), mouse (Saijo et al., 1990), human (Heck et al., 1989; Kroll and Rowe, 1991), and the sponge *Geodia cydonium* (Rottmann et al., 1987).

a. Kinases Involved in Topo II Phosphorylation

Casein kinase II (CKII) has been identified as the primary candidate involved in the phosphorylation of topo II *in vivo*. The role of CKII in the phosphorylation of topo II has been substantiated in *S. cerevisiae* (Cardenas et al., 1992) and *Drosophila* (Ackerman et al., 1985, 1988). In the budding yeast, CKII copurifies with topo II and reactivates the dephosphorylated, inactive form of the enzyme *in vitro* (Cardenas et al., 1993). In addition, immunoprecipitation with anti-topo II or anti-CKII antibodies showed that topo II exists as a complex with CKII *in vivo*, and both the components of this complex are catalytically active (Bojanowski et al., 1993). Protein kinase C (PKC) also phosphorylates topo II of *S. pombe* (Shiozaki and Yanagida, 1992), *G. cydonium* (Rottmann et al., 1987), and humans (Wells et al., 1995) *in vivo*. In addition to CKII and PKC, kinases such as Ca^{2+}/calmodulin-dependent protein kinase, $p34^{cdc2}$ kinase and several MAP kinases phosphorylate topo II in various systems (Cardenas et al., 1992; Wells and Hickson, 1995). Mammalian extracellular-signal-regulated kinases, ERK1 and ERK2, have also been shown to phosphorylate topo IIα *in vitro* and *in vivo* (Shapiro et al., 1999).

b. Phosphorylation Sites on Topo II

In all organisms studied so far, topo II is phosphorylated primarily on serine and threonine residues in its species-specific C-terminal domain. In *S. cerevisiae* and *Drosophila*, both CKII and PKC phosphorylate topo II at closely juxtaposed sites (Cardenas et al., 1992, 1993; Corbett et al., 1992b, 1993). The modification of human topo IIα by proline-directed MAP kinases also occurs in the C-terminal domain (Wells and Hickson, 1995). While some residues remain phosphorylated at all times, the status of other sites varies depending on the stage of the cell cycle (discussed below). A notable exception to the above generalization is the PKC-mediated phosphorylation of *S. pombe* topo II (Shiozaki and Yanagida, 1992) and human topo IIα (Wells et al., 1995). In both these cases, the modified serine residue resides in the N-terminal domain.

c. Cell Cycle Stage-Specific Regulation of Phosphorylation

Topo II phosphorylation varies as a function of the phase of the cell cycle with maximal modification detectable in the M phase. A detailed characterization of this phenomenon has been performed with *S. cerevisiae* and human topo II. Phosphorylation of *S. cerevisiae* topo II is maximal during mitosis with the increase being mainly quantitative, that is, more number of molecules are phosphorylated at the same sites (Cardenas et al., 1992). Few minor mitosis-specific sites, which appear to be targets of $p34^{cdc2}$ kinase are present, but have not yet been characterized. In humans, the situation differs due to the presence of

two isoforms of topo II: topo IIα and topo IIβ. Phosphorylation of topo IIα is both qualitatively and quantitatively different during various stages of the cell cycle (Wells et al., 1995; Wells and Hickson, 1995). For example, threonine-1342, serine-1376, and serine-1524 are phosphorylated to similar extents in mitotic as well as in interphase cells (Wells et al., 1994; Ishida et al., 1996), while serine-1212, -1246, -1353, -1360, and -1392 are modified only in M phase (Wells and Hickson, 1995). The latter group of residues is believed to be the target of proline-directed MAP kinases. Interestingly, PKC-mediated phosphorylation of serine-29 is also mitosis specific (Wells et al., 1995). Topo IIβ is phosphorylated *in vivo* (Kimura et al., 1994a, 1994b) and interacts with the mitotic phosphoprotein antibody MPM-2 (Taagepera et al., 1993). Mitosis-specific phosphorylation of topo IIβ leads to an increase in its apparent molecular weight as detected by denaturing gel electrophoresis (Kimura et al., 1994a, 1994b).

d. Effect of Phosphorylation on the Functional Activity of Topo II

The effect of phosphorylation on topo II catalytic activity appears to be species specific. Dephosphorylation of *S. cerevisiae* topo II leads to the complete loss of catalytic activity. However, a C-terminal truncated form of the enzyme that lacks all of the phosphorylation sites is fully active (Cardenas et al., 1993). This observation has given rise to the speculation that the C-terminal domain may be playing a negative regulatory role and phosphorylation is required to alleviate this inhibition (Cardenas and Gasser, 1993). Phosphorylation also promotes multimerization of *S. cerevisiae* topo II (Vassetzky et al., 1994) and modulates its interactions with DNA (Dang et al., 1994). In contrast to budding yeast topo II, phosphorylation apparently does not play any discernible role in regulating the catalytic activity of *S. pombe* topo II (Shiozaki and Yanagida, 1992).

The mechanism by which phosphorylation enhances catalytic activity has been elucidated in detail using *Drosophila* topo II. Modification leads to an increase in the rate of ATP hydrolysis, which causes an increase in enzyme turnover (Corbett et al., 1992b, 1993). The situation in higher eukaryotes is somewhat complex, partly due to the existence of two isoforms of topo II. Phosphorylation of mouse topo IIα led to significant increase in catalytic activity (Saijo et al., 1990). A four- to sevenfold increase in catalytic activity of topo IIα was also observed after phosphorylation by ERK1 and ERK2 (Shapiro et al., 1999). However, others have found that phosphorylation has no role in this process, and that the increase observed could be attributed to the high concentrations of glycerol and topo IIα used in these assays (Kimura et al., 1996a). It has been shown that phosphorylation differentially affects the binding of human topo IIα and topo IIβ to the mitotic chromosome (Kimura et al., 1996b). These results suggest that phosphorylation may modulate topo II catalytic activity by influencing the interaction of topo II with DNA. Taken together these observations indicate that the effects of phosphorylation on topo II are species specific and exert their influence at multiple stages of the reaction cycle.

3. Regulation of Topo II by Protein-Protein Interactions

A significant body of evidence suggests that topo II activity may be regulated by homologous and heterologous protein-protein

interactions *in vivo*. *S. cerevisiae* topo II is associated with casein kinase II *in vivo*. Phosphorylation of *S. cerevisiae* topo II by casein kinase II is required for activity and also regulates its oligomeric status. Multimerization of topo II is believed to play a vital role in its ability to participate in chromatin condensation. Topo II physically interacts with Sgs1p (Watt et al., 1995), a homolog of RecQ helicase in a functionally relevant form. *Sgs1* mutants are defective in chromosome segregation and show increased levels of recombination (Watt et al., 1995). Topo II also interacts with Pat1p via its C-terminal domain (Wang et al., 1996). *pat1* mutants display phenotypes similar to those of *top2*^{ts} mutants at restrictive temperatures. *Drosophila* topo II interacts with the *barren* gene product, and apparently the interaction is necessary for proper chromosome segregation (Bhat et al., 1996). Human topo IIα heterodimerizes with topo IIβ *in vivo* (Biersack et al., 1996). αβ heterodimers are biochemically distinct from both αα and ββ homodimers; however, their physiological role is unclear (Gromova et al., 1998). Human topo IIα also associates with CREB, ATF2, and c-Jun. This interaction affects the catalytic activity of topo II by increasing the rate of DNA religation (Kroll et al., 1993). In contrast, the association of human topo IIα with 14-3-3ε (Kurz et al., 2000) and retinoblastoma protein (Bhat et al., 1999) negatively modulates topo II activity. Interaction of human topo IIα with casein kinase II stabilizes topo II activity in a phosphorylation-independent manner (Redwood et al., 1998). Mammalian topo IIα also associates with ERK1 and ERK2 *in vivo* (Shapiro et al., 1999). The association with the diphosphorylated form of ERK1, in the absence of topo IIα phosphorylation, leads to an increase in topo II catalytic activity. Human topo IIα interacts with CAD nuclease, in an association that enhances its decatenation activity *in vitro* and plays a role in apoptotic execution (Durrieu et al., 2000). Recently, it has been demonstrated that both the isoforms of human topo II interact with p53 (Cowell et al., 2000). p53 stimulates the catalytic activity of human topo IIα by enhancing the rate of ATP hydrolysis (Kwon et al., 2000). This phenomenon is intriguing in light of the observation that p53 downregulates transcription from the topo IIα promoter (Sandri et al., 1996; Wang et al., 1997). Topo IIβ has been shown to interact with CD3ε. The significance of this association is obscure (Nakano et al., 1996). It is evident that topo II interacts with a plethora of proteins *in vivo*. It is possible that these interactions may be crucial in the regulation of topo II function, in response to various signaling molecules, in different cell types.

VI. SUMMARY AND PROSPECTS

Topo II is an essential nuclear enzyme that plays key role(s) in DNA metabolism and chromosome organization. Its major function is to regulate the topological state of DNA during replication, recombination, and chromosome condensation and segregation. A combination of genetic, biochemical, molecular biological, and computer analyses of the structural and evolutionary relationships have helped establish the universality of topo II in eukaryotes. From the foregoing discussion, it is clear that recent years have witnessed tremendous progress in our understanding of the molecular basis by which topo II is regulated in eukaryotic cells. Several new mechanisms, including structural and enzyme modifications of DNA and acidic pH environment, have been shown to stimulate topo II-mediated DNA cleavage, suggesting that topo II is highly vulnerable to chemical and physical assaults. Of particular importance is the observation that topo II is involved in both cell proliferation and apoptosis (Li et al., 1999; Durrieu et al., 2000). The de-

tails of how topo II activity is coordinated between these two dissimilar cellular processes remain to be defined. X-ray crystal structure of the 92-kDa *S. cerevisiae* topo II has revealed interactions between different domains and has provided insights into topo II reaction mechanism. However, the crystal structures of topo II holoenzyme, a complex of holoenzyme with its substrate DNA and the "cleavable complex", remain to be determined. In yeast, topo II appears to be essential for chromosome segregation in both vegetatively dividing and meiotic cells. The role of topo II in chromosome segregation in vertebrate cells remains obscure: this function could be established by using null or dominant-negative mutants of mouse topo II. On the biomedical front, many antitumor drugs are known to poison topo II, resulting in cell death. These findings further emphasize the importance of developing potent topo II-based pharmaceuticals for cancer treatment. Thus, chemists and pharmacologists have much more incitement to develop potent compounds that would interfere with the catalytic cycle of topo II. There is persuasive evidence that topo IIα is intrinsically the major isoform in proliferating mammalian cells; therefore, one can develop compounds that interact preferentially with different isoforms of topo II. In yeast, *SGS1* (*E. coli* RecQ homologue believed to be involved in aging) interacts with topo II (Watt et al., 1995; Sinclair and Guarente, 1997). The exact physiological relevance of the interaction between Sgs1p and topoisomerases in humans is not yet clear. Considering the growing interest of structural biologists, cancer researchers, and investigators focusing on various aspects of DNA metabolism, a clearer vision of specific and specialized functions of topo II awaits us in the near future.

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