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Review

Roles of DNA topoisomerases in chromosome segregation and mitosis

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Abstract

DNA topoisomerases are highly specialized nuclear enzymes that perform topological changes in the DNA molecule in a very precise and unique fashion. Taking into account their fundamental roles in many events during DNA metabolism such as replication, transcription, recombination, condensation or segregation, it is no wonder that the last decade has witnessed an exponential interest on topoisomerases, mainly after the discovery of their potential role as targets in novel antitumor therapy. The difficulty of the lack of topoisomerase II mutants in higher eukaryotes has been partly overcome by the availability of drugs that act as either poisons or true catalytic inhibitors of the enzyme. These chemical tools have provided strong evidence that accurate performance of topoisomerase II is essential for chromosome segregation before anaphase, and this in turn constitutes a prerequisite for the development of normal mitosis. In the absence of cytokinesis, cells become polyploid or endoreduplicated.

Keywords: DNA topoisomerase II; Chromosome segregation; Cell cycle; Polyploidy; Endoreduplication

1. Introduction

The double-stranded nature of DNA poses important topological problems that arise during DNA metabolic processes such as replication, transcription and segregation of daughter molecules. An example is during the progress of the replication fork machinery along the DNA molecule, that inevitably results in the generation of positive supercoiling ahead of the fork, while the already replicated parental strands in its wake become negatively supercoiled.

DNA topoisomerases are conserved nuclear enzymes that catalyze a variety of interconversions that take place between topological isoforms of DNA through transient DNA cleavage, strand passing and religation (for a recent review, see [1]). So far, at least five different topoisomerases have been reported to be present in higher eukaryotes, namely topoisomerase I and topoisomerases IIIα and IIIβ, which are type I, and topoisomerases IIα and IIβ, two isozymes belonging to the type II family [1,2] (Fig. 1).

Type I enzymes, which do not require ATP, act by forming a transient single-strand break (ssb) through which the other DNA strand passes to achieve relaxation; while type II, usually ATP-dependent, is able to do so with the two strands that make up duplex DNA, creating a DNA-linked protein gate through which another intact duplex passes [3]. Both type I and type II enzymes are proficient in relaxing supercoiled DNA, while only topoisomerase II can decatenate intertwined DNA molecules. Whereas the biological functions of topoisomerases III and IIβ are poorly

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DNA Topoisomerase	Type	Structure	M.W. (kD)	DNA cleavage	Gene localization (Human chromosome)	Function
I	IB	Monomer	100	ssb	20	Replication, transcription, recombination
IIIα	IA	2 isoforms (alternative splicing)	110	ssb	17	Recombination, rDNA metabolism
IIIβ	IA	3 isoforms (alternative splicing)	96	ssb	22	Recombination
IIα	II	Homodimer	170	dsb	17	Chromosome condensation and segregation. Replication
IIβ	II	Homodimer	180	dsb	3	Not well defined

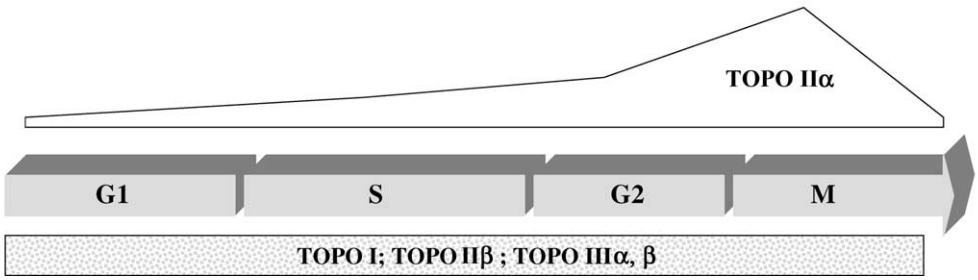

Fig. 1. Human DNA topoisomerases and their expression throughout the cell cycle. Human type IA topoisomerases (topoisomerases IIIα and IIIβ) are homologous to bacterial type I topoisomerases (bacterial topoisomerases I and III) and, like these bacterial enzymes, have activity toward negatively supercoiled DNA, but not positively supercoiled DNA substrate. Topoisomerases IIα and IIβ are isozymes. As to their mode of DNA cleavage, type I enzymes act by forming a transient single-strand break (ssb) in DNA to achieve relaxation of the supercoiled molecule before resealing, while type II topoisomerases form double-strand breaks (dsb) to facilitate unknotting or decatenation of entangled DNA molecules. Levels of topoisomerase IIα mRNA increase several-fold (normally over 10 times) in late S and G_2/M, while other topoisomerases are expressed constitutively in a less cycle-dependent fashion.

understood, many investigations have dealt with the roles of both topoisomerase I and topoisomerase IIα.

Topoisomerases are required during DNA replication, transcription (mainly topoisomerase I) and homologous recombination [3–5] and a specific and unique role for topoisomerase II in segregation of daughter chromatids after DNA replication as well as in chromatin condensation and anaphase segregation during mitosis has been proposed [6,7]. Contrasting with topoisomerase I and topoisomerase IIβ, for which the amount and stability show no significant fluctuations through the cell cycle, topoisomerase IIα protein levels vary naturally as a function of the proliferative stage (higher in cancer cells than in normal ones) and cell cycle position (Fig. 1). This particular behavior has made topoisomerase II the primary cellular target for a number of widely used antineoplastic drugs considerably more lethal to cells that contain high levels of topoisomerase II and which are undergoing high rates of DNA replication [8–10]. Levels of topoisomerase IIα mRNA peak in late S and G_2/M several-fold (normally over 10 times) over the amount detected in G_1 cells. These high levels are consistent with the idea that topoisomerase IIα is required mainly during the final stages of DNA replication to facilitate chromosome untangling, condensation and mitotic segregation. A variety of drugs that interfere with topoisomerase II have been reported to induce polyploidy and endoreduplication to different degrees [7,11–16], providing indirect evidence that the enzyme is required for separation of sister chromatids. This review will focus on topoisomerase II as the nuclear

enzyme that plays an essential role during chromosome segregation, an absolute prerequisite for the development of normal mitosis and the proper distribution of the genetic material between daughter cells.

2. Essential function of topoisomerase II for anaphase separation of chromosomes

As concluded from reports on temperature sensitive yeast mutants that are defective in one or more topoisomerases, type I and type II enzymes are able to substitute for each other to some extent in many functions such as DNA replication and transcription, while the only topoisomerase that is essential for cell viability as a whole seems to be the type II enzyme [2]. Yeast topoisomerase II, encoded by the *top2* gene, is absolutely required to carry out separation of completely replicated chromosomes before cell division. Topoisomerase type I is basically unable to fully separate double-stranded DNA molecules. Lending support to this proposed role of the enzyme, it has been reported that in yeast cells with no topoisomerase II activity plasmids are fully replicated but accumulate as catenated dimers [6]. When it comes to higher eukaryotes, the lack of topoisomerase II mutants has made it difficult to prove a similar mitotic function of the enzyme. As an alternative approach, topoisomerase II poisons and catalytic inhibitors have been employed. In good agreement with the observations made in yeast [4], reports from studies carried out in *Drosophila* [17], amphibia [18] and mammals [3,7,14,19] indicate that the conserved nuclear enzyme topoisomerase II plays a major role in chromosome segregation during mitosis. A consistent observation that lends support to the idea of the importance of the enzyme for anaphase segregation is that if topoisomerase II function is blocked after chromosome condensation, the cells are arrested at metaphase and the chromatids fail to separate [4,7,14,20,21]. Taken as a whole, these results seem to support the idea that supercoils generated in initiation and elongation steps of replication can be removed by topoisomerase I activity, while the final step of decatenation of intertwined daughter molecules can only be carried out by topoisomerase II. A consistent observation is that inactivation of DNA topoisomerase II does not result in an inhibition of DNA synthesis [22–24].

A difficulty commonly encountered when using topoisomerase II poisons such as etoposide to stabilize the normally transient covalent intermediate DNA–enzyme cleavable complex, is that rather high cytotoxic doses, that induce of DNA damage with subsequent G_2 delay have to be used to block enzyme function [25–27]. This drawback is overcome by the use of topoisomerase II catalytic inhibitors which do not cause DNA and chromosome damage, in order to determine the relative importance of the enzyme in promoting chromosome segregation at metaphase–anaphase transition [14]. Focusing on topoisomerase II catalytic inhibitors, even though they have been available for quite a long time and despite their usefulness and promise, reports on induced polyploidization and endoreduplication as a result of topoisomerase II malfunction are scarce. Human leukemic CEM cells continuously exposed to the topoisomerase II catalytic inhibitors merbarone and SN22995 first accumulated in G_2 but then escaped the G_2 block and proceeded into mitosis. Failure to divide led to re-replication, and the cells accumulated at the 8N DNA stage [12]. Inactivation of topoisomerase II by merbarone also resulted in polyploidy in male mouse meiotic cells [16]. Among other classes of catalytic topoisomerase II inhibitors, the bisdioxopiperazines (ICRF-154, ICRF-187, ICRF-193, etc.) have been the most studied to analyze their effect on chromosome segregation. Andoh et al. [28,29] first reported that ICRF-193, considered a catalytic non-cleavable-complex-forming-type topoisomerase II inhibitor [30] led to an absence of chromosome segregation at mitosis with further accumulation of polyploid cells with 8C DNA complements. Formation of polyploid nuclei as a consequence of failure of chromosome segregation in the presence of ICRF-193 was also reported in HeLa cells [31]. Similar results on endoreduplication resulting in large highly polyploid cells have been recently obtained after treatment of human leukemia cells with dexrazoxane (ICRF-187) [32]. While the notion that bisdioxopiperazines are pure catalytic inhibitors of DNA topoisomerase II has been recently challenged, with reports on their possible poisoning mechanism [33,34] treatment of cultured Chinese hamster ovary (CHO) cells with ICRF-193 also results in a high yield of endoreduplicated cells, as reported recently from our laboratory [35] (Fig. 2). Interestingly, the repair defective CHO

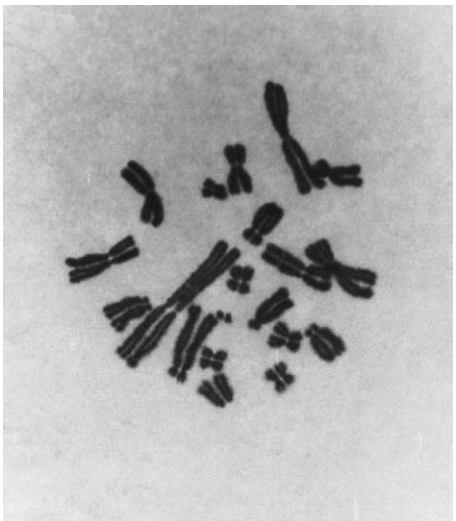

Fig. 2. Chinese hamster ovary (CHO) metaphase cell showing the characteristic diplochromosomes, made up of four chromatids instead of the normal two, as a consequence of a treatment with the bisdioxopiperazine ICRF-193, a topoisomerase II catalytic inhibitor.

mutant EM9 [36] that exhibits a relatively high rate of spontaneous endoreduplication [37], appears as highly sensitive to ICRF-193 treatment as compared with its parental line AA8 [35].

A great deal of the above observations can be explained, at the molecular and mechanistic levels, as a consequence of the recently proposed notion of the "decatenation checkpoint" [38]. In human cells, it has been reported that chromatid catenation is actively monitored as cells progress from G_2 to mitosis, in such a way that entry into mitosis is inhibited when chromatids are insufficiently decatenated [38]. This failure results in a delayed mitosis until chromatids are sufficiently decatenated by topoisomerase II [39]. Sustained chromatid catenation as that induced by ICRF-193 and other topoisomerase II inhibitors may stress chromosomes and end up in aneuploidy or polyploidy through aberrant mitosis, causing genetic instability. As to the genes involved, ICRF-193-induced mitotic delay has been shown to be ATM-independent, unlike the G_2 checkpoint response to DNA damage, while the products of the *ATR* and *BRCA* genes seem to be required [38].

A role for topoisomerase II in meiotic chromosome condensation and segregation in the yeast *Schizosaccharomyces pombe* [5] and mice [16,40] has also been reported. In male mice, topoisomerase II exhibited a higher level of activity in meiotic spermatocytes than in round spermatids, while the enzyme inhibitors teniposide (VM-26) and ICRF-193 dramatically inhibited the formation of metaphase chromosomes in cells induced to progress from prophase to metaphase. Interestingly, however, the disassembly of the synaptonemal complex was not prevented, indicating that mechanistically this latter process could be uncoupled from condensation of chromatin [40]. Also in male mouse meiotic divisions, the topoisomerase II inhibitor merbarone was reported to induce cell cycle arrest and aneuploidy. Both polyploid and hypoploid metaphase II spermatocytes were observed after merbarone treatment of the first division diplotene–diakinesis spermatocytes [16]. Taken as a whole, these observations seem to support a similar meiotic role for topoisomerase II as that played in mitosis for the segregation of sister chromatids after completion of DNA replication. In the case of meiosis, for separation in metaphase I of homologous chromosomes having one or more crossovers, topoisomerase II has to carry out the same task as in mitosis: separation of sister chromatids that are entangled because of meiotic DNA replication. Failure of this process should lead to arrest at the first meiotic division [16,40].

3. Gene products that may associate with topoisomerase II in the chromosomal segregation process

Focusing exclusively on topoisomerase II as the enzyme responsible for chromosome segregation at mitosis is too simplistic, given the high complexity of molecular events occurring during cell division. To ensure a proper progression from metaphase to anaphase, a number of processes such as ubiquitin-dependent proteolysis, protein dephosphorylation, a poorly understood function by the TPR repeat proteins, chromosome transport by microtubule-based motor proteins and DNA topological change by topoisomerase II are all necessary [41]. In addition, chromosome condensation, mitotic kinetochore function and spindle formation require a large number of proteins, which are also prerequisites for successful sister chromatid separation [41]. Here we will only deal with gene

products reported to interact with topoisomerase II and play a role in association with the enzyme for its ability to decatenate intertwined fully replicated DNA molecules before their segregation.

Separation of sister chromatids in mitosis, though highly dependent on topoisomerase II, seems to require, to different extent, a number of other gene products. For instance, a gene isolated from *Drosophila*, the so-called barren (barr) gene has been shown to be required for anaphasic segregation of chromosomes [42]. The protein encoded by barr is present in proliferating cells, localizes to chromatin throughout mitosis, and has homologs in yeast and human [43]. Co-localization and biochemical experiments have indicated that the barren protein associates with topoisomerase II during mitosis and alters the activity of the enzyme, in a manner suggesting a cooperative role for proper chromosomal segregation by facilitating chromatid decatenation [42,43]. Another protein that has been proposed to interact with yeast topoisomerase II is the protein encoded by the *PAT1* gene [44]. This gene product does not appear to be essential for viability, but *pat1* mutants present a slow growth rate and exhibit a phenotype that resembles that of yeast cells grown under limiting amounts of topoisomerase II. The mutants show a reduced fidelity of chromosome segregation during both mitosis and meiosis, suggesting that this protein plays a key, but non-essential role in accurate chromosome transmission during cell division. The precise function of the *PAT1* gene, however, remains to be determined [2].

Contrasting with topoisomerases I and II, whose function in eukaryotes are well established (Fig. 1), the role of DNA topoisomerase III (a type I enzyme that in mammals exists as α and β isoforms) remains poorly defined. Notwithstanding, it has been proposed that topoisomerase III may play a role in chromosomal segregation [45]. In bacteria, topoisomerase III, in conjunction with the RecQ helicase, is capable of decatenating completely double-stranded interlinked DNA molecules [46] presumably via two sequential strand passage reactions. This proposal challenges the previous suggestion that only type II topoisomerases had the ability to completely decatenate double-stranded interlinked circular DNA molecules [47].

Both in yeast [48] and higher eukaryotes including human [49,50], a body of data seem to support a model in which the association of a RecQ helicase and topoisomerase III might be important to facilitate decatenation of late stage replicons to permit faithful chromosome segregation during anaphase. Interestingly, the human Bloom's and Werner's syndrome gene products, which belong to the RecQ family of DNA helicases, have been shown to be associated with topoisomerase IIIα [51,52]. Nevertheless, it has been recently proposed that type I topoisomerases such as topoisomerases I and III are primarily involved in recombination as opposed to being directly involved in chromosome decatenation [46,47,53], again suggesting that only topoisomerase II is an essential enzyme for DNA segregation.

On the other hand, active DNA topoisomerase IIα has been also recently found to be a component of the centrosome, a critical component of the chromosome segregation apparatus [54,55]. Isolated centrosomes exhibited DNA decatenation and relaxation activity that correlated to the amount of topoisomerase IIα protein [54]. While it has been proposed that cytokinesis can be blocked as a result of inhibition of topoisomerase IIα [55], the exact significance of the active enzyme in centrosomes is as yet poorly understood.

4. Conclusions

Although many unanswered questions still remain, the essential role of topoisomerase II in chromosome segregation at mitosis seems to be well established. In higher eukaryotes, despite the lack of topoisomerase mutants comparable to those isolated in yeast, the use of topoisomerase II poisons as well as catalytic inhibitors of the enzyme has provided a powerful tool to investigate the degree of involvement of topoisomerase II in chromosome segregation as compared with type I enzymes. As can be seen in Fig. 3, as a consequence of topoisomerase II malfunction, despite the presence of all the necessary growth factors, cyclins and cyclin-dependent kinases (CDKs), daughter chromosomes are unable to undergo anaphase separation towards the cell poles. As a consequence of this failure to achieve anaphase separation, cells become polyploid and, if a new replication (S) period takes place, the endoreduplicated cell can reach the next mitosis showing diplochromosomes made up of four chromatids (Figs. 2 and 3) instead of the normal two. However, even though these aberrant cells

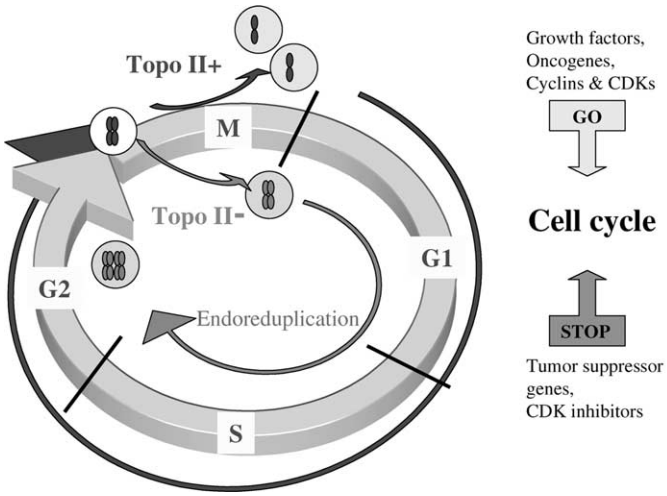

Fig. 3. Topoisomerase II and chromosome segregation at mitosis. Even in the presence of all the factors that favor progression through G_2 and M phases, such as cyclins and cyclin-dependent kinases (CDKs), oncogene products, and growth factors, malfunction of topoisomerase II leads to an aberrant mitosis as anaphase separation fails. As a result, cells become polyploid as a consequence of failed cytokinesis. Should a new replication (endoreduplication) proceed, the cell can enter the next mitosis with diplochromosomes (made up of four chromatids).

that underwent mitosis with a non-functional topoisomerase II can proceed through the cell cycle up to a given point, they eventually are bound to die.

The open questions mainly concern the gene products other than topoisomerase II that could influence the proper development of the segregation process, mainly through association with the enzyme in a cooperative manner. In this context, we have mentioned the barren product first isolated from *Drosophila* but with homologs in yeast and humans [42,43], as well as the product of the *pat1* gene [44], though undoubtedly new gene products are yet to be discovered.

The most controversial question maybe whether or not topoisomerase II is the exclusive topoisomerase involved in decatenation of replicated DNA. Topoisomerase III, a type I enzyme, in association with the RecQ helicase, has been proposed to be important to facilitate decatenation of late stage replicons to permit faithful chromosome segregation during anaphase [45,46]. However, type I topoisomerases may be more likely to be involved with recombination rather than chromosome decatenation [46,47,53].

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