

REVIEW

DNA Topoisomerase I in Oncology: Dr Jekyll or Mr Hyde?

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Mammalian DNA topoisomerase I is a multifunctional enzyme which is essential for embryonal development. In addition to its classical DNA nicking-closing activities which are needed for relaxation of supercoiled DNA, topoisomerase I can phosphorylate certain splicing factors. The enzyme is also involved in transcriptional regulation through its ability to associate with other proteins in the TFIID-, and possibly TFIIF-, transcription complexes, and is implicated in the recognition of DNA lesions. Finally, topoisomerase I is a recombinase which can mediate illegitimate recombination. A crucial reaction intermediate during relaxation of DNA is the formation of a DNA-topoisomerase I complex (the cleavable complex) where topoisomerase I is covalently linked to a 3'-end of DNA thereby creating a single stranded DNA break. Cleavable complexes are also formed in the vicinity of DNA lesions and in the presence of the antitumor agent, camptothecin. While formation of

cleavable complexes may be necessary for the initial stages of the DNA damage response, these complexes are also potentially dangerous to the cell due to their ability to mediate illegitimate recombination, which can lead to genomic instability and oncogenesis. Thus the levels and stability of these complexes have to be strictly regulated. This is obtained by maintaining the enzyme levels relatively constant, by limiting the stability of the cleavable complexes through physical interaction with the oncogene suppressor protein p53 and by degradation of the topoisomerase I by the proteasome system. Emerging evidence suggest that these regulatory functions are perturbed in tumor cells, explaining at the same time why topoisomerase I activities so often are increased in certain human tumors, and why these cells are sensitized to the cytotoxic effects of camptothecins. (Pathology Oncology Research Vol 5, No 3, 171-178, 1999)

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Introduction

Despite many reviews on DNA topoisomerases, little is available concerning the biological properties of eukaryotic topoisomerase I alone. In general, the two major classes of DNA topoisomerases, types I and II, tend to be considered as different variants of a group of enzymes which exclusively serve to change DNA conformation. Nevertheless, topoisomerase I and topoisomerase II are different proteins with their own distinct properties and biological functions. Recent results suggest that topoisomerase I is a multifunctional protein with at least two different catalytic activities, whereas topoisomerase II, in addition to its catalytic roles, is a structural protein which serves as a chromatin-docking protein for different molecular partners. This review will cover our current knowledge on the biological functions and activities of eukaryotic topoisomerase I as well as its implications in tumor progression and genetic instability.

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Type I topoisomerases

Eukaryotic topoisomerase I, prokaryotic topoisomerase I and topoisomerase III are all type I enzymes. These monomeric proteins catalyze interconversions between different topological states of DNA by transiently breaking the phosphodiester backbone of one strand of DNA thereby allowing the passage of the intact DNA strand through

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the break. This is followed by religation of the nicked DNA strand leading to reformation of an intact DNA helix. Recent studies show, that topoisomerase I also has a kinase activity and can phosphorylate certain splicing factors, such as SF2/ASF.¹

The initial discovery of topoisomerase I was made in 1971 in *Escherichia coli* followed by the discovery of topoisomerase I activity in extracts from eukaryotic cells.^{2,3} Since then topoisomerase I enzymes have been isolated from numerous organisms including calf thymus, yeast, *Drosophila melanogaster*, human HeLa cells, *Trypanosoma cruzi* and vaccinia virus.⁴⁻⁹ Recently, another type of eukaryotic topoisomerase I, topoisomerase III, has been isolated from yeast and human cells.^{10,11}

Although type I enzymes are heterogeneous in size and amino acid sequence, eukaryotic topoisomerase I proteins are organized in a similar way. Limited proteolysis and crystallographic structure determination indicate that eukaryotic topoisomerase I consists of four major regions, the NH₂-terminal, the core, the linker and the C-terminal domain.^{12,13}

- the N-terminal part (aa 1-212 for human topoisomerase I) is highly charged, contains very few hydrophobic amino acids and is largely disordered. This region contains four putative nuclear localization sequences (NLS) as well as a binding domain for the SF2/ASF splicing factor.^{14,15}
- the globular core region (aa 213 to 635) is highly conserved and is needed for DNA binding. This region exhibits preferential binding to superhelical as compared to relaxed DNA.
- the positively-charged globular linker domain (aa 636-712) is poorly conserved and is not obligatory for catalytic activity.
- the globular C-terminal domain (aa 713-765), is highly conserved and contains the active site tyrosine (Tyr 723) which is absolutely required for DNA relaxation. This tyrosine residue forms a covalent linkage between the enzyme and a 3'-end of DNA, thereby creating the single-stranded DNA nick which permits strand passage. As illustrated in *Figure 1*, the covalent DNA-topoisomerase complex (often called the cleavable complex) is crucial not only for DNA relaxation, but also for other topoisomerase I-mediated reactions such as recombination and recognition of DNA damage. In addition, it is the target for clinically used antineoplastic agents of the camptothecin family, which act by preventing the religation step, thereby resulting in the accumulation of cleavable complexes. In contrast, Tyr 723 is neither required for transcriptional modulation nor for kinase activity. The C-terminal region also contains the binding site for ATP which is necessary for the kinase activity of topoisomerase I.¹⁶

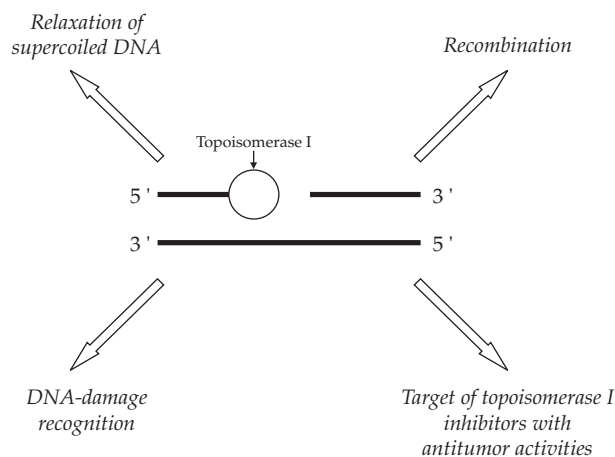


Figure 1. The covalent DNA-topoisomerase I complex (cleavable complex). During the catalytic cycle, the active site tyrosine of topoisomerase I forms a covalent bond with a 3'-end of DNA, thereby creating a single-strand break (nick) in the DNA, which permits passage of the intact DNA strand. After strand passage, topoisomerase I will usually reseal the two ends of the nicked DNA recreating an intact DNA helix. However, the enzyme is also able to religate the covalently bound DNA with a 5'-end of a different DNA strand thereby creating recombinant DNA. While the covalent reaction intermediate usually is very short-lived, it can be stabilized in the presence of DNA lesions or by the antitumor agent camptothecin resulting in an overall increase in the cellular levels of cleavable complexes.

Expression and cellular localization

The human topoisomerase I gene maps to chromosome region 20q12.¹⁷ The coding sequence is particularly complex with 21 exons distributed over at least 85 kilobase pairs of human genomic DNA.¹⁸ Since all 20 exons contain the sequence elements known to be required for pre-mRNA splicing, it is possible that different splice variants of topoisomerase I may exist. The promoter region of human topoisomerase I does not contain TATA or CCAAT regulatory elements.¹⁹ However, the promoter contains binding sites for other transcription factors including Sp1, octamer transcription factor (OTF), cAMP-responsive-element-binding protein (CREB/ATF), NF- κ B and Myc-related proteins.^{19,20} While Sp1 and OTF serve as general transcription factors, the other transcription factors are known to be activated as a response to external stimuli or under conditions of cellular stress. This is consistent with the observation that the levels of topoisomerase I mRNA and protein increase during the initial phase of human T lymphocyte proliferation, whereas protein levels decrease during myeloid maturation.^{21,22} Similarly, topoisomerase I protein levels are decreased in growth factor deprived fibroblast.²³ The decrease in protein levels is likely mediated by the 26S proteasome, since the proteolysis is preceded by multi-ubiquitination of topoisomerase I.²⁴

Topoisomerase I does not show significant cell cycle-dependent alterations in both amounts and stability during the eukaryotic cell cycle.^{25,26} Furthermore, the catalytic activity of topoisomerase I in all normal mouse and human tissues examined including liver, kidney, brain, heart, lung, ovary, testis, tongue and tonsil were found to be very comparable.²⁷

In contrast, topoisomerase I activities vary significantly between different tumor types as well as between patients with the same tumor type. Increased topoisomerase I activity is usually associated with increased protein and mRNA levels as well as an increase in gene copy number.^{28,29} Lung and breast cancers as well as ganglioneuroblastomas show only low catalytic activities whereas increased topoisomerase activities have been observed for cervix cancer and in metastatic malignant melanoma.^{29,32} Comparison between topoisomerase I activities in tumors and normal tissue from the same patient showed no differences for kidney tumors whereas topoisomerase I protein levels and catalytic activities were markedly higher in colorectal and prostate cancer and in immature neuroblastomas than in the corresponding normal tissues.^{28,32,33} Interestingly, increased topoisomerase I activity in melanomas is often correlated with abnormal p53 function.³⁰

Topoisomerase I is concentrated in the nucleolus in many interphase cells.^{26,34-36} This is consistent with the observation, that formation and maintenance of a functional nucleolus in yeast require active RNA polymerase I as well as DNA topoisomerases I and II.³⁷ This is also observed for mammalian cells where inhibition of RNA synthesis during differentiation or as a result of drug treatment is accompanied by translocation of topoisomerase I from the nucleolus into the nucleoplasm.^{22,26} In S phase cells, topoisomerase I is localized in a spotted pattern in the nucleus consistent with its presence at the replication fork.³⁶ During mitosis, topoisomerase I remains attached to the DNA and is present on mitotic chromosomes in a diffuse pattern.³⁶

Activities and biological functions

Initial studies showed that topoisomerase I was not required for viability in *Schizosaccharomyces pombe* or *Saccharomyces cerevisiae*.^{38,39} However, absence of topoisomerase I resulted in delayed onset of DNA elongation during early S phase as well as decreased rates of RNA transcription.^{39,40} In contrast, further studies have shown that topoisomerase I is essential for the viability of dividing, multicellular organisms. Thus, topoisomerase I is necessary for development in *Drosophila melanogaster* past the blastocyst stage while disruption of the topoisomerase I gene in mice results in death of the embryos between the 4- and 16-cell stage.^{41,42} These results suggest, that besides the biological activities observed in yeast models, topoisomerase I may have additional functions in multicellular

organisms. This is supported by recent findings which indicate that topoisomerase I is a multifunctional protein with different biological activities of which some, like the phosphorylation of splicing factors, do not play a role in yeast, which do not have alternative splicing.

1. DNA nicking-closing activity

The ability of topoisomerase I to relax supercoiled DNA through transient DNA nicking-closing is needed during DNA replication to relieve the superhelical stress created by the replication fork.⁴³ Relaxation of supercoiled DNA is also required during transcription, which is coherent with the preferential association of topoisomerase I with actively transcribed DNA regions.⁴⁴ Furthermore, microinjection of topoisomerase I-directed antibodies into the nucleus reduces the transcription of both ribosomal and messenger RNA whereas subsequent addition of topoisomerase I restores the transcriptional activity.⁴⁵ However, these functions are not unique for topoisomerase I but can equally be carried out by topoisomerase II which takes over if topoisomerase I is lacking.³⁸⁻⁴⁰

2. Transcriptional modulation

In addition to its catalytic activities, topoisomerase I can serve as a transcriptional modulator. This effect is mediated through physical interaction with other proteins in the transcription complexes, and does not require catalytic activity or even DNA binding.^{46,47} Topoisomerase I can both repress basal transcription and stimulate transcription in the presence of transcriptional activators. This effect is limited to genes which contains a TATA box in their promoter region and is mediated by physical interaction between topoisomerase I and different constituents of the general transcription factor TFIID complex.^{46,48} The repression is observed both with full size TFIID and with one of the TFIID subunits, the TATA-box binding protein (TBP). In contrast, transcriptional activation depends on interaction between topoisomerase I and other subunits of the TFIID complex, the TATA-binding protein-associated-factors (TAFs). The stimulatory effect of topoisomerase I on transcriptional activation, is due to enhancement of the formation of complexes between TFIID and another transcription factor, TFIIA which is needed for transcriptional initiation.⁴⁷

3. Kinase activity

It has recently been shown that topoisomerase I possesses a kinase activity towards proteins involved in RNA processing.¹ This activity requires binding of the splicing factor to the N-terminal part of topoisomerase I whereas ATP binds to the conserved, C-terminal part of the molecule.^{15,16} The kinase activity of topoisomerase I likely

affects gene expression, since phosphorylation of splicing factors is believed to affect splice choice resulting in alternative splicing of pre-mRNA transcripts and thereby in different proteins.

4. Recombination and viral integration

Illegitimate recombination joins two molecules of DNA which shares no homology. Illegitimate recombination leads to genetic instability and chromosome aberrations and is closely associated with the development of neoplasia. As discussed above, during relaxation of supercoiled DNA, topoisomerase I forms a covalent DNA-protein complex with the 3'-end of one DNA strand. This 3'-end is usually resealed with the 5'-end of the same strand at the end of the reaction. Alternatively, religation may occur with a 5'-hydroxyl end of a heterogeneous DNA molecule resulting in recombinant DNA.⁴⁹ Topoisomerase I shows structural and functional similarities to site-specific recombinases⁵⁰ and increased topoisomerase I levels are associated with recombination in several experimental models. In yeast, increased topoisomerase I activity is accompanied by increased levels of illegitimate recombination, which predominantly occurs at preferred topoisomerase I cleavage sites.⁵¹ Topoisomerase I is also implicated in the integration of viral DNA from Simian virus 40 (SV40), hepatitis B virus and woodchuck hepatitis virus into cellular DNA^{49,52-54} and it has been proposed, that viruses that do not contain an integrase gene rely on cellular topoisomerase I for viral integration. Furthermore, treatment of mammalian cells with camptothecin and other topoisomerase I-directed antitumor drugs which leads to accumulation of covalent DNA-topoisomerase I complexes also results in sister chromatid exchange and chromosomal aberrations.⁵⁵ Maybe the best illustration of the potent recombinase activity of topoisomerase I is illustrated by the fact, that several commercial available kits use topoisomerase I instead of DNA ligase to integrate PCR-products or other DNA fragments into plasmid DNA.

5. DNA damage response

Purified topoisomerase I is able to recognize endogenous DNA lesions such as mismatched bases, abasic sites and deaminated cytosines. This is associated with nicking of DNA at the first phosphodiester bond 5' to the DNA lesion and formation of stable DNA-topoisomerase I complexes similar to those which accumulate in the presence of camptothecin.^{56,57} Covalent DNA-topoisomerase I complexes are also formed in the proximity of cyclopyrimidine dimer lesions as well as in living cells within a few minutes after UV-irradiation.^{56,58,59} Generally, the creation of such complexes correlates with enhanced cellular survival after UV irradiation.⁵⁸

Although topoisomerase I is able to recognize at least some DNA lesions *in vitro*, it is likely that the recruitment of topoisomerase I to the site of DNA lesions in living cells is mediated by interaction with other proteins. Topoisomerase I is known to interact with both isolated and TFIID-associated TBP.⁴⁶⁻⁴⁸ It has recently has been shown that TBP/TFIID binds selectively to, and is sequestered by, cisplatin- or UV-damaged DNA in the context of a multi-protein complex that includes TFIIH.⁶⁰ Since it has been shown that topoisomerase I is recruited to the DNA template by TFIID during transcriptional initiation,⁴⁷ it is likely, that it is also TFIID which recruits topoisomerase I to the sites of DNA damage. Unexpectedly, covalent topoisomerase I binding to DNA lesions also depends on one of the components of the TFIIH complex, the XPD helicase, since cells lacking XPD are strongly deficient in their ability to recruit topoisomerase I into covalent DNA-protein complexes following UV exposure.⁵⁹ This can not be attributed to alterations of topoisomerase I activity or protein levels, since the formation of covalent DNA-topoisomerase I complexes in the presence of camptothecin is comparable with wild-type cells. At this time, it is not clear if the requirement for the XPD helicase is based on protein-protein interactions or if it is the ability of the helicase to modify the DNA structure locally which is necessary for topoisomerase I recruitment.

6. Mitotic functions

The association of topoisomerase I with mitotic chromosomes suggests a possible mitotic function for the enzyme. This is supported by genetic studies in yeast, where the catalytic activity of topoisomerase I was shown to be required for chromosome condensation.⁶¹ Similar results have been reported for *Xenopus* egg extracts, where 13S condensin, a multi-subunit protein essential for chromosome condensation, is able to introduce positive supercoils into closed circular DNA in the presence of topoisomerase I. This reaction is likely to represent a key mechanism underlying the compaction of chromatin fibers during mitosis.⁶²

Regulation of topoisomerase I activity

1. Phosphorylation/dephosphorylation

Topoisomerase I exists as a phosphoprotein in living cells, and is a substrate for casein kinase II and protein kinase C *in vitro*.⁶³⁻⁶⁶ The catalytic activity of topoisomerase I is strongly dependent on the phosphorylation state, since dephosphorylation of purified topoisomerase I abolishes the capacity of topoisomerase I to relax supercoiled DNA whereas subsequent rephosphorylation with casein kinase II or protein kinase C restores the activity.⁶³⁻⁶⁷ Finally, a hyper-phosphorylated form of topoisomerase I appears during mitosis suggesting that topoisomerase I is a substrate for one or several mitotic kinases.⁶⁸

2. Poly(ADP-ribosylation)

Poly(ADP-ribosylation) is a posttranslational modification of chromosomal proteins that is activated by DNA strand breaks caused by a variety of toxic agents.⁶⁹ Topoisomerase I is poly(ADP-ribosylated) within minutes after the occurrence of DNA lesions consistent with its involvement in the early response to DNA damage.^{69,70} This is not a permanent modification, since the poly(ADP-ribosylation) of topoisomerase I returns to control levels after 60 minutes of incubation. Poly(ADP-ribosylation) of topoisomerase I is accompanied by a decrease in the catalytic activity *in vitro* as in living cells.⁷⁰⁻⁷³ This is particularly the case for modifications with long poly(ADPR) chains while short poly(ADPR) chains do not seem to inactivate the enzyme.⁷⁴ The inhibition of topoisomerase I by poly(ADP-ribosylation) is probably strongest in the vicinity of DNA strand breakage and might serve to locally shut down DNA replication until repair has occurred.⁷⁵

3. Physical interactions with other proteins

As previously described, topoisomerase I is part of different multi-protein complexes involved in replication, transcription and DNA repair. It is therefore not surprising, that topoisomerase I can associate physically with numerous molecular partners, of which some are able to modulate the catalytic activity of the enzyme. Chromatin-associated proteins such as several high mobility group proteins (HMG1, HMG2 and, HMG17) as well as histone H1 co-sediments with eukaryotic topoisomerase I and enhance its catalytic activity.⁷⁶ Two nucleolar proteins, RNA polymerase I and nucleolin, also associate with topoisomerase I, consistent with the known role of topoisomerase I in the formation and maintenance of a functional nucleolus.^{77,78}

Both protein kinase C and casein kinase II copurify with topoisomerase I, and the interaction with casein kinase II dramatically increases the catalytic activity of topoisomerase I even in the absence of phosphorylation.^{66,79}

Of particular interest is the physical interaction between topoisomerase I and the oncogene suppressor protein p53. The activation of p53 following DNA damage is a slow response that takes several hours. In parallel with the upregulation and nuclear translocation of p53 the catalytic activity of topoisomerase I is stimulated. At least part of the activation of topoisomerase I is due to association with p53, since topoisomerase I was shown to coprecipitate with p53 in living cells. An interesting aspect of the interaction between the two proteins is that although both mutant and wild-type p53 bind to topoisomerase I, the interaction between the topoisomerase I and p53 is differently regulated in cells with wild-type and mutant p53. In cells with wild-type p53, the association between the two

proteins is tightly regulated in a spatial and temporal manner and takes place only during brief periods of genotoxic stress. In marked contrast, topoisomerase I and p53 are constitutively associated in cells, which have mutant p53. As we will discuss in the next section, these findings have important implications for both cellular stress response and genomic stability given the ability of topoisomerase I to recognize DNA lesions as well as to cause illegitimate recombination.⁸⁰⁻⁸³

Topoisomerase I in oncology: Dr Jekyll or Mr Hyde?

Topoisomerase I seems to play a double role in human oncology. On one hand, it participates in the protection of cells toward DNA damage through the formation of covalent DNA-protein complexes at, or in the vicinity of DNA lesions. The formation of these DNA-protein complexes may be necessary for the subsequent recruitment of repair enzymes since the absence of such complexes is associated with highly increased sensitivity to genotoxic agents and UV light. However, the covalent DNA-topoisomerase I complexes are also potentially dangerous to the cell due to their ability to mediate illegitimate recombination, which can lead to genomic instability and oncogenesis. Thus, the level and duration of these complexes must be tightly controlled in normal cells. One way to obtain this is to keep the cellular levels of topoisomerase I relatively constant. As discussed previously, this appears indeed to be the case for most non-transformed cells, where the number of topoisomerase I molecules stays around one million copies per cell.¹⁸ However, this is clearly not the case for transformed cells, where topoisomerase I protein levels and activities may vary over more than two logs of magnitude between different tumor types.²⁷

Some mechanisms capable of regulating the duration of the covalent DNA-topoisomerase I complex start to emerge. Upregulation and translocation of wild-type p53 is maximal 4-8 hours after DNA damage and is accompanied by stimulation of the catalytic activity of topoisomerase I and increased topoisomerase I-mediated DNA religation.⁸³ As a result, we expect the amounts of covalent DNA-topoisomerase I complexes immobilized on the damaged DNA to decrease.

It has also been suggested that the proteasome-mediated degradation of topoisomerase I is regulated differently in normal and neoplastic cells since the topoisomerase I present in covalent DNA-topoisomerase complexes is much more stable in certain tumor cells than in non-transformed cells. Since the multi-ubiquitination of topoisomerase I apparently is normal, the defect in tumor cells must lie downstream from the ubiquitin conjugation step in the ubiquitin-proteasome pathway.⁸⁴

If dysregulated topoisomerase I is a source of genetic instability this might explain why topoisomerase activities

and levels are so often altered in human tumors. Interestingly, the altered properties of the covalent DNA-topoisomerase I complexes in malignant cells may also explain why tumor cells are more sensitive to topoisomerase I inhibitors of the camptothecin family than are most normal cells. As illustrated in *Figure 1*, the formation of the cleavable complex is a central event not only in DNA damage recognition and recombination, but also in the cytotoxic action of camptothecins. These antineoplastic agents act by preventing the religation step of the catalytic cycle leading to accumulation of covalent DNA-topoisomerase I complexes which are potentially cytotoxic (for recent review, see ⁸⁵). If the topoisomerase I levels are elevated in tumor cells we would expect more drug-induced complexes to be formed, whereas the increased stability of the complexes would allow them to persist longer. Thus, the same properties of topoisomerase I may at the same time explain why this enzyme is so often dysregulated in human tumors and why tumor cells are more sensitive to the cytotoxic action of topoisomerase I-directed antitumor drugs than are normal cells, thereby providing a unifying theme for the dual role of topoisomerase I in oncogenesis and cancer therapy.

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