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A Human TOP2A Core DNA Binding X-ray Structure Reveals Topoisomerase Subunit Dynamics and a Potential Mechanism for SUMO Modulation of Decatenation

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In this issue of the *Journal of Molecular Biology*, Wendorff *et al.* describe the first structure of the active-site core of the human TOP2A protein in complex with DNA. This structure complements the X-ray model of the active core of the paralogous human protein TOP2B, which was published last year.[1] Together, these two structures provide key information that will be relevant to understanding how these closely related proteins perform different roles during cell growth and tissue development. The new structure suggests a potential rationale to selectively target drugs to a single enzyme isotype and a functional role for SUMOylation.

Enzyme structure

In organisms from bacteria to man, topoisomerases are classified by structure and mechanism as either type I enzymes that break and rejoin a single strand of DNA without a nucleotide cofactor or type II topoisomerases that are ATP dependent and break both strands simultaneously. All cellular type II topoisomerases studied to date have highly conserved structural motifs that form a two-chamber molecular machine. Linear maps comparing human TOP2A, *Escherichia coli* Top-IV, and DNA gyrase are shown in Fig. 1. The human TOP2A enzyme is composed of two homodimers with an ATP binding domain near the N-terminus that acts as a gate (ATP gate). In *E. coli*, Top-IV adopts a similar structure but is formed by a tetramer of two polypeptides, $ParC_2$–$ParE_2$. In both cases, these enzymes can catalytically remove negative (–) or positive (+) supercoils from DNA, and they are efficient at unknotting and disentangling catenated DNA circles. In step 1 of a decatenation reaction, a DNA strand called the gate or G segment (aqua) loads through the ATP gate and binds at the base of the chamber that is formed by the WHD and TOWER domains (Fig. 1, left side). The bound DNA G segment adopts a sharp bend with arms that project at angles of 130–150° in different prokaryotic and eukaryotic crystal structures. In step 2, ATP binding stimulates a second DNA strand called the transfer or T segment (green) to localize above the G segment in the upper chamber. ATP also induces ATP-gate closure by dimerization of the two ATP-bound head domains of the enzyme. The C-terminal domains or CTD (shown as gray spines) help organize the chiral crossing of DNA substrates. Whereas both (+) and (–) supercoils are relaxed by these topoisomerases, the reaction rate is faster on (+) supercoiled substrates. In step 3, the TOPRIM domain coordinates binding of magnesium ions. The metals trigger formation of a transient, covalent protein–DNA complex that involves a reversible phosphotyrosine intermediate with the same chemical energy as a phosphodiester bond. Wang was prescient in predicting this easily reversible mechanism for breaking and forming DNA phosphodiester bonds in the characterization of the ω protein, which is now known as *E. coli* TopI.[2] Formation of the covalent complex opens a gap in DNA that allows passage of the T segment into the lower chamber. This conformation shift also opens the C gate for release of the transferred strand at the bottom. In step 4, reversal of the tyrosine–DNA intermediate re-forms both phosphodiester bonds and hydrolysis of ATP resets the enzyme. In Fig. 1, two double-strand substrate circles entangled by a single catenane link

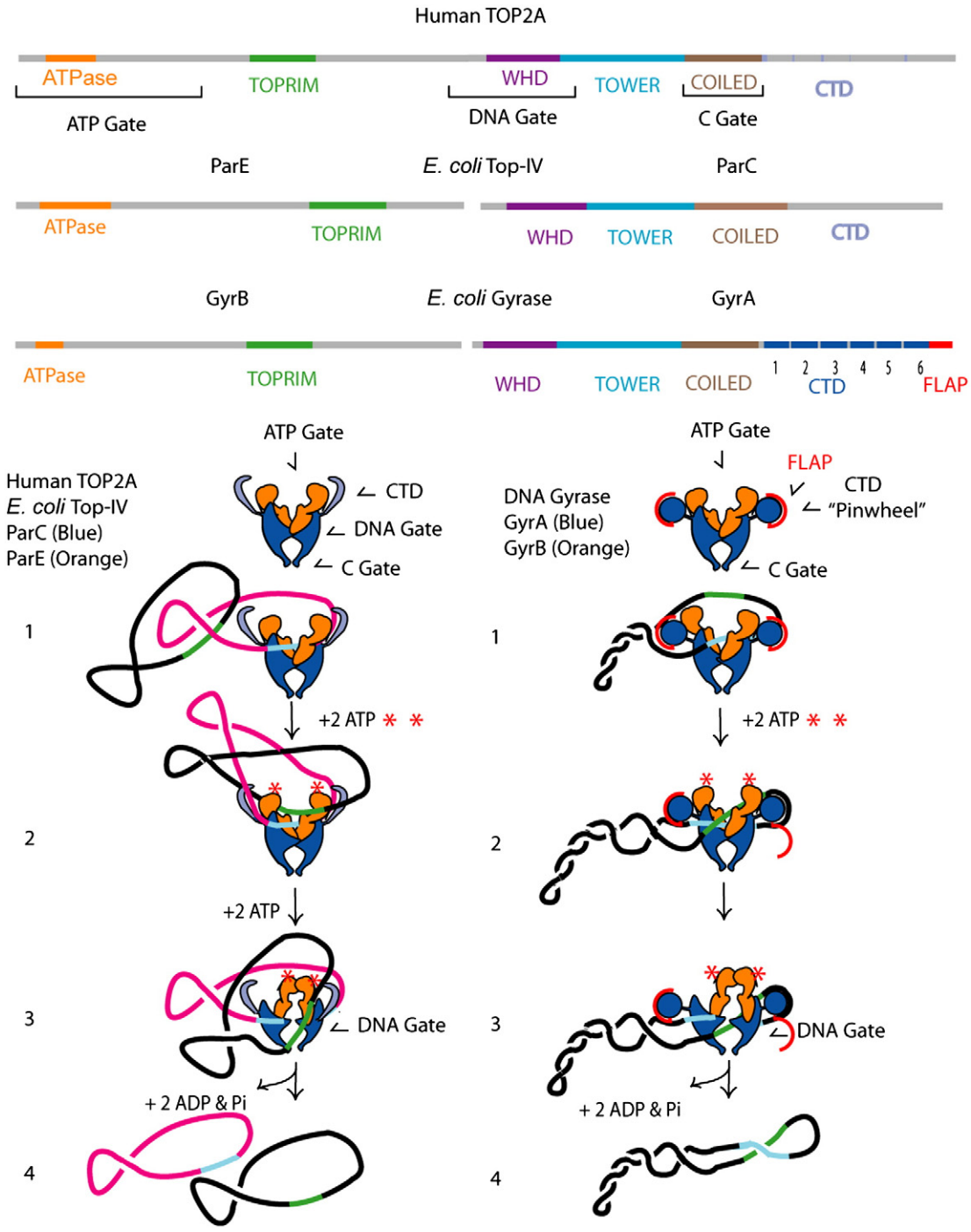

Fig. 1. Structural motifs and catalytic cycle for type II topoisomerases. Reaction steps for the catalytic cycle of human TOP2A and *E. coli* Top-IV are shown on the left and DNA gyrase is shown on the right. The unique C-terminal domain (CTD) of GyrB includes six "pinwheel" elements and an acidic terminal flap (red) that regulates tight DNA wrapping to control (–) supercoiling production (see the text for description).

are liberated as unlinked molecules. This decatenation reaction is necessary to fully separate the strands of circular bacterial chromosomes and long eukaryotic linear chromosomes that are pulled apart in metaphase.

Enzyme mechanics and modification

In the structure of TOP2A shown here, the authors note that the C gate is open. A survey of published structures of different type II topoisomerases from

bacterial and eukaryotic sources revealed that the open C-gate conformation and a rotation of the TOWER complex was correlated with structures that have a covalent attachment to DNA or that contained DNA mimics of the covalent intermediate, such as the staggered nick species that occupies the gate position in this study. Modeling suggests that rigid-body, *en bloc* movements of the WHD and TOPRIM domains may be correlated with the opening of both the DNA gate and the C gate. This would explain how a non-hydrolyzable ATP analog such as AppNHp supports a single round of supercoiling by gyrase because after covalent attachment and transfer to the lower chamber, the T segment could escape without the hydrolytic step that reopens the ATP gate.

A second feature of this structure involves the covalent addition of SUMO to lysine residues on many nuclear eukaryotic enzymes, including type I and type II topoisomerases.[3] SUMO modification can change a target protein's location, activity, stability, or ability to interact with other proteins. But this modification is removed by isopeptidase activity of multiple SUMO proteases so that it is typically only found on a small subset of any specific protein. A recent paper showed that the *Xenopus laevis* TOP2A could be SUMOylated on Lys660 by PIAS-γ SUMO ligase.[4] Interestingly, SUMOylation occurred *in vitro* only when TOP2A was bound to DNA and the modified complex is inhibited for decatenation. The Lys660 residue is in the DNA-binding site and accessible to modification in this X-ray structure. One explanation is that SUMO is designed to control decatenation in mitotic chromosomes. Following replication, sister chromosome bivalents are held together by cohesin and by catenated links between sister chromatids. At metaphase, each chromosome pair is aligned on the metaphase plate and attached to spindle fibers that pull each pair apart in anaphase and telophase to form new nuclei in the daughter cells. Cohesin is released from sister chromatids by a protease called separase, and a SUMO isopeptidase may be necessary to trigger decatenation activity of SUMO-blocked TOP2A complexes.

Enzyme specialization: replication *versus* transcription

The type II topoisomerase paralogs of both bacteria and higher eukaryotes appear to have specialized to either support DNA dynamics of chromosome replication and segregation or ameliorate topological problems related to transcription. The replication problem involves eliminating (+) supercoils that accumulate ahead of replisomes and untangling the sister chromatids to allow segregation. A topological problem with transcription was predicted by Liu and Wang, who suggested that the DNA duplex must rotate (relative to the cytoplasm) due to the movement and inertial mass of RNA polymerase.[5] This model predicted that RNA polymerase would generate "twin domains" of supercoiling with (–) supercoils accumulating in DNA segment behind the promoter and (+) supercoils appearing downstream of transcription terminators in highly expressed loci. This prediction was recently confirmed at specific regions of high transcription in live bacterial cells.[6]

The human TOP2A enzyme appears to be specialized for replication. It is most abundant in rapidly dividing and undifferentiated cells. Etoposides that stabilize the TOP2A covalent intermediate lead to chromosome breaks during DNA replication; these compounds are leading chemotherapeutic drugs for killing rapidly dividing and relatively undifferentiated cancer cells. But there is a downside to etoposide treatment. These drugs also block TOP2B, which appears designed for transcription. In a mouse model, the incidence of VP-16-induced melanoma was much higher in TOP2B+ cells than in TOP2B knockouts, strongly suggesting that some form of topoisomerase lesion can cause as well as cure cancer.[7] Transcription is implicated in the process that stimulates chromosome breaks in these differentiated skin cell populations. More recently, the formation of etoposide-induced, therapy-related acute myeloid leukemia was discovered to involve balanced chromosome translocations. The model that best explained this result is that gene pairs undergoing expression in transcription "factories" became trapped in TOP2B–drug complexes, and these breaks cause balanced translocations that lead to development of the rare MLL.[8] Therefore, TOP2A causes etoposide-related damage during DNA replication and TOP2B causes etoposide-stimulated chromosome breaks during transcription.

Recent work in bacteria suggests a similar division of labor between Top-IV and DNA gyrase. Similar to human TOP2A, *E. coli* Top-IV is the primary decatenation enzyme in bacteria—the enzyme selectively eliminates (+) supercoils that accumulate before the fork in bacterial replication and untangles catenated links between sister chromosomes. Gyrase has recently been linked to transcription. Gyrase introduces negative supercoils by virtue of a specialized C-terminal domain that contains five segments called pinwheel elements (blue) and a terminal acidic flap (red) shown in the Fig. 1 map. The gyrase supercoiling cycle differs from the TOP2B/TOP-IV pathway in one important way. Step 1 is the same, but in step 2, the binding of ATP (*) rearranges the C-terminal flap that exposes the DNA-binding activity of the C-terminal pinwheels.[9,10] Wrapping of DNA in a tight loop over one CTD (blue circle) positions the T segment (green) as a (+) chiral cross above the G segment

(aqua). Steps 3 and 4 are the same as the TOP-IV reaction except that each gyrase cycle adds two (–) supercoils to the DNA substrate.

The speed of RNA transcription during bacterial growth varies with temperature. In wild-type *E. coli*, the transcription elongation rate in rich medium is 90 nt/s at 37 °C but is only 50 nt/s at 30 °C. These rates obey the Q10 rule, where chemical reactions increase twofold for each 10 K increase in temperature. At 30 °C when the average transcription rate is 50 nt/s, wild-type gyrase can processively supercoil DNA at four to five supercoils per second.[10] Together, the rates of RNA polymerase elongation and gyrase-catalyzed supercoiling establish the average supercoil density across the genome. In gyrase mutants that have a slow catalytic rate, growth at the permissive temperature of 30 °C causes the disappearance of (–) supercoiling from the chromosome.[11] In chromosomes lacking supercoils, the transcription rate falls from 50 to 30 nt/s, showing that the rates of transcription and supercoiling are linked. The separation of Watson and Crick strands appears to be a barrier to forward movement of RNA polymerase, and negative supercoiling reduces the burden, allowing transcription and growth to proceed at faster rate limits. Different species of bacteria have considerably different average levels of (–) supercoiling[12] and different maximal doubling times. Thus, in *E. coli* as in elephants, one type II topoisomerase (Top-IV and TOP2A, respectively) is specialized for replication/segregation while the other topoisomerase (gyrase and TOP2B) is optimized to manage the topological problems of transcription.[11]

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