PROSPECT
Journal of Cellular Biochemistry 110:35–43 (2010)

Journal of Cellular Biochemistry

Replication Initiation and DNA Topology: The Twisted Life of the Origin

E. Rampakakis, C. Gkogkas, D. Di Paola, and M. Zannis-Hadjopoulos*

Goodman Cancer Centre, Department of Biochemistry, 3655 Promenade Sir William Osler, Montreal, Quebec, Canada H3G 1Y6

ABSTRACT

Genomic propagation in both prokaryotes and eukaryotes is tightly regulated at the level of initiation, ensuring that the genome is accurately replicated and equally segregated to the daughter cells. Even though replication origins and the proteins that bind onto them (initiator proteins) have diverged throughout the course of evolution, the mechanism of initiation has been conserved, consisting of origin recognition, multi-protein complex assembly, helicase activation and loading of the replicative machinery. Recruitment of the multiprotein initiation complexes onto the replication origins is constrained by the dense packing of the DNA within the nucleus and unusual structures such as knots and supercoils. In this review, we focus on the DNA topological barriers that the multi-protein complexes have to overcome in order to access the replication origins and how the topological state of the origins changes during origin firing. Recent advances in the available methodologies to study DNA topology and their clinical significance are also discussed. J. Cell. Biochem. 110: 35–43, 2010.

KEY WORDS: DNA REPLICATION; REPLICATION ORIGINS; DNA TOPOLOGY; TOPOISOMERASE; CHROMATIN STRUCTURE

The initial description of the double helical structure of the DNA molecule by Watson and Crick indicated a possible mechanism of duplication for the cellular genetic material, but also suggested another dimension of complexity of the genetic code, which expands beyond the linear succession of nucleotides and includes the unique structural and topological features of DNA [Watson and Crick, 1953]. Under physiological conditions, the two complementary DNA strands form a right-handed helix with a pitch of 10.5 base pairs, each one of which is rotated by 36 degrees from its adjacent pair (B-DNA). Rotation of the helix around its axis leads to the addition or subtraction of twists, which may result in the overwinding (positive supercoiling) or unwinding (negative supercoiling) of the molecule (Fig. 1). In the case of circular DNA or DNA molecules with fixed nuclear attachments supercoiling can be manifested either as a local twist (Tw) or as a three-dimensional writhe (Wr) of the helix and is described by the linking number (Lk), which represents the number of times one of the two strands wraps around the other on a planar surface ($\Delta Lk = \Delta Tw + \Delta Wr$) [Boles et al., 1990]. These contortions along with the organization of DNA into nucleosomes result in the tight packaging of the genetic material and render its confinement to the nucleus possible. Supercoiling further contributes to the maintenance of DNA in a knot-free conformation [Burnier et al., 2008] into the nucleus opposing its propensity to become knotted [Arsuaga et al., 2002; Raymer and Smith, 2007].

Negative supercoiling is physiologically important since it facilitates the local melting of the DNA duplex, allowing access to *trans*-acting factors such as transcription, DNA replication and DNA repair factors. Various physiological cellular processes, such as the DNA strand unwinding associated with the function of DNA and RNA polymerases, chromosome segregation during cell division, and chromatin assembly and remodeling, generate strong torsional forces along the DNA axis, resulting in positive supercoiling. Notwithstanding, DNA is homeostatically maintained in an underwound state in all species, facilitating access to the genetic code [Zechiedrich et al., 2000; Travers and Muskhelishvili, 2007]. How do the cells manage to reset the topological status of the DNA and revert it to an underwound state? The answer was revealed with the discovery of DNA topoisomerases, first in *Escherichia coli* [Wang, 1971] and afterwards in all species throughout evolution.

DNA TOPOISOMERASES

DNA topoisomerases are ubiquitous nuclear enzymes that manage the topological state of DNA [reviewed in Nitiss, 2009a]. They

*Correspondence to: Dr. M. Zannis-Hadjopoulos, Goodman Cancer Centre, McIntyre Building, room 709, 3665 Promenade Sir William Osler, Montreal, Quebec, Canada H3G 1Y6. E-mail: maria.zannis@mcgill.ca
Received 27 January 2010; Accepted 29 January 2010 • DOI 10.1002/jcb.22557 • © 2010 Wiley-Liss, Inc.
Published online 8 March 2010 in Wiley InterScience (www.interscience.wiley.com).

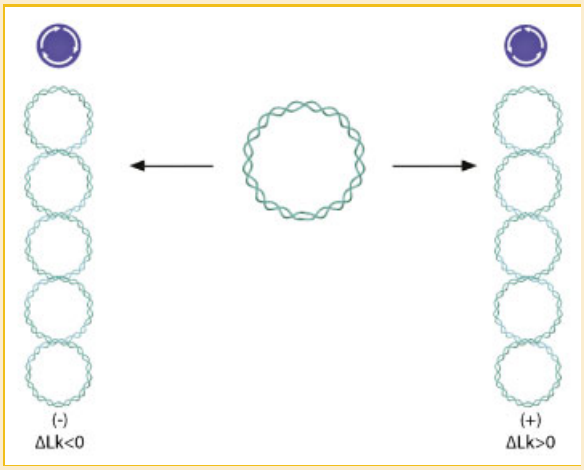

Fig. 1. DNA Superhelicity. Generation of torsional stress along the DNA helix induces rotational forces which result in the subtraction ($\Delta L_k < 0$) or addition ($\Delta L_k > 0$) of helical twists. Schematics of negatively (−) and positively (+) supercoiled forms of DNA are depicted, however intermediate states of supercoiling are also observed under physiologic conditions (not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

achieve this through their unique ability to transiently cleave and reseal the phosphodiester backbone via a transesterification reaction. This reaction involves two sequential nucleophilic attacks: (i) an initial nucleophilic attack of an active-site tyrosyl oxygen of the enzyme on a DNA phosphate, followed by the formation of a covalent 3′ phosphotyrosine bond and the simultaneous breakage of the phosphodiester bond; and (ii) strand rejoining through the nucleophilic attack of the 5′-hydroxyl group, generated in the first transesterification reaction, on the 3′ phosphotyrosine link and subsequent restoration of the DNA backbone bond.

There are two families of DNA topoisomerases, type I and type II, which create a single- or double-strand DNA break respectively. Type I topoisomerases do not need ATP for their activity and are subdivided into IA and IB subfamilies, depending on the DNA backbone phosphate they attach to (5′ and 3′ respectively). Enzymes of the IA subfamily have an "enzyme-bridging" mechanism, which involves the passage of a single DNA strand through the enzyme-bridged cleavage of the opposite strand [Tse and Wang, 1980], while type IB topoisomerases use a "strand rotation" mechanism, whereby the duplex rotates freely around its axis [Stewart et al., 1998]. Type II topoisomerases (IIA and IIB), on the other hand, function via a "cross-inversion" mechanism, involving the passage of a double-stranded DNA region through a double-stranded gap of the same or different DNA molecule, which requires ATP hydrolysis [Roca et al., 1996]. The aforementioned mechanistic differences of the various topoisomerases are nicely mirrored in their structure, with enzymes belonging to the same subfamily structurally resembling each other, while members of distinct subfamilies differing. For an excellent review of the biochemical characteristics and structures of the various topoisomerases please refer to Champoux [2001].

INITIATION OF DNA REPLICATION AND DNA TOPOLOGY

Although the role of DNA topoisomerases in replication fork progression, chromosome segregation, and transcription has been extensively studied [reviewed in Nitiss, 2009a], recent molecular studies have significantly expanded their repertoire of functions. In this review we focus on their less characterized role in the initiation of DNA replication, scanning throughout evolution in order to possibly uncover an evolutionarily conserved function of DNA topoisomerases.

EUBACTERIA

Chromosome replication in bacteria initiates from a single origin, which is recognized by the initiator protein DnaA, a member of the AAA+ (ATPases associated with various cellular activities) protein superfamily. Multiple DnaA molecules bind a series of defined 9-bp DnaA boxes [Messer, 2002] and recruit the DnaB replicative helicase onto the replication origin [Funnell et al., 1987], assisted by the DnaC helicase-loader protein [Davey et al., 2002]. Subsequent binding of the DnaG primase and replisome assembly leads to origin activation and initiation of bi-directional replication [Fang et al., 1999].

DNA replication initiation in plasmid DNA requires a negatively supercoiled template conformation [Funnell et al., 1987; Crooke et al., 1991] and the transcription-induced introduction of negative supercoils within the *E. coli* oriC enhances its activation [Asai et al., 1992]. Furthermore, inhibition of the enzymatic activity of bacterial gyrase, the only known topoisomerase able to generate negative supercoiling, results in decreased initiation of DNA replication in *Bacillus subtilis* [Ogasawara et al., 1979] and *E. coli* [Gellert et al., 1976]. Formation of a DnaA-negatively supercoiled DNA nucleoprotein complex leads to the unwinding of a nearby AT-rich DNA unwinding element, which is thought to facilitate the assembly of the multi-protein replisome. The resolution of the crystal structure of the ATP-bound DnaA provided much insight into the underlying mechanism [Erzberger et al., 2006]; binding of ATP by DnaA was found to induce a conformational switch, which resulted in the formation of a right-handed helical filament by multiple DnaA molecules arranged in head-to-tail manner. This right-handed ATP-DnaA spiral is able to stabilize DNA into a positive-handed wrap, which is thought to induce a compensatory negative writhe and relaxation of the neighboring unstable DUE. This strand separation process is antagonized by the binding of the SeqA protein, which restrains the negative supercoils induced by DnaA at the OriC plasmid and inhibits the initiation of DNA replication [Torheim and Skarstad, 1999].

VIRAL SYSTEMS

Simian virus 40 (SV-40). Replication of the SV-40 DNA involves an 8–10 min-lag before initiation, which is known as the pre-synthesis stage. During this stage, formation of an initiation complex containing the virally encoded large tumor (T) antigen, replication protein A (RPA) and topoisomerase I and/or II, takes place onto the origin [Tsurimoto et al., 1989]. Subsequent

recruitment of additional host proteins such as polymerase α-primase, PCNA and RFC results in the initiation of DNA synthesis.

The process of initiation leading to the assembly of the replication machinery in SV-40 is associated with changes in the topological conformation of the origin DNA, which resemble the DnaA/B/C assembly in *E. coli* [Roberts, 1989]. In the presence of ATP, T-antigen interacts with the origin core, forming a double-hexamer structure [Mastrangelo et al., 1989], which results in the untwisting and partial melting of the origin [Parsons et al., 1990; Dean and Hurwitz, 1991]. Addition of purified human topoisomerase I to an SV-40 T-antigen-driven in vitro replication system resulted in a threefold induction of DNA synthesis due to enhanced initiation of DNA replication. Part of the stimulation of origin activation induced by topoisomerase I was found to be mediated through direct interaction with T-antigen [Trowbridge et al., 1999]. Origin-association of T-antigen however, was found to be unaffected by the addition of DNA topoisomerases [Halmer et al., 1998]. Instead, topoisomerase I was found to bind to T-antigen double-hexamers already associated with SV-40 origin DNA, and participate in the unwinding reaction [Gai et al., 2000] and RPA recruitment [Simmons et al., 2004] during initiation complex formation. Interestingly, Halmer et al. [1998] showed that the addition of topoisomerases stimulated the initiation of SV-40 DNA replication from chromatin but not from protein-naked DNA molecules, leading them to suggest that the effect of topoisomerases on initiation complex assembly may be exerted through their ability to diffuse the positive supercoils accumulated at nucleosome-rich origin regions due to the unwinding of origin DNA by T-antigen.

Papillomavirus (PV). Replication of the small double-stranded PV genome requires two virally encoded proteins, E1 and E2 [Ustav and Stenlund, 1991], and a number of factors of the host replication machinery such as RPA and DNA polymerase α-primase [Masterson et al., 1998; Loo and Melendy, 2004]. The E1 protein in its double-hexameric form represents the PV replicative helicase, which unwinds the double-stranded DNA ahead of the replication fork [Sedman and Stenlund, 1998], and its origin recruitment is affected by the E2 protein which binds to DNA in proximity and increases the sequence-specificity of E1 for the origin [Seo et al., 1993].

Human topoisomerase I was shown to associate with E2 and this interaction resulted in the stimulation of the topoisomerase I relaxation activity by three- to fourfold, while E2 origin binding remained unaffected [Clower et al., 2006]. Furthermore, Hu et al. [2006] demonstrated that topoisomerase I and E1 form a complex in the absence of DNA, which leads to the stimulation of the origin-binding of E1 by several fold. This effect was found to be highly specific, since E1 binding to non-origin DNA remained unaffected, and non-synergistic with the stimulation by E2. Altogether, these results suggest that topoisomerase I may participate in the assembly of the PV initiation complex in a dual way, affecting the topological relaxation of the origin as well as altering the E1 structure into a more favorable origin-binding conformation.

Herpesvirus. Lytic DNA replication of a herpesvirus initiates at an origin (*ori-Lyt*), which is recognized and bound both by virally-encoded and host cellular *trans*-acting factors. Kaposi's sarcoma-associated herpesvirus (KSHV) contains two copies of *ori-Lyt*, referred to as *ori-Lyt (L)* and *ori-Lyt (R)* [AuCoin et al., 2002], onto which a replication initiaton complex is recruited, containing a DNA polymerase (POL), a polymerase processivity factor (PPF), a single-stranded DNA binding protein (SSB), a trimeric helicase-primase complex (HEL, PRI, PAF) and the K8 and RTA regulatory proteins [Wu et al., 2001; AuCoin et al., 2004].

A recent study, using a DNA-affinity purification procedure to isolate host cellular proteins that bind to the KSHV *ori-Lyt*, identified both topoisomerases I and II among other proteins [Wang et al., 2008]. All the aforementioned identified proteins were found to accumulate in viral replication compartments in the nucleus, suggesting a role in the viral DNA replication. Furthermore, inhibition of the topoisomerase I and topoisomerase II activities blocked *ori-Lyt*-dependent DNA replication, indicating an essential role in the initiation of KSHV lytic DNA replication.

A similar role for topoisomerases I and II was also suggested for the replication of the closely related Epstein–Barr virus (EBV); blockage of their enzymatic activity using campthotecin and ellipticine, respectively, also led to inhibition of progeny EBV DNA in superinfected Raji cells [Kawanishi, 1993]. However, this study was not able to differentiate whether the initiation or elongation stage of DNA replication, or both, was affected by these treatments.

YEAST

***Schizosaccharomyces pombe* (Sp).** Eukaryotic DNA replication initiates with the stepwise assembly of a multiprotein initiation complex onto the origins, the pre-Replication Complex (pre-RC). The origins are first bound by the hexameric Origin Recognition Complex (ORC), which, in turn, recruits upon the chromatin the Cdc18 (yeast homologue of the metazoan Cdc6) and Cdt1 proteins. The resulting complex is then responsible for the ATP-dependent loading of the replicative helicase, the minichromosome maintenance protein complex (MCM2-7), giving rise to a functional pre-RC.

Although SpORC does not bind replication origins sequence-specifically it exhibits a clear preference for A/T rich tracks [Chuang et al., 2002]. This preference is due to the N-terminal binding domain of the ORC subunit 4 (SpORC4), which contains nine copies of the HMG-I (Y)-related AT-hook motif, known to bind to the minor groove of A/T-rich DNA stretches in a sequence-non-specific manner [Chuang and Kelly, 1999]. In agreement, the *A*utonomously *R*eplicating *S*equences (ARS) in *S. pombe* have an unusually high A/T content [Kelly and Brown, 2000]. SpORC binds preferentially to negatively supercoiled DNA and its recruitment to the ars1 replication origin occurs in a biphasic manner [Houchens et al., 2008], whereby an initial salt-sensitive SpORC-DNA complex is formed rapidly, presumably due to tethering of the SpORC to the origin DNA by the SpORC4 N-terminus, followed by a more-stable, salt-resistant protein-DNA complex that involves additional non-electrostatic interactions. Origin association of SpORC was associated with the induction of a topologic distortion at the origin area, possibly through the wrapping of DNA around SpORC [Gaczynska et al., 2004], which was further enhanced by the recruitment of SpCdc18 and SpCdt1, facilitating localized DNA unwinding and potentially the loading of the MCM helicase.

Altogether, these data indicate that SpORC exhibits a high preference for negatively supercoiled DNA and that pre-RC

assembly in *S. pombe* is associated with drastic changes in the topologic structure of the origin, reminiscent of the *E. coli* DNA replication.

***Saccharomyces cerevisiae* (Sc).** In contrast to SpORC, ScORC binds in a sequence-specific manner to the *A*RS *C*onsensus *S*equence (ACS) [Broach et al., 1983], and mutation of this element results in reduced ScORC recruitment and origin activation [Bell and Stillman, 1992]. This intrinsic biochemical difference ensures tethering of ScORC to replication origins and obviates the necessity for affinity to negatively supercoiled DNA in *S. cerevisiae*. Nonetheless, the ARS1 region covered by ScORC in vivo is under torsional strain [Diffley and Cocker, 1992]. Furthermore, using an in vivo UV photofootprinting method, Fujita et al. showed that topologic changes in the structure of replication origins take place during pre-RC assembly in *S. cerevisiae* as well, as a result of the ORC–ACS interaction, which are further stimulated by the Cdc6 and MCM5 recruitment [Fujita et al., 1998]. Altogether, these findings indicate that certain aspects of this topologic remodeling mechanism have been conserved in *S. cerevisiae*, albeit downstream of ScORC binding onto the origin.

DROSOPHILA MELANOGASTER

Similar to SpORC, *Drosophila melanogaster* ORC (*Dm*ORC) was shown to exhibit only mild sequence specificity, with relative affinity for DNA fragments of different sequence varying from one- to sixfold [Remus et al., 2004]. However, at physiologically relevant conditions, binding of DmORC to DNA was dependent on the degree of superhelicity, exhibiting a ~30-fold preference for negatively supercoiled DNA over linear or relaxed DNA. This preference was found to be highly specific for locally twisted, negatively supercoiled DNA, since the affinity of DmORC for positively supercoiled DNA of the same writhe was comparable to that for linear DNA. Finally, Remus et al. demonstrated that the association of DmORC to negatively supercoiled DNA is accompanied by topological changes in the DNA corresponding to ΔL_k equivalent to -1. These changes may contribute to local unwinding of the origin DNA, assisting in the recruitment of the replicative helicase (MCM2-7), similar to DnaB in eubacteria. Using a nuclease digestion approach for the detection of single-stranded DNA (ssDNA), the authors were unable to detect DmORC-dependent unwinding of negatively supercoiled DNA; this result, however, may reflect the coverage of ssDNA by ORC or the inability of this experimental approach to detect short stretches of ssDNA.

XENOPUS

Initiation of DNA replication in *Xenopus laevis* follows the common eukaryotic scheme, with ORC conducting the pre-RC assembly, which is followed by transition to a pre-Initiation Complex (pre-IC) and loading of the replisome. Similar to SpORC, XlORC does not exhibit strict DNA sequence specificity and preferentially associates with A/T-rich regions [Kong et al., 2003]. Using the Xenopus egg in vitro system Kong et al. demonstrated that SpORC and XlORC compete for the same AT-rich DNA sequences and SpORC could recruit XlCdc6 and XlMCM onto these sequences, suggesting surprising similarities in the properties of ORC between the two organisms.

During early embryonic development, when rapid proliferation takes place, *Xenopus* cell cycles consist of alternating S and M phases, without G_1 and G_2. At this stage, major rearrangements of chromatin organization occur during mitosis in each embryonic cell division, which include the shortening of the DNA loops attached to the nuclear matrix [Lemaitre et al., 2005], previously known to participate in the localization of replication origins during eukaryotic replication [Vogelstein et al., 1980; Dijkwel et al., 1991]. Lemaitre et al. showed that this mitotic remodeling prepares chromatin for the subsequent S phase by enhancing its ability to bind ORC, and is topoisomerase II-dependent since it is blocked by the addition of the topoisomerase II inhibitor ICRF 193 [Lemaitre et al., 2005].

In a subsequent study, topoisomerase II was found to participate in the resetting of replicons in *Xenopus* eggs during the S-M transition by clearing ORC1/2 from chromatin, through interacting with the peptidyl-prolyl isomerase *Pin1* [Cuvier et al., 2008]. However, using DNA combing the authors found that addition of ICRF during early S phase did not affect the initiation of DNA synthesis and the initial rates of DNA synthesis, suggesting that topoisomerase II activity is not required at this stage. On the other hand, a different study using intercalating agents, which release supercoiling of the DNA, showed a negative effect for DNA relaxation on the initiation of DNA replication [Krasinska and Fisher, 2009]; addition of ethidium bromide at high doses or doxorubicin for prolonged periods of time, disrupted the assembly of the nuclear envelope and lamina as well as the decondensation of DNA, and resulted in decreased initiation of DNA replication due to defective activation, but not loading, of the pre-IC. Interestingly, addition of echinomycin, a bis-intercalating quinoxaline, previously known to remove negative supercoils [Wakelin and Waring, 1976], which resulted in weaker intercalation and did not affect nuclear envelope formation, still inhibited DNA replication in a dose-dependent manner, indicating that additional parallel mechanisms of inhibition of replication initiation may exist. In agreement, addition of ethidium bromide at 60 min, upon incubation of DNA with extracts to allow prior assembly of the pre-RC, had a smaller effect on DNA synthesis, compared to its addition from the beginning, before pre-RC and pre-IC assembly. The results of this study could be interpreted by a model where disruption of negative DNA supercoiling may delay pre-RC and/or pre-IC assembly; at later stages however, upon prolonged treatment with ethidium bromide, when assembly of the multiprotein complexes has finished, but formation of the nuclear envelope is blocked, defective activation of pre-IC becomes the limiting factor for the initiation of DNA replication.

Altogether, these results indicate a role for topologic remodeling of the DNA during both the initiation and termination of DNA replication in *Xenopus*. The inability of Cuvier et al. to detect an impact on the initiation of DNA synthesis by DNA combing, when using ICRF in early S phase, could be due to the fact that topoisomerase II is required at a previous stage, such as during pre-RC assembly. However, when ICRF was added in the in vitro reaction from the beginning, total DNA synthesis was unaffected, suggesting that DNA topology does not participate in this process. Alternatively, DNA synthesis may be unaffected by topoisomerase II

inhibition due to the functional redundancy between DNA topoisomerases I and II (topoisomerase I substituting in the absence of the topoisomerase II enzymatic activity), which is, however, unmasked when using intercalating reagents that affect global DNA supercoiling.

MAMMALS

Purified recombinant human ORC (HsORC) does not exhibit any sequence-specific binding but, similar to SpORC, it does show a preference for A/T-rich sequences [Vashee et al., 2003]. Although a requirement for negatively supercoiled DNA has not been demonstrated for origin binding by mammalian ORC, a role for DNA topology during origin activation has been shown.

Addition of a DNA gyrase inhibitor to Chinese hamster ovary cells resulted in inhibition of replication initiation, while having little effect on chain elongation [Mattern and Painter, 1979]. More recently, Abdurashidova et al. [2007] demonstrated that both topoisomerases I and II bind onto the lamin B2 replication origin in a cell cycle modulated manner. This interaction was found to be very dynamic, with the two enzymes being recruited specifically at the origin at different times during G_1 phase, but never being present onto it at same time. Topoisomerase I was detected on the lamin B2 origin during early G_1 phase and at the G_1/S transition, while topoisomerase II appeared at the region covered by the pre-RC during mid-G_1 phase. These results led the authors to suggest a model, whereby topoisomerase I plays a role in origin definition during early G_1 phase by affecting the ORC2 targeting to the origin, while topoisomerase II participates in the maintenance of a favorable local DNA topology during pre-RC assembly [Falaschi et al., 2007]. Moreover, the association of topoisomerase I with the origin was essential for the initiation of DNA replication at the G_1/S border, probably by participating in the origin unwinding and commencement of fork movement. In agreement, activation of both the early-firing lamin B2 and the late-firing hOrs8 origins was found to be associated with the generation of topoisomerase II-dependent transient dsDNA breaks, specifically at the origin area, during early- and mid-G_1 phase [Rampakakis and Zannis-Hadjopoulos, 2009]. Pharmacologic inhibition of topoisomerase II blocked the formation of these breaks and resulted in prolonged G_1 phase due to defective ORC assembly and Cdt1 recruitment. When the topoisomerase II inhibitor was added after the recruitment of these proteins, no effect was observed in the activation of the two origins, suggesting the involvement of topoisomerase II in the pre-RC assembly step. These results were further corroborated by another recent study, which showed that the association of topoisomerase II and HsORC with chromatin occurs within a proximity of 600 bp, and that both proteins are enriched at the UPR replication origin of the human MCM4 gene, compared to origin-distal sequences [Hu et al., 2009].

Altogether, these results indicate a role for DNA topoisomerases in mammalian DNA replication, with topoisomerases I and II participating in distinct but complementary phases during the initiation process. Topoisomerase I seems to be mainly implicated in establishing a favorable topologic configuration for origin selection and activation during early- and late-G_1 phase, respectively. Topoisomerase II, on the other hand, functions in maintaining this permissive structure for the completion of the pre-RC assembly during the origin decision point, either by maintaining a basal level of supercoiling necessary for recruitment of initiator proteins or by relaxing the negative supercoiling known to be induced by HsORC during origin binding [Houchens et al., 2008].

MECHANISM OF ACTION

A number of possible mechanisms can be postulated for the establishment of an origin-characteristic topological structure, which affects the recruitment of replication initiator proteins (Fig. 2). In *E. coli*, as mentioned above, negative supercoiling is maintained by the enzymatic activity of gyrase (Fig. 2A), but a similar mechanism does not exist in eukaryotes, since no enzymatic

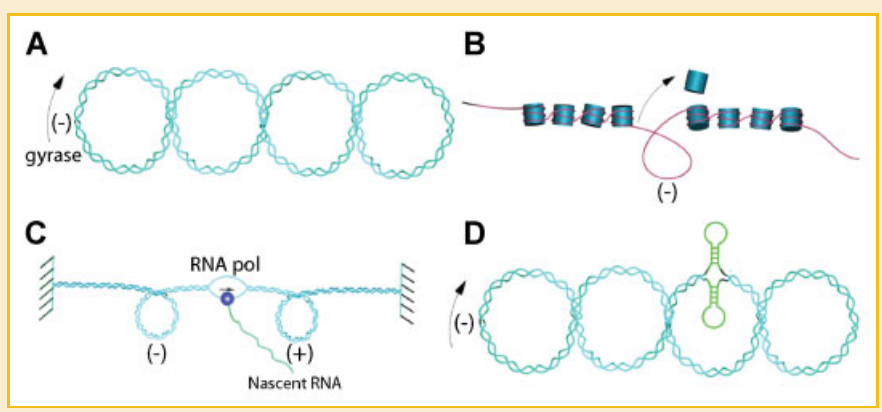

Fig. 2. Possible mechanisms of replication initiator protein recruitment. A: Enzyme-mediated introduction of negative DNA supercoils. Bacterial DNA gyrase and archaeal reverse gyrase are examples of enzymes with such properties. B: Displacement of nucleosomes from a chromosomal region by chromatin remodeling factors may induce the transient formation of negatively supercoiled DNA. C: Transcription of origin-proximal genes by RNA polymerase creates a positive superhelical stress ahead of the transcription machinery, which may result in the transmission of compensatory negative supercoils to the region behind it. Adopted from Hirose and Ohta [1990]. D: Generation of torsional stress may stabilize alternative DNA structures such as cruciforms forming at or near replication origins, which serve as signals for protein recognition [reviewed in Pearson et al., 1996]. Upon cruciform extrusion, supercoiled DNA is in turn partially relaxed at a rate of one superhelical turn per 10.5 bp of DNA [Sinden, 1994]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

activity with the property to introduce negative supercoils in the DNA has been yet discovered [Champoux, 2001]. Instead, transient local alterations in the topology of eukaryotic chromosomal DNA may be accomplished through nucleosome displacement by chromatin remodeling complexes (Fig. 2B). Removal of a nucleosome might lead to the transient formation of negative supercoils, which would in turn act as bait for initiator proteins with high affinity for negatively-supercoiled DNA, such as *Dm*ORC or *Sp*ORC [Remus et al., 2004; Houchens et al., 2008]. Alternatively, DNA unwinding during other biologic processes, such as transcription of nearby genes, might lead to the generation of positive supercoils in circular DNA molecules as well as in linear molecules with nuclear attachments or high nucleosomal density, which inhibit the free rotation of the DNA helix (Fig. 2C). Although affinity for positively supercoiled DNA has not been reported yet for any of the replication factors, rotation of the transcription machinery around the helical axis would result in the transmission of negative supercoils to the region behind it, thus enabling the binding of initiator proteins to origins that are located in this region.

Recruitment of replication licensing factors might also be mediated by indirect mechanisms, instead of direct interaction with supercoiled DNA. The torsional energy stored in supercoiled DNA can influence the geometry of the DNA either locally or globally [reviewed in Sinden, 1994]. At the local level, negatively supercoiled DNA has been shown to stabilize alternative DNA structures such as cruciforms, left-handed Z-DNA and triple-helical H-DNA, which serve as targets for protein binding. Among these, cruciforms have been shown to dynamically extrude during G_1 phase [Ward et al., 1990] and to localize at or near replication origins [Bell et al., 1991; Pearson et al., 1996]. It is therefore reasonable to hypothesize that the transient stabilization of cruciforms during the cell cycle may serve as regulatory signals for initiator protein(s) binding during DNA replication (Fig. 2D), such as topoisomerase II which has been shown to specifically recognize cruciforms [West and Austin, 1999; Rene et al., 2007].

TECHNIQUES

The development of new techniques to study the interaction of topoisomerases with replication origins and their activity there has been recently described, allowing for a more detailed analysis of their function during DNA replication. Topoisomerase poisons inhibit the strand-rejoining reaction involved in the enzymes' mechanism of action (see DNA Topoisomerases Section above), resulting in the stabilization of covalent DNA phosphate-enzyme intermediates (3′-phosphate-enzyme and 5′-phosphate-enzyme intermediates generated by topoisomerase I and topoisomerase II poisons, respectively). Using a combination of enzyme-freezing with topoisomerase poisons and PCR-based amplification procedures, Abdurashidova et al. [2007] were able to map, with nucleotide precision, the interaction of topoisomerases with replication origin sites [Falaschi, 2009]. In the case of topoisomerase I mapping, the experimental procedure entails a multi-step, ligation-mediated polymerase chain reaction (LM-PCR), involving the ligation of an asymmetric linker to the DNA intermediate, template amplification, hot extension, and final visualization of the product by autoradiography upon DNA sequencing electrophoresis. Similarly, for the determination of the topoisomerase II sites, the DNA intermediate is ribotailed at the 3′-end by terminal deoxytransferase (TdT), ligated to an asymmetric linker complementary for the rG tail, followed by PCR amplification, hot extension and sequencing, as described above [Falaschi, 2009]. This technique may be modified to use any topoisomerase poison and be applied to any origin with known DNA sequence, representing a powerful tool for the mapping of topoisomerase sites at replication origins.

In a different study, Ju et al. [2006] developed a protocol, which is able to detect formation of DNA breaks at specific chromosomal sites. This approach involves the labeling of DNA ends with biotin-deoxyuridine triphosphate (dUTP), using the enzymatic activity of TdT, and subsequent ChIP analysis of the biotinylated DNA. Incorporation of biotin during this process at specific DNA regions can then be determined by PCR using sequence-specific primers. Finally, blockage of these breaks upon inclusion of a topoisomerase inhibitor indicates the involvement of these enzymes in regulating the DNA topology at this chromosomal area. Although this method was initially used to demonstrate the estrogen-dependent restructuring of the pS2 gene promoter by topoisomerase IIβ during its activation, it could be extended to any region of the genome with known sequence. Recently, a slightly modified version of this method was used to detect the topoisomerase II-dependent generation of transient DNA breaks at the human lamin B2 and hOrs8 replication origins during pre-RC assembly [Rampakakis and Zannis-Hadjopoulos, 2009], validating this technique as a useful tool for the study of the function of topoisomerases during the initiation of DNA replication.

In addition to the above aforementioned techniques, a number of recently developed single-molecule approaches are already in use for the study of structural changes in DNA molecules under torsional stress, as well as the characterization of the function of topoisomerases [reviewed in Charvin et al., 2005]. In principle, these methodologies involve the tethering of the two ends of a single DNA molecule to a fixed surface and a force generator/sensor. Upon generation of torsional stress and/or the addition of a DNA-remodeling protein, their effect on DNA structure and dynamics can be determined by measuring the changes in the length or conformation of the DNA molecule. Using single-molecule nanomanipulation, Koster et al. [2007] showed that inhibition of the human topoisomerase I enzymatic activity by camptothecin results in a significant delay in the uncoiling of mechanically supercoiled DNA, with a more pronounced effect on the relaxation of positive compared to negative supercoils. Altogether, the results led the authors to suggest that topoisomerase I participates in the uncoiling of positive supercoils that accumulate ahead of the replication fork. In another recent study, Randall et al. [2009] simulated the effect of a comprehensive range of physiologically relevant supercoiling on the structure of the DNA helix. The authors demonstrated that underwinding of a DNA molecule under conditions where writhe is prevented, led to the induction of sequence-specific base flipping and denaturation, while overwinding resulted in the formation of Pauling-like DNA (P-DNA). As a consequence, they suggested that torsional stress may influence

DNA sequence recognition and binding of DNA replication proteins through these localized transitions. It is conceivable that application of such methodologies, with certain modifications, might be utilized to study the effect of torsional stress on the DNA topology of various replication origins, providing some insight into the structural transitions that may occur during initiation of DNA replication. Identification of possible intrinsic differences in the behavior of different origins might explain why certain metazoan origins are well defined, while others occur in the form of wide replication zones. Finally, similar to DNA topoisomerases, the effect of initiator proteins or their inhibitors on the origin DNA topology could be tested in order to gain insight in their mechanistic involvement during the topologic remodeling occurring during replication origin activation.

IMPLICATIONS AND FUTURE DIRECTIONS

The clinical potential of DNA topoisomerase inhibitors, due to their ability to induce enzyme-mediated DNA damage, was rapidly identified by researchers, leading to the generation of a number of antimicrobial and anticancer agents. Topoisomerases are the target of several classes of anticancer drugs either as monotherapy or in combination regimens. However, recent molecular studies have increased our understanding of the function of these remarkable enzymes, allowing for their utilization in additional clinical applications as well as the fine-tuning of existing therapeutic strategies.

A major challenge in nuclear-transfer experiments is the decreased cloning efficiency due to the failure of differentiated nuclei to sustain proper embryonic development. Transfer of sperm nuclei into *Xenopus* interphase egg extracts is followed by rapid replication, in contrast with erythrocyte nuclei where DNA replication is compromised [Blow and Laskey, 1986; Lu et al., 1999]. Lemaitre et al. showed that initial incubation of permeabilized erythrocyte nuclei with extracts from M phase eggs renders them equally competent for DNA replication as sperm chromatin, and found that this effect is due to a chromatin remodeling reaction involving the topoisomerase II-dependent replicon resetting at M phase, which facilitates ORC recruitment [Lemaitre et al., 2005]. Furthermore, the authors demonstrated that, while pre-fertilization sperm and egg nuclei are blocked at metaphase with short chromatin loops, competent for rapid replication, embryonic development is associated with a progressive increase in loop size, which impairs ORC recruitment and the efficiency of DNA replication. In summary, these findings indicate an important role for topoisomerase II in replicon resetting during mitosis and, more importantly, suggest that topoisomerase II is a critical determinant of the efficiency of nuclear cloning experiments.

Drugs targeting topoisomerases such as etoposide, doxorubicin, irinotecan, and camptothecin, have been clinically proven as highly active anticancer agents in a variety of clinical settings [Pommier, 2006; Nitiss, 2009b]. However, a serious adverse event accompanying topoisomerase targeting is the formation of secondary malignancies due to chromosomal translocations [Felix, 1998]. Using a mouse skin carcinogenesis model, Azarova et al. [2007] showed that skin-specific topoisomerase IIβ-knockout mice exhibit higher levels of VP-16-induced double-strand breaks (DSBs) and chromosomal rearrangements, as well as higher incidence of melanomas compared to topoisomerase IIα-knockout mice. These results suggested that topoisomerase II targeting is associated with isoform-specific types or extent of DNA damage. Recently, topoisomerase IIβ was shown to participate in the DNA remodeling during pre-RC assembly at replication origins by generating origin-specific transient DSBs [Rampakakis and Zannis-Hadjopoulos, 2009]. In light of this finding, a plausible model explaining the isoform-specific effect of topoisomerase II-targeting is that trapping of topoisomerase IIβ at replication origins leads to the induction of extended DNA damage and chromosomal instability, resulting in secondary malignancies. Altogether, these studies suggest that development of isotype-specific topoisomerase inhibitors, such as the recently described NK314, which targets topoisomerase IIα [Toyoda et al., 2008], may offer considerable benefits in cancer treatment.

CONCLUSIONS

Since their discovery, DNA topoisomerases have attracted the attention of researchers due to their unique properties and their applicability to the clinic in the treatment of a variety of pathological conditions. The recent insights in their role in the initiation of DNA replication have revived the interest in these remarkable enzymes, and open the doors to new biological questions as well as exciting therapeutic opportunities. These issues arise in a timely fashion with the development of pioneering techniques for the study of the function of DNA topoisomerases, preparing the ground for promising discoveries in the near future.

REFERENCES

Abdurashidova G, Radulescu S, Sandoval O, Zahariev S, Danailov MB, Demidovich A, Santamaria L, Biamonti G, Riva S, Falaschi A. 2007. Functional interactions of DNA topoisomerases with a human replication origin. EMBO J 26:998–1009.

Arsuaga J, Vazquez M, Trigueros S, Sumners de W, Roca J. 2002. Knotting probability of DNA molecules confined in restricted volumes: DNA knotting in phage capsids. Proc Natl Acad Sci USA 99:5373–5377.

Asai T, Chen CP, Nagata T, Takanami M, Imai M. 1992. Transcription in vivo within the replication origin of the *Escherichia coli* chromosome: A mechanism for activating initiation of replication. Mol Gen Genet 231:169–178.

AuCoin DP, Colletti KS, Xu Y, Cei SA, Pari GS. 2002. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) contains two functional lytic origins of DNA replication. J Virol 76:7890–7896.

AuCoin DP, Colletti KS, Cei SA, Papouskova I, Tarrant M, Pari GS. 2004. Amplification of the Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 lytic origin of DNA replication is dependent upon a cis-acting AT-rich region and an ORF50 response element and the trans-acting factors ORF50 (K-Rta) and K8 (K-bZIP). Virology 318:542–555.

Azarova AM, Lyu YL, Lin CP, Tsai YC, Lau JY, Wang JC, Liu LF. 2007. Roles of DNA topoisomerase II isozymes in chemotherapy and secondary malignancies. Proc Natl Acad Sci USA 104:11014–11019.

Bell SP, Stillman B. 1992. ATP-dependent recognition of eukaryotic recognition of origins of DNA replication by multiprotein complex. Nature 357:128–134.

Bell D, Sabloff M, Zannis-Hadjopoulos M, Price GB. 1991. Anti-cruciform DNA affinity purification of active mammalian origins of replication. Biochim Biophys Acta 1089:299–308.

Blow JJ, Laskey RA. 1986. Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of Xenopus eggs. Cell 47:577–587.

Boles TC, White JH, Cozzarelli NR. 1990. Structure of plectonemically supercoiled DNA. J Mol Biol 213:931–951.

Broach JR, Li YY, Feldman J, Jayaram M, Abraham J, Nasmyth KA, Hicks JB. 1983. Localization and sequence analysis of yeast origins of DNA replication. Cold Spring Harb Symp Quant Biol 47(Pt 2):1165–1173.

Burnier Y, Dorier J, Stasiak A. 2008. DNA supercoiling inhibits DNA knotting. Nucleic Acids Res 36:4956–4963.

Champoux JJ. 2001. DNA topoisomerases: Structure, function, and mechanism. Annu Rev Biochem 70:369–413.

Charvin G, Strick TR, Bensimon D, Croquette V. 2005. Tracking topoisomerase activity at the single-molecule level. Annu Rev Biophys Biomol Struct 34:201–219.

Chuang RY, Kelly TJ. 1999. The fission yeast homologue of Orc4p binds to replication origin DNA via multiple AT-hooks. Proc Natl Acad Sci USA 96: 2656–2661.

Chuang RY, Chretien L, Dai J, Kelly TJ. 2002. Purification and characterization of the Schizosaccharomyces pombe origin recognition complex: Interaction with origin DNA and Cdc18 protein. J Biol Chem 277:16920–16927.

Clower RV, Hu Y, Melendy T. 2006. Papillomavirus E2 protein interacts with and stimulates human topoisomerase I. Virology 348:13–18.

Crooke E, Hwang DS, Skarstad K, Thony B, Kornberg A. 1991. E. coli minichromosome replication: Regulation of initiation at oriC. Res Microbiol 142:127–130.

Cuvier O, Stanojcic S, Lemaitre JM, Mechali M. 2008. A topoisomerase II-dependent mechanism for resetting replicons at the S-M-phase transition. Genes Dev 22:860–865.

Davey MJ, Fang L, McInerney P, Georgescu RE, O'Donnell M. 2002. The DnaC helicase loader is a dual ATP/ADP switch protein. EMBO J 21:3148–3159.

Dean FB, Hurwitz J. 1991. Simian virus 40 large T antigen untwists DNA at the origin of DNA replication. J Biol Chem 266:5062–5071.

Diffley JFX, Cocker JH. 1992. Protein-DNA interactions at a yeast replication origin. Nature 357:169–172.

Dijkwel PA, Vaughn JP, Hamlin JL. 1991. Mapping of replication initiation sites in mammalian genomes by two-dimensional gel analysis: Stabilization and enrichment of replication intermediates by isolation on the nuclear matrix. Mol Cell Biol 11:3850–3859.

Erzberger JP, Mott ML, Berger JM. 2006. Structural basis for ATP-dependent DnaA assembly and replication-origin remodeling. Nat Struct Mol Biol 13:676–683.

Falaschi A. 2009. Binding of DNA topoisomerases I and II to replication origins. Methods Mol Biol 582:131–143.

Falaschi A, Abdurashidova G, Sandoval O, Radulescu S, Biamonti G, Riva S. 2007. Molecular and structural transactions at human DNA replication origins. Cell Cycle 6:1705–1712.

Fang L, Davey MJ, O'Donnell M. 1999. Replisome assembly at oriC, the replication origin of E. coli, reveals an explanation for initiation sites outside an origin. Mol Cell 4:541–553.

Felix CA. 1998. Secondary leukemias induced by topoisomerase-targeted drugs. Biochim Biophys Acta 1400:233–255.

Fujita M, Hori Y, Shirahige K, Tsurimoto T, Yoshikawa H, Obuse C. 1998. Cell cycle dependent topological changes of chromosomal replication origins in Saccharomyces cerevisiae. Genes Cells 3:737–749.

Funnell BE, Baker TA, Kornberg A. 1987. In vitro assembly of a prepriming complex at the origin of the *Escherichia coli* chromosome. J Biol Chem 262:10327–10334.

Gaczynska M, Osmulski PA, Jiang Y, Lee JK, Bermudez V, Hurwitz J. 2004. Atomic force microscopic analysis of the binding of the Schizosaccharomyces pombe origin recognition complex and the spOrc4 protein with origin DNA. Proc Natl Acad Sci USA 101:17952–17957.

Gai D, Roy R, Wu C, Simmons DT. 2000. Topoisomerase I associates specifically with simian virus 40 large-T-antigen double hexamer-origin complexes. J Virol 74:5224–5232.

Gellert M, O'Dea MH, Itoh T, Tomizawa J. 1976. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc Natl Acad Sci USA 73:4474–4478.

Halmer L, Vestner B, Gruss C. 1998. Involvement of topoisomerases in the initiation of simian virus 40 minichromosome replication. J Biol Chem 273:34792–34798.

Hirose S, Ohta T. 1990. DNA supercoiling and eukaryotic transcription—Cause and effect. Cell Struct Funct 15:133–135.

Houchens CR, Lu W, Chuang RY, Frattini MG, Fuller A, Simancek P, Kelly TJ. 2008. Multiple mechanisms contribute to Schizosaccharomyces pombe origin recognition complex-DNA interactions. J Biol Chem 283:30216–30224.

Hu Y, Clower RV, Melendy T. 2006. Cellular topoisomerase I modulates origin binding by bovine papillomavirus type 1 E1. J Virol 80:4363–4371.

Hu HG, Baack M, Knippers R. 2009. Proteins of the origin recognition complex (ORC) and DNA topoisomerases on mammalian chromatin. BMC Mol Biol 10:36.

Ju BG, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, Rosenfeld MG. 2006. A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. Science 312:1798–1802.

Kawanishi M. 1993. Topoisomerase I and II activities are required for Epstein–Barr virus replication. J Gen Virol 74(Pt 10):2263–2268.

Kelly TJ, Brown GW. 2000. Regulation of chromosome replication. Annu Rev Biochem 69:829–880.

Kong D, Coleman TR, DePamphilis ML. 2003. Xenopus origin recognition complex (ORC) initiates DNA replication preferentially at sequences targeted by Schizosaccharomyces pombe ORC. EMBO J 22:3441–3450.

Koster DA, Palle K, Bot ES, Bjornsti MA, Dekker NH. 2007. Antitumour drugs impede DNA uncoiling by topoisomerase I. Nature 448:213–217.

Krasinska L, Fisher D. 2009. Replication initiation complex formation in the absence of nuclear function in Xenopus. Nucleic Acids Res 37:2238–2248.

Lemaitre JM, Danis E, Pasero P, Vassetzky Y, Mechali M. 2005. Mitotic remodeling of the replicon and chromosome structure. Cell 123:787–801.

Loo YM, Melendy T. 2004. Recruitment of replication protein A by the papillomavirus E1 protein and modulation by single-stranded DNA. J Virol 78:1605–1615.

Lu ZH, Xu H, Leno GH. 1999. DNA replication in quiescent cell nuclei: Regulation by the nuclear envelope and chromatin structure. Mol Biol Cell 10:4091–4106.

Masterson PJ, Stanley MA, Lewis AP, Romanos MA. 1998. A C-terminal helicase domain of the human papillomavirus el protein binds E2 and the DNA polymerase a-primase p68 subunit. J Virol 72:7407–7419.

Mastrangelo IA, Hough PV, Wall JS, Dodson M, Dean FB, Hurwitz J. 1989. ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication. Nature 338:658–662.

Mattern MR, Painter RB. 1979. Dependence of mammalian DNA replication on DNA supercoiling. II. Effects of novobiocin on DNA synthesis in Chinese hamster ovary cells. Biochim Biophys Acta 563:306–312.

Messer W. 2002. The bacterial replication initiator DnaA. DnaA and oriC, the bacterial mode to initiate DNA replication. FEMS Microbiol Rev 26:355–374.

Nitiss JL. 2009a. DNA topoisomerase II and its growing repertoire of biological functions. Nat Rev Cancer 9:327–337.

Nitiss JL. 2009b. Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev Cancer 9:338–350.

Ogasawara N, Seiki M, Yoshikawa H. 1979. Effect of novobiocin on initiation of DNA replication in Bacillus subtilis. Nature 281:702–704.

Parsons R, Anderson ME, Tegtmeyer P. 1990. Three domains in the simian virus 40 core origin orchestrate the binding, melting, and DNA helicase activities of T antigen. J Virol 64:509–518.

Pearson CE, Zorbas H, Price GB, Zannis-Hadjopoulos M. 1996. Inverted repeats, stem-loops, and cruciforms: Significance for initiation of DNA replication. J Cell Biochem 63:1–22.

Pommier Y. 2006. Topoisomerase I inhibitors: Camptothecins and beyond. Nat Rev Cancer 6:789–802.

Rampakakis E, Zannis-Hadjopoulos M. 2009. Transient dsDNA breaks during pre-replication complex assembly. Nucleic Acids Res 37:5714–5724.

Randall GL, Zechiedrich L, Pettitt BM. 2009. In the absence of writhe, DNA relieves torsional stress with localized, sequence-dependent structural failure to preserve B-form. Nucleic Acids Res 37:5568–5577.

Raymer DM, Smith DE. 2007. Spontaneous knotting of an agitated string. Proc Natl Acad Sci USA 104:16432–16437.

Remus D, Beall EL, Botchan MR. 2004. DNA topology, not DNA sequence, is a critical determinant for Drosophila ORC-DNA binding. EMBO J 23:897–907.

Rene B, Fermandjian S, Mauffret O. 2007. Does topoisomerase II specifically recognize and cleave hairpins, cruciforms and crossovers of DNA? Biochimie 89:508–515.

Roberts JM. 1989. Simian virus 40 (SV40) large tumor antigen causes stepwise changes in SV40 origin structure during initiation of DNA replication. Proc Natl Acad Sci USA 86:3939–3943.

Roca J, Berger JM, Harrison SC, Wang JC. 1996. DNA transport by a type II topoisomerase: Direct evidence for a two-gate mechanism. Proc Natl Acad Sci USA 93:4057–4062.

Sedman J, Stenlund A. 1998. The papillomavirus E1 protein forms a DNA-dependent hexameric complex with ATPase and DNA helicase activities. J Virol 72:6893–6897.

Seo YS, Muller F, Lusky M, Gibbs E, Kim HY, Phillips B, Hurwitz J. 1993. Bovine papilloma virus (BPV)-encoded E2 protein enhances binding of E1 protein to the BPV replication origin. Proc Natl Acad Sci USA 90:2865–2869.

Simmons DT, Gai D, Parsons R, Debes A, Roy R. 2004. Assembly of the replication initiation complex on SV40 origin DNA. Nucleic Acids Res 32: 1103–1112.

Sinden R. 1994. DNA structure and function. San Diego: Academic Press, Inc.

Stewart L, Redinbo MR, Qiu X, Hol WG, Champoux JJ. 1998. A model for the mechanism of human topoisomerase I. Science 279:1534–1541.

Torheim NK, Skarstad K. 1999. *Escherichia coli* SeqA protein affects DNA topology and inhibits open complex formation at oriC. EMBO J 18:4882–4888.

Toyoda E, Kagaya S, Cowell IG, Kurosawa A, Kamoshita K, Nishikawa K, Iiizumi S, Koyama H, Austin CA, Adachi N. 2008. NK314, a topoisomerase II inhibitor that specifically targets the alpha isoform. J Biol Chem 283:23711–23720.

Travers A, Muskhelishvili G. 2007. A common topology for bacterial and eukaryotic transcription initiation? EMBO Rep 8:147–151.

Trowbridge PW, Roy R, Simmons DT. 1999. Human topoisomerase I promotes initiation of simian virus 40 DNA replication in vitro. Mol Cell Biol 19:1686–1694.

Tse Y, Wang JC. 1980. *E. coli* and *M. luteus* DNA topoisomerase I can catalyze catenation of decatenation of double-stranded DNA rings. Cell 22:269–276.

Tsurimoto T, Fairman MP, Stillman B. 1989. Simian virus 40 DNA replication in vitro: Identification of multiple stages of initiation. Mol Cell Biol 9:3839–3849.

Ustav M, Stenlund A. 1991. Transient replication of BPV-1 requires two viral polypeptides encoded by the E1 and E2 open reading frames. EMBO J 10:449–457.

Vashee S, Cvetic C, Lu W, Simancek P, Kelly TJ, Walter JC. 2003. Sequence-independent DNA binding and replication initiation by the human origin recognition complex. Genes Dev 17:1894–1908.

Vogelstein B, Pardoll DM, Coffey DS. 1980. Supercoiled loops and eucaryotic DNA replicaton. Cell 22:79–85.

Wakelin SP, Waring MJ. 1976. The binding of echinomycin to deoxyribonucleic acid. Biochem J 157:721–740.

Wang JC. 1971. Interaction between DNA and an *Escherichia coli* protein omega. J Mol Biol 55:523–533.

Wang Y, Li H, Tang Q, Maul GG, Yuan Y. 2008. Kaposi's sarcoma-associated herpesvirus ori-Lyt-dependent DNA replication: Involvement of host cellular factors. J Virol 82:2867–2882.

Ward GK, McKenzie R, Zannis-Hadjopoulos M, Price GB. 1990. Dynamic distribution and quantification of DNA cruciforms in eukaryotic nuclei. Exp Cell Res 188:235–246.

Watson JD, Crick FHC. 1953. Molecular structure of nucleic acids: A structure for dexoyribose nucleic acid. Nature 171:737–741.

West KL, Austin CA. 1999. Human DNA topoisomerase IIbeta binds and cleaves four-way junction DNA in vitro. Nucleic Acids Res 27:984–992.

Wu FY, Ahn JH, Alcendor DJ, Jang WJ, Xiao J, Hayward SD, Hayward GS. 2001. Origin-independent assembly of Kaposi's sarcoma-associated herpesvirus DNA replication compartments in transient cotransfection assays and association with the ORF-K8 protein and cellular PML. J Virol 75:1487–1506.

Zechiedrich EL, Khodursky AB, Bachellier S, Schneider R, Chen D, Lilley DM, Cozzarelli NR. 2000. Roles of topoisomerases in maintaining steady-state DNA supercoiling in *Escherichia coli*. J Biol Chem 275:8103–8113.