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RecQ Helicase and Topoisomerase III Comprise a Novel DNA Strand Passage Function: A Conserved Mechanism for Control of DNA Recombination

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Summary

***E. coli* RecQ protein is a multifunctional helicase with homologs that include the *S. cerevisiae* Sgs1 helicase and the *H. sapiens* Wrn and Blm helicases. Here we show that RecQ helicase unwinds a covalently closed double-stranded DNA (dsDNA) substrate and that this activity specifically stimulates *E. coli* topoisomerase III (Topo III) to fully catenate dsDNA molecules. We propose that these proteins functionally interact and that their shared activity is responsible for control of DNA recombination. RecQ helicase has a comparable effect on the Topo III homolog of *S. cerevisiae*, consistent with other RecQ and Topo III homologs acting together in a similar capacity. These findings highlight a novel, conserved activity that offers insight into the function of the other RecQ-like helicases.**

Introduction

DNA helicases are protein motors capable of separating the individual strands of dsDNA (for review, see Lohman and Bjornson, 1996). These enzymes play vital roles in replication, recombination, repair, and segregation of chromosomes (for review, see Matson and Kaiser-Rogers, 1990). Given their importance, it is not surprising that some mutations in genes encoding helicases are deleterious and are implicated in several human diseases, including Bloom's and Werner's syndromes (Ellis et al., 1995; Yu et al., 1996). These two disorders are rare, inherited diseases that share similar features, including pronounced genomic instability. In addition, Werner's syndrome results in premature aging and therefore is believed to mimic many of the aspects of the natural aging process.

Bloom's and Werner's syndromes arise from mutations in the Blm and Wrn helicases, respectively (Ellis et al., 1995; Yu et al., 1996). These two proteins fall into a growing and important class of helicases, the RecQ helicase family (Gorbalenya et al., 1989). RecQ-like helicases are widespread, having been found in bacteria (Irino et al., 1986), yeast (Gangloff et al., 1994; Watt et al., 1995; Stewart et al., 1997), and humans (Puranam and Blackshear, 1994; Ellis et al., 1995; Yu et al., 1996). Members of this family share both significant amino acid sequence similarity and, in those that have been studied, 3′ to 5′ DNA helicase activity (Umezu et al., 1990; Gray et al., 1997; Karow et al., 1997; Bennett et al., 1998).

The prototype helicase of this family is the RecQ helicase from *Escherichia coli*. Null mutations at the *recQ* locus, in conjunction with other mutations, result in a 100-fold reduction in homologous recombination proficiency as well as an increase in sensitivity to UV irradiation (Nakayama et al., 1984, 1985). In addition, loss of RecQ helicase function in wild-type cells results in a 30-fold increase in illegitimate recombination (Hanada et al., 1997). In vitro, RecQ helicase initiates homologous recombination and unwinds a wide variety of DNA substrates, including homologous pairing intermediates (Harmon and Kowalczykowski, 1998). Thus, RecQ helicase is a multifunctional protein capable of both initiating homologous recombination and suppressing illegitimate recombination (Hanada et al., 1997; Harmon and Kowalczykowski, 1998).

Sgs1 helicase, a RecQ-like helicase from *Saccharomyces cerevisiae*, is important for segregation of chromosomes, control of aging, and regulation of recombination (Gangloff et al., 1994; Watt et al., 1995, 1996; Sinclair et al., 1997). Null mutations at the *SGS1* locus suppress the slow growth phenotype of Δ*top3* (Topo III) mutants (Gangloff et al., 1994), confer a hyperrecombination phenotype (Gangloff et al., 1994; Watt et al., 1995, 1996), and result in an increase in both meiotic and mitotic chromosomal nondisjunction (Watt et al., 1995). In addition, Δ*sgs1* strains have a marked decrease in life span relative to wild-type *S. cerevisiae* (Sinclair et al., 1997). The decrease in life span observed in these yeast is the result of an accumulation of extrachromosomal ribosomal DNA (rDNA) circles, which arise from increased recombination events between repeats within the rDNA (Sinclair and Guarente, 1997). The increased recombination frequency in this background suggests that Sgs1 helicase is required to inhibit this type of recombination.

Sgs1 protein physically interacts with both yeast topoisomerase II (Topo II) and yeast Topo III (Gangloff et al., 1994; Watt et al., 1995). *E. coli* possess a structural and functional homolog of the yeast Topo III. In general, both the yeast and bacterial enzymes have a distinct requirement for a region of ssDNA in their DNA substrate: each removes supercoils from negatively supercoiled DNA (scDNA) poorly unless single-stranded DNA (ssDNA) character is introduced into the DNA substrate (DiGate and Marians, 1988; Kim and Wang, 1992). In addition, *E. coli* Topo III possesses decatenation activity, but this activity also requires the presence of ssDNA in one of the linked DNA molecules (Dean et al., 1983; DiGate and Marians, 1988). Based on the physical interaction between Sgs1 helicase and Topo III, Rothstein and coworkers proposed a model in which the helicase activity of Sgs1 protein would provide Topo

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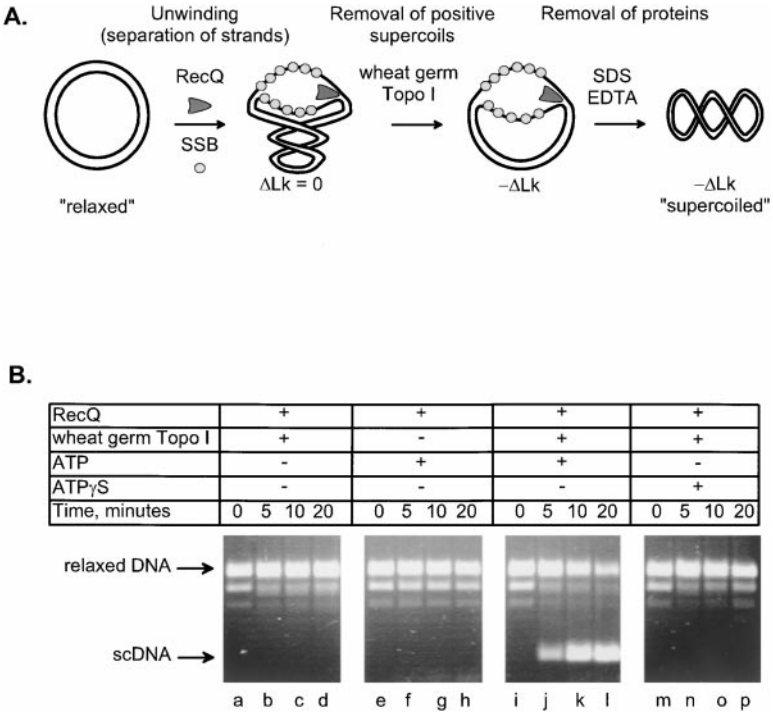

Figure 1. RecQ Helicase Unwinds Covalently Closed dsDNA

(A) "Supercoiling" assay for unwinding of relaxed dsDNA by RecQ helicase. See text for details.

(B) The supercoiling reaction applied to RecQ helicase. All reactions were carried out under standard conditions except lanes a–d, ATP omitted; lanes e–h, wheat germ Topo I omitted; and lanes m–p, ATPγS substituted for ATP.

III with a ssDNA-binding site (Gangloff et al., 1994; Rothstein and Gangloff, 1995).

Because of the similarities between the yeast and bacterial proteins (both biochemical and structural), we chose to investigate whether *E. coli* Topo III and RecQ helicase displayed a physical or functional interaction. We discovered RecQ helicase has a profoundly stimulatory and specific effect on the DNA strand passage activity of Topo III. We demonstrate that RecQ helicase unwinds covalently closed dsDNA in an ATP-dependent fashion, and in combination with Topo III, this DNA is converted into extensive catenanes. The findings demonstrate that RecQ helicase and Topo III functionally interact to promote DNA strand passage and suggest a conserved means to effect a full dsDNA strand passage from the concerted actions of a Topo III–like topoisomerase and a RecQ-like helicase. This novel discovery provides insight into the underlying causes of the genomic instability observed in both yeast cells lacking Sgs1 function and in the human diseases Bloom's and Werner's syndromes.

Results

RecQ Helicase Unwinds Covalently Closed dsDNA

A topological assay was used to investigate the possibility that RecQ helicase unwinds covalently closed dsDNA (Figure 1A; Wold et al., 1987). Separation of strands in a relaxed dsDNA substrate by RecQ helicase partitions the DNA substrate into negatively (i.e., unwound) and positively supercoiled domains without an overall change in linking number ($\Delta Lk = 0$). Treatment of this product with wheat germ topoisomerase I (wheat germ Topo I), which can relax both positively and negatively supercoiled DNA, should result in the selective removal of the supercoils in the positively supercoiled domain (net -ΔLk). This is the case because the ssDNA comprising the unwound domain is expected to be bound by both RecQ helicase and *E. coli* single-stranded DNA-binding (SSB) protein. Therefore, the relaxed dsDNA substrate is converted to negatively scDNA (overall -ΔLk) products, which can be distinguished from one another by agarose gel electrophoresis.

The results obtained for RecQ helicase in this supercoiling assay appear in Figure 1B. When RecQ helicase is supplied with ATP, the pUC19 relaxed dsDNA substrate is rapidly converted to a highly supercoiled species; greater than 90% of the relaxed DNA is converted to this product within 10 min (lanes i–l). The resultant scDNA product is negatively supercoiled: both *E. coli* Topo I and Topo III were capable of relaxing purified aliquots of this DNA (data not shown). As expected, supercoiled product does not accumulate in the absence of wheat germ Topo I (lanes e–h). RecQ helicase requires ATP to convert the relaxed dsDNA substrate to the scDNA product (lanes a–d). Additionally, ATPγS, a nonhydrolyzable ATP analog that inhibits the helicase activity of RecQ helicase but not its dsDNA-binding activity (F. G. H. and S. C. K., unpublished observations), did not substitute as nucleotide cofactor (lanes m–p). In addition, RecQ helicase unwound the relaxed DNA poorly in the absence of SSB protein (F. G. H. and S. C. K., unpublished observations), an indication that SSB protein is needed to trap the ssDNA strands produced by unwinding. Thus, RecQ helicase is actively separating the strands of the DNA substrate and not simply binding to it.

RecQ Helicase Stimulates Topo III to Fully Catenate dsDNA

Since RecQ helicase unwinds covalently closed dsDNA and therefore produces a potential substrate for *E. coli* Topo III, we explored the possibility that RecQ helicase

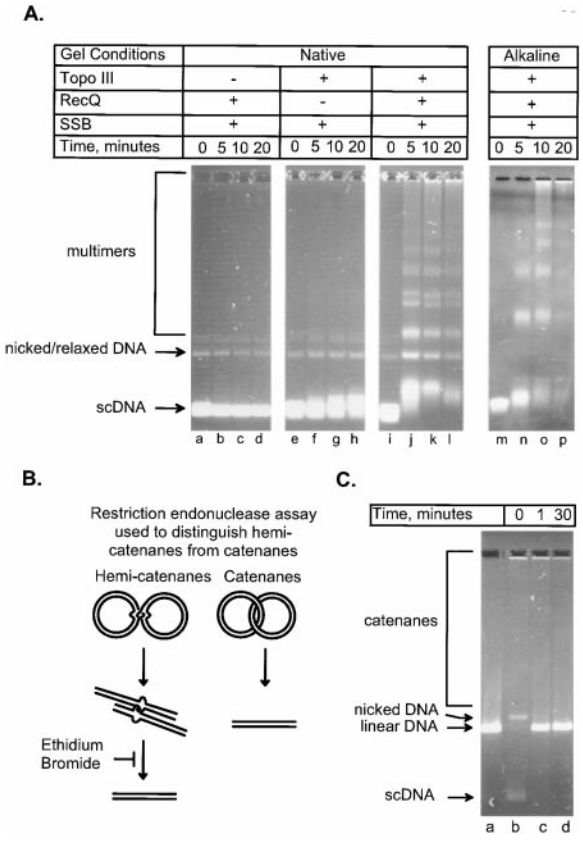

Figure 2. RecQ Helicase Stimulates Topo III to Form DNA Multimers that Are Full Catenanes

(A) Together, RecQ helicase and Topo III produce large DNA multimers. All reactions were carried under standard conditions with the following exceptions: lanes a–d, Topo III omitted; lanes e–h, RecQ helicase omitted. Samples in lanes a–l were run under native (i.e., nondenaturing) conditions, and those in lanes m–p under alkaline (i.e., denaturing) conditions.

(B) Restriction endonuclease assay to distinguish hemicatenanes from full catenanes. See text for details.

(C) The restriction enzyme assay applied to purified, protein-free DNA multimers formed by Topo III and RecQ helicase. Lane a represents a control for pUC19 linear dsDNA. In lanes b–d, approximately 15 μM of the purified multimers was treated with HindIII. Samples were taken at the indicated times and analyzed by native agarose gel electrophoresis. Ethidium bromide was present at 0.5 μg/ml during all manipulations to block thermal branch migration.

could affect the activity of Topo III. Surprisingly, a combination of RecQ helicase and Topo III (with SSB protein), acting on pUC19 negatively scDNA, generated a set of slower migrating DNA multimer products (Figure 2A, lanes i–l); in addition, these larger DNA species grew in size and complexity over the reaction time course (in lanes k and l, note the significant amount of DNA trapped in the wells). The same DNA species were formed, but to a lesser extent, when 10-fold less Topo III and/or RecQ helicase were present in similar reactions (F. G. H. and S. C. K., unpublished observations). Under standard conditions, Topo III alone (with SSB protein) was unable to convert a substantial amount of the scDNA substrate to similar high-molecular-weight species (Figure 2A, lanes e–h). Similarly, RecQ helicase and SSB protein incubated together with the scDNA substrate did not yield the same DNA species (lanes a–d). SSB protein was an important stimulatory factor for catenation: in the absence of SSB protein, Topo III and RecQ helicase converted less of the scDNA substrate to higher-molecular-weight species, and the resultant DNA multimers were less complex (F. G. H. and S. C. K., unpublished observations). Therefore, these DNA multimers are products of the combined activities of Topo III, RecQ helicase, and SSB protein.

Since Topo III is a highly active decatenase, it was possible that the multimers produced by the combined activities of Topo III and RecQ helicase represented extensively catenated DNA. To determine whether these unique DNA species were covalently linked, samples were subjected to denaturing alkaline agarose gel electrophoresis. As can be seen in Figure 2A, lanes m–p, the DNA multimers produced by Topo III and RecQ helicase were stable under denaturing conditions. Thus, these species represented covalently linked, possibly catenated DNA monomers and were not composed of transiently paired DNA molecules.

From the preceding analysis, it was clear that Topo III and RecQ helicase worked together to covalently link dsDNA monomers into species consistent with covalently linked catenanes. Two different types of catenanes were possible (Figure 2B): (1) hemicatenanes, in which two DNA molecules were linked by a single DNA strand, and (2) full catenanes, which resulted from the passage of both strands of one DNA molecule across a second dsDNA molecule. Hemi- and full catenanes can be distinguished from one another by treating the suspect species with a restriction enzyme that cuts the DNA once, followed by agarose gel electrophoresis of the products (Droge and Cozzarelli, 1992). Digestion of fully catenated DNA molecules yields linear dsDNA as the sole product. On the other hand, complete cleavage of hemicatenanes generates a heterogeneous distribution of linked cleavage products that separate only after thermal branch migration. Previously, Tse and Wang used this technique to verify the makeup of catenanes formed by Topo I from *E. coli* and *Micrococcus luteus* (Tse and Wang, 1980). In the analysis used here, ethidium bromide was present during both digestion and electrophoresis to block thermal branch migration and, hence, halt dissociation of any linked cleavage products (Panyutin and Hsieh, 1994).

The structure of the DNA multimers formed from pUC19 scDNA by the combined actions of RecQ helicase and Topo III (with SSB protein) was probed using the restriction enzyme HindIII (Figure 2C). Following addition of HindIII to DNA purified from a standard Topo III–RecQ helicase reaction, all of the linked DNA that was digested within the first minute (which represents the bulk of the DNA present in the reaction) appears as linear dsDNA (lane c). At no time is there a significant fraction of an intermediate DNA species (lanes c and d), which would indicate the production of linked cleavage products. Comparable results were obtained when HindIII was added directly to an ongoing standard Topo III–RecQ helicase reaction (data not shown). Furthermore, direct visualization by atomic force microscopy demonstrated that the majority of the species that could be clearly resolved were fully catenated (F. G. H., J. P. Brockman, and S. C. K., unpublished observations). This analysis demonstrates that the majority of the DNA multimers are full catenanes, rather than hemicatenanes. Additionally, recovery of unit length linear dsDNA in this

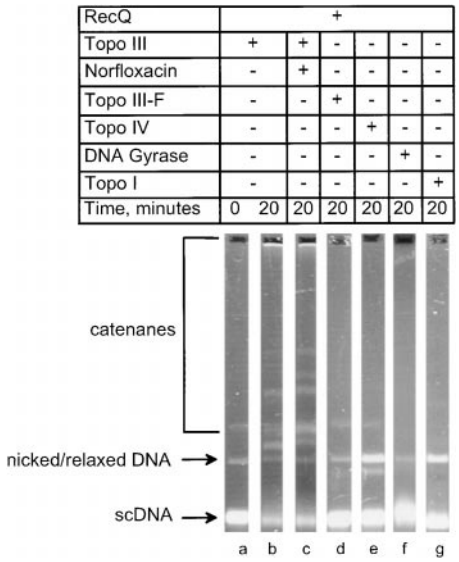

Figure 3. Topo III Is Responsible for Catenation

Reactions were carried out under standard conditions except as indicated: lane c, 100 μM norfloxacin was included; lane d, native Topo III substituted with 100 nM Topo III-F; lanes e and f, either Topo IV (lane e), DNA gyrase (lane f), or *E. coli* Topo I (lane g) were substituted for Topo III.

assay confirms that the DNA associated in the multimers is composed of intact, covalently closed dsDNA monomers; therefore, the multimers did not arise from the aberrant ligation of DNA molecules by Topo III. Thus, Topo III and RecQ helicase act together to form fully catenated DNA.

Topo III Is Responsible for Catenation

To rule out the possibility that catenation is due to a minor contaminant in either protein preparation, we tested the sensitivity of the catenation reaction to norfloxacin, a fluoroquinolone that is a potent inhibitor of type II topoisomerases (Sugino et al., 1977; Peng and Marians, 1993). Inclusion of 100 μM norfloxacin, which is sufficient to inhibit both *E. coli* topoisomerase IV (Topo IV) and DNA gyrase (Khodursky et al., 1995), in the standard Topo III–RecQ helicase catenation reaction had essentially no effect on the yield of catenanes (Figure 3, compare lane c to b). Hence, catenation is not mediated by a type II topoisomerase. Additionally, an inactive mutant of Topo III, Topo III-F, in which the catalytic Tyr-328 is replaced by phenylalanine (Wang et al., 1996), does not substitute for native Topo III in a standard catenation reaction (Figure 3, compare lane d to b). Since both native Topo III and Topo III-F were purified using the same protocol, each preparation should contain the same contaminating activities. Finally, RecQ helicase incubated alone with the pUC19 scDNA substrate did not form catenanes (Figure 2A, lane d). Thus, catenation is specific to the combined inherent activities of Topo III and RecQ helicase.

To address the possibility that RecQ helicase is a general topoisomerase stimulatory factor, we determined the effect of RecQ helicase on the activity of another type I topoisomerase, Topo I, and two distinct type II topoisomerases, DNA gyrase and Topo IV. In the presence of RecQ helicase, neither Topo IV nor DNA gyrase produced high-molecular-weight species from pUC19 scDNA (Figure 3, lanes e and f, respectively). Similarly, RecQ helicase did not induce Topo I to form catenanes (lane g) but instead inhibited the topoisomerase activity of Topo I (data not shown). Thus, RecQ helicase is not a general topoisomerase stimulatory factor. These results further affirm our conclusion that the Topo III–mediated catenation activity is an intrinsic property of Topo III and that catenation does not arise from trace amounts of either Topo IV or DNA gyrase. Most importantly, however, these results clearly demonstrate that RecQ helicase specifically stimulates Topo III to form catenated DNA species. A simple explanation for the specificity of this stimulation would be provided by the existence of specific protein–protein interactions between RecQ helicase and Topo III. However, we were unable to detect a stable physical interaction between these two proteins using IASys biosensor analysis, affinity chromatography, and gel filtration chromatography (data not shown).

The Helicase Activity of RecQ Helicase Is Required for Catenane Formation

Previously, it was found that for Topo III to separate two catenated DNA molecules, one of the linked DNA molecules must contain a region of ssDNA (DiGate and Marians, 1988). Therefore, the simplest mechanism by which RecQ helicase could stimulate Topo III is by providing it with a ssDNA substrate. Alternatively, RecQ helicase may direct Topo III to the DNA without first unwinding it. To distinguish between these two possibilities, we used the ability of RecQ helicase to bind to dsDNA in the absence of ATP, a condition where no unwinding is observed (Harmon and Kowalczykowski, 1998). When ATP was omitted, little of the DNA substrate was converted to slower migrating species (data not shown); the results were very similar to those obtained when RecQ helicase was omitted (Figure 2A, lanes e–h). Also, when ATP was substituted with ATPγS, a combination of RecQ helicase and Topo III failed to form the expected catenated species (data not shown). Thus, it is the helicase activity of RecQ helicase that is required for Topo III–dependent formation of catenanes, which argues that unwinding of the DNA by RecQ helicase provides a DNA substrate for Topo III.

Inhibition of RecQ Helicase Leads to Decatenation by Topo III

Clearly, the helicase activity of RecQ helicase was required by Topo III to initiate catenane formation, but the importance of active unwinding later in the reaction, once Topo III had acquired a ssDNA-binding site, remained unclear. To determine whether continued unwinding by RecQ helicase influenced the outcome of this reaction, excess ATPγS (i.e., 5 mM) was used to inhibit the helicase activity of RecQ helicase following an initial 10 min incubation with Topo III, SSB protein, and pUC19 scDNA; this treatment will halt unwinding, leaving a complex of RecQ helicase, SSB protein, and unwound DNA.

In the absence of added ATPγS, RecQ helicase and Topo III incorporated the scDNA into multiply catenated

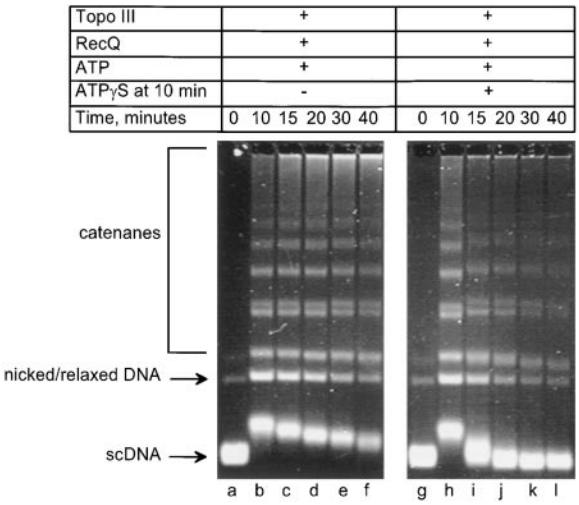

Figure 4. RecQ Helicase "Drives" Topo III to Catenate DNA

Two standard reactions were performed for 10 min. At this time, aliquots were removed and 5 mM ATPγS was added to the indicated reaction to inhibit the helicase activity of RecQ helicase (lanes g–l). Further samples were taken at the indicated times and analyzed by native agarose gel electrophoresis.

DNA products (Figure 4, lanes a–f). After addition of ATPγS, the amount of DNA trapped in the wells and the overall number of intermediate-sized products declined over the same time course (lanes g–l). The loss of these catenated DNA species coincided with the appearance of scDNA monomers. Thus, Topo III could decatenate the individual DNA monomers associated in the catenanes, following inactivation of RecQ helicase. Comparable results were obtained from similar reactions in which RecQ helicase activity was inhibited using an increase in magnesium concentration (data not shown). The recovery of scDNA monomers as products of decatenation from these catenated structures demonstrates further that the molecules participating in catenation are covalently closed, rather than nicked. These data are consistent with a model in which Topo III is "driven" by the helicase activity of RecQ helicase to form and maintain a steady-state level of catenated DNA, and upon removal of this activity, Topo III will decatenate the DNA molecules. In agreement, Topo III was unable to decatenate purified deproteinized catenanes that were created by RecQ helicase and Topo III (F. G. H. and S. C. K., unpublished observations). Therefore, Topo III requires the presence of RecQ helicase, or a structure that it introduces into DNA by unwinding, to effect a dsDNA strand passage.

The Presence of ssDNA Is Not Sufficient to Stimulate Topo III–Promoted Catenation

To confirm that Topo III is stimulated by a unique attribute of RecQ helicase and not simply its ssDNA product, we determined whether Topo III is capable of catenating DNA substrates possessing extensive ssDNA character. If RecQ helicase only provides a region of ssDNA for Topo III, then these DNA substrates should fully mimic the effects of RecQ helicase on Topo III–mediated catenation. On the other hand, if the presence of both RecQ helicase and its unwinding product are equally important, then catenation will occur exclusively in the presence of RecQ helicase.

To determine whether ssDNA was sufficient to stimulate Topo III–mediated catenation, Topo III (with SSB protein) was incubated with pUC19 scDNA and either ϕX174 dsDNA having a 162 nucleotide gap between the PstI and AvaI restriction sites (Figure 5, gDNA [gapped DNA]) or M13mp7 circular ssDNA (Figure 5, ssDNA). With the mixture of ϕX174 gDNA and scDNA, Topo III produced few catenated DNA species (Figure 5, lanes a–d). Thus, Topo III was capable of catenating DNA substrates possessing a region of ssDNA, but this reaction was inefficient relative to that promoted by Topo III in the presence of RecQ helicase (lanes e–h). Similarly, Topo III alone provided with M13mp7 circular ssDNA, as the ssDNA species produced a few higher mobility species (lanes i–l), but Topo III and RecQ helicase acting together converted the ss- and scDNA substrates to catenanes (lanes m–p). Substitution of pUC19 scDNA in either case with the cognate homologous scDNA (i.e., ϕX174 or M13mp7 RFI) yielded identical results (data not shown); hence, Topo III does not require homology between the two DNA species to effect catenation. Thus, the presence of ssDNA in a DNA substrate is not sufficient to stimulate extensive Topo III–dependent catenation. These data clearly indicate that the presence of RecQ helicase is equally important to the catenation activity of Topo III as is its ssDNA substrate.

E. coli Helicase II Does Not Stimulate Topo III to Catenate DNA

An alternative explanation for the stimulation of Topo III–promoted catenation by RecQ helicase is that the act of strand separation in dsDNA produces a preferential DNA substrate for the topoisomerase that is not present in either fully ssDNA or gDNA. If this is the case, then any helicase with mechanistic similarities to RecQ helicase may induce catenation by Topo III. To investigate this possibility, *E. coli* helicase II was assayed for its ability to stimulate the catenation activity of Topo III. Helicase II, a component of the DNA excision repair system (Taucher-Scholz et al., 1983), was chosen because RecQ helicase is thought to unwind dsDNA in a manner similar to this helicase (Umezu and Nakayama, 1993). A combination of helicase II and Topo III, however, did not form catenanes when provided with a substrate consisting of either pUC19 scDNA or pBluescriptSK$^-$ (pBSKM) dsDNA containing a single, site-specific nick (data not shown). On the other hand, RecQ helicase and Topo III produced the expected catenane products with both of these dsDNA substrates (F. G. H. and S. C. K., unpublished observations). These findings clearly indicate that Topo III requires the action of RecQ helicase to promote catenation. Thus, it is only RecQ helicase and Topo III together that will reconstitute the observed potent DNA strand passage activity.

S. cerevisiae Topo III Catenates scDNA in the Presence of RecQ Helicase

To further define the protein specificity of the catenation reaction, we sought to determine whether a heterologous but similar protein would substitute for *E. coli* Topo III. In these experiments, *E. coli* Topo III was substituted

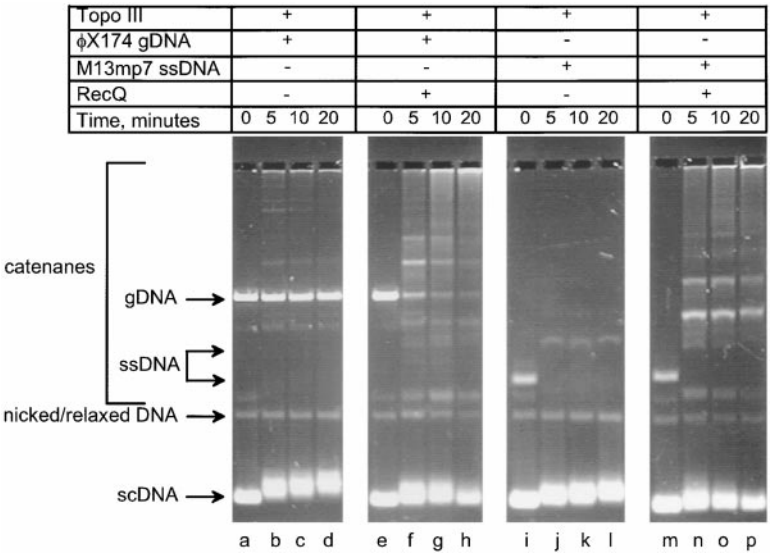

Figure 5. The Presence of ssDNA Is Not Sufficient to Stimulate Topo III–Promoted Catenation

Standard reactions with pUC19 scDNA and either ϕX174 containing a ssDNA gap (gDNA, lanes a–h) or M13mp7 circular ssDNA (lanes i–p) were incubated with Topo III and SSB protein. RecQ helicase was present for those reactions depicted in lanes e–h and m–p.

with *S. cerevisiae* Topo III. If the unwound RecQ helicase–DNA complex is a preferential substrate for catenation by Topo III–type topoisomerases, then yeast Topo III might also catenate scDNA unwound by RecQ helicase.

Yeast Topo III, RecQ helicase, and the *S. cerevisiae* equivalent of SSB protein, replication protein A (RPA), produced catenated products similar to those observed with the bacterial enzyme when incubated with pUC19 scDNA under standard conditions (Figure 6, lanes f–j). In the absence of RecQ helicase, none of the scDNA substrate was converted to DNA multimers (data not shown). Therefore, the action of RecQ helicase on the scDNA substrate produces a suitable substrate for catenation promoted by yeast Topo III. Interestingly, when RPA was replaced with SSB protein, DNA catenanes accumulated later, and less of the scDNA substrate was taken up into the catenane products (Figure 8, compare lanes a–e to f–j). Reactions without an SSB protein did not yield catenanes (data not shown). These results suggest that yeast Topo III may act in conjunction with RPA. Since yeast Topo III is not expected to specifically recognize RecQ helicase, these findings support the idea that the structure of the unwound RecQ helicase–DNA complex is the unique species that allows a Topo III–type topoisomerase to catenate DNA. Moreover, these data demonstrate another similarity between the yeast and bacterial topoisomerases: namely, the ability to catenate DNA that was unwound by RecQ helicase.

Discussion

Here we demonstrate that RecQ helicase unwinds covalently closed dsDNA in an ATP-dependent fashion. This unwound RecQ helicase–DNA complex represents a novel DNA substrate for *E. coli* Topo III because, when coupled with the strand passage activity of Topo III, dsDNA monomers are converted into complex, covalently linked catenanes. Neither Topo III nor RecQ helicase alone are able to construct similar DNA species. The dsDNA monomers incorporated into these unique products are fully catenated. Topo III alone does not effectively catenate DNA substrates that are either partially or fully single stranded, an indication that Topo III responds to a unique attribute of RecQ helicase itself or of the RecQ helicase–DNA complex. In support, *E. coli* helicase II, which is mechanistically similar to RecQ helicase, does not stimulate Topo III to catenate either a singly nicked or an scDNA substrate. Also, these effects of RecQ helicase are specific to Topo III, as RecQ helicase does not stimulate the activity of either *E. coli* Topo I, Topo IV, or DNA gyrase. Thus, RecQ helicase is a specific stimulatory factor for the DNA strand passage activity of *E. coli* Topo III, producing fully catenated DNA by concerted ssDNA strand passage events. Interestingly, RecQ helicase has an identical effect on Topo III from *S. cerevisiae*: a marked stimulation of DNA strand passage functions to produce catenated DNA. These findings suggest that yeast Topo III and the Sgs1 helicase have the same shared activity as their bacterial counterparts. Further, the parallels between the *E. coli* and *S. cerevisiae* enzymes suggest that these proteins may have a conserved function in DNA metabolism.

Model for Catenation Promoted by the Combined Activities of RecQ Helicase and Topo III

We propose that RecQ helicase induces Topo III to fully catenate dsDNA molecules by providing a DNA binding focus for Topo III. In this view, two Topo III monomers bound to opposite DNA strands at the unwound region of the DNA could sequentially pass each ssDNA strand across a second DNA molecule to generate fully catenated DNA molecules (Figure 7). Initially, RecQ helicase binds to an internal region in a covalently closed dsDNA molecule and unwinds the duplex DNA to yield ssDNA (Figure 7A). In this model, an unknown feature of the ssDNA–RecQ helicase complex targets Topo III to the region of DNA being actively unwound. In doing so, RecQ helicase acts to focus individual Topo III monomers to a region of the unwound dsDNA substrate (Figure 7B). Full catenation of the first DNA molecule with a second is accomplished when two adjacent Topo III

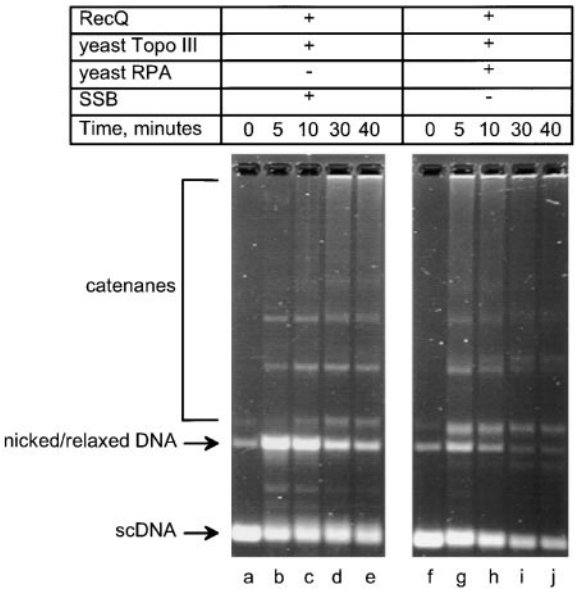

Figure 6. *S. cerevisiae* Topo III Catenates scDNA in the Presence of RecQ Helicase

Standard reactions containing yeast Topo III, RecQ helicase, and the indicated single-stranded DNA binding protein were performed with pUC19 scDNA. *E. coli* SSB protein was present in the reactions shown in lanes a–e and *S. cerevisiae* RPA in the reactions shown in lanes f–j.

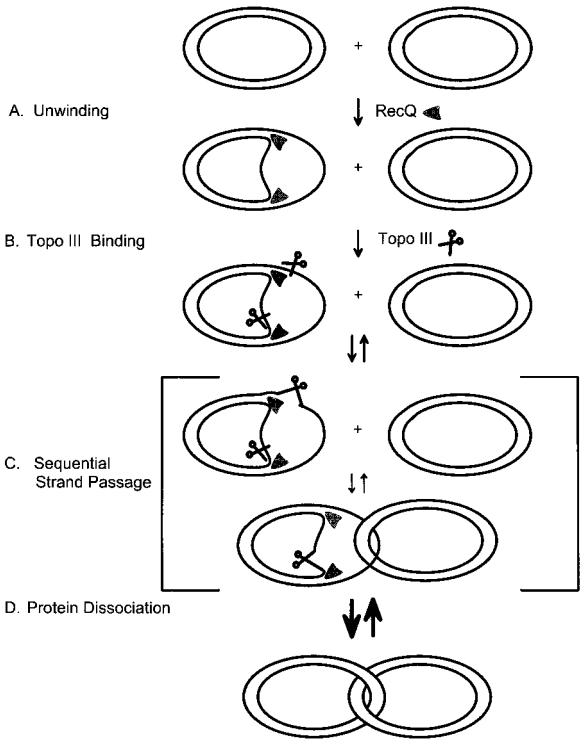

Figure 7. Model for Catenation Promoted by the Combined Activities of RecQ Helicase and Topo III

(A) RecQ helicase unwinds a covalently closed dsDNA molecule to yield ssDNA, a preferential DNA-binding site for Topo III.

(B) An unknown feature at the RecQ helicase–dsDNA complex targets two Topo III monomers to opposite strands. Each Topo III monomer is now positioned to promote a ssDNA strand passage event.

(C) Two concerted ssDNA strand passage reactions produce the equivalent of a dsDNA strand passage. In this way, sequential strand passage by each Topo III monomer leads to catenation of a second dsDNA molecule.

(D) Catenated dsDNA molecules result after dissociation of proteins. Decatenation is the reverse of this process.

complexes promote sequential, perhaps concerted, passage of the second dsDNA through each bound strand (Figure 7C). After dissociation of the proteins, the two covalently closed dsDNA molecules are fully catenated as a consequence of the two ssDNA passage events (Figure 7D). Since unlinking of catenated dsDNA molecules is mechanistically the same process, decatenation presumably occurs by a reversal of this process.

RecQ Helicase and Topo III as a Means to Control Recombination in *E. coli*

Based on this and previous work, it is clear that RecQ helicase is a multifunctional protein in *E. coli*. The different roles that RecQ helicase plays in DNA metabolism can be separated into two categories: (1) those that require only the helicase function of RecQ helicase, and (2) those that involve the DNA strand passage activity of Topo III acting in concert with RecQ helicase.

A clear role for the helicase function of RecQ protein is to initiate homologous recombination either at dsDNA breaks in the absence of the primary initiator of recombination, the RecBCD enzyme (Nakayama et al., 1984, 1985), or at ssDNA regions (Lanzov et al., 1991; Lloyd and Buckman, 1995). Moreover, RecQ helicase, in conjunction with the RecA and SSB proteins, is capable of initiating homologous recombination in vitro (Harmon and Kowalczykowski, 1998). Thus, the accumulated genetic and biochemical evidence are consistent with RecQ helicase acting to initiate homologous recombination.

In addition to its initiation role in homologous recombination, RecQ helicase also suppresses illegitimate recombination. A null *recQ* mutation results in a 20- to 300-fold increase in nonhomologous recombination, involving an average of nine base pairs of homology, between lambda phage and the *E. coli* chromosome (Hanada et al., 1997). RecQ helicase can also unwind a variety of DNA substrates in vitro, including recombination intermediates, consistent with its participation in this activity (Harmon and Kowalczykowski, 1998). Taken together, these data support the idea that the helicase activity of RecQ protein acts to disrupt joint molecules formed by illegitimate recombination.

Based on the data presented here, we propose that an alternative role for RecQ helicase is to act together with Topo III to control the levels of both homologous and nonhomologous recombination. Wang and coworkers were the first to suggest that the DNA strand passage activity of Topo III was a potential means of recombination control (Wang et al., 1990). Our findings extend the original proposal by demonstrating that RecQ helicase is a specific factor that enhances the DNA strand passage activity of Topo III, and therefore, it is the concerted

actions of RecQ helicase and Topo III that are responsible for the control of recombination in *E. coli*. The expected outcome of recombination between two homologous sequences in separate chromosomal domains is the excision or loss of intervening DNA or markers. If Topo III and RecQ are important for the suppression of this type of recombination, then loss of their combined activities should elevate the frequency of recombination-mediated deletions between repeated DNA sequences. In agreement, loss of Topo III function in wild-type cells results in a substantial increase in the number of deletions arising from intramolecular recombination events between direct repeats of the *lacI* gene (Whoriskey et al., 1991; Schofield et al., 1992).

Implications for the Function of the Topo III–Sgs1 Helicase Complex in *S. cerevisiae*

S. cerevisiae also appears to suppress recombination using a similar mechanism, employing a RecQ-like helicase and a Topo III–type topoisomerase. Topo III from *S. cerevisiae*, encoded by the *TOP3* gene, is structurally and functionally homologous to the bacterial protein (see above). Null mutations at the *TOP3* locus result in decreased growth rate, a sporulation defect, and increased recombination frequency within the rDNA cluster and δ sequences (Wallis et al., 1989; Gangloff et al., 1994). Expression of *E. coli* Topo I in *top3* null mutants alleviates the slow growth and sporulation defect in these cells but not the hyperrecombination phenotype (Wallis et al., 1989). Thus, Topo I cannot participate in the recombination-dependent functions of Topo III. Interestingly, *E. coli* Topo I, unlike bacterial Topo III, relaxes scDNA very effectively (Wang, 1971) but decatenates DNA poorly (Hiasa et al., 1994) and, as we show here, is unaffected by RecQ helicase. Therefore, the catenation/decatenation activity of yeast Topo III, not its relaxation activity, is required to suppress recombination between repetitive sequences.

Recombination frequency at the rDNA locus is also elevated in the absence of Sgs1 helicase (Gangloff et al., 1994). The product of these recombination events is the extrachromosomal rDNA circles responsible for aging in yeast (Sinclair and Guarente, 1997). As indicated above, yeast Topo III and Sgs1 helicase physically interact and, therefore, are thought to function as a complex. Further, *SGS1* is epistatic to *TOP3*, consistent with Sgs1 helicase acting before Topo III in the same pathway. On the whole, the accumulated data support the idea that Sgs1 helicase and yeast Topo III are integral components of a pathway needed to suppress recombination. Moreover, the data presented here, taken together with the similarities between the yeast and bacterial proteins, strongly suggest that recombination suppression is accomplished by a strand passage activity composed of yeast Topo III and Sgs1 helicase.

Sgs1 helicase and yeast Topo III may also play a vital role in separating newly replicated daughter chromosomes (Wang, 1991). This model envisions that as replication forks approach one another, the remaining unreplicated region between the forks becomes highly overwound and, as a result, inaccessible to the ssDNA-specific yeast Topo III (Gangloff et al., 1994). Therefore, Sgs1 helicase is needed to provide yeast Topo III access to the interfork region, so that it can decatenate the newly replicated chromosomes to allow proper segregation of the daughter chromosomes. This model is supported by the findings that Δ*sgs1* strains display pronounced genome instability, including elevated meiotic and mitotic chromosome nondisjunction (Watt et al., 1995, 1996). Indirect support for this model is also provided by the data presented here in which we demonstrate that the strand passage activity of yeast Topo III is stimulated by a relative of Sgs1 helicase, RecQ helicase. If Sgs1 helicase in fact exerts a similar effect on the strand passage activity of yeast Topo III, then these two proteins could clearly participate in the decatenation of newly replicated daughter chromosomes. However, Sgs1 helicase also physically interacts with yeast Topo II, a type II topoisomerase that is also an active decatenase (Watt et al., 1995). Therefore, it remains to be determined which combination of helicase and topoisomerase actually take part in chromosome decatenation in *S. cerevisiae*.

Implications for the Function of the Blm and Wrn Helicases in Mammalian Cells

Helicase–topoisomerase combinations may also be important for control of recombination and decatenation of chromosomes in human cells. Recent investigations demonstrated that mutations in two human RecQ helicase homologs, the Wrn and Blm helicases, result in the diseases Werner's and Bloom's syndromes, respectively (Ellis et al., 1995; Yu et al., 1996). Although each is a distinct disease, cells isolated from both Werner's syndrome and Bloom's syndrome patients share the common phenotypic feature of pronounced genomic instability (Ellis et al., 1995; Yu et al., 1996). According to the accumulated data, it is highly likely that the Wrn and Blm helicases are intimately involved in the orderly recombination and segregation of chromosomes. Although no evidence yet exists that Topo III–type topoisomerases are needed for maintenance of chromosome stability in human cells, two variants of Topo III have been identified in human cells (Hanai et al., 1996; Kawasaki et al., 1997). It will be interesting to see whether these two proteins, like their bacterial and yeast counterparts, function with helicases such as Wrn or Blm in a manner similar to that described here for *E. coli* Topo III and RecQ helicase.

Experimental Procedures

Proteins and Reagents

RecQ helicase and SSB protein were purified as described previously (Harmon and Kowalczykowski, 1998). *E. coli* Topo III and Topo I were purified as described previously (DiGate and Marians, 1988; Hiasa and Marians, 1994). *E. coli* Topo IV was kindly provided by Dr. Kenneth J. Marians (Memorial Sloan-Kettering Cancer Center, New York, NY). *E. coli* helicase II was a kind gift of Dr. Steve Matson (University of North Carolina, Chapel Hill, NC). *S. cerevisiae* Topo III was a kind gift of Dr. James C. Wang (Harvard University, Cambridge, MA). *S. cerevisiae* RPA was a kind gift of Dr. Tomohiko Sugiyama (University of California, Davis, CA). Wheat germ Topo I was purchased from Promega and DNA gyrase from GIBCO-BRL. Norfloxacin (Sigma) was dissolved and diluted in 10 mM NaOH.

DNA

pUC19 supercoiled DNA was purified using alkaline lysis followed by CsCl–ethidium bromide equilibrium centrifugation (Sambrook et

al., 1989). The pUC19 relaxed dsDNA substrate was produced by treatment with wheat germ Topo I as described by the manufacturer. Relaxed dsDNA was recovered by ethanol precipitation following phenol extraction. pBSKM nicked dsDNA and ϕX174 gDNA with a 162 nucleotide gap between the PstI and AvaI sites were the kind gift of Dr. Eugene Zaitsev (University of California, Davis, CA). M13mp7 circular ssDNA was purified as described previously (Messing, 1983).

Supercoiling Assay

All reactions were carried out under standard conditions, which consisted of 25 mM Tris acetate (pH 7.5), 1 mM magnesium acetate, 0.1 mM DTT, 1 mM PEP, 80 U ml^{-1} pyruvate kinase, 0.1 mg ml^{-1} BSA, and 1 mM nucleotide cofactor. Standard reactions consisted of 10 μM pUC19 relaxed dsDNA, 2 μM SSB protein, 7 U wheat germ Topo I, and 0.25 μM RecQ helicase. All components except for wheat germ Topo I and RecQ helicase were incubated at 37°C for 2 min prior to the taking of a zero time point and start of the reaction with the addition of the topoisomerase, followed by RecQ helicase. Aliquots were stopped with 50 mM EDTA and 1% SDS, deproteinized with 1.5 μg μl^{-1} proteinase K at 37°C for 5 min prior to loading on a 1% agarose gel, and run in 1× TAE buffer at 1.5 V cm^{-1} for 12 hr. Gels were stained with 0.5 μg ml^{-1} ethidium bromide in water for 1 hr followed by extensive (>2 hr) destaining with water.

RecQ Helicase–Stimulated Topo III Catenation Assay

All reactions were carried under standard conditions as described under "Supercoiling Assay," with the addition of 10% (wt/vol) polyethylene glycol (PEG) to the buffer. The PEG is not essential, however, since reducing its concentration from 10% to 0% monotonically reduced the yield of DNA multimers to approximately 5% of the standard reaction (F. G. H. and S. C. K., unpublished observations). Where indicated, the buffer also contained 100 μM norfloxacin. Standard reactions consisted of 15 μM pUC19 scDNA, 1.4 μM SSB protein, 0.1 μM Topo III, and 1.0 μM RecQ helicase. Where indicated, Topo III was substituted with either 0.1 μM Topo III-F, 0.1 μM Topo IV, 14 U DNA gyrase, or 0.1 μM *E. coli* Topo I. In addition, 5 μM ϕX174 gDNA or 7.5 μM M13mp7 ssDNA was present where indicated. Reactions having yeast Topo III were carried out as above, except the protein concentrations were as follows: 0.1 μM yeast Topo III and 0.7 μM SSB protein or 0.23 μM RPA.

All components, except for topoisomerase and ATP, were equilibrated at 37°C for 2 min. Reactions were started by the sequential addition of topoisomerase and ATP. In reactions containing either ϕX174 gDNA or M13mp7 ssDNA, reactions were initiated by addition of a mixture of Topo III and SSB protein to prevent SSB protein from blocking the binding of Topo III to the ssDNA. Reactions were stopped and analyzed as described under "Supercoiling Assay," except that SDS was omitted from the stop buffer.

Protein-free multimers for restriction digest analysis were isolated from a standard catenation reaction after 60 min by ethanol precipitation subsequent to digestion of the proteins with proteinase K at 65°C for 20 min. Proteinase K was inactivated prior to ethanol precipitation by incubation at 75°C for 20 min. Digestion of the purified multimers was carried out in the buffer supplied with Hind III enzyme (New England Biolabs) supplemented with 0.5 μg ml^{-1} ethidium bromide. Reactions were terminated and analyzed as above, except all solutions contained 0.5 μg ml^{-1} ethidium bromide.

Reactions with helicase II were carried out in the standard buffer with 0.3 μM SSB protein and either 15 μM pUC19 scDNA or 10 μM pBSKM nicked DNA. Reactions were initiated by the sequential addition of 0.1 μM Topo III and 0.25 μM of helicase II. Aliquots were treated as above, except that incubation with proteinase K was at 55°C for 5 min.

Alkaline agarose gel electrophoresis was carried out as described (Sambrook et al., 1989). Gels of 1% agarose were run for 1.5 V cm^{-1} for 20 hr and then neutralized by soaking in 1 M Tris–HCl for 1 hr. The DNA was visualized by ethidium bromide staining as above.

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