Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 785–789, January 1996
Biochemistry

Resolution of Holliday junctions by eukaryotic DNA topoisomerase I

JoAnn Sekiguchi*, Nadrian C. Seeman†, and Stewart Shuman*

*Molecular Biology Program, Sloan–Kettering Institute, New York, NY 10021; and †Chemistry Department, New York University, New York, NY 10003

Communicated by Kenneth I. Berns, Cornell University Medical College, New York, NY, October 11, 1995 (received for review August 29, 1995)

ABSTRACT **The Holliday junction, a key intermediate in both homologous and site-specific recombination, is generated by the reciprocal exchange of single strands between two DNA duplexes. Resolution of the junctions can occur in two directions with respect to flanking markers, either restoring the parental DNA configuration or generating a genetic crossover. Recombination can be regulated, in principle, by factors that influence the directionality of the resolution step. We demonstrate that the vaccinia virus DNA topoisomerase, a eukaryotic type I enzyme, catalyzes resolution of synthetic Holliday junctions *in vitro*. The mechanism entails concerted transesterifications at two recognition sites, 5′-CCCTT↓, that are opposed within a partially mobile four-way junction. Cruciforms are resolved unidirectionally and with high efficiency into two linear duplexes. These findings suggest a model whereby type I topoisomerases may either promote or suppress genetic recombination *in vivo*.**

Type I topoisomerases have been considered candidate agents in regulating genetic recombination ever since their discovery. One can easily envision a catalytic function in promoting recombination whereby topoisomerase I, which forms a covalent adduct with one strand of the DNA duplex, would religate the bound DNA to a heterologous acceptor strand. Indeed, purified type I topoisomerases catalyze such strand transfer reactions *in vitro* (1–6), and the vaccinia virus enzyme can do so when expressed in a heterologous system *in vivo* (7, 8). Recent genetic studies suggest that type I topoisomerases can also act as suppressors of recombination *in vivo*. When specific bacterial or yeast topoisomerases are inactivated, there is an increase in recombination between direct repeats in the bacterial or yeast genome (9–13). This has prompted the suggestion that topoisomerases may actively disengage recombination intermediates (14). A plausible target for positive or negative regulation of recombination by topoisomerases is the Holliday junction.

Holliday junctions have been extensively studied as model intermediates in homologous and site-specific recombination (15, 16). Enzymes that resolve these structures have been isolated from a variety of sources. The best-studied examples are *Escherichia coli* RuvC (17–19), *E. coli* Rus (20), phage T4 endonuclease VII, and phage T7 endonuclease I (21–24). These enzymes are endonucleases that recognize the junction and make pairwise hydrolytic nicks to liberate nicked linear duplexes. Members of the recombinase family (Flp, Int, Cre) generate and resolve Holliday intermediates during site-specific recombination; these proteins can resolve synthetic four-way DNA junctions *in vitro*, provided that the substrates contain recombinase binding sites in the four arms (25–28). The recombinase mechanism entails transesterification, not hydrolysis, and is essentially identical to the reaction mechanism of the eukaryotic type I DNA topoisomerases.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Can the eukaryotic type I topoisomerases act on Holliday junctions? We examined this issue by using purified vaccinia virus DNA topoisomerase and synthetic Holliday junctions. The 314-amino acid type I topoisomerase encoded by vaccinia virus is mechanistically and structurally similar to the nuclear type I enzymes and has served as a prototype for structure–function analysis of the eukaryotic topoisomerase I family (29–33). A distinctive feature of the vaccinia enzyme is its specificity for cleavage at the sequence 5′-YCCTT↓ (Y = C or T) in duplex DNA (34–38). The vaccinia enzyme is thus ideally suited to study topoisomerase action on four-way junctions because the substrates are readily designed on the basis of the CCCTT cleavage site. We report herein that the vaccinia topoisomerase is indeed a Holliday junction "resolvase."

RESULTS

A synthetic Holliday junction was formed by annealing four DNA oligonucleotides, 59, 66, 75, and 68 nucleotides in length. This Holliday junction contains 10 complementary bases flanking the branch point, permitting branch migration across the 10-bp homologous core (Fig. 1). Topoisomerase cleavage sites are situated on strands 2 and 4 within the core. The substrates used in the junction resolution assay were uniquely 5′-end-labeled on one of the four constituent strands; this allowed the fate of each strand to be followed separately. The junctions were incubated with a 10-fold molar excess of vaccinia topoisomerase [purified to homogeneity as described (31)], then the DNA was deproteinized and analyzed by electrophoresis through a nondenaturing polyacrylamide gel. Successful and highly efficient resolution was evinced by a conversion of the radiolabeled junctions, which migrate slowly relative to linear DNA under native conditions, into one of two linear duplex products of 59 or 75 bp (Fig. 2). Junctions labeled on either strand 1 or strand 2 were resolved to a 59-bp duplex, whereas junctions labeled on strand 3 or 4 were converted to duplexes of 75 bp (Fig. 2). This showed that topoisomerase-catalyzed resolution was unidirectional and occurred by action of the enzyme on the CCCTT-containing strands (Fig. 1).

According to the proposed reaction pathway (Fig. 1), each 5′-labeled CCCTT-containing strand should be converted in the process of resolution into a novel species of predictable size. In contrast, the chain length of the AAGGG-containing strands should not change. This was exactly what was observed when the reaction products were electrophoresed under denaturing conditions. Strands 1 and 3 were unaffected by incubation with topoisomerase, whereas strand 2, originally 66 nt, was converted into a product of 59 nt, and strand 4 was converted from 68 nt to 75 nt (Fig. 3). Note that an increase in chain length of any strand is incompatible with a hydrolytic mechanism of resolution but is diagnostic of a cleavage-religation event. Additional reaction products derived from strand 2 and 4 corresponded to strands that were linked covalently to topoisomerase. The size of these species (denoted by Cl in Fig. 3) was consistent with cleavage of strands 2 and 4 at the CCCTT sites; heterogeneity was caused by a variable

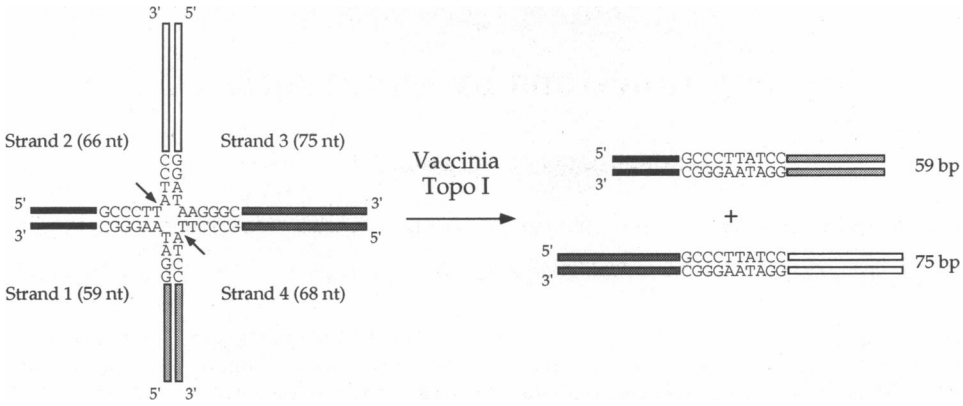

FIG. 1. Synthetic Holliday junction substrate. The junction was designed by using the program SEQUIN (39) and consisted of synthetic 59-, 66-, 75-, and 68-nucleotide DNA strands. The sequences of the oligonucleotides were as follows: strand 1, GGTTGACGTTCTAGTGTGACCGCAATACGGATAAGGGCTGAGCACGCCGACGAACATAC; strand 2, GTATGTTCGTCGGCGTGCTCAGCCCTTATCCCAGAATGCACCAACAGTTCCTCAAGATAGAGACTC; strand 3, GAGTCTCTATCTTGAGGAACTGTTGGTGCATTCTGGGATAAGGGCACTATGGCTCCACTGATGTCGTAAGCATCC; and strand 4, GGATGCTTACGACATCAGTGGAGCCATAGTGCCCTTATCCGTATTGCGGTCACACTAGAACGTCAACC. In preparing the junction, one strand was 5′-end-labeled at high specific activity with polynucleotide kinase and [γ-^{32}P]ATP prior to hybridization to the three other constitutent strands, each of which had been 5′-end-labeled at 1% the specific activity of the "hot" strand. All oligonucleotides were gel-purified after the kinase reaction to free them of ATP. The hot strand was annealed with a 4-fold excess of each of the other three strands in 10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.2 M NaCl by heating at 95°C for 2 min, followed by incubation at 65°C for 10 min, 37°C for 10 min, and 22°C for 10 min. The four-strand junctions were then purified away from the single-strand and two- or three-strand molecules by electrophoresis through a 4% polyacrylamide gel. The junctions were excised from the gel and eluted into 0.4 ml of 10 mM Tris·HCl, pH 8.0/1 mM EDTA/5 mM $MgCl_2$. The structure of the junction is diagrammed at the left. The sequence of the 10-bp homologous core is shown with the topoisomerase cleavage sites positioned at the crossover point. The arrows indicate the scissile bonds on strands 2 and 4. The nonhomologous arms flanking the central core are depicted as shaded rectangles. Resolution by the vaccinia topoisomerase (Topo I) by concerted cleavage at the two CCCTT sites and religation to the heterologous strand should produce two linear duplexes of 59 and 75 bp.

number of residual amino acids linked to the 3′ phosphate of the cleaved strand after digestion with proteinase K (35). Religated recombinant strands predominated over the covalent intermediate.

The resolved product of the junction originally end-labeled in CCCTT-containing strand 2 was gel-purified and subjected to Maxam–Gilbert chemical sequencing. The 59-nt product contained sequence 5′ of the CCCTT-containing core region that derived from strand 2 and sequence 3′ of the core derived from strand 4 (data not shown). Similar sequence analysis of the resolved product of the junction labeled originally in strand 4 showed that the 75-mer recombinant strand contained 5′ sequence contributed by strand 4 and sequence 3′ of the core region contributed by strand 2 (not shown). No gain or loss of

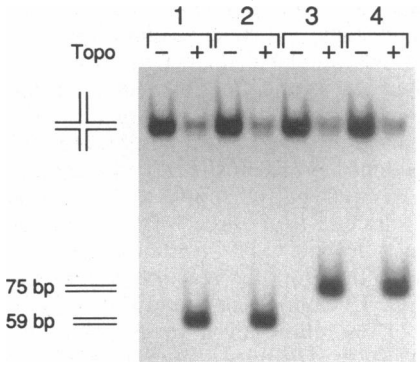

FIG. 2. Holliday junction resolution by purified topoisomerase. Reaction mixtures (20 μl) containing 50 mM Tris·HCl at pH 8.0, 1.5 mM $MgCl_2$, 0.5 pmol of junction DNA, and 0 (− lanes) or 5 pmol (+ lanes) of topoisomerase were incubated at 37°C for 15 min. Reactions were terminated by adjustment to 0.2% SDS and proteinase K at 0.5 mg/ml. After incubation at 37°C for 1 h, the DNA products were extracted with phenol and then phenol/chloroform. The aqueous phase was removed and adjusted to 5% glycerol, then the samples were electrophoresed through a 4% polyacrylamide gel in 0.25× TBE (22.5 mM Tris borate, pH 8.3/6.3 mM EDTA) at 100 V for 3 h. The gels were dried and then subjected to autoradiography. The numbers above the bracketed lanes indicate the strand which was uniquely 5′-labeled in the junction substrate. The positions of the synthetic Holliday junctions and the linear resolution products are indicated at the left.

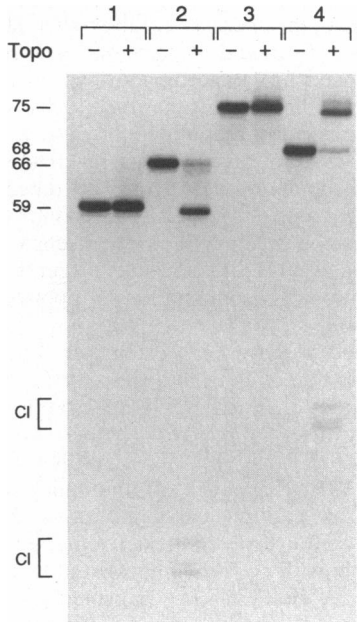

FIG. 3. Analysis of recombinant DNA molecules by denaturing gel electrophoresis. The deproteinized products of the resolution reaction were precipitated with ethanol, resuspended in formamide, heat-denatured, and then electrophoresed through a 12% polyacrylamide gel containing 7 M urea in TBE (90 mM Tris borate, pH 8.3/25 mM EDTA) at 60 W for 2.5 h. An autoradiograph of the gel is shown. The numbers above the bracketed lanes indicate the strand which was uniquely 5′-labeled in the junction substrate. The positions and chain lengths of the component strands are indicated at the left. Cleaved DNA strands (Cl) are indicated by brackets.

nucleotides occurred during strand exchange. These findings confirm that topoisomerase resolved the junctions by strand cleavage and conservative strand transfer within the CCCTT-containing 10-bp core region.

The extent of resolution increased as a function of the amount of topoisomerase added (Fig. 4*A*). Resolved linears accumulated with increasing time of incubation (Fig. 4*B*). Nearly 80% of the junctions were resolved after 10–20 min. Resolution was refractory to added salt at concentrations up to 100–150 mM, but it declined sharply at >200 mM NaCl (Fig. 4*C*). These results underscore the remarkably high efficiency of the topoisomerase as a Holliday junction resolvase.

At 25 nM input junction DNA, optimal resolution was achieved at 250 nM input enzyme—i.e., at a 10:1 ratio of enzyme to junction molecules, or 5:1 enzyme to cleavage sites (Fig. 4*A*). The titration curve deviated from linearity at low topoisomerase concentrations, as might be expected in a reaction that requires the binding of the topoisomerase monomers to two different sites on the substrate. Note that binding and cleavage by topoisomerase at only one CCCTT site leaves no option for strand transfer and thus does not score in the assay for resolution. Binding of the topoisomerase to the junction was examined directly by using a nondenaturing gel mobility-shift assay (Fig. 5). At 10 nM input DNA, increasing concentrations of enzyme resulted in formation of discrete complexes of retarded mobility. A shifted species observed at 10–20 nM enzyme appears to be a closely spaced doublet; these bands may represent junction molecules bound by a single topoisomerase molecule at one or the other CCCTT site. At 50 nM enzyme, the input junctions were bound quantitatively and several new species were detected. These include a more slowly migrating complex (presumably containing two enzyme molecules bound to the junction) and the more rapidly migrating products of junction resolution. The resolved molecules migrated either as a 59-nt linear duplex or as shifted duplexes containing bound topoisomerase (Fig. 5). At 100 nM enzyme, most of the input DNA was resolved and the linear product remained associated with enzyme. Thus, this nondenaturing gel assay demonstrated clearly that topoisomerase-catalyzed resolution was in no way dependent on the addition of protein denaturants to the reaction. We detected at 100 nM enzyme a higher-order shifted complex that may correspond to junction DNAs with more than two bound enzyme molecules. At higher enzyme concentrations, the vaccinia topoisomerase is known to bind noncovalently at nonspecific sites on duplex DNA (40).

Parallel binding experiments were performed with [Phe^{274}]-topoisomerase, a mutated version of vaccinia topoisomerase in which the active-site nucleophile, Tyr-274, has been replaced by phenylalanine. [Phe^{274}]Topoisomerase binds DNA noncovalently but is unable to catalyze transesterification (36, 40). As shown in Fig. 5, [Phe^{274}]topoisomerase bound to the Holliday junction DNA with the same affinity as wild-type enzyme and formed a similar series of retarded complexes. However, [Phe^{274}]topoisomerase was not able to resolve the junction (Fig. 5). (A lesser amount of the putative 2:1 enzyme DNA complex was seen for [Phe^{274}]topoisomerase than for the wild-type topoisomerase at 50 nM enzyme in this experiment, but other titration experiments confirmed that this complex was formed by [Phe^{274}]topoisomerase.) We conclude that binding of topoisomerase to the Holliday junction is independent of strand cleavage.

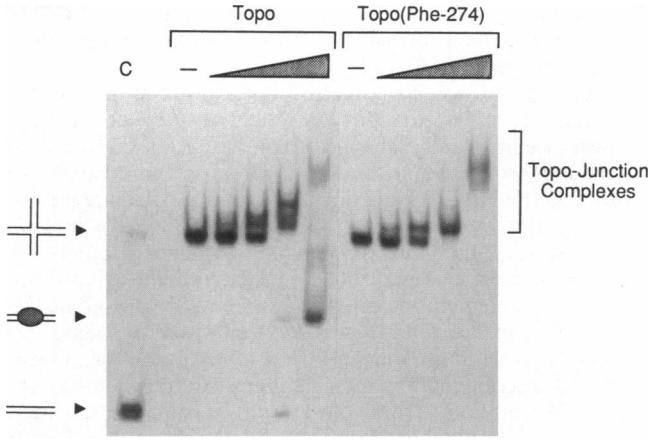

FIG. 5. Binding of topoisomerase to the Holliday junction. Reaction mixtures (20 μl) contained 50 mM Tris·HCl at pH 8.0, 1.5 mM $MgCl_2$, 10 nM junction DNA (strand 2 labeled), and increasing concentrations of wild-type vaccinia topoisomerase or active-site-mutant [Phe^{274}]topoisomerase [Topo(Phe-274)]—10, 20, 50, and 100 nM from left to right in each titration series. Control reaction mixtures (–) lacked enzyme. After incubation at 37°C for 15 min, glycerol was added to 5% and the samples were electrophoresed through 4% polyacrylamide gels in 0.25× TBE at 80 V for 4 h. The gels were dried and subjected to autoradiography. The deproteinized linear duplex resolution product is shown in lane C. The positions of the input junction, the enzyme-bound resolution product, and the free duplex resolution product are indicated at the left. Complexes of topoisomerase bound to the Holliday junction are denoted at the right.

DISCUSSION

Our results demonstrate that a eukaryotic type I topoisomerase is able to resolve Holliday junctions. The finding has specific implications for viral recombination. Two distinct classes of vaccinia DNA rearrangements occur in vivo—(*i*) general homologous recombination and (*ii*) resolution of the

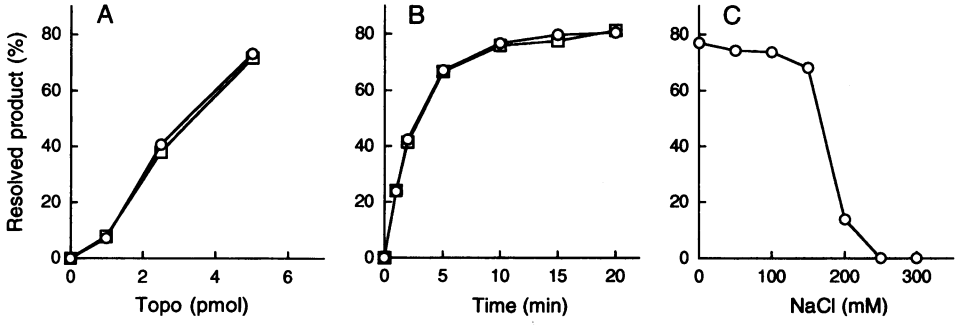

FIG. 4. Characterization of the resolution reaction. (*A*) Enzyme dependence. Reaction mixtures containing 0.5 pmol of junction DNA 5′-labeled in either strand 2 (○) or strand 4 (□) and topoisomerase as indicated were incubated for 15 min at 37°C. The percentage of input DNA resolved was determined by scanning the gel with a FUJIX (Tokyo) BAS1000 phosphorimager and is plotted as a function of input enzyme. (*B*) Kinetics. Reaction mixtures contained 25 nM junction DNA labeled in strand 2 (○) or strand 4 (□) and 250 nM topoisomerase; aliquots were withdrawn at the indicated times and processed for electrophoresis. (C) Salt effect. Reaction mixtures contained 1 pmol of junction DNA labeled in strand 2, 10 pmol of topoisomerase, and NaCl at the concentrations indicated. Incubation was for 15 min at 37°C.

vaccinia hairpin telomeres. Both recombination reactions require viral gene expression and both involve heteroduplex DNA intermediates (41, 42). Virtually nothing is known about the identity of specific viral (or host) proteins involved in poxvirus recombination, but any model must include a strand transferase or strand exchange factor that promotes formation of Holliday intermediates, as well as an enzyme capable of resolving the Holliday junctions. Vaccinia topoisomerase now emerges as a clear candidate for the role of Holliday junction resolvase in homologous recombination and/or telomere resolution. The presence of >1000 pentapyrimidine topoisomerase cleavage elements within the 192-kbp vaccinia genome (43) provides ample potential sites for topoisomerase action. In principle, the topoisomerase may also catalyze formation of Holliday recombination intermediates from two linear duplexes. Formation of the four-strand intermediate by topoisomerase would require protein-mediated synapsis and reciprocal strand exchange, essentially as carried out by the site-specific recombinases. This is a more stringent reaction than junction resolution, which does not entail DNA synapsis.

A key feature of the resolution reaction catalyzed by the vaccinia topoisomerase is that it is unidirectional. This stems from the fact that the vaccinia enzyme binds and cleaves duplex DNA as a monomer and does so at specific pentapyrimidine DNA sequences that that lack internal symmetry. Two binding sites are clearly sufficient for optimal junction resolution by vaccinia topoisomerase. This is in contrast to the recombinases, which normally bind to specific recognition sites in all four DNA arms flanking the crossover point; bacteriophage λ integrase is unable to resolve synthetic junctions containing only two Int binding sites (44, 45). Recombinases usually resolve synthetic junctions bidirectionally, but directional bias can be introduced into the system by manipulating the crossover point of the junction (46–48). In its inherent unidirectionality and sequence specificity, the vaccinia topoisomerase is akin to RuvC, the bacterial Holliday resolvase. RuvC is a sequence-specific hydrolase that resolves junctions unidirectionally as it encounters its recognition site 5′-WTT↓S (W = A or T; S = G or C) at the junction during branch migration (49).

Our finding that vaccinia topoisomerase resolves Holliday junctions suggests that cellular type I topoisomerases may catalyze similar reactions. Our data also provide a plausible biochemical framework for how type I enzymes might influence genome stability. If potential sites of topoisomerase cleavage occur with similar frequency on both strands of duplex DNA, and there is no conformational restriction on the junction, then the action of topoisomerase I on Holliday intermediates can be expected to yield an equal distribution of parental and recombinant products. However, recombination directionality *in vivo* may show strong bias. In yeast, recombination between tandem rRNA gene (rDNA) repeats is suppressed by the action of Top1 (9). Preferential resolution of Holliday intermediates by these enzymes in favor of the parental marker configuration might provide an explanation for this effect. This can occur if recombination initiates with a strand bias and if the topoisomerase cleavage sites are situated predominantly on one DNA strand within the direct repeat sequence (i.e., the strand that initially exchanges). There is some basis for this scenario insofar as (*i*) yeast rDNA contains a sequence element, *HOT1*, that stimulates mitotic recombination between direct repeats and does so in an orientation-dependent fashion (50); and (*ii*) high-affinity cleavage sites for topoisomerase I are located exclusively on the noncoding strands of the rDNA repeat of *Tetrahymena* (51). The 16-bp cleavage sequence is recognized by topoisomerase I from mammalian, avian, insect and yeast cells; related sequences are found in the rDNAs of diverse organisms, including yeast (52). The location of high-affinity sites for topoisomerase I within the ribosomal gene cluster may explain why Top1-suppression of mitotic recombination in yeast is specific to rDNA. Top3 also suppresses recombination between rDNA repeats, but the Top3 effect is not restricted to rDNA (53).

Interaction of topoisomerase with other proteins at the junction might also influence the efficiency and perhaps the directionality of resolution. In bacteria, the directionality of branch migration is established by assembly of the RuvAB helicase complex such that RuvB engages only two of the junction arms. Current models invoke association of RuvC with the branch migration complex; this might direct the resolvase to one pair of strands at the branch point (49, 54, 55). Yeast Top3 interacts genetically and physically with the helicase-like protein Sgs1 (53), which, by analogy, may be involved in branch migration in yeast and may therefore target topoisomerases to the junction.

It appears that different type I enzymes can exert distinctive and even opposite effects on recombination *in vivo*—e.g., whereas yeast Top1 and Top3 suppress recombination between rDNA repeats, overexpression in yeast of the vaccinia topoisomerase stimulates excisive recombination between rDNA repeats (56). This makes sense, given that the vaccinia protein has a distinctive sequence specificity and that the resolvase activity of the vaccinia topoisomerase ought not to be biased by virtue of interaction with components of the yeast recombination apparatus. Differential action of type I enzymes on Holliday junctions suggests how topoisomerases may wield a "double-edged sword" (14) in controlling genome stability.

We are grateful to Arthur Lustig for critical commentary on the manuscript and to Ann Knab and MaryAnn Bjornsti for sharing their findings on the effects of vaccinia topoisomerase on rDNA recombination in yeast. This work was supported by National Institutes of Health Grants GM46330 to S.S. and GM29554 to N.C.S.

1. Been, M. D. & Champoux, J. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2883–2887.
2. Halligan, B. D., Davis, J. L., Edwards, K. A. & Liu, L. F. (1982) *J. Biol. Chem.* **257,** 3995–4000.
3. Tse-Dinh, Y. (1986) *J. Biol. Chem.* **261,** 10931–10935.
4. Shuman, S. (1992) *J. Biol. Chem.* **267,** 8620–8627.
5. Shuman, S. (1992) *J. Biol. Chem.* **267,** 16755–16758.
6. Christiansen, K. & Westergaard, O. (1994) *J. Biol. Chem.* **269,** 721–729.
7. Shuman, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3489–3493.
8. Shuman, S. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10104–10108.
9. Christman, M. F., Dietrich, F. S. & Fink, G. R. (1988) *Cell* **55,** 413–425.
10. Kim, R. A. & Wang, J. C. (1989) *Cell* **57,** 975–985.
11. Wallis, J. W., Chrebet, G. Brodsky, G., Rolfe, M. & Rothstein, R. (1989) *Cell* **58,** 409–419.
12. Kim, R. A., Caron, P. R. & Wang, J. C. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2667–2671.
13. Schofield, M. A., Agbunag, R., Michaels, M. L. & Miller, J. H. (1992) *J. Bacteriol.* **174,** 5168–5170.
14. Wang, J. C., Caron, P. R. & Kim, R. A. (1990) *Cell* **62,** 403–406.
15. Lilley, D. M. J. & Clegg, R. M. (1993) *Annu. Rev. Biophys. Biomol. Struct.* **22,** 299–328.
16. Seeman, N. C. & Kallenbach, N. R. (1994) *Annu. Rev. Biophys. Biomol. Struct.* **23,** 53–86.
17. Dunderdale, H. J., Benson, F. E., Parsons, C. A., Sharples, G. J., Lloyd, R. G. & West, S. C. (1991) *Nature (London)* **354,** 506–510.
18. Iwasaki, H., Takahagi, M., Shiba, T., Nakata, A. & Shinagawa, H. (1991) *EMBO J.* **10,** 4381–4389.
19. Ariyoshi, M., Vassylyev, D. G., Iwasaki, H., Nakamura, H., Shinagawa, H. & Morikawa, K. (1994) *Cell* **78,** 1063–1072.
20. Sharples, G. J., Chan, S. N., Mahdi, A. A., Whitby, M. C. & Lloyd, R. G. (1994) *EMBO J.* **13,** 6133–6142.
21. Duckett, D. R., Murchie, A. I. H., Diekmann, S., von Kitzing, E., Kemper, B. & Lilley, D. M. J. (1988) *Cell* **55,** 79–89.
22. Mueller, J. E., Kemper, B., Cunningham, R. P., Kallenbach, N. R. & Seeman, N. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9441–9445.

23. Picksley, S. M., Parsons, C. A., Kemper, B. & West, S. C. (1990) *J. Mol. Biol.* **212,** 723–735.
24. Pottmeyer, S. & Kemper, B. (1992) *J. Mol. Biol.* **223,** 607–615.
25. Hsu, P. & Landy, A. (1984) *Nature (London)* **311,** 721–726.
26. Kitts, P. A. & Nash, H. A. (1987) *Nature (London)* **329,** 346–348.
27. Hoess, R., Wierzbicki, A. & Abremski, K. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6840–6844.
28. Dixon, J. E. & Sadowski, P. D. (1993) *J. Mol. Biol.* **230,** 522–533.
29. Morham, S. G. & Shuman, S. (1990) *Genes Dev.* **4,** 515–524.
30. Wittschieben, J. & Shuman, S. (1994) *J. Biol. Chem.* **269,** 29978–29983.
31. Sekiguchi, J. & Shuman, S. (1995) *J. Biol. Chem.* **270,** 11636–11645.
32. Sharma, A., Hanai, R. & Mondragon, A. (1994) *Structure* **2,** 767–777.
33. Hanai, R. & Wang, J. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11904–11908.
34. Shuman, S. & Prescott, J. (1990) *J. Biol. Chem.* **265,** 17826–17836.
35. Shuman, S. (1991) *J. Biol. Chem.* **266,** 1796–1803.
36. Shuman, S. (1991) *J. Biol. Chem.* **266,** 11372–11379.
37. Shuman, S. & Turner, J. (1993) *J. Biol. Chem.* **268,** 18943–18950.
38. Sekiguchi, J. & Shuman, S. (1994) *J. Biol. Chem.* **269,** 31731–31734.
39. Seeman, N. C. (1990) *J. Biomol. Struct. Dynam.* **8,** 573–581.
40. Sekiguchi, J. & Shuman, S. (1994) *Nucleic Acids Res.* **22,** 5360–5365.
41. Merchlinsky, M. (1990) *J. Virol.* **64,** 3437–3446.
42. Fisher, C., Parks, R. J., Lauzon, M. L. & Evans, D. H. (1991) *Genetics* **129,** 7–18.
43. Goebel, S. J., Johnson, G. P., Perkus, M. E., Davis, S. W., Winslow, J. P. & Paoletti, E. (1990) *Virology* **179,** 517–563.
44. Franz, B. & Landy, A. (1990) *J. Mol. Biol.* **215,** 523–535.
45. Kho, S. H. & Landy, A. (1994) *EMBO J.* **13,** 2714–2724.
46. Nunes-Duby, S. E., Azaro, M. A. & Landy, A. (1995) *Curr. Biol.* **5,** 139–148.
47. Dixon, J. E. & Sadowski, P. D. (1994) *J. Mol. Biol.* **243,** 199–207.
48. Lee, J., Lee, J. & Jayaram, M. (1995) *J. Biol. Chem.* **270,** 19086–19092.
49. Shah, R., Bennet, R. J. & West, S. C. (1994) *Cell* **79,** 853–864.
50. Voelkel-Meiman, K., Keil, R. L. & Roeder, G. S. (1987) *Cell* **48,** 1071–1079.
51. Bonven, B. J., Gocke, E., & Westergaard O. (1985) *Cell* **41,** 541–551.
52. Christiansen, K., Bonven, B. J. & Westergaard, O. (1987) *J. Mol. Biol.* **193,** 517–525.
53. Gangloff, S., McDonald, J. P., Bendixen, C., Arthur, L. & Rothstein, R. (1994) *Mol. Cell. Biol.* **14,** 8391–8398.
54. Parsons, C. A., Stasiak, A., Bennet, R. J. & West, S. C. (1995) *Nature (London)* **374,** 375–378.
55. Hiom, K. & West, S. C. (1995) *Cell* **80,** 787–793.
56. Knab, A. M. (1994) Ph.D. thesis (Thomas Jefferson Univ., Philadelphia).