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Supplemental Information

Decatenation of DNA by the *S. cerevisiae* Sgs1-Top3-Rmi1 and RPA Complex: A Mechanism for Disentangling Chromosomes

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Figure S1

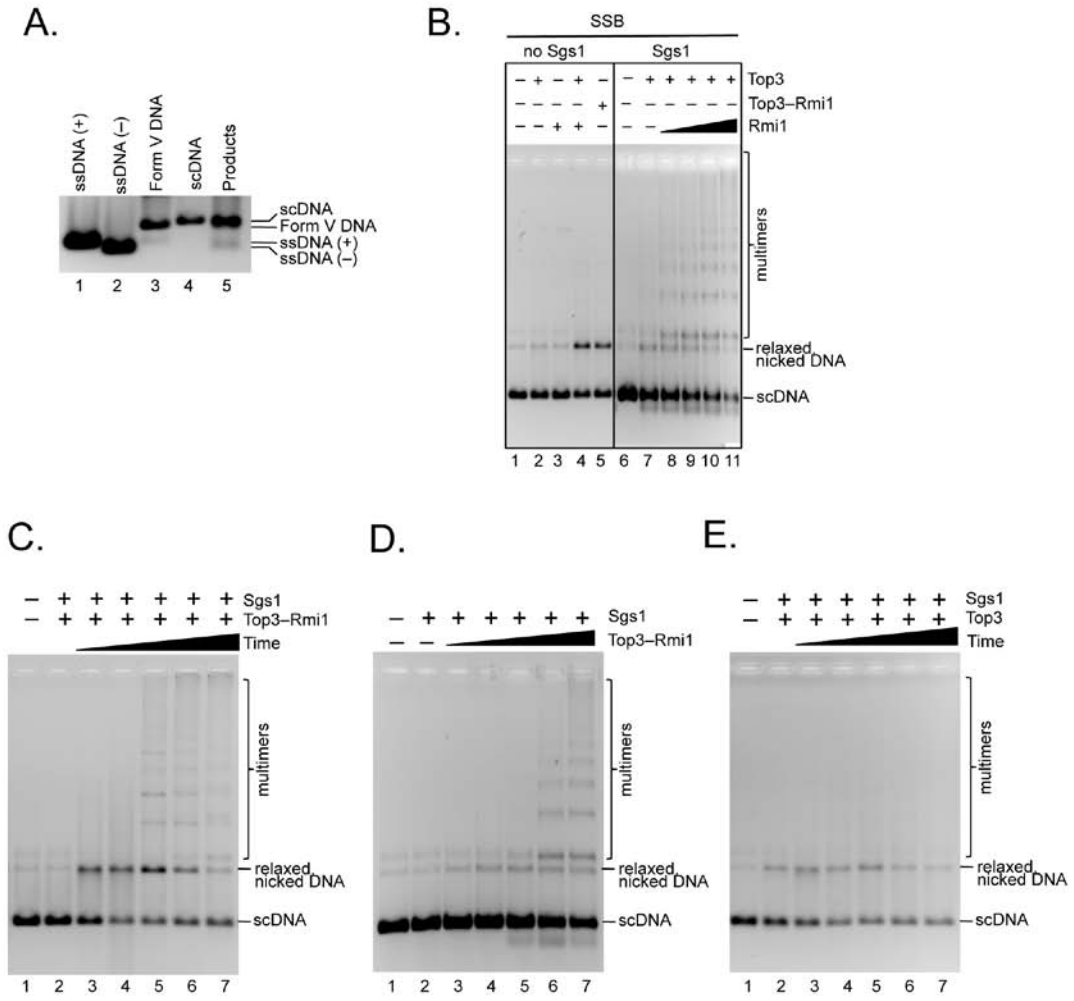

Figure S1 (related to Figure 1). Rmi1 stimulates catenation of dsDNA by Sgs1–Top3. (A) Sgs1 and Top3 can fully unlink plasmid-length dsDNA. Negatively supercoiled plasmid dsDNA (scDNA, shown in lane 4) was incubated with Sgs1 (100 nM), Top3 (400 nM), and RPA (3 μM). As seen in lane 5, a DNA band with faster electrophoretic mobility appears below the negative scDNA. The two respective ssDNA molecule markers are shown in lanes 1 and 2. The position of DNA Form V, resulting from annealing of the two complementary ssDNA molecules, is shown in lane 3. The reaction products were analyzed by 1% agarose electrophoresis, and stained with 0.5 μg/ml ethidium bromide. (B) Rmi1 stimulates catenation of dsDNA by Sgs1–Top3 in the presence of SSB. Catenation assay was carried out with Sgs1 (100 nM), Top3-Rmi1 heterodimer (300 nM), Top3 (lanes 2, 4, 7, 9: 300 nM; lanes 7-11: 400 nM), Rmi1 (300 nM, or 30, 100, 400 and 800 in lanes 8-11, respectively), and SSB (3.7 μM). (C) DNA multimers accumulate over time. Catenation assay was carried out with Sgs1 (100 nM), Top3-Rmi1 heterodimer (400 nM), and SSB (3.7 μM), for 1, 5, 10, 15 and 60 minutes, lanes 3-7, respectively. (D) DNA multimers accumulate in a Top3-Rmi1 concentration-dependent manner. Catenation assay was performed with Sgs1 (100 nM), Top3-Rmi1 heterodimer (10, 30, 100, 200, and 400 nM, lanes 3-7, respectively), and SSB (3.7 μM). (E) DNA multimers do not accumulate in the absence of Rmi1. Catenation assay as in (C), except for Top3 was used instead of Top3-Rmi1 heterodimer.

Figure S2

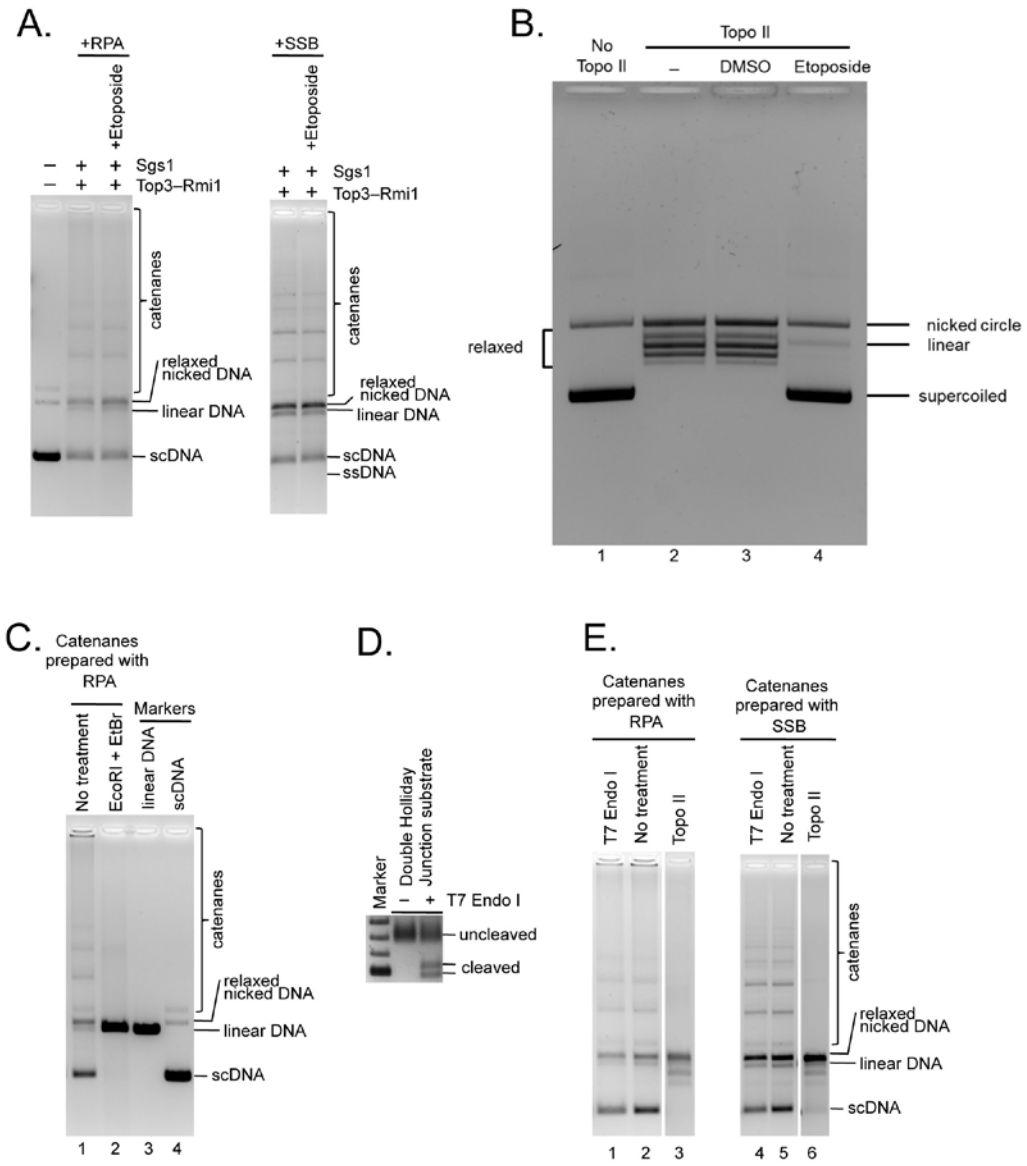

Figure S2 (related to Figure 2). Sgs1, Top3, and Rmi1 produce full dsDNA catenanes. (A) The topoisomerase II inhibitor, etoposide, does not inhibit dsDNA catenation by Sgs1–Top3–Rmi1. Catenation assay was carried out with Sgs1 (60 nM), Top3–Rmi1 (400 nM), and either RPA (1 μM) or SSB (3.7 μM). Where indicated, the reaction buffer was supplemented with etoposide (100 μM). The fact that etoposide does not inhibit the catenation reaction proves that the activity is not a result of a Topo II contamination. (B) Etoposide is active, inhibiting Topo II activity: negatively supercoiled pBlueScript SK+ plasmid (Lane 1) is relaxed by *Drosophila* Topoisomerase II (0.5 U/μl, 0.5 nM dimer) (Topo II, Lane 2). Topo II is unaffected by 0.23% DMSO (Lane 3), but is inhibited by 100 μM etoposide, 0.23% DMSO (Lane 4); a small fraction of plasmid substrate is linearized and nicked in the presence of the drug. (C) Sgs1, Top3, and Rmi1 form full dsDNA catenanes in the presence of RPA. Products of a catenation assay carried out with Sgs1 (60 nM), Top3-Rmi1 (400 nM), and RPA (1 μM), lane 1, were digested with EcoRI (10 U per reaction, 15 minutes at 37 °C, lane 2) in the presence of 0.5 μg/ml ethidium bromide. The corresponding monomer DNA linearized with EcoRI is shown in lane 3, and monomer supercoiled DNA in lane 4. (D) The activity of T7 Endo I was tested on a double HJ substrate (0.025 U per reaction, 15 minutes at 37 °C). (E) Products of a catenation assay carried out with Sgs1 (100 nM), Top3-Rmi1 (400 nM), and RPA (1 μM), lane 2, or SSB (3.7 μM), lane 5, were incubated with T7 Endo I (0.025 U per reaction, 15 minutes at 37 °C, lanes 1 and 4, respectively), or with *Drosophila* Topo II (20 U per reaction, 15 minutes at 37 °C, lanes 3 and 6, respectively). Lane 2 is identical to lane 1 in panel C; lane 5 is identical to Figure 2C, lane 1.

Figure S3

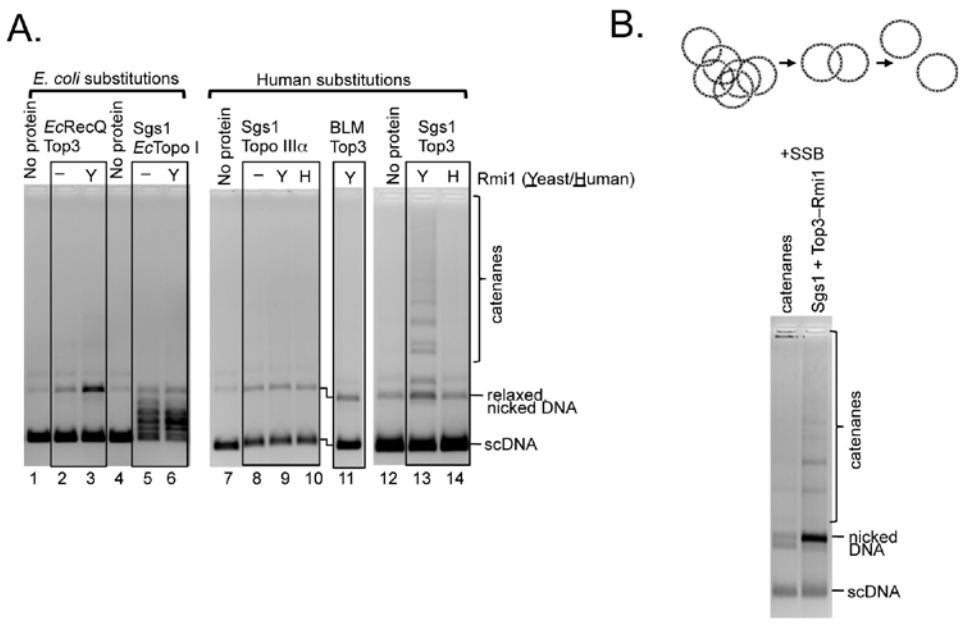

Figure S3 (related to Figure 3). DNA catenation and decatenation in the presence of heterologous proteins. (A) Catenation of dsDNA by Sgs1, Top3, and Rmi1 is specific to the yeast proteins. Catenation assay was carried out with Sgs1 (100 nM), Top3 (400 nM), Rmi1 (400 nM), Top3-Rmi1 (400 nM), *E. coli* RecQ (1000 nM), *E. coli* Topo I (5 U per reaction), human Topo IIIα (400 nM), human RMI1 (400 nM), and/or human BLM (70 nM). All reactions contained SSB (3.7 μM). (B) Sgs1, Top3 and Rmi1 proteins can decatenate DNA catenanes in the presence of SSB. Products of a catenation reaction carried out with Sgs1 (60 nM), Top3-Rmi1 (400 nM), and RPA (1 μM), lane 1, were decatenated with Sgs1 (10 nM), Top3-Rmi1 (40 nM), and SSB (2.6 μM).

Figure S4

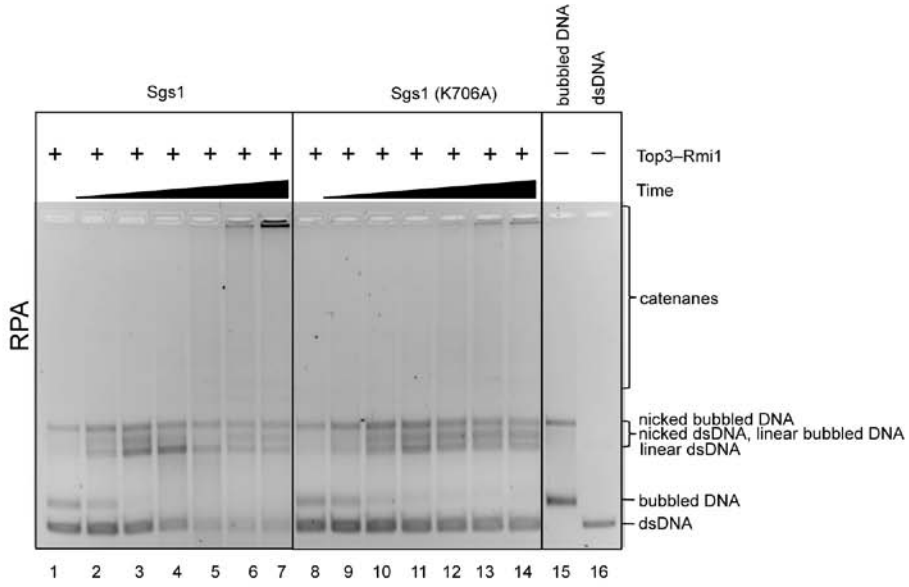

Figure S4 (related to Figure 5). Kinetic analysis of catenation of double-stranded and 'bubbled' DNA. The reactions contained both 'bubbled' and fully duplex DNA (total 10 ng/μl), Sgs1 or Sgs1(K706A), as indicated (60 nM), Top3-Rmi1 (400 nM), and RPA (1 μM), and were terminated at 1, 2, 4, 8, 15 and 30 minutes, respectively, with SDS/EDTA and Proteinase K. Panel shows a representative experiment. Quantification is shown in Figure 5C.

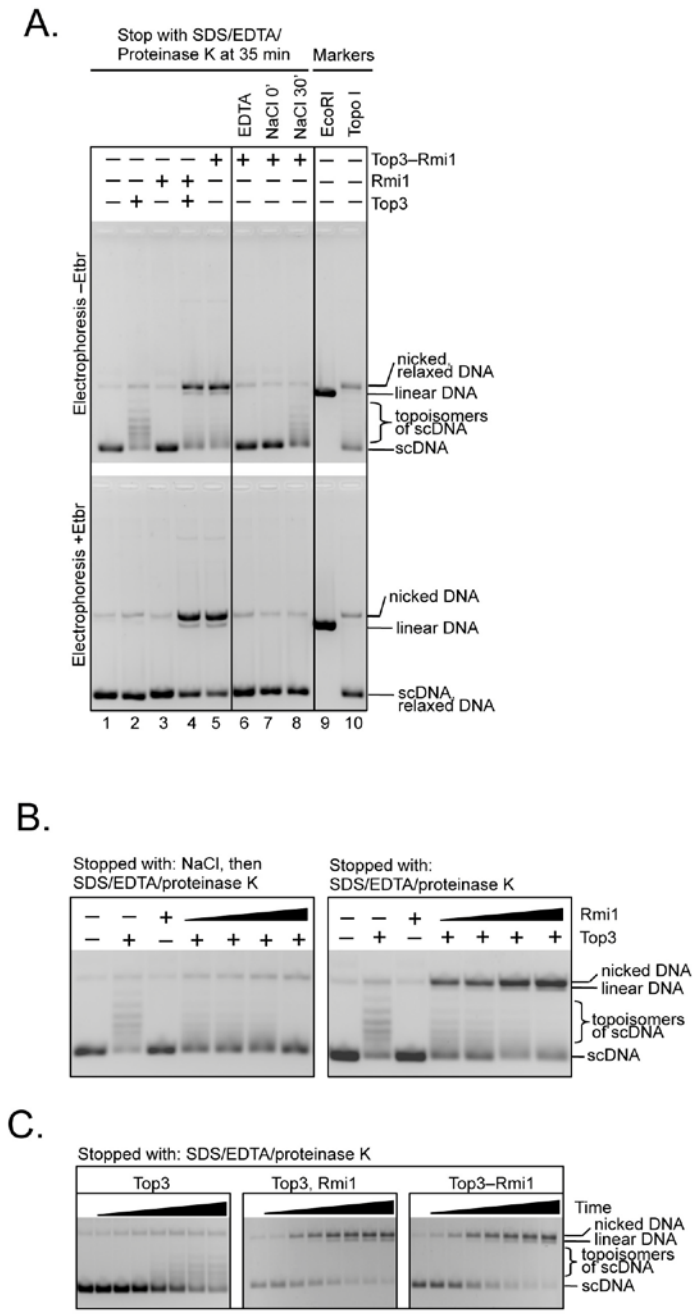

Figure S5 (related to Figure 6). Rmi1 slows Top3-dependent relaxation of negatively supercoiled DNA and increases the steady-state amount of Top3-DNA covalent intermediates. (A) Panel is identical to Figure 6A, except that the image of the entire gel is shown to show that there are no catenanes at the top of the gel. (B) Standard relaxation assay was with Top3 (100 nM) and Rmi1 (100 nM, where indicated with "+", and 50, 100, 200 and 400 nM, where indicated by the wedge, respectively). Reaction products were incubated with NaCl (500 mM, 5 min, left panel only), and all reactions were terminated with SDS, EDTA and Proteinase K as described in Experimental Procedures. The products were analyzed by 1% agarose electrophoresis, and stained with ethidium bromide (0.5 μg/ml). Quantification is shown in Figure 6B and C. (C) Standard relaxation assay was with Top3 (left, 100 nM); Top3 and Rmi1 (middle, 100 nM each); and Top3-Rmi1 heterodimer (right, 100 nM). The reactions were terminated at various times (1, 2, 4, 8, 15, 30, and 60 minutes, respectively). The products were analyzed by 1% agarose electrophoresis, and stained with ethidium bromide (0.5 μg/ml).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Recombinant proteins

S. cerevisiae Sgs1, Top3, Rmi1, Top3–Rmi1 heterodimer, and RPA; *E. coli* SSB and RecQ; and human BLM were purified as described previously (Harmon et al., 1999; Harmon and Kowalczykowski, 1998; Kantake et al., 2003; Karow et al., 1997). Human Topo IIIα was a gift from I. Hickson (University of Oxford) and P. Janscak (University of Zurich), human RMI1 from I. Hickson, and Drosophila Topo II from T. Hsieh (Duke University).

SUPPLEMENTAL REFERENCES

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