Supporting Information

Nimonkar et al. 10.1073/pnas.0809380105


Fig. S1. Analysis of DNA resection by hExo1 using alkaline agarose-gel electrophoresis. Nuclease reactions were performed as described in Materials and Methods, except that products were analyzed by electrophoresis using a 1% alkaline agarose gel at 4.5 V/cm for 12 h. (A) DNA substrate labeled at the 3' end and (B) substrate labeled at the 5' end. Shown are time courses with BLM alone (lanes 1–3), hExo1 and BLM (lanes 4–6), and hExo1 alone (lanes 7–9). The positions of the intact substrate (2.7 kbp), resection products, and molecular size standards (5' end-labeled PCR fragments) are indicated.
Fig. S2. RPA lowers the extent of BLM-stimulated resection of DNA by hExo1. Nuclease reactions were performed as described in Materials and Methods, except that the hExo1 and BLM concentrations were 40 nM and 80 nM, respectively, in the absence or presence of added hRPA (200 nM). Image shows reaction products: time course with hExo1 and BLM (lanes 1–4) and with hExo1, BLM and hRPA (lanes 5–8). The positions of the intact substrate (2.7 kbp), resection products, and molecular size standards are indicated. The asterisk marks position of higher molecular mass resection intermediates seen in the presence of RPA.
Fig. S3. Human Exo1 is not stimulated by *E. coli* RecQ. Nuclease reactions were performed as described in Materials and Methods, except BLM was replaced with an equimolar amount of *E. coli* RecQ. Image shows reactions products: time course with hExo1 alone (lanes 1–3), hExo1 and BLM (lanes 4–6), and hExo1 and *E. coli* RecQ (lanes 7–9). The positions of the intact substrate (2.7 kbp), resection products, and molecular size standards are indicated.
Fig. S4. The rate and extent of joint molecule formation are a function of hRad51 concentration. Joint molecule reactions were performed as described in Materials and Methods. Image shows reaction products: time course with 1.0 μM hRad51 (lanes 1–3), 2.5 μM hRad51 (lanes 4–6), and 5.0 μM hRad51 (lanes 7–9). The positions of intact DNA (2.7 kbp), resection products, and joint molecules are indicated.
Fig. S5. RPA negligibly enhances hRad51-mediated joint molecule formation. Joint molecule reactions were performed as described in Materials and Methods. Image shows reaction products: time course with hRad51 alone (lanes 1–3), hRad51 and hRPA (lanes 4–6), and hRPA alone (lanes 7–9). The positions of intact DNA (2.7 kbp), resection products, and joint molecules are indicated.
Fig. S6. hRad51 can mediate joint molecule formation in the presence of a heterologous RPA. Joint molecule reactions were performed as described in Materials and Methods. Lane 1, control, substrate incubated in the absence of any protein; lanes 2-5, pairing reactions in the presence of hRPA, yRPA, and no RPA, respectively. The positions of intact DNA (2.7 kbp), resection products, and joint molecules are indicated.