Supplemental Material to:

RNase H2-Initiated Ribonucleotide Excision Repair

Justin L. Sparks[1], Hyongi Chon[2], Susana M. Cerritelli[2], Tom A. Kunkel[3], Erik Johansson[4], Robert J. Crouch[2] and Peter M. Burgers[1]*

[1]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110; [2]Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda MD 20892; [3]NIEHS; [4]Department of Medical Biochemistry and Biophysics, Umeå University, SE-901 87, Umeå, Sweden.

EXPERIMENTAL PROCEDURES

Proteins **-** RPA (Henricksen et al., 1994), PCNA (Eissenberg et al., 1997), RFC (Gomes et al., 2000), FEN1 (Gomes and Burgers, 2000), RNase H1 (Cerritelli et al., 1993), and RNase H2 (Jeong et al., 2004), and RNase H2 mutants (Chon et al., 2009) were purified from *E. coli* overexpression systems, while Pol δ (Fortune et al., 2006), Pol ε (Chilkova et al., 2003), Dna2 (Ayyagari et al., 2003), Exo1 (Gelperin et al., 2005), and DNA ligase (Ayyagari et al., 2003) were purified from yeast overexpression systems.

Determination of average ribonucleotide misincorporation frequency - This frequency follows from the median size of alkali-resistant replication products. First, the radioactivity distribution, between 10 kb and 0.2 kb, was divided up in ~500 segments and the radioactivity value in each segment divided by the size of the DNA in each segment (determined from size markers). This essentially converts the radioactivity distribution into a size distribution of alkali-resistant DNA molecules, from which the median length of the DNA population can be determined. Second, we need to account for the presence of a nick in the completely replicated DNA, and for DNA breakage due to alkaline treatment and handling that is unrelated to the presence of incorporated ribonucleotides. Since the size distributions, on a neutral gel, of M13mp8 DNA replicated with or without rNTPs are the same (Fig. S1B), the median size of alkali-resistant replication products synthesized without rNTPs forms a suitable control. From this we can determine the frequency of ribonucleotide incorporation as:

a=median size with dNTPs, b=median size with dNTPs + rNTPs.
rNTP incorporation frequency = a/(a/b - 1).

Under the conditions used:
for Pol δ: a = 4.1 kb, b = 0.61 kb --> rNTP incorporation frequency = 1/720
for Pol ε: a = 3.5 kb, b = 0.54 kb --> rNTP incorporation frequency = 1/640

SUPPLEMENTARY FIGURES

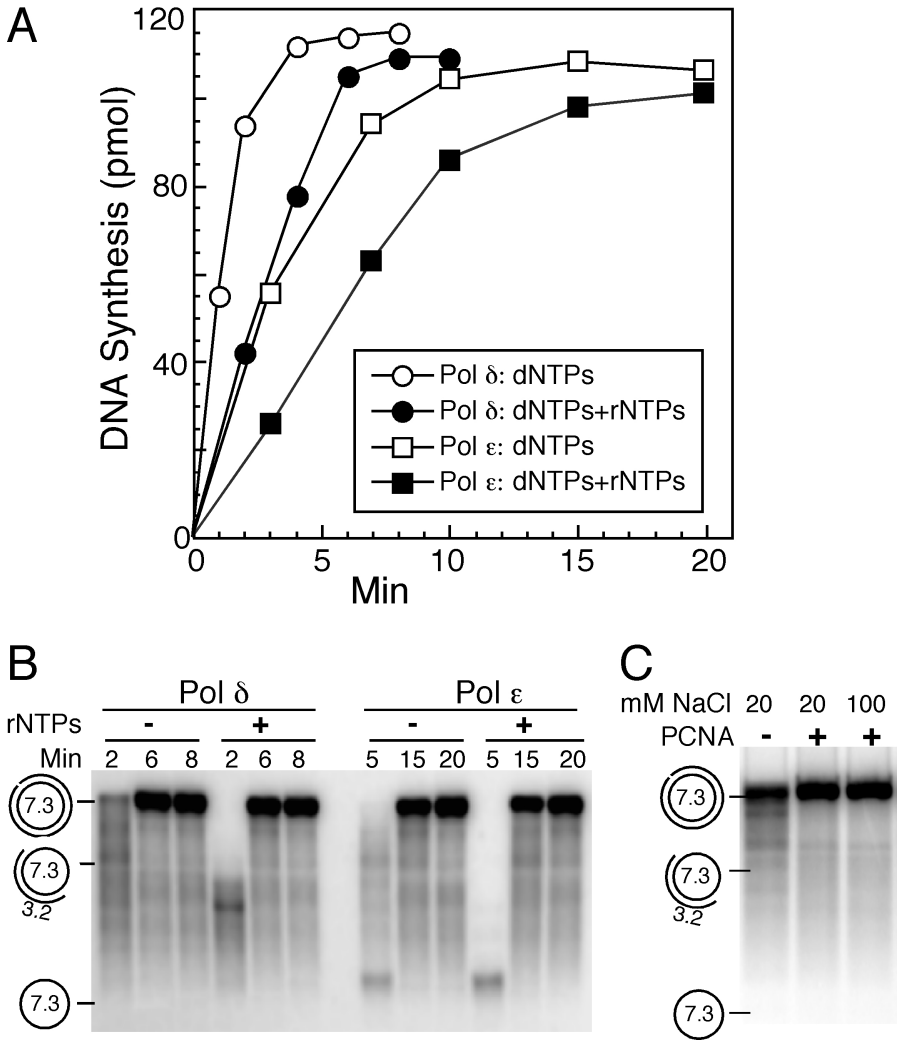

Fig. S1. Associated with Fig. 1. Replication of M13mp18 DNA in the presence of rNTPs. (A) rNTPs slow down replication rates. DNA replication assays were carried out as described in Materials and Methods under standard conditions with PCNA and either Pol δ or Pol ε, with or without rNTPs. Aliquots were taken and acid-precipitable radioactivity determined. **(B,C) Complete replication of M13mp18 DNA. (B)** Selected aliquots from (A) were electrophoresed on a 1% agarose gel. Note the decrease in the size of replication products at the early time points in the presence of rNTPs. Marker positions are SS-M13mp18 DNA and nicked DS-M13mp18 DNA, and gapped DS-M13mp18 DNA, as shown. **(C)** The standard assay (lane 3) contains dNTPs plus rNTPs, 100 mM NaCl, 1.5 nM primed M13mp18 DNA, RPA, 15 nM PCNA, 3 nM RFC and 3 nM of Pol δ for 8 min. In lane 1, PCNA and RFC were omitted, NaCl was reduced to 20 mM, Pol δ increased to 500 nM, and the incubation time to 180 min. In lane 2, the assay was the same as standard except for 20 mM NaCl. Replication products were analyzed on a 1% neutral agarose gel (shown here), or digested with NaOH and analyzed on an alkaline agarose gel (shown in Fig. 1C). Marker positions are as indicated.

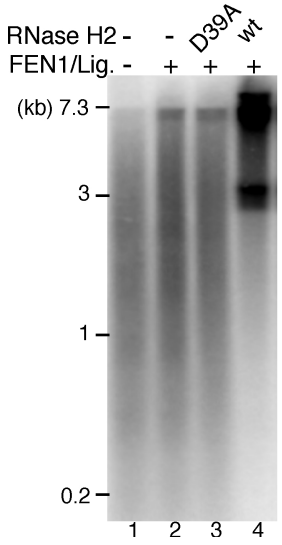

Fig. S2. Associated with Fig. 2. RER requires the catalytic function of RNase H2. Primed M13mp18 DNA was replicated with dNTPs plus rNTPs, RPA, PCNA, RFC and Pol δ for 8 min. Then FEN1, DNA ligase and either wild-type RNase H2 or catalytic-dead RNase H2-D39A was added as indicated, and incubation continued for an additional 4 min. Replication products were digested with NaOH and analyzed on an 1% alkaline agarose gel. Size markers are shown.

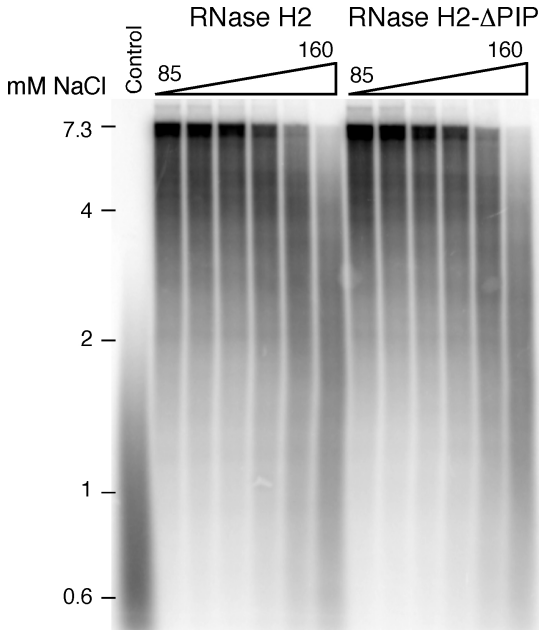

Fig. S3. Associated with Fig. 3. The PCNA-interaction motif of RNase H2 is not required for efficient RER. Primed M13mp18 DNA was replicated with dNTPs plus rNTPs, RPA, PCNA, RFC and Pol δ for 8 min. Then, the indicated levels of NaCl were added followed by 3 nM FEN1, 15 nM DNA ligase I, and 0.6 nM RNase H2 or RNase H2-ΔPIP, and incubation continued for 3 min. Replication products were digested with NaOH and analyzed on an 1% alkaline agarose gel. Size markers are shown.

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