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Supplementary Materials for

Overcoming a nucleosomal barrier to replication

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This PDF file includes:

Supplementary Figures

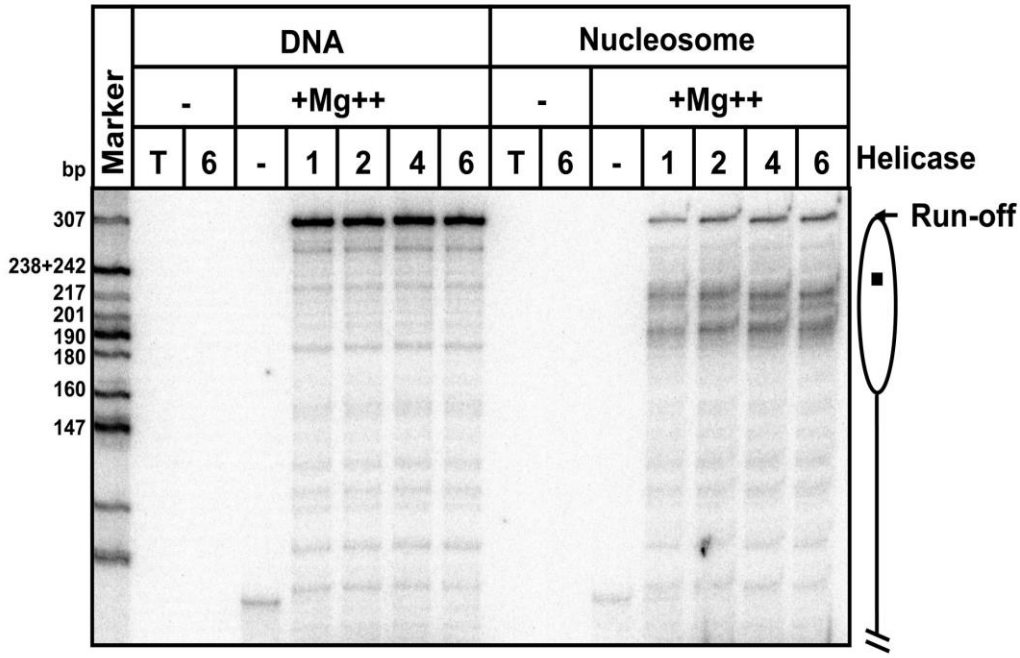

fig. S1. Helicase activity is essential for processive replication by the T7 replisome. T7 replisome complexes were formed at different molar ratios (1, 2, 4 or 6) of helicase to DNA polymerase on DNA or nucleosome templates. Replication was conducted for 4 minutes as described in Fig. 1.

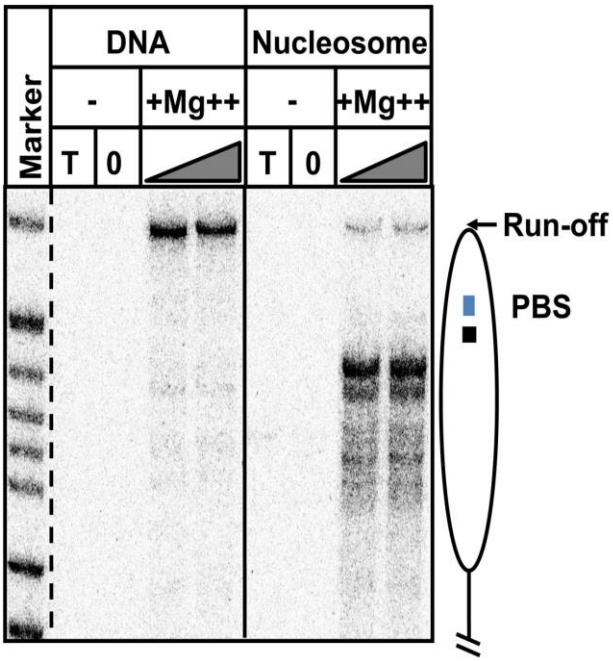

fig. S2. Strong nucleosomal barrier affects the processivity of the T7 replisome. Analysis of the nucleosome barrier formed after the replication of 601 DNA (histone-free or organized in a nucleosome) for different time intervals (0, 120, 240 seconds). Other designations as in Fig. 1. Note the different efficiencies of replication through the permissive (Fig. 1B) and non-permissive nucleosomes.

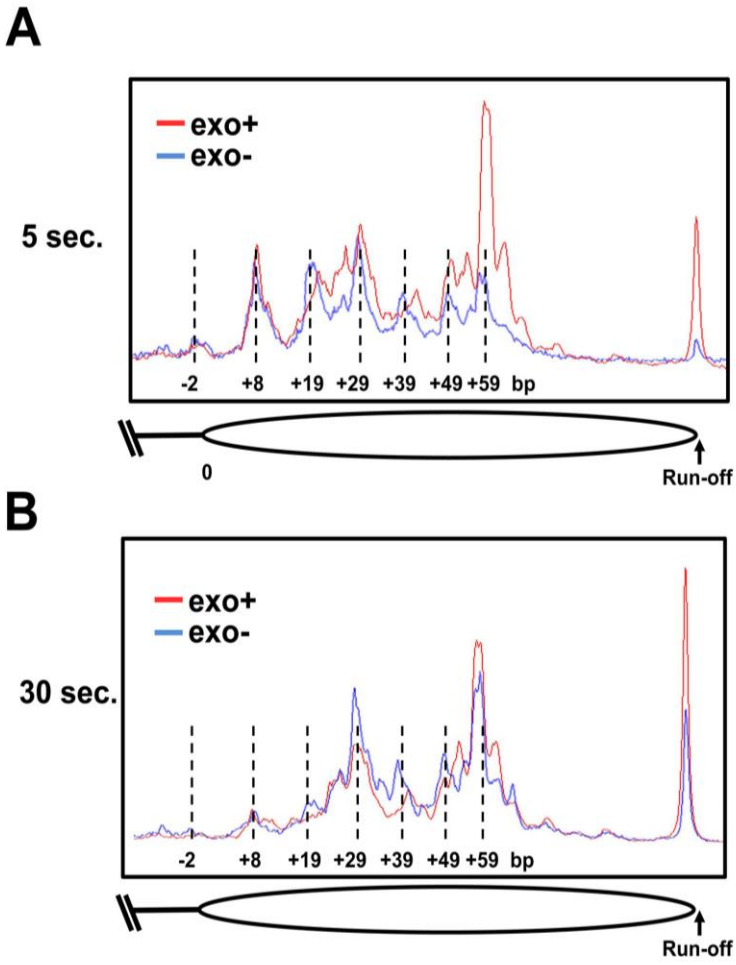

fig. S3. Analysis of the nucleosomal pausing patterns formed during replication by exo$^+$ and exo$^-$ replisomes. Lanes in Fig. 3A, corresponding to replication by exo+ or exo- replisomes for 5 (**A**) or 30 seconds (**B**) were scanned using a PhosphorImager. The scans were aligned pairwise. The ~10-bp periodic pausing pattern detected after replication for 5 seconds by exo- replisome and the positions of the pauses on nucleosomal DNA (distances from the proximal nucleosomal boundary) are indicated by dashed lines.

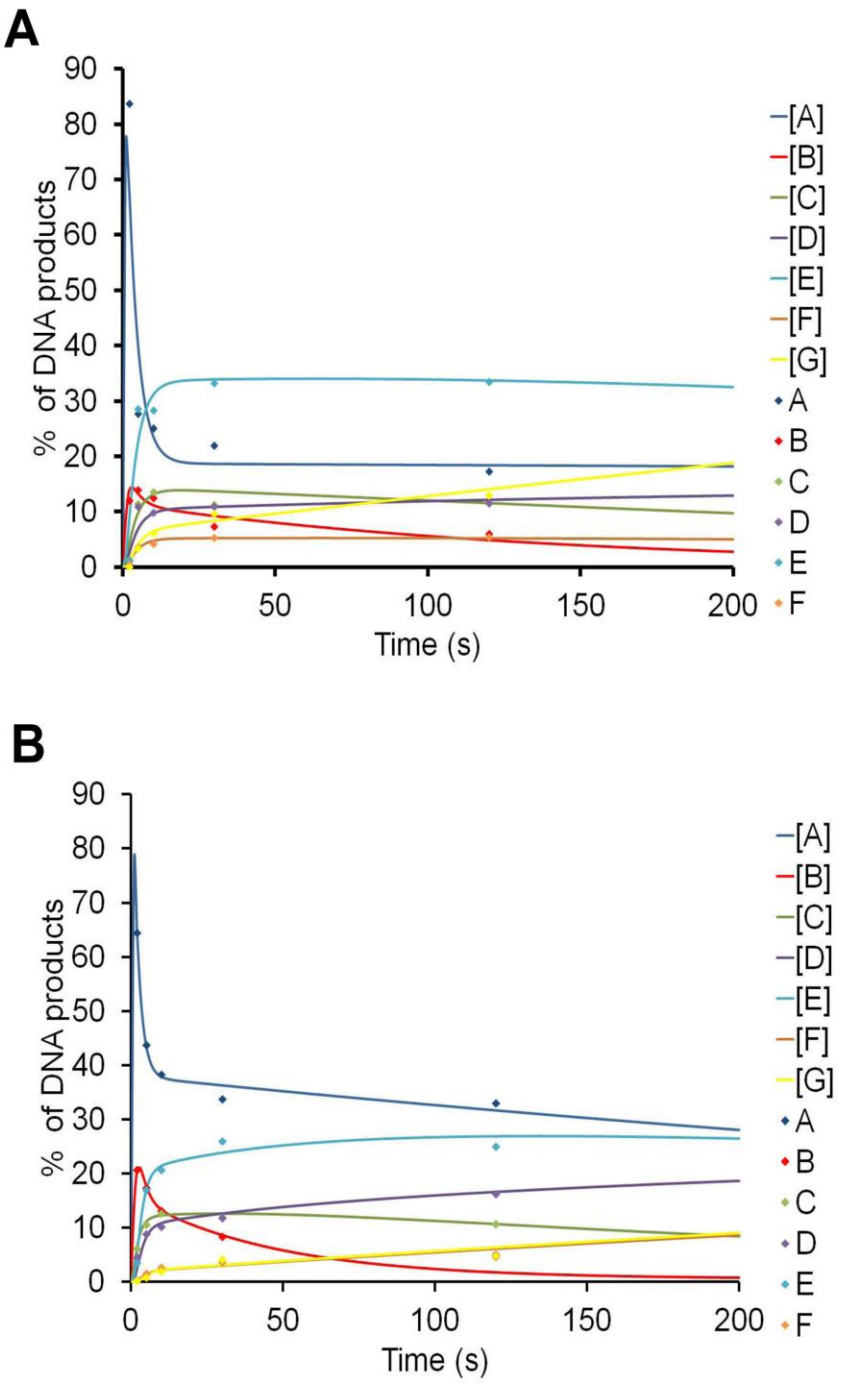

fig. S4. Analysis of time courses of replication through chromatin by the exo$^+$ (A) or exo$^-$ replisome (B) using KinTek Explorer software. Fit with the model.

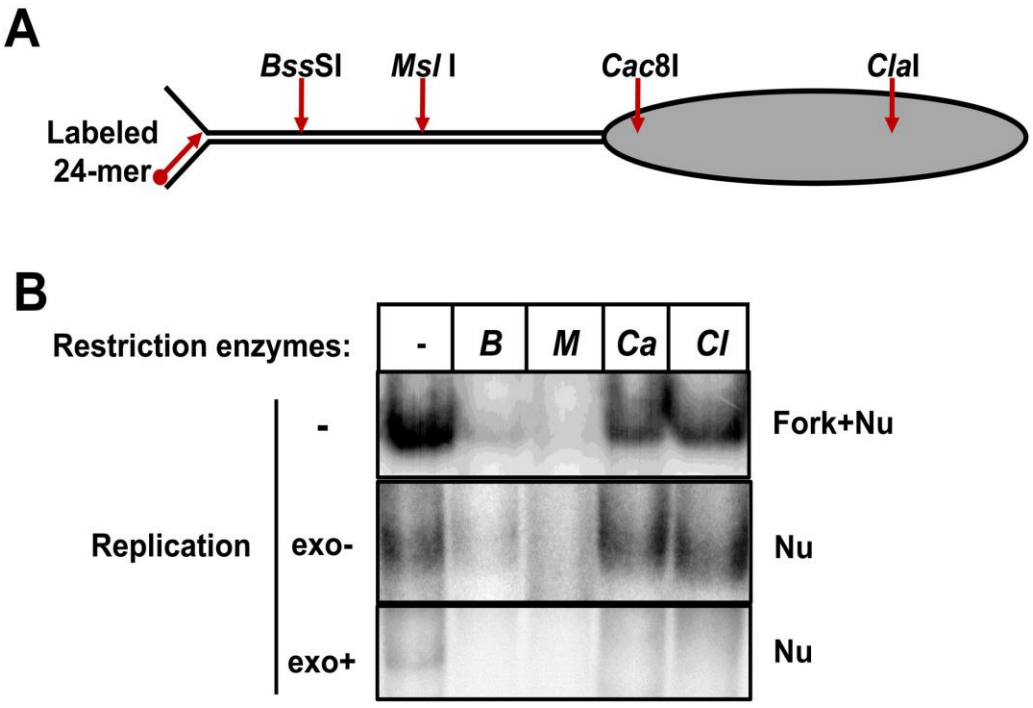

fig. S5. Mapping of nucleosome positions after replication by the T7 replisome using restriction enzyme sensitivity assay. Labeled templates were incubated in the presence of an excess of indicated restriction enzymes (B: *Bss*SI; M: *Msl*I; Ca: *Cac*8I; Cl: *Cla*I) before and after replication by exo+ or exo- T7 replisome for 4 minutes. Note that the nucleosomes after replication by the exo+ enzyme are sensitive to all restriction enzymes.

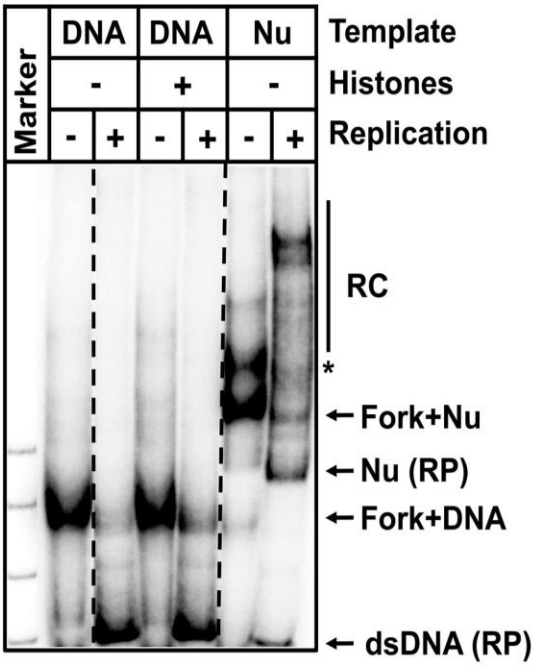

fig. S6. Nucleosomes are not formed de novo during or after T7 replication. ^{32}P-labeled DNA and nucleosomal (Nu) templates were replicated by T7 replisome for 4 minutes. A mixture of histone H2A/H2B dimers and H3/H4 tetramers present at the same molar concentration as nucleosomes was added to DNA before replication. After replication, all products were analyzed by native PAGE. Note that no nucleosomes are formed in the presence of DNA-free histones after DNA replication. M: pBR322-*Msp*I digest; * - unknown contamination; RP: replication products.

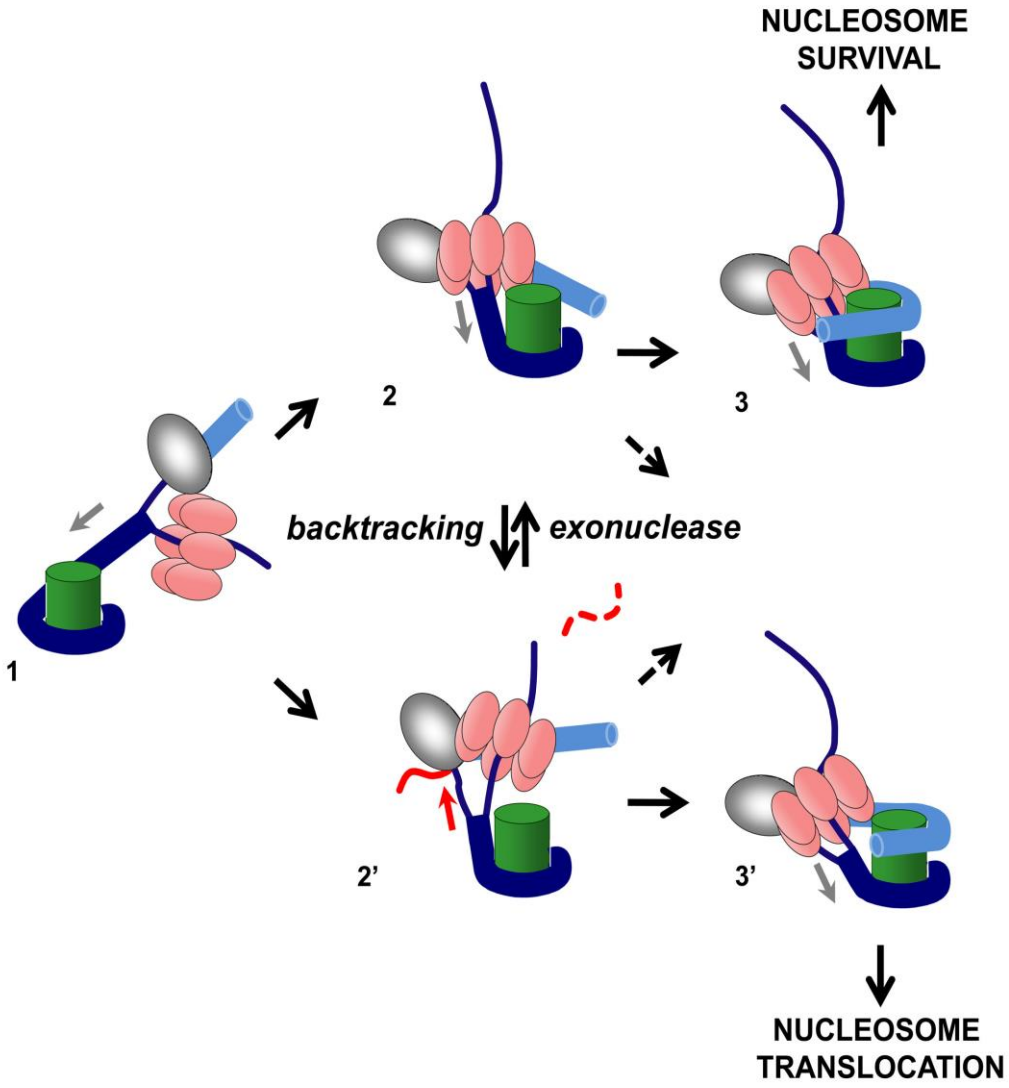

fig. S7. Proposed role for exonuclease activity during replication through a nucleosome. When T7 replisome enters into a nucleosome, nucleosomal DNA is partially uncoiled from the histone octamer (complex 1). We propose that exonuclease activity of T7 DNAP can resolve the backtracking intermediate (complex 2') and thus facilitate T7 replisome progression through a nucleosome (complex 2). The minimal intranucleosomal DNA loop (complex 3) is likely formed and nucleosome survives at original position on the DNA. A larger intranucleosomal DNA loop likely forms after backtracking (complex 3'), resulting in more efficient nucleosome translocation after replication by the exo- enzyme.

table S1. Sequences of oligonucleotides and DNA templates.

24-mer primer	**GCGCTGGTTAGTGGAAGAGATTCA**
100 bp fork	**TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTACGTAGGAGCATCACAAGA CTCTCTCGTGACTCATCTGGACACCGGCACTGGGCACACACAGG**
90 bp fork	**CCTGTGTGTGCCCAGTGCCGGTGTCCAGATGAGTCACGAGAGAGTCTTGTG ATGCTCCTACGTAGTTGAATCTCTTCCACTAACCAGCGC**
603 NPS	**CCCAGTTCGCGCGCCCACCTACCGTGTGAAGTCGTCACTCGGGCTTCTAAG TACGCTTAGCGCACGGTAGAGCGCAATCCAAGGCTAACCACCGTGCATCGA TGTTGAAAGAGGCCCTCCGTCCTTATTACTTCAAGTCCCTGGGGTA**
601 NPS	**ATAGGATGTATATATGTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTG GCGGTTAAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGCGGTGCTAGA GCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTCTCCAG**
Linker 1*	**ACTACGTAGGAGCATCACAAGACTCTCTCGTGACTCATCTGGACACCGGCA CTGGGCGAGACATACACGAATATGGCGTTTTCCTAGTACAAATCACCCCAGC GTGACGCGTAAAATAATCGACACTCTCGGGTGC**
Linker 2*	**ACTACGTAGGAGCATCACAAGACTCTCTCGTGACTCATCTGGACACCGGCA CTGGGCATCTCTGCATGGGCTTTTTTCTCCGTCAATTCTCTGATGCTTCGCGC TTTTTATCCGTAAAAAGCTATAATGCACTAAAATGGTGCAACCTGTTCAGGAG ACTGCTTTATGGCAACAGGCACGCAGCCCGATGCTGGGCAGATCCTCAACT CGCTGATTAACAGTATTTTGAACGAACAGATCGAATACCTGATCCACAACCC AATCAGGACGGCACTGGGG**
Linker 3*	**ACTACGTAGGAGCATCACAAGACTCTCTCGTGACTCATCTGGACACCGGCA CTGGGTTTGAGCTGTACTACAGCGTCTAAGTGTTTTAGTTGCCGTGGAAACT TTTCGCCTGTCTCTGGCAGGCCTGGGATCGGTGGCAAGCACATCACGCCGG ATGCGACGCAAATGCGTCTTATCCGGCCTACACGGTGATGATGTGGTAGGCC GGAGCAGGTGAGTCGCTCTCCAACGTGAAGTTTGTCAGCTATCTGTAGCCC ATCTCTGCATGGGCTTTTTTCTCCGTCAATTCTCTGATGCTTCGCGCTTTTTAT CCGTAAAAAGCTATAATGCACTAAAATGGTGCAACCTGTTCAGGAGACTGCT TTATGGCAACAGGCACGCAGCCCGATGCTGGGCAGATCCTCAACTCGCTGA TTAACAGTATTTTGAACGAACAGATCGAATACCTGATCCACAACCCAATCAG GACGGCACTGGGG**

Note: Linkers 1-3 are DNA sequences between the fork sequence and 603 NPS for different templates.