Effects of Bulky End-Termini on T7 RNA Polymerase Transcription Across Single-Strand Breaks in the DNA Template: Strong Difference Between 5' and 3' Adducts

Alexander J. Neil, Boris P. Belotserkovskii, Philip C. Hanawalt*

*Department of Biology, Stanford University, Stanford, CA 94305, USA
hanawalt@stanford.edu
(650) 723-2424

Funding
National Cancer Institute; National Institutes of Health (grant CA077712 to P.C.H); UAR Major grant (to A.J.N. at Stanford University)

Supplementary Information

Figure Legends

Figure S1 – (A) Sequence of the three insert oligonucleotides. The black dot indicates extra thymine residue that is present in substrates containing SSBs with a 1nt gap. Black triangles indicate sites of SalI restriction. The bold nucleotides on the 3' end of the 5' TS oligo were only present in RNAP II substrates. They allowed for ligation to BseRI sticky ends. (B) Results of a SalI restriction assay with T7 inserts; even lanes are assays without enzyme, odd lanes are assays with enzyme. The black chevron indicates full length NTS oligo (87nt in this case because all substrates contain a 1nt gap, even lanes). The white chevron indicates NTS oligo after SalI restriction (78nt, odd lanes). DNA fragments between 40nt and 46nt correspond to free, labeled TS oligonucleotides, which were present in excess during annealing reactions (see materials and methods). Similar results were obtained for insert containing SSBs with no gap and RNAP II substrates. (C) Table listing the SalI restriction percentage for all SSB-containing annealed inserts tested. For 5'-PO_4|3'-OH SSBs (not listed in table), restriction was carried out on final transcription constructs. For these substrates, the majority of the sample was restricted, but precise quantitation was complicated by the very small signal from uncut NTS oligo. SalI cleavage was calculated as the radioactive volume of 77/78nt NTS fragment divided by that volume plus the radioactive volume of 86/87nt NTS oligo.

Figure S2 – Analysis of T7 transcription substrates by gel electrophoresis following EcoRI restriction. Final constructs (343bp) migrate as expected following EcoRI restriction (odd numbered lanes). Intermediate constructs (686bp) are present in assays

without restriction enzyme (even numbered lanes). Short fragments at the bottom of the gel represent free annealed insert, which was present in significant excess during ligation reactions to ensure that the majority of ligated substrates consisted of promoter fragments bound to inserts rather than self-ligated promoter fragments (see "minor product" figure 1). These free inserts were not present when similar gels were run for RNAP II substrates, which did not require excess insert.

Figure S3 – Effect of HeLa extract on various substrates. Radiolabeled RNAP II substrate inserts (without promoters) were incubated in HeLa nuclear cell extract (odd lanes) or with 1x transcription buffer (even lanes) under the same conditions as RNAP II transcription experiments then run on a 20% denaturing gel. The white arrow indicates unmodified (5'-OH) 5' TS oligos, which were 5' labeled with radioactive phosphate during labeling reactions. There is a significant loss of signal in odd lanes, indicating a likely phosphatase activity in HeLa nuclear extracts. The black arrow and dashed line indicate 3' TS oligos that are labeled on the 5' end with radioactive phosphate. Lanes 1, 2, 11 and 12 contain unmodified (3'-OH) 3' TS oligos. Lanes 9 and 10 contain 3'-biotinylated oligos, which illustrate a significant gel shift. This shifted signal is decreased in the presence of HeLa cell nuclear extract (lane 9), indicating enzymatic removal of biotin from SSB oligos. This removal of biotin may be caused by the nuclear enzyme biotinidase, which recognizes and cleaves biocytin, which is similar in structure to our biotinylated DNA end termini. Lanes 7 and 8 contain 3'-C6 TS oligos, which also show a slight gel shift without reduction in the presence of HeLa cell nuclear extract. 3'-C3 modification does not show gel shift in 20% gel (lanes 3-6).