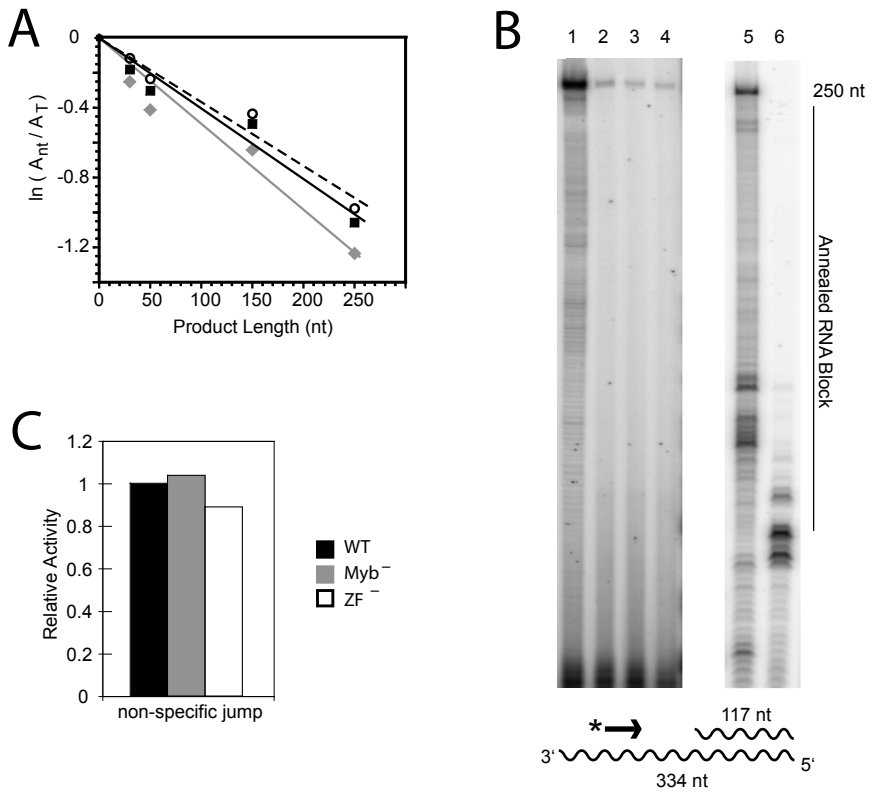
Supplemental Figure 2

Affects of ZF$^-$ and Myb$^-$ mutants on the reverse transcriptase activity of the R2 protein



(**Panel A**) Processivity of the R2 reverse transcriptase was assayed by primer extension with a ^{32}P-labeled primer AB.23 d(GGGGTACCGACAGGTTTCCCGACTG) annealed to the 3' end of a 334 nt Bluescript (Stratagene)-derived RNA template (1). Sixty fmole of R2 protein, either WT, ZF$^-$ or Myb$^-$, was preincubated with the 300 fmole of the primer/template complex for 5 minutes at 37ºC in 50 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 10 mM $MgCl_2$, 2.5 mM DTT, 0.01% Triton X-100. Primer extension was initiated by the addition of both 250 μM dNTP (final concentration) and 20 μg of heparin. The heparin functioned as a trap inhibiting reinitiation by the enzyme (1). The extension reactions were incubated at 37º C for 5 minutes, and the reaction products analyzed on denaturing 7% polyacrylamide gels. By quantitating the amount of product

as a function of distance along the RNA template, the probability for dissociation of the ternary complex (P_{off}) was calculated (1). The data was logarithmically transformed, ln (A_{nt} /A_T), and fitted to a linear function where the slope represents the probability for premature dissociation of the ternary complex (P_{off}) (2). Black squares, WT R2 protein; white circles, ZF^- protein; and gray diamonds, Myb^- protein. The P_{off} of each of the R2 proteins, given by the slopes of the linear regressions, differed only slightly (4.1×10^{-3} for WT, 4.9×10^{-3} for ZF^-, and 3.9×10^{-3} for Myb^-) suggesting that the N-terminal mutations do not significantly affect the stability of the RT/ RNA template during polymerization. Other primer extension analyses of the three proteins (WT, ZF^-, Myb^-) revealed that the RT activity of the mutant proteins were similar to wild type when calculated per unit protein (data not shown).

(**Panel B**) A modified primer extension assay was used to study RNA/RNA duplex displacement in which a 117 nt RNA was annealed to the 5' end of the 334 nt template RNA as diagrammed at the bottom of the panel (1). The end-labeled primer used to prime synthesis, AB.21 d (CTATGGCAGCTGGAGCT), was annealed nearer the 3' end of the 334 nt template RNA. All other assay conditions were as described in the processivity assay. The primer extension reaction using MMLV reverse transcriptase was performed in the buffer recommended by the supplier (Promega). Lane 1, WT protein without the duplex RNA; lane 2, WT protein with the duplex RNA; lane 3, ZF^- protein with the duplex RNA; lane 4, Myb^- protein with the duplex RNA; lane 5, MMLV reverse transcriptase without the duplex RNA; and lane 6, MMLV reverse transcriptase with the duplex RNA. To generate full-length cDNA products from this template (250 nt), the R2 protein had to displace the 117 nt annealed RNA. Similar to our previous findings (1) reverse transcription by WT R2 was not inhibited by the RNA:RNA duplex whereas the retroviral reverse transcriptase was blocked by the duplex (compare lanes 1, 2 with lanes 5,

6). Mutations of the R2 protein ZF motif or Myb motif did not affect the ability of protein to displace RNA duplex during polymerization. The reduction in total cDNA accumulation with WT R2 RT in the presence of the annealed RNA (lanes 2-4) compared to the absence of this RNA (lane 1) was a result of the excess 117 nt RNA in the reaction priming the synthesis reaction thereby lowering the observed signal from the labeled DNA primer (1).

(**Panel C**) The template jumping reactions (homology independent template switching) were conducted as previously described (3) using a ^{32}P-labeled DNA primer annealed to a 177 nt fragment of 28S rRNA used as the donor RNA. The acceptor RNA was a 182 nt RNA derived from pBluescript II SK+/- (Stratagene). Jumping activity was quantified by the level of ~360 nt and larger cDNA products divided by the level of the 177 nt extension product. Black bar, WT R2 protein set at 100%; gray bar, Myb$^-$ protein; and white bar, ZF$^-$ protein. The end-to-end jumping activities of the WT, ZF$^-$ and Myb$^-$ proteins were similar (average of three experiments).

These combined experiments suggest that the N-terminal nucleic acid binding motifs do not interact with the RNA template to the extent that they affect either the processivity, duplex displacement, or template jumping of the R2 RT. Additional evidence to suggest that the N-terminal domain is not involved in RNA binding was the lack of an observable RNA:protein complex in band shift assays using labeled 3' UTR RNA and the 229 polypeptide (data not shown). Finally, these experiments also served as a critical control for the DNA binding and cleavage reactions (Figures 5 and 6 of this study) to indicate that the ZF- and Myb- R2 proteins were soluble, stable and could be as enzymatically reactive as WT R2 protein.

References

1. Bibillo,A. and Eickbush,T.H. (2002) High processivity of the reverse transcriptase from a

non-long terminal repeat retrotransposon. *J Biol Chem* **277**, 34836-34845.

2. Bibillo,A. and Eickbush,T.H. (2002) The reverse transcriptase of the R2 non-LTR retrotransposon: continuous synthesis of cDNA on non-continuous RNA templates. *J Mol Biol* **316**, 459-473.
3. Bibillo,A. and Eickbush,T.H. (2004) End-to-end template jumping by the reverse transcriptase encoded by the R2 retrotransposon. *J Biol Chem* **279**, 14945-14953.