LEGEND TO SUPPLEMENTAL FIGURE 1

Oligo dA chains contain 5-phosphate ends - Oligo dA products were prepared as described in Fig. 4B from reactions that were increased 10-fold. Following incubation, the mixture was supplemented with 100 μg of BSA, 100 μl H_20 and treated with 100 μl of 7% $HClO_4$. After 20 min on ice, the mixture was centrifuged for 30 min at 4ºC in an Eppendorf centrifuge. The pellet was dissolved in 100 μl of 0.1 N NaOH and then precipitated with 100 μl of 7% $HClO_4$. The pellet was collected and the procedure repeated twice; the final pellet was dissolved in 100 μl of 0.05 N NaOH. The ^{32}P present in oligo dA (determined by DEAE paper) pre- and post-acid precipitation were identical. Degradation of the DNA product was carried out as follows: a reaction mixture (25 μl) containing 20 mM Tris-HCl (pH 8.0), 2 mM $CaCl_2$, 10 μl of the oligo dA ($1.5x10^6$ cpm, equivalent to ~1.5 nmol of ^{32}P-dAMP) and micrococcal nuclease (125U, Worthington) was incubated at 37ºC for 60 min. The mixture was then adjusted to pH ~7.0 with 0.93 μl of 1N HCl and treated with 0.5 U of spleen phosphodiesterase. Following 30 min at 37ºC, an additional 0.25 U of spleen phosphodiesterase was added and the mixture incubated for an additional 30 min. We verified that the hydrolysis of the oligo dA chains was complete (monitored by CIP treatment which indicated that more than 95% of the radioactivity was converted to ^{32}Pi). Aliquots from the nuclease digested material were then incubated with and without CIP as follows: reactions mixtures (5μl) containing 2.5 μl of the digested material, 1 mM magnesium acetate, 20 mM Tris-HCl (pH 8.0) were incubated with and without CIP (2U) for 30 min at 37ºC. Aliquots (1μl) of the treated material was subjected to TLC separation on separate PEI strips that were developed in 0.5 M ammonium formate (pH 3.5). Prior to loading, pAp (3') and dAp (3') (5 μmol each) were added to the reaction as markers. The markers were located on the plates and the distribution of ^{32}P monitored by counting of 1 cm strips of the PEI plate. This analysis revealed ^{32}P peaks corresponding to the pdAp and dAp markers only in the digest not treated with CIP. Quantitation of the ^{32}P present in the dA and pdAp regions revealed the presence of 70,000 and 2200 cpm, respectively. The oligo dA chains formed by Tk DNA primase, determined by size and amount of [$\alpha^{32}P$]-dATP incorporated, were calculated to be between 30 and 40 nt in length. Hence, based on the amount of ^{32}P detected in the monophosphate region and the length of the oligo dA chains, we calculated that the pdAp region (possessing two phosphate residues) should contain 4660 (30 nt chain) or 3500 cpm (40 nt chain) if we assume that all termini contained a 5' phosphate end. The observed recovery (2200 cpm) represents 47-63% of the calculated value (~50%).

Supplemental figure 1