SUPPLEMENTAL TABLE 4

Solvent used in TLC separation	Region Isolated	³² P content (cpm)
1) 0.5 M ammonium formate, pH 3.5	Ap (2', 3' mixture) pAp (2', 3' mixture)	64,450 3,100
2) 1 M HCOOH, 0.3 M LiCl	Ap (2', 3' mixture) pAp (2', 3' mixture)	62,600 2,800

TLC separation of products formed after alkaline hydrolyse of ³²P-oligo rA - Oligo rA was synthesized as described in Fig. 4A (lane 6) with $[\alpha^{32}P]$ -ATP and the products precipitated with alcohol. The alcohol precipitated material was washed twice with 70% alcohol and dried in vacuo. The pellet was suspended in 20 µl of 0.3 M KOH and incubated at 37°C for 18 h and then neutralized with 1N HCl. The mixture was supplemented with 5 µmol each of Ap (2', 3' mixture), pAp (2', 3' mixture) and adenosine tetraphosphate (ppppA). Aliquots (1.0 µl) were added to PEI cellulose strips and subjected to TLC separation in 0.5 M ammonium formate (pH 3.5) (solvent 1) or 1 M HCOOH, 0.3 M LiCl (solvent 2). Regions coincident with the added markers were excised and counted. Reactions lacking oligo dT₃₀ or enzyme were also carried through the same procedure; no radioactivity was detected in the Ap (2', 3') or pAp (2', 3' mixture) region while the ppppA region contained ~600 cpm in the control and in the incubated samples. We interpret this to represent contamination with $[\alpha^{32}P]$ -ATP and for this reason analyses of the tetraphosphate region are not presented. Based on the data presented above, the ³²P present in the pAp (2', 3' mixture) regions represents 4.8% and 4.5% (in 1 and 2), respectively, of the label present in the Ap (2', 3' mixture) regions. As pAp (2', 3' mixture) includes two phosphate residues present in the oligo rA chains, 50% of the ³²P recovered in this region represents the 5'-end. Thus, based on the findings that oligo rA chains formed were ~20-nt long (average), they contained ~50% of the calculated 5'-phosphate ends.