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Initiation of DNA Synthesis by Oligoribonucleotides

G. Feix

Institute of Genetics
University of Freiburg
Freiburg, Germany

The known DNA polymerases are unable to initiate the synthesis of new DNA chains without a primer (1). DNA synthesis begins by the addition of nucleotide residues to the 3′-OH terminus of a pre-existing chain which has always been of the deoxy type. Recently, however, it was discovered that oligoribonucleotides also can be used for the priming of DNA synthesis. Verma *et al.* (2) found that DNA synthesis by reverse transcriptase from avian myeloblastosis virus is initiated by an oligoribonucleotide, and, further, it has been suggested that an oligoribonucleotide might be involved in the initiation of DNA synthesis by *Escherichia coli* polymerase (3).

Oligoribonucleotides, if needed, can be produced easily by transcription and might exert a kind of positive control in initiation of DNA synthesis by virtue of their specific priming capacity. Therefore, it is of interest to study the various DNA-synthesizing enzymes with respect to their behavior toward an oligoribonucleotide primer.

I would like to give a short account on some experiments done in this connection with terminal deoxynucleotidyl transferase from calf thymus. This rather small enzyme (mol wt 32,000), which displays primer dependency, has been extensively purified by Chang and Bollum (4). It is suggested that this enzyme might be part of a larger DNA-synthesizing enzyme complex which disintegrates during enzyme purification (5).

So far, the enzyme always has been primed by either oligodeoxynucleotides or DNA (6,7). If an oligoribonucleotide such as $(A\text{-}A\text{-}A\text{-}A)_r$ is offered as primer

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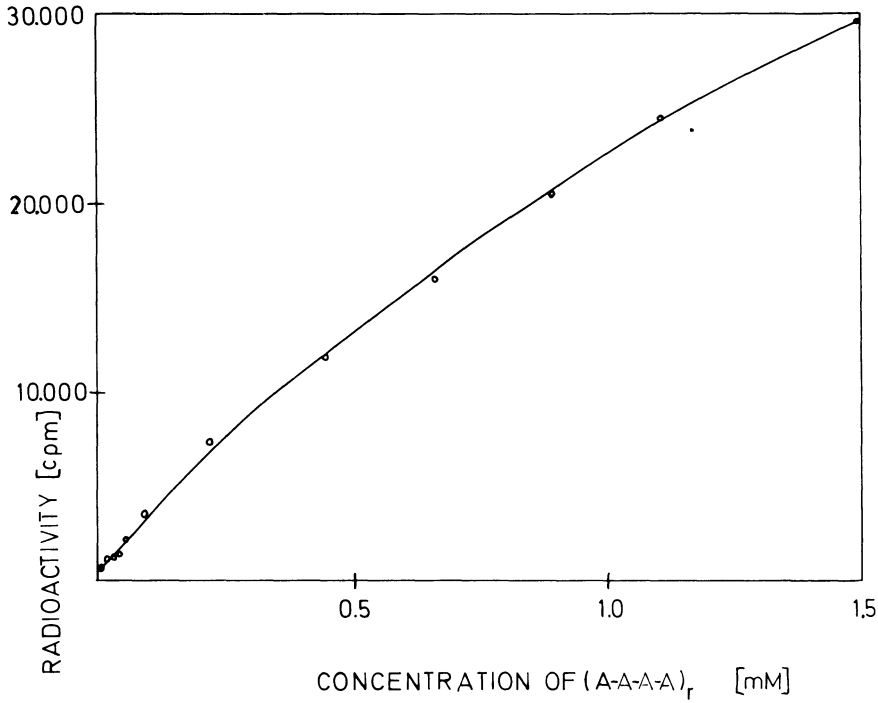

Fig. 1. Stimulation of DNA synthesis. Enzyme assays (30 µl) containing 6 µmoles potassium cacodylate (*p*H 7.0), 30 nmoles $CoCl_2$, 0.6 µmoles KCl, 30 nmoles mercaptoethanol, 30 nmoles ^{3}H-dCTP (specific activity 11,000 cpm/nmole), 7 µg enzyme [9000 units per milligram protein as measured with dCTP and $CoCl_2$ (4)], and $(A\text{-}A\text{-}A\text{-}A)_r$ as primer in the amounts indicated in the figure were incubated at 37° for 30 min. Then 15 µl aliquots were taken, diluted into a mixture of 0.05 ml 8% $Na_4P_2O_7$ (*p*H 7.0) and 0.1% bovine serum albumin, and acid precipitated with 6% TCA. The acid-precipitated radioactivity was collected on membrane filters, and after washing of the filters with 6% TCA and drying, the radioactivity was measured in a Beckman liquid scintillation spectrometer using a toluene-based scintillator.

for the polymerization of dCTP at a concentration of 10 µM (sufficient for substantial synthesis if primed by a oligodeoxynucleotide), no detectable polymerization takes place as tested by the incorporation of radioactive substrate into acid-insoluble material (8). If, however, the concentration of $(A\text{-}A\text{-}A\text{-}A)_r$ is considerably increased, DNA synthesis is stimulated as shown in Fig. 1, which illustrates the concentration dependency of $(A\text{-}A\text{-}A\text{-}A)_r$ as primer for the polymerization of dCTP.

Since a considerable primer concentration is needed for a significant incorporation of deoxynucleotides into acid-insoluble polymers, it is necessary to