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Preparation of capped RNA transcripts using T7 RNA polymerase*

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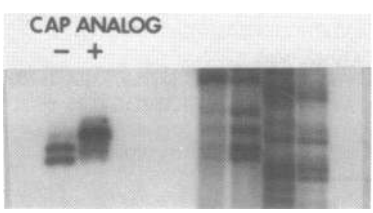
The use of capped RNAs, synthesized in vitro using SP6 RNA polymerase, has been shown to be advantageous in studies of RNA processing, in vitro protein synthesis, and RNAs microinjected into Xenopus oocytes (1-3). In this report we show that bacteriophage T7 RNA polymerase, which has been cloned and is available from overproducing cells, efficiently incorporates the cap analog 5' 7meGpppG 3' into RNAs to yield capped transcripts.

The plasmid pGEM3 was linearized with AvaI and transcribed in the presence or absence of the cap analog and analyzed on a sequencing gel (see fig. legend). The uncapped transcript yields two bands which may indicate initiation at adjacent nucleotides. A capped transcript will exhibit an increase of 1 nucleotide in size and a decrease in charge from -32 to -31. Over 80% of the transcript produced in the presence of the cap analog migrates as a single band with a slower mobility - which is consistent with the production of a 5' capped transcript. The fact that the transcription reaction containing the cap analog exhibits a single band, and an increased yield of 1.9 fold, suggests that the cap analog may enhance the fidelity of initiation. We conclude that T7 RNA polymerase can be used to efficiently synthesize a relatively homogeneous population of capped RNA transcripts.



Autoradiography of labelled RNA transcripts produced by T7 RNA polymerase. RNA was transcribed either in the presence (+) or absence (-) of 500 μM 5' 7meGpppG 3' cap analog in a 50 μl reaction mixture containing 3 μg AvaI linearized pGEM3, 100 units T7 RNA polymerase, 40 mM Tris pH 7.9, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 80 units RNasin, 500 μM each of ATP, CTP and UTP, 50 μM GTP and 12.5 μCi α-^{32}P-UTP for 60 min at 37°C, phenol extracted, ethanol precipitated and resuspended in H_2O and fractionated by electrophoresis on a 20% acrylamide-urea sequencing gel with a DNA sequencing ladder as a standard.

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