Non-processive transcription of poly[d(A–T)] by wheat germ RNA polymerase II

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Received 18 October; revision received 17 November 1982

RNA product distribution obtained during the transcription of poly[d(A–T)] by wheat germ RNA polymerase IIA under various experimental conditions was analyzed by high resolution polyacrylamide gel electrophoresis. Poly[r(A–U)] synthesis proceeded as if wheat germ RNA polymerase II was a non-processive enzyme: a ladder of RNA products of increasing lengths was obtained, which apparently, terminated at every other nucleotide. RNA release was not dependent upon nucleoside triphosphate substrate concentrations. A likely explanation would be that ternary complexes enzyme : DNA : RNA were very much unstable; moreover, oligonucleotides released were not re-used for further elongation by the enzyme.

Eukaryotic RNA polymerase *Poly[d(A–T)]transcription* *Kinetic processivity*

1. INTRODUCTION

RNA synthesis catalyzed by DNA-dependent RNA polymerase can be divided into several processes [1]:

(i) Binding of the enzyme to the DNA template;
(ii) Initiation of RNA synthesis;
(iii) Elongation of the RNA chain;
(iv) Termination of the RNA synthesis.

Recent results obtained with *E. coli* RNA polymerase, where the results are best documented, indicate that the initiation process is far more complex than anticipated: DNA–RNA polymerase complexes undergo an abortive initiation reaction involving the release of short oligonucleotides, even in the presence of all 4 nucleoside triphosphates [2]. A recycling model for initiation was proposed to account for the high yield of oligonucleotides compared to long RNA transcripts [3]. This model suggests that after each phosphodiester bond is formed a finite probability of dissociating the product exists (at least for the first 7–9 steps).

This behaviour is interesting in terms of regulation of transcription for several reasons. It has been suggested that the half-time of productive initiation (formation of long RNA transcripts) is directly related to the amount of abortive initiation or recycling (formation of oligonucleotides) that can occur [4], and that the oligonucleotides produced during abortive initiation could possibly be used as primers to amplify gene expression of genes with complementary sequences [5]. Furthermore, it appears that the strength of a promoter site on DNA depends on the amount of abortive initiation that can occur at this site [4]. Since abortive synthesis is also seen using synthetic templates such as poly[d(A–T)] [6], we were encouraged to investigate the behaviour of an eukaryotic RNA polymerase. The RNA product distribution obtained during the transcription of poly[d(A–T)] by wheat germ RNA polymerase IIA under various experimental conditions was analyzed by using high resolution polyacrylamide gel electrophoresis, which allows detection of any oligonucleotides. The results presented in this paper show that poly[r(A–U)] synthesis proceeds as if the wheat germ RNA polymerase II was a non-processive enzyme [7].

Published by Elsevier Biomedical Press
00145793/82/0000—0000/$2.75

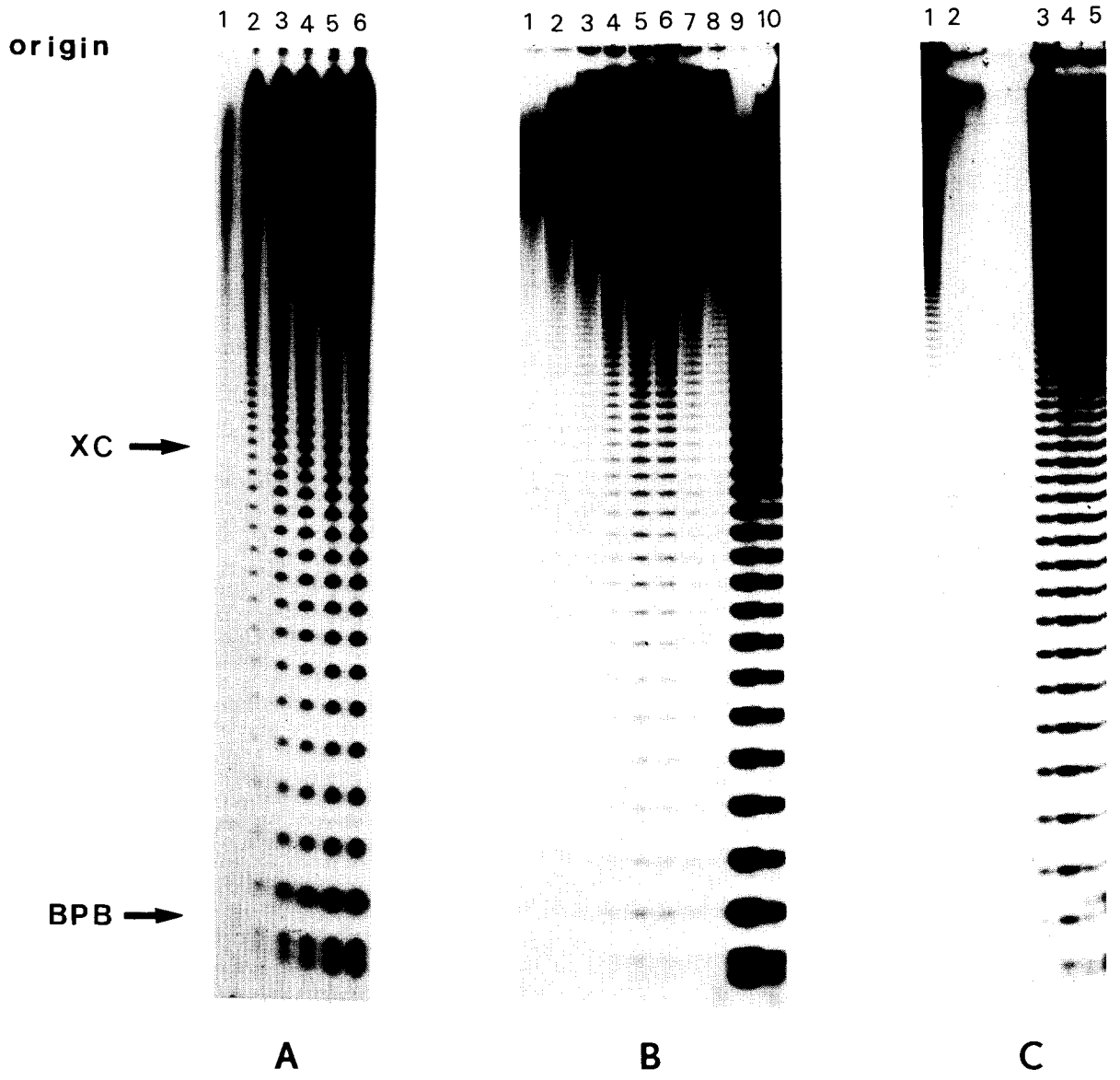

Fig. 1. Transcription of poly[d(A–T)] by wheat germ RNA polymerase II. Reactions were performed and analyzed by gel electrophoresis as in section 2. The final substrate concentrations and the incubation times were as indicated; XC = xylene cyanol, BPB = bromophenol blue are electrophoresis marker dyes. In a 15% denaturing polyacrylamide gel, such as those of fig. 1, these marker dyes comigrate with oligonucleotides of lengths 40 and 10 nucleotides for XC and BPB, respectively [11]. (A) Kinetics of UMP incorporation: Final UTP (8 μCi) and ATP concentrations were 5 and 50 μM, respectively. Incubation times were 3, 9, 18, 25, 40 and 60 min for lanes 1–6, respectively. (B) Effect of substrate concentration: Incubation time was 30 min. Lanes 1–6: UTP was 5 μM (8 μCi) and ATP was 1, 2, 5, 15, 50 and 100 μM, respectively; lane 7, ATP was 10 μM and UTP 5 μM (8 μCi); lane 8, ATP was 10 μM and UTP 50 μM (24 μCi); lane 9, ATP was 100 μM and UTP 5 μM (8 μCi); lane 10, ATP was 100 μM and UTP 50 μM (24 μCi). (C) Lanes 1 and 2: Analysis of RNA after molecular sieving of reaction mixture. A reaction mixture (20 μl) containing 200 μM ATP and 10 μM UTP (8 μCi) was incubated for 2 h, then the reaction was stopped by mixing with 40 μl of stop buffer. The 60 μl were applied onto a 3 ml column of Bio-Gel P2 equilibrated with 80% formamide, 1 mM EDTA; 30 fractions of 120 μl were collected and counted by Cerenkov radiation; 1 μl fractions corresponding to the void volume and to the included volume (lanes 1 and 2, respectively) were analyzed. (D) Lanes 3–5: Stability of RNA products upon time of incubation at 35°C. A reaction mixture (18 μl) containing 5 μM UTP (8 μCi) and 105 μM ATP was incubated for 20 min. The reaction was stopped by adding 1 μl α-amanitin (400 μg/ml) and incubation was continued at 35°C; 2 μl aliquots of the mixture were withdrawn at various times and analyzed. Incubation times after α-amanitin addition were 0, 30 and 60 min for lanes 3–5, respectively.

2. MATERIALS AND METHODS

ATP, UTP and poly[d(A–T)] were purchased from Sigma. [α^{32}P]UTP (3000 Ci/mmol) was from New England Nuclear. Wheat germ RNA polymerase IIA was purified as in [8], as modified [9].

The reaction mixtures contained 0.17 μM RNA polymerase, 20 μg/ml poly[d(A–T)], 12.5% (v/v glycerol, 50 mM ammonium sulfate, 1 mM $MnSO_4$, 50 mM Tris–HCl (pH 7.8) buffer, 0.75 mM NaF and 1.5 mM dithiothreitol. Usually the final volume was 6 μl and the medium contained also [α^{32}P]UTP, ATP and UTP as appropriate, incubations were performed at 35°C. Reactions were stopped by mixing 1 μl reaction medium with 2 μl stop buffer containing 1 mM EDTA, 80% formamide, 0.1% of each xylene cyanol and bromophenol blue.

RNA products (1 μl aliquots) were analyzed by gel electrophoresis on 15% (or 8%) acrylamide, 7 M urea, 50 mM Tris–borate (pH 8.0) gels (0.03 × 20 × 40 cm). Electrophoresis was conducted at a constant 35 W until the [α^{32}P]UTP had migrated into the lower buffer chamber. The gels were autoradiographed at −80°C on X-O-Mat films (Kodak). The X-ray films were scanned using a Vernon recording densitometer.

3. RESULTS

Fig. 1A shows the kinetics of [α^{32}P]UMP incorporation into poly[r(A–U)]. Analysis of the product distribution by gel electrophoresis indicates that RNA synthesis proceeds by the release of a large number of oligonucleotides. A ladder of products is obtained, and in between the position of xylene cyanol and of bromophenol blue, about 25 bands can be counted on a 8% gel and ~15 on a 15% gel. Moreover, above the position of the xylene cyanol marker, up to 80–100 bands can be seen on a 8% gel (not shown).

As suggested in [7], the coupling between nucleoside triphosphates binding and the distributive behaviour of wheat germ RNA polymerase was investigated. Fig. 1B shows that varying [ATP] from 1–100 μM, or [UTP] from 5–50 μM did not result in a qualitative change in the product distribution pattern.

To investigate whether the distribution pattern was due to some artifactual process, a set of experiments was performed.

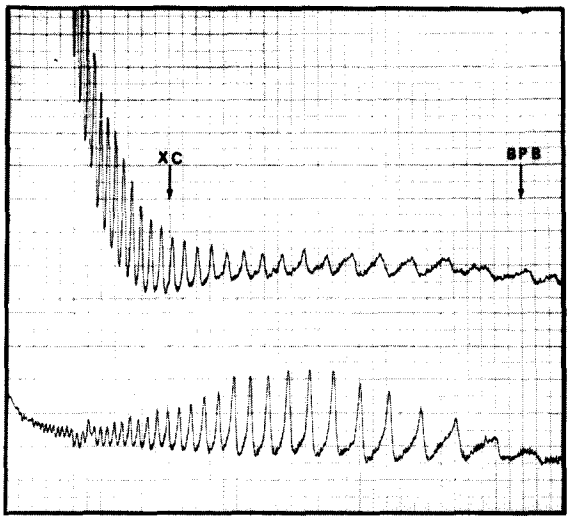

Fig. 2. Densitometric recordings of lanes 1 and 2 of fig. 1C: upper trace, lane 1 of fig. 1C, corresponding to the void volume of the Bio-Gel P2 column; lower trace, lane 2 of fig. 1C corresponding to the included volume of the column. Arrows indicate the migrations of the electrophoresis marker dyes.

RNA with distribution such as that of fig. 1B were synthesized, then α-amanitin was added in order to stop RNA synthesis, and the mixture was incubated at 35°C. Fig. 1C (lanes 3–5) shows that there was no difference, neither qualitatively nor quantitatively in the product distribution pattern after incubation of the reaction mixture for up to 1 h. Therefore, it appears that the short oligonucleotides did not originate from the longest products by the presence of some contaminating RNase activity in the reaction components.

RNA with distribution such as that of fig. 1B were synthesized, then chromatographed onto a Bio-Gel P2 column. Fractions eluting in the void volume and in the included volume of the column were analyzed by gel electrophoresis (fig. 1C, lanes 1 and 2, densitometer recordings in fig. 2). One can see that:

(i) Small RNA products were absent in the fractions collected in the void volume;
(ii) Large RNA products were absent in the fractions collected in the included volume.

Thus, we conclude that the small RNA products were not generated from the largest ones during the electrophoresis run.

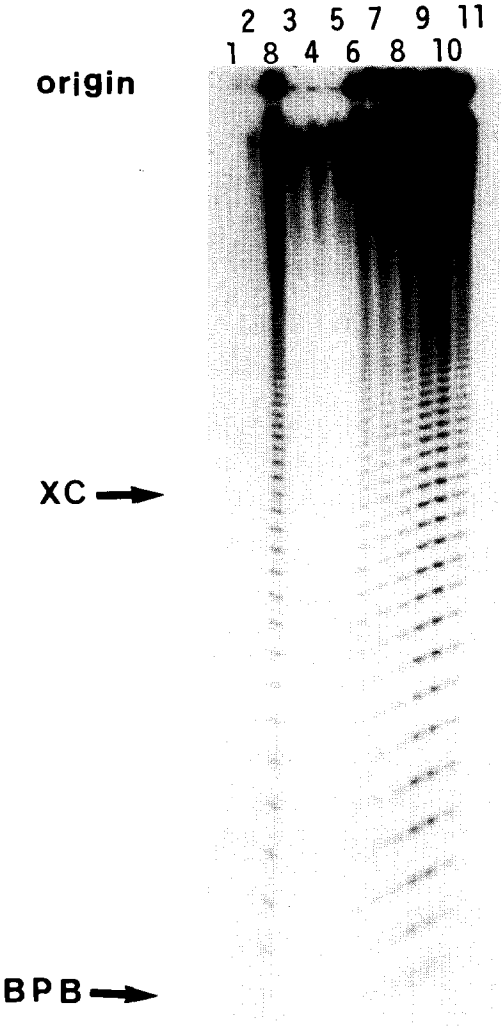

Fig. 3. Pulse-chase of preformed labelled RNA. A reaction mixture (18 μl) containing 100 μM ATP and 0.36 μM UTP (3 μCi) was incubated for 10 min, then 3 μl 3 mM unlabelled UTP were added, which resulted in a 1200-fold dilution of the labelled substrate. Incubation was allowed to proceed at 35°C; 3 μl aliquots were withdrawn at various intervals of time and analyzed. Incubation times after label dilution were 0.6, 7, 23, 33, 44 and 55 min for lanes 6–11, respectively. In another identical reaction mixture unlabelled UTP was added at zero time. Incubation was allowed to proceed at 35°C; 3 μl aliquots were withdrawn at various intervals of time and analyzed. Incubation times were 0.6, 7, 21, 31 and 46 min for lanes 1–5, respectively.

To investigate whether the oligonucleotides produced during an experiment such as one of those in fig. 1A,B, were in the process of being elongated, a pulse-chase was performed (fig. 3). Labelled RNA were synthesized for 10 min in the presence of 0.36 μM [α^{32}P]UTP and 100 μM ATP, then [UTP] was brought to 430 μM using unlabelled substrate. RNA synthesis was allowed to continue for 45 min at 35°C; aliquots were withdrawn at various intervals of time and analyzed by gel electrophoresis. A control experiment where the cold UTP was introduced in the reaction medium at zero time showed the effectiveness of the chase experiment (fig. 3, lanes 1–5). For the products analyzed in lanes 6–11, there were no changes in the distribution pattern, indicating that the preformed RNA products were not elongated further.

4. DISCUSSION

As stressed in [7], the first question that should be answered in a kinetic study of a nucleic acid polymerase is: how processive is it? Our results clearly show that RNA synthesis catalyzed by wheat germ RNA polymerase II, using poly[d(A–T)] as template, proceeds as if the wheat germ enzyme was a non-processive enzyme [7]. Moreover, the behaviour exhibited by the eukaryotic enzyme is much more distributive than that seen with the prokaryotic enzyme, since for the latter oligonucleotide synthesis is limited to the synthesis of products 8–10 nucleotides long [3,4,6].

When analyzed by gel electrophoresis, the RNA synthesized by wheat germ RNA polymerase II constitute a ladder of products of increasing lengths. From the known migrations of the dye markers [10,11], it can be estimated that each RNA is two nucleotides longer than its immediate neighbour in the gel. At least two different explanations are a priori available to account for this process of RNA release:

(1) After rapid incorporation of two nucleoside triphosphate substrates a slower step will occur (e.g., translocation) during which the RNA synthesized could dissociate from the ternary complexes enzyme:DNA:RNA with a finite probability.

(2) RNA release is dependent on DNA sequence; the ternary complex *E. coli* RNA polymerase: poly[d(A–T)] : trinucleotide is much more stable with the trinucleotide ApUpA than with UpApU [5]. Furthermore, from the in vitro studies on the transcription at the lac UV5 and at the transposon Tn 5 promoters by *E. coli* RNA polymerase, it appears that short oligonucleotides are mostly released after a pyrimidine base during the cycling of the enzyme in the initiation process [3,4].

Therefore, it might be expected that the non-processive transcription of a strictly alternating template, such as poly[d(A–T)], would lead to the formation of a ladder of RNA products of increasing lengths terminating at every other nucleotide.

As observed in the case of the transcription at the lac UV5 promoter, distributive synthesis persists over a wide range of nucleoside triphosphate concentrations [3], (fig. 1B), which indicates that the step at which RNA release occurs is not directly coupled to the step at which substrate incorporation occurs.

The main cause for the occurence of a distributive RNA synthesis seems to rely upon the stability of the ternary complex enzyme : template : RNA. In the case of *E. coli* RNA polymerase, the formation of a stable ternary complex is considered to occur when short abortive products cease to be produced and elongation proceeds to the normal termination point. The length of product at which this stable ternary complex is achieved is ~6–10 nucleotides long [3,4,6,12]. Sigma factor release occurs concomitantly [6]. These results would indicate that the ternary complexes are much less stable when formed with the wheat germ enzyme than with the bacterial enzyme. Fig. 1C and 2 support this contention: if ternary complexes were stable enough they should elute in the void volume of the Bio-Gel P2 column. Therefore, the RNA distribution in the ternary complexes might have been identical to that obtained from an unseparated reaction mixture, such as those of fig. 1A,B, a result already observed using *E. coli* RNA polymerase [6]. In fact, for the products analyzed in lane 1 of fig. 1C oligonucleotides of length up to 40 nucleotides are absent, these oligonucleotides being mostly found in the included volume of the column (fig. 1C, lane 2 and fig. 2). Another piece of evidence was provided by the pulse-chase experiment of preformed RNA products.

In view of the potential role of oligonucleotide synthesis for prokaryotic RNA polymerase, one may wonder if such a behaviour exhibited by the enzyme of class II from wheat germ has practical implications during the transcription of eukaryotic genes. Further experiments are needed to elucidate this point.

ACKNOWLEDGEMENTS

This work was supported by grants from the CNRS (ATP Biologie Moléculaire Végétale). We wish to thank Dr R. Cooke and Dr B. Jacq for help in performing high-resolution gel electrophoresis experiments.

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