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Transcription by SP6 RNA polymerase exhibits an ATP dependence that is influenced by promoter topology

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ABSTRACT

Transcription of linearized DNA templates by SP6 RNA polymerase requires a higher concentration of ATP than of the other three nucleotides. This requirement is not shared by T7 RNA polymerase. The ATP requirement is partially relieved when the SP6 template is supercoiled but not when it is relaxed circular DNA. The effect of supercoiling is eliminated by replacement of the A·T rich sequence downstream from the SP6 promoter with a G·C rich sequence. Examination of the reaction products indicates that the ATP dependence of transcription from a linear template is not due to an ATPase activity or to the premature termination of transcription at low ATP concentration. These data suggest that the initiation of transcription by SP6 RNA polymerase requires partial denaturation of the template in the promoter-proximal region, and that this requirement can be satisfied by negative supercoiling or by increasing the ATP concentration. ATP also reduces, but does not eliminate, the abortive transcription that leads to the production of short, prematurely terminated transcripts by SP6 polymerase from supercoiled templates.

INTRODUCTION

Bacteriophage SP6 from *Salmonella typhimurium* encodes an RNA polymerase which has been studied intensively (1,2). Like the corresponding enzymes from the *E.coli* phages T7 and T3 (3,4), SP6 RNA polymerase transcribes the viral genome with high efficiency and specificity. All of these polymerases are stable and readily isolated, and maintain high activity and strong promoter specificity *in vitro*. Their respective promoters have been isolated and characterized and have been incorporated into a wide range of cloning vehicles. Because of the relative ease and economy with which the vectors and polymerases can be prepared, they are useful for synthesizing defined RNA molecules in abundance (5,6). Moreover, when radioactive nucleotides are included in the transcription reaction, labeled transcripts can be produced with the chosen specific activity (7,8).

The nucleotide dependence of the SP6 enzyme displays some unusual features that have not been fully explained. Transcription by SP6 RNA polymerase is inefficient when the concentration of ATP is limited (7). Since *in vitro* transcription reactions are routinely performed in the presence of a limiting concentration of the radiolabeled nucleotide, in the 1−20μM range (8), it is difficult to label RNA probes to very high specific activity with ATP. Surprisingly, the K_m for ATP is not greatly different from that for the other nucleoside triphosphates: 67 μM for ATP compared to 29.9 and 31.3μM for CTP and UTP, respectively (9). The K_m for GTP could only be approximated because the double reciprocal plot is non-linear for reasons that are not readily apparent, but is also ca. 50μM.

This study addresses the ATP requirement, showing that it is a property of SP6 RNA polymerase which is not shared by T7 RNA polymerase. The ATP requirement is reduced if a negatively supercoiled template is substituted for the linear DNA template that is normally used. Transcription of a linear template that lacks A residues in the immediately promoter-proximal region is not facilitated by elevated ATP concentrations, however. Since neither ATP hydrolysis nor elevated levels of premature termination of transcription are observed with the linear template, we suggest that the ATP dependence reflects the need for partial denaturation of the template at the site of transcription initiation.

MATERIALS AND METHODS

SP6 and T7 RNA polymerases, RNasin ribonuclease inhibitor, topoisomerase I, pSP64 DNA, pGEM-1 DNA, and Riboprobe Gemini positive control template were purchased from Promega. Ribonucleoside triphosphates were obtained from Pharmacia. Radiolabeled nucleotides were from ICN Radiochemicals. Restriction enzymes were purchased from Boehringer-Mannheim. Plasmid pCKSP6d4 was provided by C.Kang and pSP6tt was provided by R.Gumport.

Plasmid DNA was banded in CsCl (10). The pGEM-1 vector contains SP6 and T7 promoters in opposite orientations flanking a polylinker sequence, and was linearized by digestion with Pvu I. Plasmid pSP64 (7) contains the SP6 promoter and was digested with Bgl I. Plasmid pCKSP6d4 contains a mutant SP6 initiation sequence (11) and was linearized with Pvu I. Plasmid pSP6tt contains the thr attenuator and the rrnC terminator (12) and was linearized with Eco R1. The Riboprobe Gemini control template was supplied as a mixture of linear DNAs with both SP6 and T7 promoters: its transcribed sequence is from bacteriophage λ.

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Table 1. Effect of nucleotide limitation. SP6 RNA polymerase transcription reactions were performed with the Riboprobe Gemini control template.

	Nucleotide concentration (μM)				RNA Synthesized
	ATP	CTP	GTP	UTP	nMoles
A.	12	500	500	500	0.02
	500	12	500	500	0.84
	500	500	12	500	0.81
	500	500	500	12	0.87
B.	120	12	120	120	0.66
	12	12	120	120	0.02
	120	12	12	120	0.58
	120	12	120	12	0.60

Plasmids pGEM-1 and pSP64 were relaxed by incubation at 37°C for 1 hr with topoisomerase I (5 units/μg DNA) in the presence of 50mM Tris−HCl (pH 7.9), 50mM NaCl, 1mM EDTA, 1mM DTT and 20% glycerol. Following treatment, DNAs were recovered by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation.

Transcription reactions were performed in a final volume of 20μl containing 40mM Tris−HCl (pH 7.5), 6mM $MgCl_2$, 2mM spermidine, 10mM NaCl, 10mM dithiothreitol, and 20 units RNasin. Standard reactions contained 500μM of each of the three unlabeled ribonucleoside triphosphates, and 12−500μM of the fourth in unlabeled form plus 50μCi labeled with [α-^{32}P] at a specific activity of 3000 Ci/mMole (i.e., ca. 0.83 μM). DNA (0.5μg) was added to the reaction at room temperature to avoid precipitation by spermidine (8) followed by 20 units of SP6 or T7 RNA polymerase. Reactions were incubated at 37°C for 1 hr (or the times indicated) and directly analyzed by trichloracetic acid precipitation, by thin layer chromatography on polyethyleneimine-cellulose plates (Brinkmann CEL300 PEI) developed with 0.75M potassium phosphate (pH3.5), or by electrophoresis in 6% or 25% polyacrylamide/8M urea gels. Time course reactions were performed as above with several exceptions. The final reaction volume was 40 μl; 5 μl was removed at each time point and mixed on ice with an equal volume of 98% formamide gel loading dye. Reaction mixtures were prewarmed for 15 min at 37°C before the RNA polymerase was added. Apparent K_m was determined by measuring initial rates of incorporation in reactions containing varying concentrations of one nucleotide and a fixed concentration (0.5mM) of the other three nucleotides.

RESULTS

We first examined the effect of reducing the concentration of each nucleoside triphosphate in turn on the efficiency of transcription of linear DNA by SP6 RNA polymerase. In agreement with the results of Melton and coworkers (7), RNA yield was decreased sharply when the ATP concentration was low (12μM) but not when the concentration of CTP, GTP, or UTP was lowered (Table 1A). Furthermore, when the concentration of CTP was held at a suboptimal level (12μM), reducing the concentration of GTP or UTP in the same reaction had little effect on RNA synthesis, but reducing the concentration of ATP impaired transcription drastically (Table 1B). Therefore the SP6 transcription system is selectively sensitive to limiting concentrations of ATP.

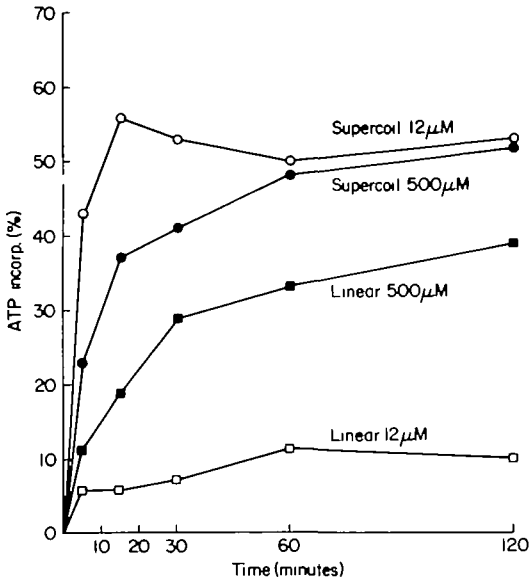

Figure 1. Kinetics of transcription by SP6 RNA polymerase. pGEM-1 plasmid DNA, linearized or supercoiled, was transcribed for the times indicated in the presence of 500μM of CTP, GTP and UTP and 12 or 500μM ATP plus [α-^{32}P] ATP.

To discover whether the ATP requirement is related to template topology, we compared the transcription of linear and supercoiled forms of pGEM-1 plasmid DNA. The time course shown in Fig. 1 reveals that all reactions were at or near completion after incubation for 60 min, regardless of the nature of the template and the ATP concentration. In agreement with the data discussed above, the linearized template allowed less incorporation when ATP was limiting (12μM) than when it was saturating (500μM). No such difference was seen with the supercoiled template, however, implying that the high ATP requirement is not eliminated by extending the reaction time but can be overcome by negative supercoiling of the DNA. Estimation of the K_m for ATP by the Lineweaver−Burke method gave a value of 77μM with supercoiled template, in close agreement with the value obtained previously with genomic SP6 DNA (9). However, a non-linear plot was obtained with linearized plasmid DNA because of its inefficiency as a template at low ATP concentration. Consequently we were unable to evaluate the K_m for ATP with linear template.

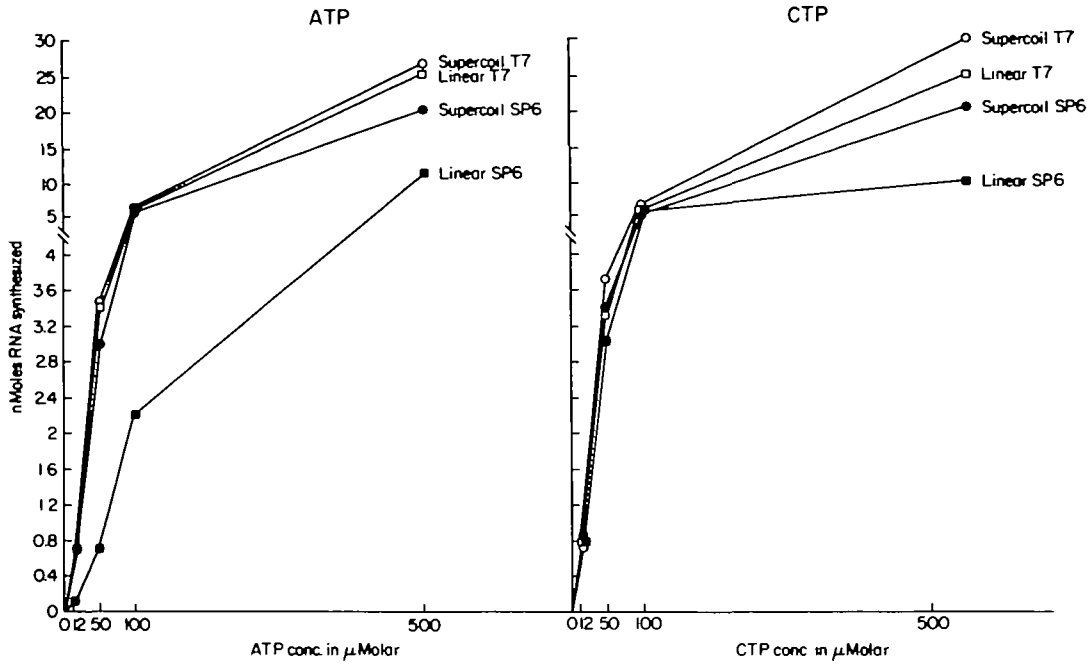

Figure 2. Dependence of SP6 and T7 RNA polymerase transcription on nucleotide concentration. Transcription reactions contained supercoiled or linearized pGEM-1 DNA and three unlabeled nucleotides at 500μM each. The concentration of the labeled nucleotide, [α-^{32}P] ATP or CTP, was varied from 12−500μM as indicated.

To assess the generality of these findings, we compared the ability of SP6 and T7 RNA polymerases to use the two DNA template conformations as a function of nucleotide concentration. As shown in Fig. 2A, transcription of the linear DNA template by SP6 RNA polymerase was relatively inefficient at ATP concentrations < 100μM. No such effect was apparent with T7 RNA polymerase, which transcribed both the linear and supercoiled templates as efficiently as the SP6 enzyme utilized supercoiled DNA. As expected, when the concentration of CTP was limiting there was no discrimination between supercoiled and linear DNA templates by either enzyme (Fig. 2B). Thus, the high ATP requirement is specific for SP6 RNA polymerase and is not shared by the T7 enzyme.

We next investigated whether the greater transcriptional efficiency seen with the supercoiled template is due to its circularity, permitting the polymerase to run for extended distances around the DNA molecule, or to its inherent torsional strain, which might relieve some topological constraint. For this purpose, pGEM-1 DNA was relaxed by incubation with topoisomerase I and tested as a template for transcription by SP6 RNA polymerase at different concentrations of ATP (Table 2). At low ATP concentration (12μM) the relaxed DNA was transcribed more efficiently than linear DNA but still much less efficiently than the supercoiled form. Transcription of the relaxed DNA, like that of the linear form, was greatly enhanced by increasing the ATP concentration to 150μM. The relaxed DNA was a somewhat better template than the linear form at both concentrations of ATP, presumably because its circularity allows more RNA to be synthesized per initiation event, but it was much less efficient than the supercoiled DNA especially at low ATP concentration. Thus, the behavior of the relaxed circular form is intermediate between that of the linear and supercoiled forms because its circularity allows extended RNA chains to be synthesized (see Fig. 5A). Over and above this effect, the presence of negative superhelical turns in the template improves the efficiency of transcription by SP6 RNA polymerase especially at low ATP concentrations.

Table 2. Effect of DNA topology and ATP concentration. SP6 RNA polymerase reactions were conducted with pGEM-1 DNA in three different conformations.

DNA	ATP incorporation, %	
	12μM ATP	150μM ATP
Negative supercoil	77	77
Linear	9	46
Relaxed circular	20	63

These observations suggested that the elevated requirement for ATP might reflect a dual role in transcription: in addition to serving as an RNA precursor, it might contribute as a source of free energy, perhaps for partially denaturing the template during initiation or for untwisting it during elongation. In this event, it should be possible to detect the hydrolysis of ATP to either P_i or PP_i in the presence of linear DNA template. Accordingly, we examined the fate of the precursor by thin layer chromatography (Fig. 3). No labeled P_i or PP_i was released from [α-^{32}P] ATP by SP6 RNA polymerase in reactions containing either linear or circular DNA template (Fig. 3A, lanes 3−5), or by T7 RNA polymerase (Fig. 3A, lanes 6−8). In reactions containing [γ-^{32}P] ATP, SP6 RNA polymerase released no P_i but did, of course, produce labeled PP_i as a result of RNA polymerization (Fig. 3A, lanes 9−11). The RNA synthesized was only weakly labeled in the reaction containing [γ-^{32}P] ATP, but was visualized as a spot remaining at the origin when the label was [α-^{32}P] ATP. Thus, no ATPase activity was detected in the SP6 RNA polymerase reactions.

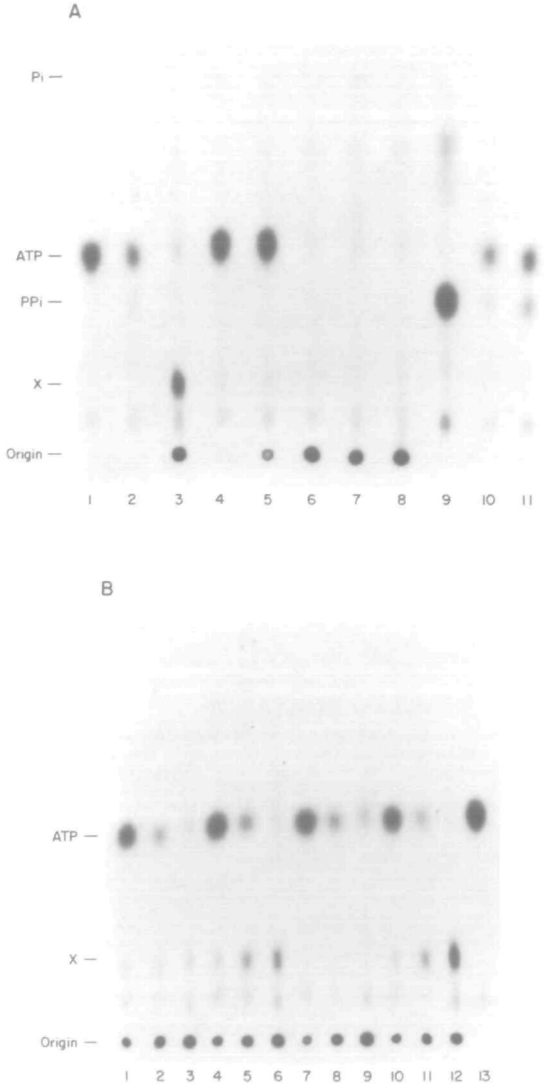

Figure 3. Fate of ATP in transcription reactions. Reactions were analyzed by thin layer chromatography on PEI plates. (A) All reactions contained three unlabeled nucleotides at 500μM each and were incubated for 60 min. before spotting. Lanes 1 and 2: [α-^{32}P] ATP and [γ-^{32}P] ATP respectively. Lanes 3–5: SP6 RNA polymerase reactions labeled with [α-^{32}P] ATP and containing supercoiled pGEM1 DNA + 12μM ATP, linear DNA + 12μM ATP, and linear DNA + 120 μM ATP, respectively. Lanes 6–8: As lanes 3–5 except with T7 RNA polymerase. Lanes 9–11: As lanes 3–5 except with [γ-^{32}P] ATP. (B) Reactions contained linear pGEM1 DNA, SP6 RNA polymerase, [α-^{32}P] ATP, and three unlabeled nucleotides at 500μM each. Lanes 7–9: Reactions containing 200μM ATP were stopped at 5, 15 and 60 mins., respectively. Lanes 10–12: Reactions containing 12μM ATP were stopped at 5, 15 and 60 mins., respectively. Lanes 1–3: As lanes 10–12, except that the ATP concentration was raised to 200μM after 5 min. Lanes 4–6: As lanes 10–12 except that the ATP concentration was raised to 200μM after 15 mins. Lane 13 contains [α-^{32}P] ATP.

A new spot (spot X) was observed in reactions containing SP6 RNA polymerase, supercoiled template, and [α-^{32}P] ATP (Fig. 3A, lane 3). The quantity of radioactivity in this spot was greater when more RNA was synthesized (compare lane 3 with lanes 4 and 5 in Fig. 3A), so spot X appears to correlate with efficient transcription rather than with an ATPase activity. In pulse-chase reactions, the accumulation of label in this spot ceased when the concentration of ATP was raised from 12μM to 200μM

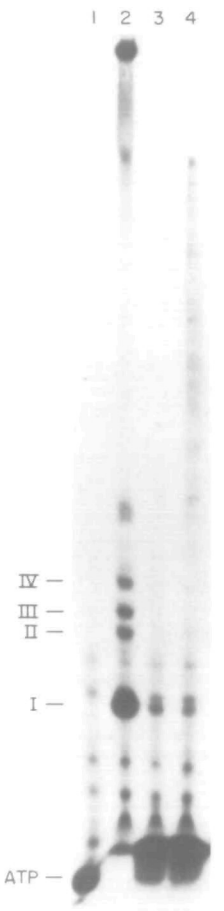

Figure 4. Electrophoretic analysis of reaction products. Products of standard SP6 RNA polymerase reactions labeled with [α-^{32}P] ATP. Lane 2, supercoiled pGEM1 DNA + 12μM ATP; lane 3, linear DNA + 12μM ATP; lane 4, linear DNA + 120μM ATP. Lane 1 contains [α-^{32}P] ATP alone.

(Fig. 3B, lanes 1–6), suggesting that it is a byproduct of transcription rather than an intermediate in RNA synthesis or a degradation product. This byproduct was not formed when reactions were initiated at 200μM ATP instead of 12μM (compare lanes 7–9 with lanes 10–12 in Fig. 3B).

Both SP6 and T7 RNA polymerases can give rise to short oligonucleotides, 2–6 bases in length, especially when the nucleoside triphosphate precursors are present at limiting concentration (6,11). To discover whether the poor yield of RNA is due to abortive transcription, we analyzed the products of SP6 RNA polymerase reactions by electrophoresis in polyacrylamide gels. The supercoiled template gave rise to high molecular weight RNA which mostly remained in the well (Fig. 4, lane 2). In a formaldehyde-agarose gel, this material was resolved as a smear (data not shown), presumably because the template lacks authentic termination signals. In addition four discrete bands of rapidly migrating material, bands I-IV, were observed. In reactions containing linear template, little long RNA was made at low ATP concentration (12μM; Fig. 4, lane 3) but longer RNAs were detected in reactions conducted at 120μM ATP (Fig. 4, lane 4). When resolved in a formaldehyde-agarose gel (not shown) this RNA migrated predominantly as a single band of the expected size (approx. 1500 nucleotides). The formation of band I was detectable with the linear template at both low and high ATP

Table 3. Effect of promoter-proximal sequence and topology. SP6 RNA polymerase reactions were conducted with two plasmids. The sequences flanking the initiation sites are illustrated.

	Initiation Sequence				
	−10	+1	+10	+20	
	•	•	•	•	
pGEM-1	ATTTAGG T	GACACTATA G	AATACAAGC	TTGGGCTGCA	GGTCGACT
pCKSP6d4	ATTTAGG T	GACACTATA G	CCCGGGCGA	GCTCGAATTC	GTAATCAT

Template	ATP concentration	% Incorporation pGEM-1	pCKSP6d4
Supercoiled	12μM	45%	25%
	120μM	67%	30%
Linear	12μM	2%	2%
	120μM	17%	3%

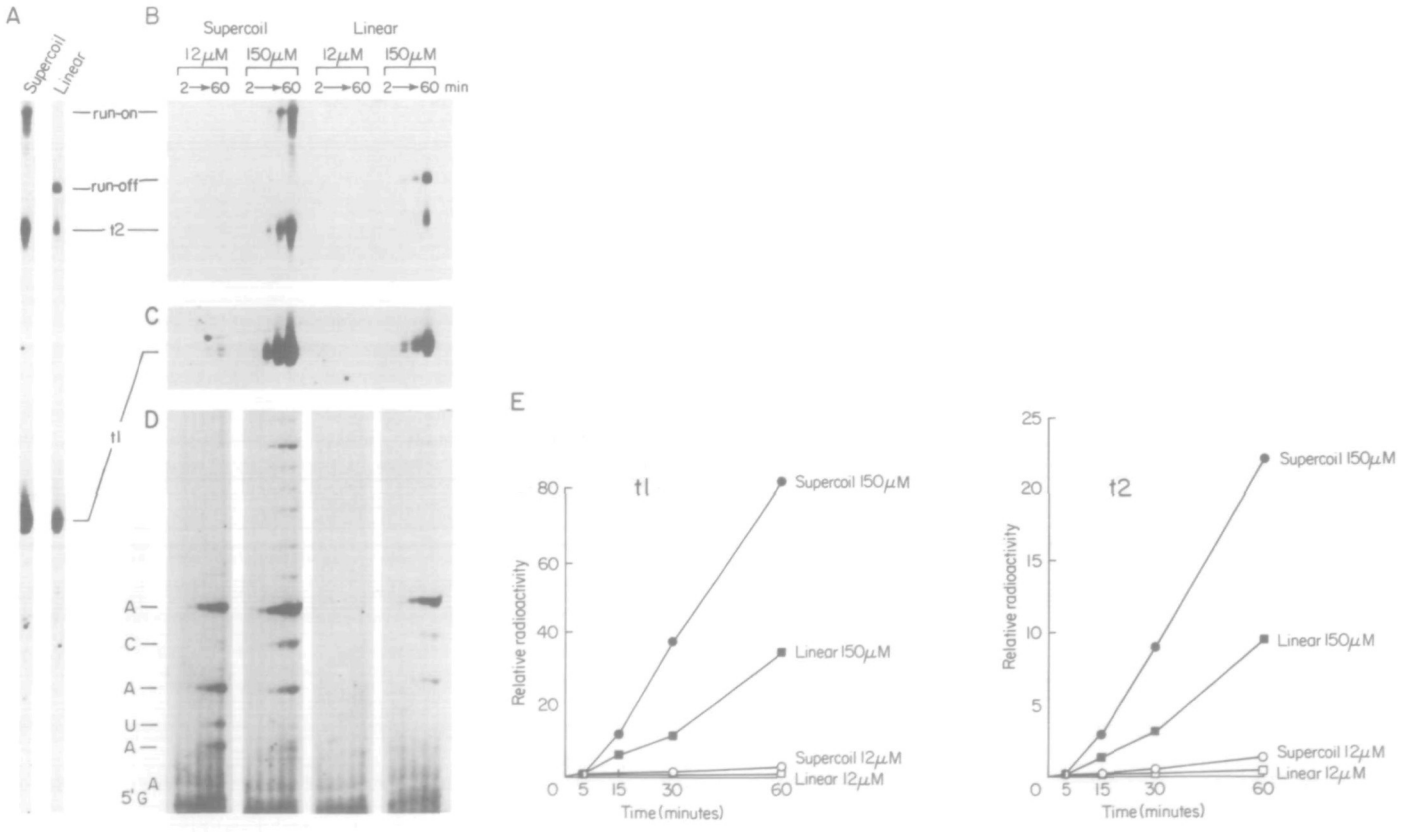

Figure 5. Analysis of initiation and elongation of terminated transcripts. (**A**) The products of 60 min reactions with SP6 RNA polymerase and either supercoiled or linear pSP6tt DNA at 150 μM ATP were examined by electrophoresis in a 6% polyacrylamide gel. Reactions contained 180 μM GTP and [γ-^{32}P] GTP. The positions of transcripts terminating at the thr attenuator (t1) and the rrnC terminator (t2), and of run-on and run-off transcripts are marked. The relative synthesis of t_1, t_2 and readthrough transcripts were 66, 19, and 15% for supercoiled DNA and 56, 18 and 26% for linear DNA. (**B,C**) Reactions containing supercoiled or linear DNA and 12 μM or 150 μM ATP were stopped at 2, 5, 15, 30 and 60 min. Transcripts were labeled with [γ-^{32}P] GTP in the presence of 180 μM unlabeled GTP and examined as in part A. (**D**) The same reaction products were analyzed by electrophoresis in a 25% polyacrylamide gel. (**E**) Quantitation of the data shown in parts B and C using a Fuji bioimage analyzer.

concentration, but it was present at much lower levels than in the reaction containing supercoiled template (Fig. 4, lane 2); bands II-IV were barely detectable in the reactions containing linear template (Fig. 4, lanes 3 and 4).

Inspection of the sequence downstream from the SP6 promoter in pGEM-1 (Table 3) suggested that bands I−IV might correspond to abortive transcripts terminating before A residues in the first few nucleotides downstream from the promoter. Thus, band I was expected to be the dinucleotide pppGpA (where the boldface **p** signifies ^{32}P label derived from the a position of ATP). In support of this view, band I material eluted from the gel comigrated with spot X upon thin-layer chromatography, and it

displayed the predicted behavior after treatment with base specific-ribonucleases (data not shown). Band I (spot X) was refractory to RNase A; it gave rise to a new slow-moving spot (presumably pppGp) after digestion with either RNase T_1 or RNase T_2, and gave rise to a spot migrating with pA (i.e., 5′ AMP) after incubation with RNase P_1. Furthemore, band I was synthesized in reactions containing [γ-^{32}P]γTP as the labeled nucleotide, and was produced in reactions lacking UTP and C-TP (data not shown). These observations prove that band I is the dinucleotide pppGpA, and suggest that bands II-IV are similar abortive transcripts. The generation of such oligonucleotides by T7, SP6 and *E.coli* RNA polymerases has been noted by others (6,11,13).

Although these abortive transcripts were formed, their scarcity in reactions containing linear DNA argues against the idea that premature termination before A sites accounts for the inefficient utilization of linear templates, but does not exclude the possibility that the ATP dependence is related to the abundance of A residues in the 5′ end of the transcript from the SP6 promoter in pGEM-1 where 5 of the first 8 residues are A. To test this hypothesis we exploited the plasmid pCKSP6d4 which contains an SP6 promoter with a mutant initiation sequence lacking A residues entirely until position +10 and possessing only one A residue in the first 15 nucleotides (Table 3). In supercoiled form, the mutant template was about half as active as pGEM-1. This value is in reasonable agreement with the results of Nam and Kang (11) who found that the mutant was 79% as active as wild-type. As with pGEM-1, the linear form of the mutant DNA was a very inefficient template at low ATP concentration (12μM). Unlike the linear wild-type template, however, transcription of the linear pCKSP6d4 template was not enhanced significantly by increasing the ATP concentration to 120μM. In this case, poor utilization of the linear template cannot be attributed to the limited availability of a nucleotide precursor as there are very few A residues in the mutant initiation sequence. On the contrary, it seems that transcription is limited by the duplex structure of the DNA template and that the limitation is relieved by high ATP concentration only if the promoter-proximal sequence contains A residues.

To examine the effect of template superhelicity on transcriptional efficiency without the complication introduced by the potentially infinite length of the products generated from a circular template, we employed a plasmid containing two transcriptional terminators, t1 and t2, in tandem. Transcription of the pSP6tt template by SP6 RNA polymerase yields RNAs of approximately 123 and 226 nucleotides as a result of termination, together with a small proportion of read-through transcripts (Fig. 5A). In this experiment, the products were labeled with [γ-^{32}P] GTP so that the incorporation of radioactivity is a measure of the number of RNA chains synthesized. Considering transcripts terminating at t1, the supercoiled template was twice as efficient as the linear template at 150 μM ATP. Reducing the ATP concentration to 12 μM reduced the rate of transcription from the supercoiled template by about 10 fold and essentially eliminated transcription from the linear template (Fig. 5C and E). Quantitatively similar results were obtained for transcripts terminating at t2, as well as for the run-off transcripts from the linear template and the run-on transcripts from the supercoiled DNA (Fig. 5B). Thus, considering the production of transcripts of the same length, the supercoiled template is more efficient than the linear template at both high and low concentrations of ATP, and transcription from the linear template is much more sensitive to ATP concentration than is transcription from the supercoiled template.

Premature termination was studied under the same condition by analyzing the reaction products in a high percentage polyacrylamide gel (Fig. 5D). Consistent with the result shown in Fig. 4, these prematurely terminated chains were produced more abundantly by the supercoiled template at 150 μM ATP than by the linear template at the same ATP concentration. As with the longer transcripts (Figs. 5B and C), no detectable RNA was made from the linear template at 12 μM ATP. Compared to the longer transcripts, abortive transcription accounted for a relatively higher percentage of the total transcription from the supercoiled template when the ATP concentration was lowered to 12 μM (Fig. 5D). Therefore, we conclude that superhelicity increases the rate of initiation, and that ATP increases initiation and suppresses premature termination.

DISCUSSION

Previous studies of the SP6 transcription system *in vitro* have noted poor incorporation at low ATP concentration when linear DNA templates are used (7,8). As shown here, short, aborted transcripts do not accumulate (Figs. 3,4 and 5), so it seems that inefficient transcription by SP6 RNA polymerase in this condition is due to weak initiation on linear templates rather than to a failure in chain elongation. This conclusion is supported by measurement of the synthesis of short, defined transcripts which terminate at specific sites within a few hundred nucleotides of the start site (Fig. 5). The limitation is overcome when the template is negatively supercoiled, suggesting that partial denaturation of the template may facilitate the initiation of transcription (Fig. 5). Similar conclusions have been drawn from studies with other RNA polymerases (14−18) although inhibitory effects of negative supercoiling have also been reported (19). Both the rate of formation and the stability of initiation complexes is enhanced on superhelical DNA (20), presumably because the template is partially single-stranded in the promoter region (21−26). Consistent with this interpretation, the template activity for SP6 RNA polymerase was reduced when the A·T-rich sequence immediately downstream from the SP6 promoter was replaced by a G·C-rich sequence which would be expected to be more resistant to denaturation (Table 3).

The limitation imposed on the transcription of linear DNA by SP6 polymerase is relieved at elevated ATP concentration. We have considered several possible explanations for this effect. First, it does not seem to be due to the A-richness of the encoded RNA because the ATP requirement was not alleviated by changing the downstream sequence for one which lacks A residues (Table 3). Moreover, as noted above, short transcripts were not produced in greater abundance when transcription was conducted on linear DNA templates at low ATP concentration (Figs. 4 and 5). Second, it is possible that SP6 RNA polymerase is associated with an ATP-dependent topoisomerase or helicase activity which untwists the DNA helix allowing the polymerase to enter. The β-γ bond of ATP is hydrolyzed by RNA polymerase II (27,28) but no hydrolysis was detected in SP6 RNA polymerase reactions (Fig. 3) and AMP-PNP was not inhibitory (data not shown). Furthermore, neither SP6 nor T7 RNA polymerases contain sequence features characteristic of such activities (2,29). Third, the polymerase might be covalently modified in an ATP-dependent reaction, for example by phosphorylation. This is unlikely because the polymerase is not detectably labeled by

incubation with either [α-^{32}P] ATP or [γ-^{32}P] ATP (data not shown). It remains possible, however, that the enzyme is non-covalently modified by binding ATP in an allosteric or cooperative manner to change the effective binding of the substrate or DNA. The presence of a second ATP binding site on the polymerase could explain the non-linearity of the Lineweaver-Burke plot. However, on this basis it is not obvious why transcription from the G·C-rich promoter was not significantly enhanced at high ATP concentration (Table 3).

On a supercoiled template, ATP increases the rate of initiation by SP6 RNA polymerase to a small extent, and greatly increases the ability of the polymerase to elongate beyond the promoter proximal region (Fig. 5). On a linear template, ATP greatly increases the rate of initiation (Fig. 5) and may also increase elongation, although our experiments were not conclusive on this point. Presumably, the elongation effect is due to the tendency of polymerases to stall and prematurely terminate at A residues when the ATP concentration is low. The nature of the initiation effect is less obvious. One possible explanation supposes that ATP favors partial denaturation of the template in the immediately promoter-proximal region. A/T rich sequences are commonly found in origins of replication and in start sites for transcription, regions of DNA that need to be melted and unwound (e.g., 23). This explanation is consistent with the similar effect of negative superhelicity, discussed above, and with the observation that replacement of the promoter-proximal A residues eliminates the effect of high concentrations of ATP (Table 3). It appears that the requirement is for ATP itself as the analogues adenosine and dATP do not overcome the requirement for high ATP concentration (data not shown).

In conclusion, SP6 RNA polymerase requires partial denaturation of the template downstream from the promoter for efficient initiation of transcription whereas T7 RNA polymerase is capable of efficient transcription from closed templates. The requisite partial denaturation can be accomplished by employing negatively superhelical template, or by supplying a high concentration of ATP, provided the promoter-proximal transcribed region is rich in A residues.

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