The EMBO Journal Vol.2 No.2 pp.179 – 184, 1983

Pausing of RNA polymerase molecules during *in vivo* transcription of the SV40 leader region

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Communicated by Y. Aloni
Received on 15 November 1982

Viral transcription complexes were isolated from SV40-infected cells and incubated *in vitro* in the presence of [α-^{32}P]UTP to allow elongation of the promoter-proximal RNA up to the attenuation sites. The 94 nucleotide attenuated RNA (spanning nucleotides 243 – 336) was purified, digested with RNase T1 and fingerprinted. The labeled oligonucleotides were then isolated, digested with RNase T2 and their base composition was determined. Based on these analyses 10 consecutive oligonucleotides, spanning residues 259 – 336, were identified. As the *in vivo* synthesized oligonucleotides are unlabeled the junctions between labeled and unlabeled oligonucleotides define the *in vivo* pause sites of RNA polymerase molecules. The characterization of the 10 radioactive spots and their relative intensities allowed the localization of two *in vivo* pause sites: one at 13 – 16 nucleotides downstream from the major initiation site presumably at the initial opening of the DNA helix and the second at ~40 nucleotides downstream from the major initiation site, just past a GC-rich region of dyad symmetry. It is postulated that pausing of RNA polymerase molecules in the leader region is an essential process in the control of SV40 late transcription.

Key words: attenuation/pausing of RNA polymerase/premature termination/SV40 transcription/transcription termination

Introduction

In procyarotes, transcription termination sites are located within, as well as at the end of, the operons. At the end of the operon, transcription termination prevents interference with expression from neighboring regions of the chromosome. Within the operon termination sites cause premature termination of the transcripts and quantitatively regulate the level of gene expression by selectively reducing the transcription of distal portions of the operons. This mechanism of regulation has been termed attenuation (Yanofsky, 1981). It is thought that regulation is achieved by altering the frequency with which transcription crosses the attenuation site (for reviews, see Adhya and Gottesman, 1978; Gottesman *et al.*, 1980).

Transcription terminations at the end and within the operon have several common features: GC-rich sequences preceding the stop site, from which a stem-and-loop structure can be formed, and uridine residues in the terminus of the RNA transcripts (for review, see Adhya and Gottesman, 1978; Biro and Weissman, 1979; Rosenberg and Court, 1979; Crawford and Stauffer, 1980; Gallupi and Richardson, 1980; Gottesman *et al.*, 1980; Yanofsky, 1981). It has been suggested that the stem-and-loop structure causes retardation of polymerase movement through the termination region, whereas the uridine residues facilitate the release of the transcript (Martin and Tinoco, 1980; Farnham and Platt, 1980). It is interesting to note that in the *trp* operon every polymerase molecule that transcribes the initial segment of the leader region pauses at the 'leader pause site', 50 nucleotides upstream of the attenuation site. The pause site is located just past a GC-rich region of dyad symmetry (Winkler and Yanofsky, 1981; Farnham and Platt, 1981).

The signals for pausing (a GC-rich region of dyad symmetry) and transcription termination (a GC-rich region of dyad symmetry followed by a string of uridylic acid residues) suggest a general mechanism for these processes based in part on the physical chemistry of nucleic acid interactions used by both the procaryotic and eucaryotic polymerases. Indeed, we have recently localized a site at which transcription of SV40 late RNA is attenuated *in vitro* (Hay *et al.*, 1982; Skolnik-David *et al.*, 1982, see accompanying paper). The properties of the DNA sequence where RNA synthesis attenuates is strikingly similar to the termination signal in procaryotes. On the basis of these observations, we have suggested that the eucaryotic RNA polymerase B can respond to a transcription termination signal similar to that of the procaryotic polymerase and we have presented a model in which attenuation and mRNA modulation in a feedback control mechanism quantitatively regulate SV40 gene expression (Hay *et al.*, 1982; Skolnik-David *et al.*, 1982; Aloni and Hay, 1983). In the above reports we have speculated that *in vivo* RNA polymerase pauses at the late promoter region of SV40 DNA. Here we provide experimental evidence that *in vivo*, during SV40 late transcription, RNA polymerase molecules indeed pause at this region. Moreover, two pause sites were located: one, 13 – 16 nucleotides downstream from the major initiation site, presumably at the open promoter complex (Saucier and Wang, 1972; Hsieh and Wang, 1978) and a second at ~40 nucleotides downstream from the major initiation site, just past a GC-rich region of dyad symmetry. It is postulated that pausing of the RNA polymerase at the leader region is an essential process in the control of SV40 late transcription.

Results

Kinetics of RNA elongation on viral transcriptional complexes

Several actively transcribing structures can be prepared from SV40-infected cells; these include isolated nuclei, minichromosomes (Laub *et al.*, 1980) and viral transcriptional complexes (VTC) (Laub and Aloni, 1976; Ferdinand *et al.*, 1977; Hartman *et al.*, 1979). All of them are capable of elongating preinitiated viral RNA *in vitro*. Whereas almost no initiation of RNA polymerase B transcripts occurs in isolated nuclei and minichromosomes (Laub *et al.*, 1980; Llopis and Stark, 1981) absolutely no initiation occurs in the VTC system (Laub and Aloni, 1976; Ferdinand *et al.*, 1977; Hartman *et al.*, 1979). The advantage of VTC over isolated nuclei is that they synthesize viral RNA exclusively.

We have recently shown that the RNA elongated *in vitro*

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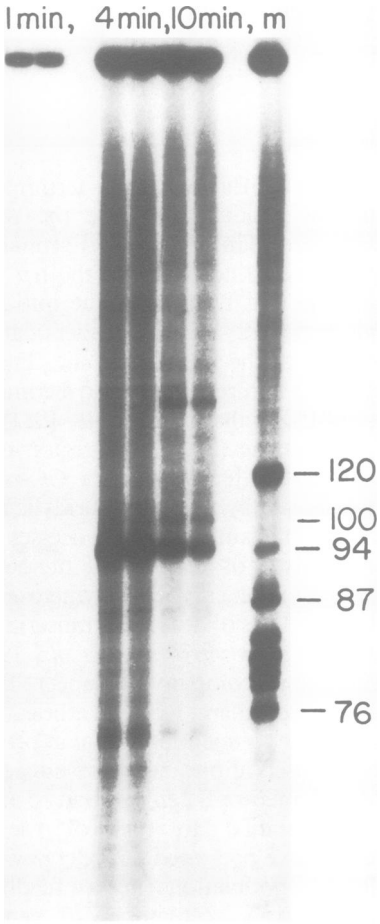

Fig. 1. Kinetics of [α-^{32}P]UMP incorporation in VTC and size analysis of viral RNA by gel electrophoresis. VTC prepared from 2 x 10^7 cells were labeled *in vitro* with 300 μCi of [α-^{32}P]UTP for 1 min, 4 min and 10 min. RNAs were extracted and subjected to electrophoresis on polyacrylamide gel in 7 M urea (Maniatis and Efstratiadis, 1980) as described in Materials and methods. Lane m - length markers of *Escherichia coli* RNA (a gift from M. David).

on VTC includes a major component of 93 – 95 nucleotides which is revealed by acrylamide gel electrophoresis. This RNA was found to map between the major initiation site at nucleotide 243 and nucleotides 335 – 337 (Hay *et al.*, 1982; Skolnik-David *et al.*, 1982) and it was defined as attenuated RNA. In the following experiment we used VTC preparations to verify the optimal time needed for maximum incorporation of [α-^{32}P]UMP into the 93 – 95 nucleotide RNA and to determine which portion of the RNA was synthesized *in vivo* and which was elongated *in vitro*.

At 52 h post-infection, VTC were prepared and incubated in the presence of [α-^{32}P]UTP for 1, 4 and 10 min. The elongated RNAs were purified and analyzed by gel electrophoresis. Figure 1 shows the occurrence in all three preparations, of one major band of RNA in a position corresponding to a molecule of 93 – 95 nucleotides (Hay *et al.*, 1982). Furthermore, it is apparent that this band is barely recognizable after 1 min of incorporation and it has a maximum radioactivity after 4 min of incorporation. After 10 min of incorporation the radioactivity in the major band is reduced. It is interesting to note that the amount of RNA in the bands of ~100 and 150 – 200 nucleotides increases with the incorporation time. Based on these and other results of similar experiments we conclude that 3 – 5 min is the optimal time needed

Table I. Radioactivity in the major band of Figure 1

Incorporation time (min)	Radioactivity (c.p.m.)
1	1180
4	8016
10	4007

The major band (94 nucleotides) from each set of two lanes in Figure 1 was excised and counted.

for the majority of the RNA polymerase molecules to reach the attenuation site located 93 – 95 nucleotides downstream from the major initiation site. In shorter incubations most of the polymerase molecules fail to reach this site, while in longer incubations some of the RNA polymerase molecules transcribe beyond this site.

The labeled RNA present in the major band of each of the three preparations was eluted from the gel and its radioactivity determined. The results of Table I are in agreement with our visual observations; after 4 min of incorporation there were seven and two times more radioactivity in the band than after 1 and 10 min of incorporation, respectively.

The locations of the in vivo pause and in vitro attenuation sites

When the labeled RNA present in the major band is digested with RNase T1 and fingerprinted (Sanger *et al.*, 1965) an oligonucleotide pattern characteristic of a transcript from the region between nucleotides 243 and 335 – 337 can be predicted. However, in an actual experiment, only those oligonucleotides which were elongated *in vitro* should produce radioactive spots, because the *in vivo* synthesized oligonucleotides are unlabeled. The junctions between labeled and unlabeled oligonucleotides define the *in vivo* pause sites of RNA polymerase molecules.

Figure 2 shows the RNase T1 fingerprints of the labeled RNA present in the major band (see Figure 1). The fingerprints of the 4 and 10 min labeled RNAs display 10 spots of which 3 and 3′ are not well resolved. In all further experiments they were analyzed together. The fingerprint of the 1 min labeled RNA displays only four clear spots (spots 6, 7, 8 and 9).

To match the oligonucleotides of each spot with the predicted T1 oligonucleotides, each spot of Figure 2A and B was eluted and digested to completion with RNase T2. The digestion results in the transfer of the labeled phosphate to the 5′ nearest nucleotide. The labeled nucleotides produced were then identified by paper electrophoresis (Figure 3A and 3B).

Figure 4 shows the base sequence spanning the major initiation site (nucleotide 243) and nucleotide 351, the predicted RNase T1 cleavage sites (following G residues) and the predicted labeled nucleotides obtained after the RNase T2 digestion of each of the oligonucleotides. Note that several oligonucleotides are not expected to be labeled. Ten consecutive oligonucleotides spanning nucleotide 259 to nucleotide 336 were identified. It is evident that spot 1 contains the two oligonucleotides CUG (nucleotides 259 – 261 and 268 – 270) because the ratio of radioactivity beteen CMP and GMP in spot 1 is 2:1 (see Figure 3). It should be noted that some polymerase molecules may have initiated elongation 13 nucleotides downstream from the transcription initiation site at

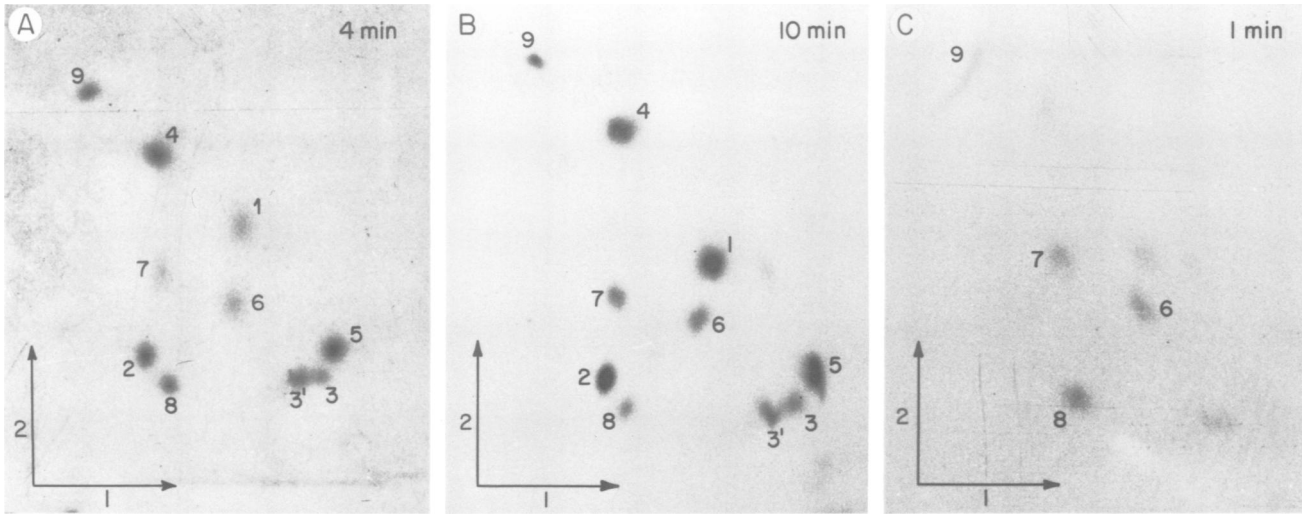

Fig. 2. Fingerprint analysis of the 1, 4 and 10 min major labeled RNA band of Figure 1. The ^{32}P-labeled RNAs recovered from the gel were digested to completion with RNase T1 and the products were separated in two dimensions as described in Materials and methods. **1** - electrophoresis, **2** - homochromatography.

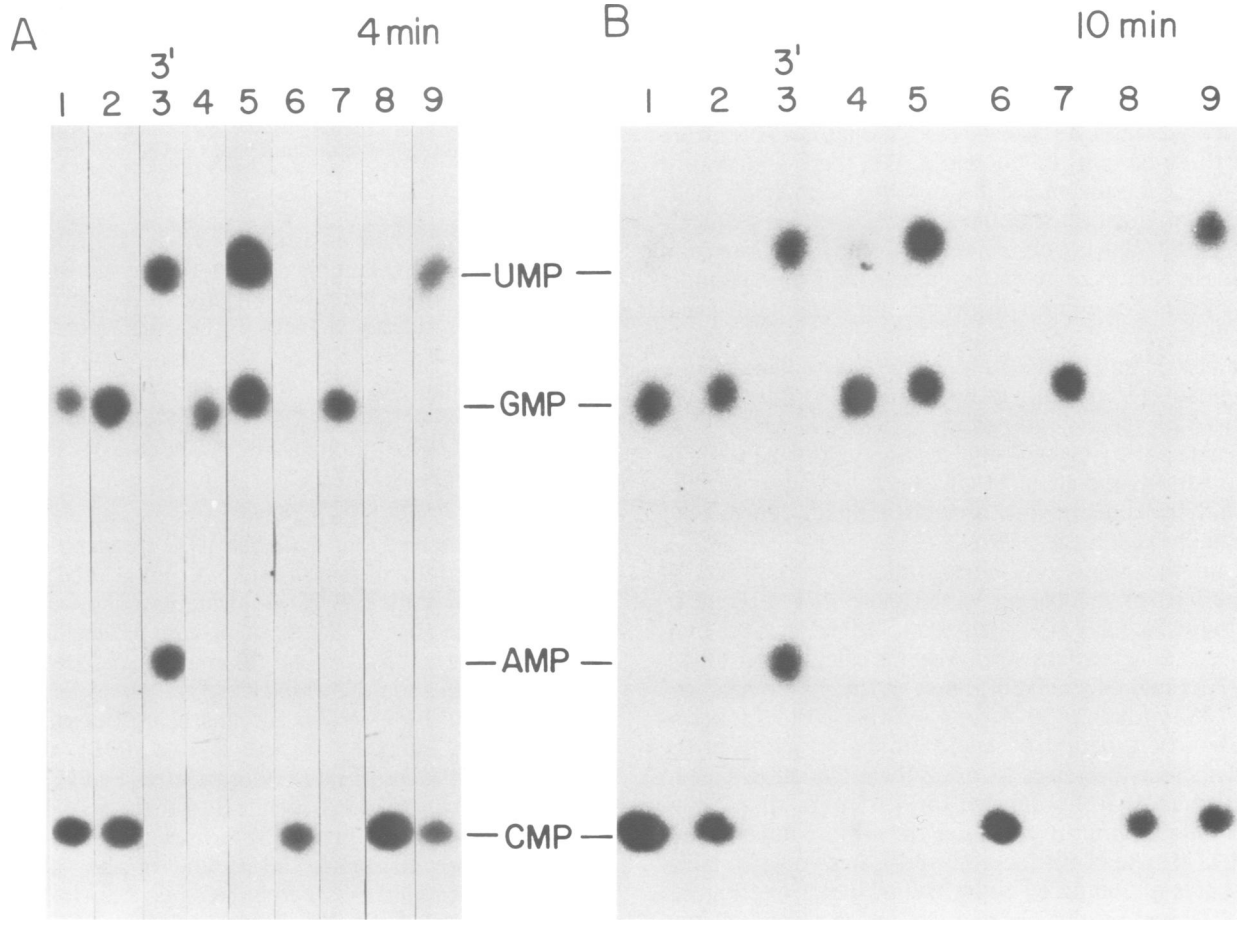

Fig. 3. Nearest-neighbor analysis by RNase T2 digestion of the various oligonucleotides in Figures 2A and B. T1 oligonucleotides were recovered from the fingerprints of Figures 2A and B and totally digested with RNase T2. The products were separated by high voltage electrophoresis as detailed in Materials and methods.

nucleotide 256 but we are unable to resolve it, because nucleotides 256–258 cannot be identified by our analyses.

Independent of the *in vitro* elongation time, in all our fingerprint analyses we have never observed an additional spot that could correspond to the oligonucleotide CCAUG (nucleotides 251–255) (see Figure 4). It appears, therefore, that *in vivo* the first pause site of active RNA polymerase molecules in VTC is located 13–16 nucleotides downstream from the transcription initiation site.

After 1 min of incorporation there is already some radio-

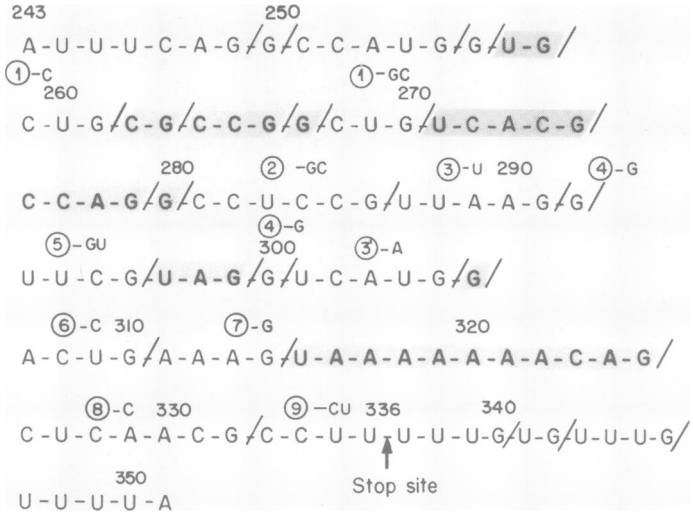

Fig. 4. The oligonucleotides otained after the RNase T1 digestion and the labeled nucleotides in each oligomer obtained following the RNase T2 digestion. Nucleotide residue numbers refer to wild-type SV40 sequence of Reddy *et al.* (1978). The slash indicates the RNase T1 cleavage site (following G). The nucleotides above each oligonucleotide are those which are labeled following the RNase T2 digestion. The shaded oligonucleotides are not labeled in the present analysis.

activity in the major band (see Figure 1 and Table I). The fingerprint analyses indicate that the labeled oligonucleotides are primarily those of spots 6, 7, 8 and 9. We, therefore, suggest that RNA polymerase molecules are also located on VTC close to the attenuation site. These molecules are relatively scarce because following an increase in incubation time from 1 to 4 min the radioactivity in the major band increased more than is expected for a linear incorporation (see Figure 1 and Table I).

The precise 3′ end of the RNA in the major band (the *in vitro* attenuation site) maps at nucleotide 336. This conclusion is based on the 1:1 ratio of radioactivity between CMP and UMP in spot 9 (Figure 3 and see Figure 4). The RNA in the major band is therefore 94 nucleotides long (nucleotides 243 – 336). This confirms our previous estimate (Hay *et al.*, 1982; Skolnik-David *et al.*, 1982).

Based on the number of nucleotides added during the various periods of incubation we estimate that the rate of RNA synthesis in our VTC system is 20 – 25 nucleotides/min. This figure is in agreement with previous calculations (Shani *et al.*, 1977; Laub *et al.*, 1980). Since in the major band the longest RNA synthesized *in vitro* is ~80 nucleotides (nucleotides 256 – 336), a period of 4 – 5 min should allow all the RNA polymerase molecules to reach the attenuation site. In the fingerprints of RNAs labeled for 4 – 5 min, spot 2 consistently contains about two to three times more radioactivity than spot 1, in spite of the fact that spot 2 has only two labeled phosphates as compared with three in spot 1 (see Figures 2A and 4). Based on this observation and the above discussion, we conclude that the highest concentration of RNA polymerase molecules on VTC is in the vicinity of the oligonucleotide CCUCCG (281 – 286) of spot 2. This could be the location of an *in vivo* pause site. If this is an exclusive pause site then the occurrence of RNA polymerase molecules upstream from it could reflect a situation in which two or more RNA polymerase molecules have initiated transcription on the same VTC and they are physically blocked by the enzyme present at the pause site. Alternatively, it is possible that RNA

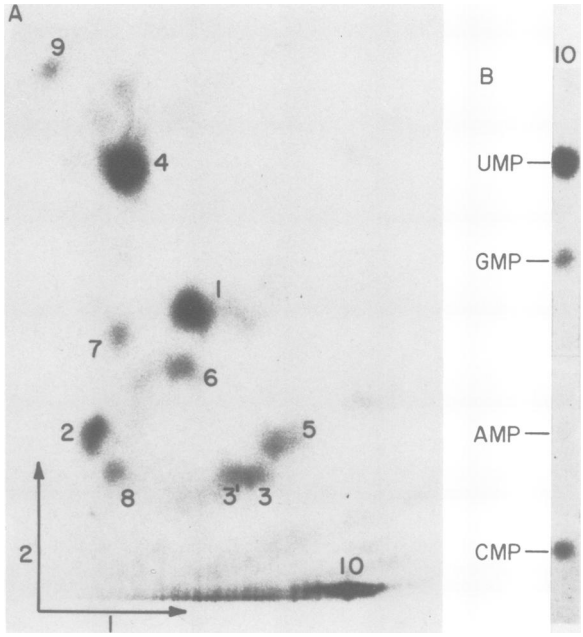

Fig. 5. Fingerprint analysis of the upper band (~100 nucleotides) as in Figure 1 (10 min pulse) and nearest-neighbor analysis by RNase T2 digestion. **(A)** The ^{32}P-labeled RNA recovered from the upper (~100 nucleotides) as in Figure 1 (10 min pulse) was digested to completion with RNase T1 and analysed as in Figure 2. **(B)** The oligonucleotide of spot 10 was recovered and totally digested with RNase T2. The products were analyzed as in Figure 3.

polymerase molecules also pause *in vivo* at the open promoter complex (see Discussion). In the fingerprint of the RNA labeled for 10 min there is an increase of radioactivity in spot 1, relative to the radioactivity in the other spots. This is expected because the RNA polymerase molecules which have initiated elongation from the oligonucleotide of spot 2 or from oligonucleotides further downstream pass the attenuation site and the RNA molecules produced are not found in the major band.

Attenuation at the U-rich region

We have noticed that when the VTC are incubated *in vitro* for short periods of time the RNA synthesized produces a band in acrylamide gels of 94 nucleotides and occasionally a diffuse region above it. With an increase of labeling time a second band is resolved above the major band (see Figure 1). Sometimes an additional band of 150 – 200 nucleotides is also recognizable. To establish the characteristics of the upper band (~100 nucleotides) the labeled RNA was eluted, digested with RNase T1 and fingerprinted. Figure 5A displays 11 spots of which 10 are identical to those found for the 94 nucleotide band (see Figure 2). However, it is apparent that spot 9, which comprises the 3′ end of the 94 nucleotide transcript (see above) is barely recognizable. Instead a new spot is revealed (spot 10). This oligonucleotide was eluted, digested with RNase T2 and the labeled nucleotides produced were identified by paper electrophoresis to be C, U and G. Figure 5B shows that UMP gives the strongest signal while GMP gives the weakest signal. We suggest that the oligonucleotide of spot 10 is CCUUUUUG (nucleotides 333 – 340, see Figure 4). Since only one CMP residue is expected to be labeled the observation that GMP gave weaker signals than CMP may reflect a situation in which only a fraction of the GMP residues in the material in spot 10 is labeled. Based on

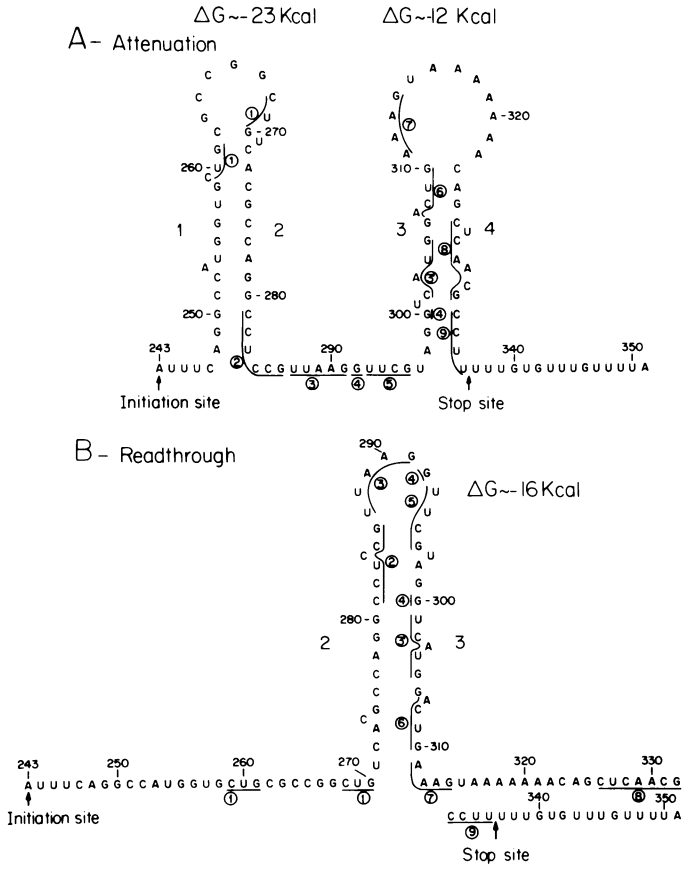

Fig. 6. Schema of alternative conformation in the attenuated RNA indicating the location of each of the oligonucleotides obtained after RNase T1 digestion. The alternative conformations are from Hay *et al.* (1982).

these observations we suggest that the 3′ end of the RNA in the upper band is at nucleotides 340 and 341 (see Figure 4). The RNA in the upper band is therefore 98 and 99 nucleotides long. It is noteworthy that when high UTP concentrations are used in incubation mixtures containing viral minichromosomes the 94 nucleotide band disappears and the upper band (~100 nucleotides) predominates (see accompanying paper; Pruzan and Aloni, unpublished results).

GC-rich dyad symmetry region in the processes of pausing and attenuation

We have recently shown that SV40 DNA sequences spanning nucleotides 243 and 336 possess dyad symmetries sufficient for the formation of alternative stable hairpin conformations. These conformations were designated 'attenuation' and 'readthrough' (Hay *et al.*, 1982). The two pairs of inverted repeat sequences 1+2; 3+4 of the 'attenuation' conformation are shown in Figure 6A. Figure 6B shows the inverted repeat sequences 2+3 of the 'readthrough' conformation. The location of the 10 oligonucleotides of the fingerprints of Figure 2 are indicated on the two alternative conformations.

It is evident that the oligonucleotide of spot 2, which we suggested above to be the position of a pause site of RNA polymerase molecules *in vivo*, is at the end of a GC-rich region of dyad symmetry (Figure 6A). The oligonucleotide of spot 6 is the first in a series of consecutive oligonucleotides that are labeled after 1 min of incorporation (see Figure 2C). This oligonucleotide is also found at the end of a GC-rich region of dyad symmetry (Figure 6B). It could be the *in vivo* location of a minor pause site. It is also evident that the major *in vitro* attenuation site is at the second uridine residue that follows a GC-rich region of dyad symmetry (Figure 6A). Based on these observations, we conclude that eucaryotic RNA polymerase B when transcribing SV40 DNA responds to signals for pausing and attenuation similar to those in procaryotes.

Discussion

Our results suggest that at least a fraction of the VTC contains nascent RNA chains 13–16 nucleotides long that were synthesized *in vivo*. One explanation for the accumulation of such ternary complexes is that RNA polymerase molecules paused *in vivo* following the synthesis of these short nascent RNA. In this regard it is noteworthy that an RNA chain of 12–14 nucleotides is involved in the formation of the first ternary complex following the opening of the DNA helix by the procaryotic RNA polymerase molecule (Melnikova *et al.*, 1978; Gamper and Hearst, 1982). We suggest that, as in the procaryotic system, the 13–16 nucleotide SV40 RNA could pertain to the open promoter complex (Saucier and Wang, 1972; Hsieh and Wang, 1978) formed by the eucaryotic RNA polymerase B transcribing the SV40 DNA molecule. We assume that to elongate an initiated transcript the RNA polymerase has to be modified from its 'initiation configuration' to 'elongation configuration'. This process can lead to a pause during transcription.

A second *in vivo* pause site was revealed at the end of a stem-and-loop structure in the viral RNA (1+2 in Figure 6). It has been shown that the strength of the hairpin actually appears to affect the length of time that polymerase remains paused at the site (Farnham and Platt, 1981). Indeed, 1+2 is the most stable hairpin in the attenuated RNA having $\Delta G = \sim -23$ Kcal (Hay *et al.*, 1982). This study thus provides an example of a eucaryotic system that supports the belief that hairpin structures may be involved in a general mechanism for regulating the elongation rate across regions of biological importance, and that this may have important implications for related aspects of transcriptional regulation (Gilbert, 1976; Kingston and Chamberlin, 1981; Winkler and Yanofsky, 1981; Farnham and Platt, 1981; Reisbig and Hearst, 1981). It has been suggested that in the *trp* leader region the function of the early (1+2) hairpin is to retard the polymerase in order to permit the translating ribosomes to catch up and remain coupled to the transcription apparatus (Winkler and Yanofsky, 1981; Farnham and Platt, 1981). This of course does not apply to the SV40 system where transcription and translation are uncoupled processes. However, there are several other potential functions for the pause in the first opening of the DNA helix and at the 1+2 hairpin structure. One of them is to help mediate interactions between RNA polymerase and other proteins such as 'attenuator' and 'anti-attenuator' factors. Another is to allow capping or methylation of the cap at the 5′ end of the RNA transcripts. In HeLa cells and adenovirus 2-infected cells the prematurely terminated transcripts (i.e., attenuated RNAs) are already capped and methylated (Salditt-Georgieff *et al.*, 1980; Tamm *et al.*, 1980) but the RNA at the pause sites has not been analyzed.

It is interesting to note that the 3+4 hairpin structure is followed by 16 nucleotides of which 13 are uridine residues (see Figures 4 and 6). It is believed that RNA polymerase can attenuate *in vitro* at various sites along this stretch of uridine

residues. In the present study, *in vitro* incubation was carried out with low UTP concentrations (1–10 μM) for a short period of time. Under these conditions RNA polymerase attenuates transcription primarily at nucleotide 336 (see Figures 4 and 6). On the other hand, when incubation was carried out for longer durations the enzyme attenuated at different uridine residues yielding transcripts of ~100 nucleotides long. In the accompanying paper we show that these transcripts can be released from the template.

Materials and methods

Cells and viruses

Growth of plaque-purified SV40 (strain 777) on BSC-1 monkey cells as well as the concentration and purification of the virus from tissue-culture lysates have been described (Laub and Aloni, 1975). BSC-1 cells were infected with 50–100 p.f.u./cell.

Preparation of VTC, in vitro elongation of RNA and purification of RNA

VTC were prepared by the Sarkosyl method as described in Laub *et al.* (1979). *In vitro* incubation was carried out in 1.0 ml buffer containing 0.15 M $(NH_4)_2SO_4$, 5 mM KCl, 1.5 mM $MnCl_2$, 1 mM $CaCl_2$, 1 mM dithiothreitol, 30 mM Hepes-NaOH at pH 8.0, 0.5 mM each of ATP, CTP and GTP and 0.15 mCi of [α-^{32}P]UTP (400 Ci/mmol, Amersham). Incubation was carried out at 26°C. [^{32}P]RNA products were extracted by an SDS-phenol chloroform procedure followed by treatment with DNase (Sigma, RNase-free) (Laub *et al.*, 1979). To remove residual RNase activity, the DNase was further purified (Maxwell *et al.*, 1977) and treated with iodoacetate.

Analysis of RNA by gel electrophoresis

The labeled RNA was dissolved in 7 μl of 10 M urea containing the dye markers and denatured at 70°C for 5 min. Electrophoresis was carried out on 12.5% polyacrylmide gel (bis:acrylamide 1:29) in 7 M urea and borate buffer (0.1 M Tris-HCl pH 8.3 0.1 M boric acid, 2 mM EDTA) (Maniatis and Efstratiadis, 1980). Electrophoresis was continued until the xylene-cyanol was 20 cm from the origin. The bands were located by autoradiography.

RNA fingerprinting

The 94 nucleotide RNA band was recovered from the gel by electroelution, mixed with 3 μg of tRNA and collected by ethanol precipitation and centrifugation. The ^{32}P-labeled RNA was resuspended in 50 mM Tris-HCl pH 7.6 and digested to completion with RNase T1 (P.L. Biochemicals) at 37°C for 6 h. The sample was lyophilized, resuspended in 2 μl of double-distilled water and fingerprinted essentially as described by Brownlee and Sanger (1969).

The separated oligonucleotides were located by autoradiography. Each spot was cut from the PEI plate and the RNA was recovered by elution of the PEI with 2 M ammonium bicarbonate. The samples were lyophilized, and for base composition analysis resuspended in 10 μl of 50 mM sodium acetate pH 4.5.

Base composition analysis

Separated oligonucleotides isolated from PEI-cellulose were digested to completion using 50 units/ml of RNase T2 at 37°C for 16 h. The products were analyzed by high voltage paper electrophoresis on Whatman No.3 paper. Electrophoresis was carried out at 4000 V in pyridine acetate buffer pH 3.5 for 45 min, side by side with the four nucleotide monophosphates as markers. The separated nucleotides were located by autoradiography.

Acknowledgements

We thank Ruchama Leizerowitz for technical assistance, M. David for helpful comments and Dr. Ann Roman for critical reading of the manuscript. This research was supported by a research grant CA 14995 from the United States Public Health Service and in part by the Norman V. Wechsler Endowment Fund.

References

Adhya,S. and Gottesman,M. (1978) *Annu. Rev. Biochem.*, **47**, 967-996.
Aloni,Y. and Hay,N. (1983) *Mol. Biol. Rep.*, in press.
Biro,P.A. and Weissman,S.M. (1979) in Taylor,J.H. (ed.), *Molecular Genetics*, Vol. 3, Academic Press, NY, pp. 177-245.
Brownlee,G. and Sanger,F. (1969) *Eur. J. Biochem.*, **11**, 395-399.
Crawford,I.P. and Stauffer,G.V. (1980) *Annu. Rev. Biochem.*, **49**, 163-195.
Farnham,P.J. and Platt,T. (1980) *Cell*, **20**, 739-748.
Farnham,P.J. and Platt,T. (1981) *Nucleic Acids Res.*, **9**, 563-577.
Ferdinand,F.J., Brown,M. and Khoury,G. (1977) *Virology*, **78**, 150-161.
Galluppi,G.R. and Richardson,J.P. (1980) *J. Mol. Biol.*, **138**, 513-539.
Gamper,H.B. and Hearst,J.E. (1982) *Cell*, **29**, 81-90.
Gilbert,W. (1976) in Losick,R. and Chamberlin,M. (eds.), Cold Spring Harbor Laboratory Press, NY, pp. 193-205.
Gottesman,M.E., Adhya,S. and Das,A. (1980) *J. Mol. Biol.*, **140**, 57-75.
Hartman,J.R., Laub,O., Aloni,Y. and Winocour,E. (1979) *Virology*, **94**, 82-94.
Hay,N., Skolnik-David,H. and Aloni,Y. (1982) *Cell*, **29**, 183-193.
Hsieh,T.-S. and Wang,J.C. (1978) *Nucleic Acids Res.*, **5**, 3337-3345.
Kingston,R.E. and Chamberlin,M.J. (1981) *Cell*, **27**, 523-531.
Laub,O. and Aloni,Y. (1975) *J. Virol.*, **16**, 1171-1183.
Laub,O. and Aloni,Y. (1976) *Virology*, **75**, 346-354.
Laub,O., Bratosin,S., Horowitz,M. and Aloni,Y. (1979) *Virology*, **92**, 310-322.
Laub,O., Jakobovits,E.B. and Aloni,Y. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3207-3301.
Llopis,R. and Stark,G.R. (1981) *J. Virol.*, **38**, 91-103.
Maniatis,R. and Efstratiadis,A. (1980) *Methods Enzymol.*, **65**, 299-305.
Martin,F.H. and Tinoco,L. (1980) *Nucleic Acids Res.*, **8**, 2295-2297.
Maxwell,I.H., Maxwell,F. and Hahn,W.E. (1977) *Nucleic Acids Res.*, **4**, 241-248.
Melnikova,A.F., Beabealashvilli,R. and Mirezabekov,A.D. (1978) *Eur. J. Biochem.*, **84**, 301-309.
Reddy,V.B., Thimmappaya,B., Dhar,R., Subramanian,K.N., Zain,B.S., Pan.J., Ghosh,P.K., Celma,M.L. and Weissman,S.M. (1978) *Science (Wash.)*, **200**, 494-502.
Reisbig,R.R. and Hearst,J.E. (1981) *Biochemistry (Wash.)*, **20**, 1907-1918.
Rosenberg,M. and Court,D. (1979) *Annu. Rev. Genet.*, **13**, 319-353.
Salditt-Georgieff,M., Harpold,M., Chen-Kiang,S. and Darnell,J.E.,Jr. (1980) *Cell*, **19**, 69-78.
Saucier,J.M. and Wang,J.C. (1972) *Nature New Biol.*, **239**, 167-170.
Sanger,F., Brownlee,G.G. and Barrell,B.G. (1965) *J. Mol. Biol.*, **13**, 373-398.
Shani,M., Birkenmeier,E., May,E. and Davidson,N. (1977) *J. Virol.*, **23**, 20-28.
Skolnik-David,H., Hay,N. and Aloni,Y. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 2743-2747.
Tamm,I., Kikuchi,T., Darnell,J. and Salditt-Georgieff,M. (1980) *Biochemistry (Wash.)*, **19**, 2743-2748.
Winkler,M.E. and Yanofsky,C. (1981) *Biochemistry (Wash.)*, **20**, 3738-3744.
Yanofsky,C. (1981) *Nature*, **289**, 751-758.