Nucleic Acids Research, Vol. 19, No. 21 5871–5875

Ara-ATP impairs 3′-end processing of pre-mRNAs by inhibiting both cleavage and polyadenylation

Kalpana Ghoshal and Samson T.Jacob*
Department of Pharmacology and Molecular Biology, The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064, USA

Received August 30, 1991; Revised and Accepted October 1, 1991

ABSTRACT

Previous studies have demonstrated that Ara-ATP can inhibit poly(A) polymerase activity by competing with ATP. To elucidate the mechanism of action of this compound, its effect on the cleavage and polyadenylation of two specific substrates, SV40L and adenovirus L3 pre-mRNAs, was studied in HeLa nuclear extracts. Unlike cordycepin 5′ triphosphate, Ara-ATP inhibited both cleavage and poly(A) addition. Addition of poly(A) polymerase fraction devoid of any other factors required for the processing reactions overcame the inhibitory effect on cleavage as well as polyadenylation of pre-mRNAs. These data suggest that Ara-ATP inhibits both cleavage and polyadenylation reactions by interacting with the ATP-binding site on poly(A) polymerase, the activity of which is essential for the cleavage reaction. Ara-ATP also blocked formation of the post-cleavage and polyadenylation-specific complexes, which further confirmed the inhibitory effect of the ATP analog on the two tightly coupled 3′-end processing reactions.

INTRODUCTION

The 3′-end processing of pre-mRNA substrates is a complex multistep reaction involving several protein components (1–3). The advent of *in vitro* polyadenylation system (4) which mimics the *in vivo* 3′-end processing with remarkable accuracy has facilitated elucidation of the mechanism of this complex process. The pre-mRNA substrate is first cleaved endonucleolytically at an adenosine residue to which 200–250 AMP residues are added. Both cleavage and polyadenylation reactions are tightly coupled and require the highly conserved hexamer sequence (AAUAAA) found 10–30 nucleotides upstream of the poly(A) site. GU or U rich elements present downstream of the cleavage site are critical but not absolutely necessary for cleavage reaction (5). The cleavage reaction can be uncoupled from polyadenylation *in vitro* by using either ATP analogs, [3′ dATP or AMP(CH_2)PP] or EDTA. Similarly, polyadenylation reaction can be studied independent of the initial cleavage by using pre-mRNA substrates whose 3′-end is the actual or very near to the poly(A) site. Until now, no inhibitor of the cleavage reaction has been reported. A few years ago, we reported that Ara-ATP (9-β-D-arabinofuranosyladenine triphosphate) derived from Ara-A, a potent antiviral agent, is an inhibitor of poly(A) synthesis *in vitro* (6). A unique observation has been the selective inhibition of poly(A) synthesis without affecting RNA synthesis (6) which was in marked contrast to that observed for cordycepin 5′ triphosphate (7). Nevertheless, both ATP analogs could inhibit the poly(A) synthesis *in vitro* at relatively low concentrations which were not inhibitory to RNA synthesis (6, 8). As observed for cordycepin 5′ triphosphate (8), the sensitivity to Ara-ATP of the initial polyadenylation reaction associated with the chromatin was significantly greater than the chain elongation reaction (6). Although these studies did not utilize specific pre-mRNA substrates, there was a remarkable similarity of the inhibitory characteristics of these compounds to those observed *in vivo* (see Discussion). The availability of the cell-free systems to study the 3′-end processing of mRNAs prompted us to examine the effect of Ara-ATP on these reactions using SV40 late and adenovirus L3 pre-mRNAs as the substrates. The present studies report the results of such an investigation.

MATERIALS AND METHODS

Materials: Adenine 9β-D-arabinofuranosyladenine 5′-triphosphate (Ara-ATP), and cordycepin triphosphate were from Sigma Chemical Co., St. Louis, MO; SP6 RNA polymerase, DNaseI, RNAsin were obtained from Promega, and 7-meGpppG was purchased from Pharmacia.

Plasmids and RNA substrates

The plasmids, pSPSV −58/+70 and its point mutant containing AAGAAA instead of the wild type AATAAA sequence, were generous gifts of M. D. Sheets and M. Wickens (9). The plasmid pSPSV contains 128 nucleotide fragment of SV40 spanning the poly(A) addition site of mRNAs for the late virion protein. Transcription of the template digested with Dra I generates an RNA of 125 nucleotide comprising 12 nucleotides of the vector followed by 113 nucleotides of SV40 sequence from −58 to +55. The same plasmid was cleaved 9 nucleotide past the poly(A) site by Bsm I and the two 3′ overhang nucleotides were removed by T4 DNA polymerase. Transcription of this template yields

* To whom correspondence should be addressed

a 77 nucleotide long RNA (−58 to +7 of SV40 along with 12 nucleotides of the vector). The plasmid PSPL3MD containing the adeno virus type 2 L3 poly(A) site was kindly provided by J. Stefano and D. Adams (10). This plasmid contains the L3 (hexon) poly(A) site region of the adeno virus type 2 transcription unit subcloned into pSP65. *In vitro* transcription of the Dra I-linearized plasmid with SP6 polymerase generates a transcript of 188 nucleotide.

5′-capped RNA substrates were prepared by run-off transcription of the linearized plasmids using SP6 polymerase and (α-^{32}P)UTP. Labelled RNA substrates were purified by elution from 4% acrylamide-8 M urea gels prior to use.

Preparation of HeLa cell nuclear extract and its fractionation

HeLa nuclear extracts were prepared essentially by the method of Dignam *et al.* (11) with the following modifications: PMSF was omitted from all the buffers, the crude nuclear pellet was resuspended in 0.7 ml buffer C/ml packed cell volume and the final dialysate was centrifuged for 5 minutes at 5,000 rpm in a microfuge.

The 20−40% fraction of HeLa nuclear extract was prepared according to Takagaki, Ryner and Manley (12). This fraction was then dialyzed in HG_{20}MEDAS [20 mM Hepes (pH 7.9), 20% glycerol, 3 mM $MgCl_2$, 0.3 mM EDTA, 0.5 mM DTT and 50 mM ammonium sulfate] and loaded onto a DEAE-Sepharose column (bed volume of 1 ml/5 mg protein) equilibriated with the same buffer. The flow-through fraction and the fraction eluted with 600 mM salt were precipitated with $(NH_4)_2SO_4$ (60% saturation), resuspended in a minimal volume of buffer D [20 mM Hepes (pH 7.9), 20% glycerol, 100 mM KCl, 0.2 mM EDTA and 0.5 mM DTT] and dialyzed against the same buffer with one change. The dialysate was aliquoted, frozen in liquid N_2 and stored at −70°C until use.

In vitro polyadenylation of pre-mRNA substrates

Polyadenylation reactions were carried out in a final volume of 12.5 or 25 μl containing 5 μl of the nuclear extract (25−30 μg protein), $2-2.5\times10^4$ cpm of ^{32}P−labelled substrates and additional components. The final concentrations of the various components were as follows: 40 mM KCl, 8 mM Hepes (pH 7.9), 0.6 mM $MgCl_2$, 0.08 mM EDTA, 0.5 mM DTT, 20 mM creatine phosphate, 1 mM ATP, 8% glycerol, polyvinyl alcohol (2.5% for SV40L pre-mRNA and 1% for adeno L3 pre-mRNA) and 40 ng/μl tRNA. To detect the cleaved product, polyadenylation was inhibited by including 1 mM 3′dATP in the reaction mixture instead of ATP. To test the effect of Ara-ATP on polyadenylation or cleavage, the reactions were performed in presence of increasing amount of the analog (0.25 mM to 2.5 mM) either in presence of ATP (for polyadenylation) or 3′dATP (for cleavage). The reaction mixture was incubated at 30°C for 60 minutes and terminated by the addition of a buffer containing 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 20 mM NaCl, 0.4% SDS and proteinase K (final concentration 200 μg/ml) to make up the volume to 200 μl followed by incubation at 30°C for 15 minutes. The deproteinized RNA was purified by phenol-chloroform-isoamyl alcohol (25:24:1::V/V/V) extraction and ethanol precipitation. RNA was analyzed by electrophoresis through 12% acrylamide−8M urea gels.

Gel shift analysis of *in vitro* polyadenylation reaction products

Polyadenylation reactions were carried out in presence of ATP or Ara-ATP as described earlier. To study the nature of RNA-protein complexes formed the reactions were stopped by adding heparin (5 mg/ml, final concentration) and the products were analyzed in non-denaturing polyacrylamide gel (4% acrylamide,

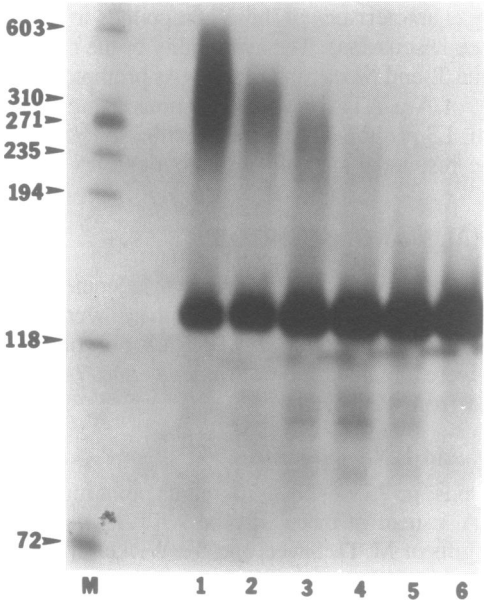

Figure 1. Inhibition of polyadenylation of SV40L pre-mRNA (−58/+70) *in vitro* by Ara-ATP: HeLa nuclear extract (~25μg protein per reaction) was incubated with increasing amounts of Ara-ATP (0, 0.25, 0.50, 1.0, 2.0 and 2.5 mM, in lanes 1−6 respectively) along with ^{32}P labelled pre-mRNA (20,000 cpm per reaction), ATP (1 mM) and other necessary components (see Materials and Methods) for 1 hour at 30°C. The reactions were terminated by the addition of a proteinase K stop buffer, phenol extracted and ethanol precipitated. The products were then separated on urea (8M)-polyacrylamide (12%) gel electrophoresis and subjected to autoradiography. Lane M corresponds to DNA size markers (^{32}P-labelled fragments from Hae III−digestion of bacteriophage ϕX174 DNA).

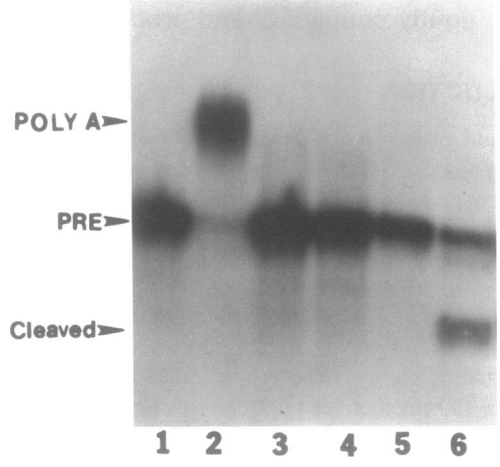

Figure 2. Inhibition of polyadenylation of adeno L3 pre-mRNA by Ara-ATP *in vitro*: HeLa nuclear extract (25mg of protein) was incubated for 1 hour at 30°C with increasing amounts of Ara-ATP (0,1.0,1.5 and 2.0 mM in lanes 2−5 respectively) along with (α-^{32}P)-UTP-labelled adeno L3 pre-mRNA, ATP (1 mM) and other necessary components and the reaction products were analyzed by urea (8M)- polyacrylamide (16%) gel electrophoresis. To detect the cleaved product, 3′dATP was used instead of ATP (lane 6). Lane 1 contains the 188 nucleotides-long pre-mRNA substrate (PRE). 'POLY A' indicates polyadenylated RNA and 'cleaved' indicates 5′ RNA fragment (147 nucleotides-long) generated upon accurate endonucleotylic cleavage at the L3 poly(A) site.

0.05% N, N' methylene bis-acrylamide) at 15 V/cm for 4 hours. Both the gel and running buffer contained 45 mM Tris, 45 mM boric acid and 0.06 mM EDTA, pH 8.3 (13). The gels were autoradiographed overnight at −70°C.

RESULTS AND DISCUSSION

Ara-ATP inhibits polyadenylation of SV40 L and Adeno L3 pre-mRNA substrates

When ^{32}P-labelled SV40L pre-mRNA substrate (−58/+55) was incubated with HeLa nuclear extract in presence of 1 mM ATP and other co-factors, it was efficiently polyadenylated (Fig. 1, lane 1). When Ara-ATP was added to this reaction mixture in increasing amounts, polyadenylation of the substrates was proportionately decreased (Fig. 1, lanes 2−6). Significant inhibition of polyadenylation was observed at 0.25 mM Ara-ATP (Fig. 1, lane 2). Polyadenylation was completely blocked at 2 mM Ara-ATP (Fig. 1, lane 5). Concomitant with inhibition of poly(A) addition, accumulation of unprocessed substrate molecules but not of the cleaved product (70 nucleotides) was observed (Fig. 1, lanes 2−6). Similar result was obtained when adeno L3 pre-mRNA was used as the substrate (Fig. 2). In presence of ATP, adeno L3 pre-mRNA was efficiently polyadenylated (Fig. 2, lane 2) in HeLa nuclear extract. When ATP was replaced by 3′ dATP, the cleaved RNA of 147 nucleotide was accumulated (Fig. 2, lane 6). But neither the cleaved nor the polyadenylated RNA was formed when Ara-ATP was added in increasing amounts to the reaction mixture (Fig. 2, lanes 3−5). Even at a concentration of 1 mM, Ara-ATP completely impaired the 3′-end processing of adeno L3 pre-mRNA (Fig. 2, lane 3). These data demonstrate that Ara-ATP can inhibit polyadenylation of pre-mRNAs and suggest that it may also block the cleavage reaction at the poly(A) site, as no cleaved product was accumulated following inhibition of polyadenylation.

Ara-ATP also inhibits the cleavage reaction

To confirm further the inhibitory effect of Ara-ATP on the cleavage of pre-mRNA, cordycepin triphosphate (3′ dATP) which blocks poly(A) chain elongation without interfering the cleavage reaction was used to detect the cleaved product. When polyadenylation was blocked by 3′dATP, the cleaved product was accumulated (Fig. 3, lane 1). Varying amounts of Ara-ATP were used in the presence of 3′ dATP to study the effect of the ATP analog on the cleavage reaction (Fig. 3, lanes 2−4). Inhibition of the cleavage of SV40L pre-mRNA was evident at 0.5 mM Ara-ATP; complete inhibition was achieved at 2 mM Ara-ATP. The extent of inhibition at concentrations below 2 mM Ara-ATP was not at the same level as that observed for polyadenylation. For example, at 0.5 mM Ara-ATP, polyadenylation was inhibited as much as 85% (Fig. 1, lane 2) whereas at the same concentration, cleavage was inhibited only about 35%. This differential sensitivity of the polyadenylation and cleavage reactions to Ara-ATP indicate that inhibiton of the former reaction is not entirely due to inhibition of cleavage and that the two reactions might be independently blocked by the ATP analog.

Ara-ATP inhibits polyadenylation of 'pre-cleaved' substrate

To confirm further that inhibition of polyadenylation could occur independent of the cleavage, the effect of the ATP analog on the polyadenylation of 'pre-cleaved' SV40L mRNA containing −58 to +7 nucleotides was studied. Polyadenylation of this substrate does not require cleavage, as the downstream sequences

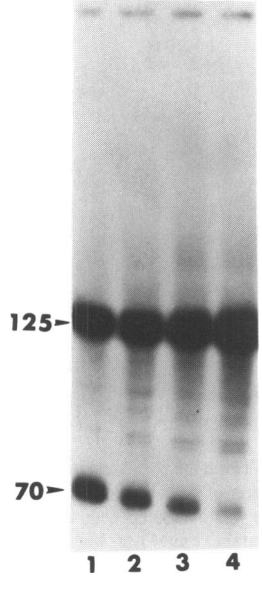

Figure 3. Effect of Ara-ATP on the endonucleolytic cleavage of SV40L pre-mRNA (−58/+70): HeLa nuclear extract (~25μg of protein per reaction) was incubated with ^{32}P-labelled substrate (~20,000 cpm per reaction), 3′ dATP (1 mM) along with other co-factors for 30 minutes at 30°C. The reactions were stopped and the products were analyzed as described in the legend to Figure 1. Lanes 1 to 4 represent reactions with increasing concentrations of Ara-ATP (0, 0.5, 1.0 and 2.0 mM respectively). Accurate endonucleotide cleavage of SV40L pre-mRNA yields 70 nucleotide-long 5′-end product.

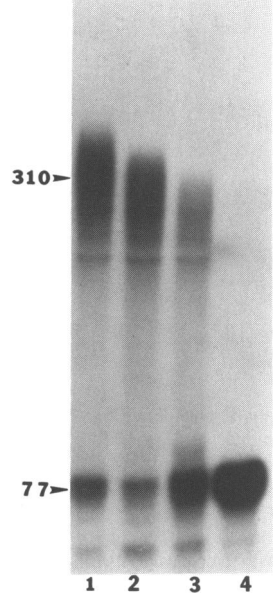

Figure 4. Inhibition of polyadenylation of 'precleaved' (−58/+7) SV40 L pre-mRNA substrate *in vitro* by Ara-ATP: HeLa nuclear extract (~25μg protein per reaction) was incubated with increasing amounts of Ara-ATP (0, 0.5, 1.0 and 2.0 mM in lanes 1−4 respectively) along with ^{32}P-labelled pre-mRNA (~20,000 cpm per reaction), ATP (1 mM) and other necessary components for 1 hour at 30°C. The reactions were stopped and the products were analyzed as described in Figure 1.

necessary for cleavage are absent in this substrate. This 'pre-cleaved' substrate was efficiently polyadenylated in HeLa nuclear extract (Fig. 4, lane 1). Ara-ATP inhibited poly(A) addition in a dose-dependent manner (Fig. 4, lanes 2−4). Complete inhibition of polyadenylation of 'full-length' and 'pre-cleaved' mRNA substrates was achieved at the same (2 mM) concentrations of Ara-ATP (Fig. 1, lane 5 and Fig. 4, lane 4, respectively). Interestingly, this analog can also inhibit Mn^{+2}-dependent polyadenylation of pre-mRNAs (data not shown), which is usually independent of cleavage (14).

Ara-ATP inhibits cleavage and polyadenylation reactions by interacting with poly(A) polymerase

Unlike cordycepin triphosphate (3′ dATP), which selectively inhibits the initial polyadenylation reaction (8), Ara-ATP inhibits both cleavage and polyadenylation. Although ATP is required for both polyadenylation and cleavage, hydrolysis of ATP is not required for cleavage, as nonhydrolyzable analog, e.g., AMP-CH_2-PP can also support cleavage but not polyadenylation (4, 14). Recent studies have shown that poly(A) polymerase is essential for both polyadenylation and cleavage reactions (1, 2, 13, 15). It is conceivable that Ara-ATP blocks the cleavage and polyadenylation reactions by interacting with ATP binding site of poly(A) polymerase. To test this possibility, poly(A) polymerase was separated from the cleavage/specificity factors by DEAE-Sepharose chromatography. Among the different factors necessary for *in vitro* polyadenylation reaction, only poly(A) polymerase elutes in the flow-through fraction of this column (1, 2). This fraction, by itself cannot catalyze the 3′end processing of mRNA (Fig. 5A, lane 5) unless supplemented with specificity and cleavage factors (Fig. 5A, lane 6). By adding the poly(A) polymerase fraction to HeLa nuclear extract, the inhibitory effect of Ara-ATP on polyadenylation was partly overcome (Fig. 5A, lane 3 vs. lane 4). Similarly, the effect of Ara-ATP on cleavage was partially reverted by supplementing with poly(A) polymerase fraction (Fig. 5B, lane 3 vs. lane 4). Poly(A) polymerase fraction alone could not catalyze cleavage reaction (Fig. 5B, lane 5) unless supplemented with cleavage-specificity fraction (Fig. 5B, lane 6). These data indicate that poly(A) polymerase is at least one of the factors mediating inhibitory activity of Ara-ATP.

Ara-ATP inhibits formation of post-cleavage and polyadenylation specific complexes

Studies from several laboratories have shown that the cleavage and polyadenylation of pre-mRNA substrates are mediated by specific RNA-protein complexes (16−19). Immediately after addition of RNA to the nuclear extract, a 'non-specific' complex A migrating much slower than free RNA is formed. This complex is non-specific, as it is formed with any RNA (18). A new complex B or 'pre-cleavage' complex is formed with increase in incubation time. As cleavage proceeds, this slower moving 'pre-cleavage' complex disappears with concomitant appearance

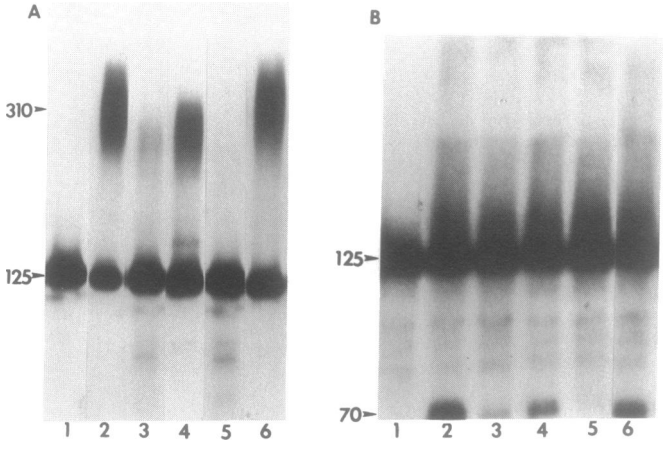

Figure 5. A. Effect of poly(A) polymerase fraction on polyadenylation of SV40L pre-mRNA, partially inhibited by Ara-ATP: HeLa nuclear extract (25 μg of protein) was incubated with ATP (1 mM) (lane 2) or ATP (1 mM) and Ara-ATP (0.5 mM) (lane 3). To the ATP (1 mM) and Ara-ATP (0.5 mM)-containing reaction mixture, poly(A) polymerase (PAP) fraction (7.5 μg of protein) was added (lane 4). The PAP fraction, by itself, could not polyadenylate pre-mRNA (lane 5) unless supplemented with the cleavage specificity fraction (600 mM salt wash of DEAE Sepharose column) (lane 6). **B.** Effect of poly(A) polymerase fraction on endonucleolytic cleavage of 'full-length' (−58/+70) SV40L pre-mRNA partially inhibited by Ara-ATP. HeLa nuclear extracts were incubated with 3′dATP (1 mM), lane 2; 3′dATP (1 mM) and Ara-ATP (0.5mM), lane 3; 3′dATP (1 mM), Ara-ATP (0.5 mM) along with poly(A) polymerase fraction (7.5 μg of protein), lane 4. PAP fraction (Fig. 5B, lane 5) alone could not cleave the pre-mRNMA unless supplemented with the cleavage specificity fraction (Fig. 5B, lane 6). After 30 minutes of incubation at 30°C, the reactions were stopped and the products were analyzed as described in Fig. 1. Lane 1 in each figure shows substrate alone incubated in absence of nuclear extract.

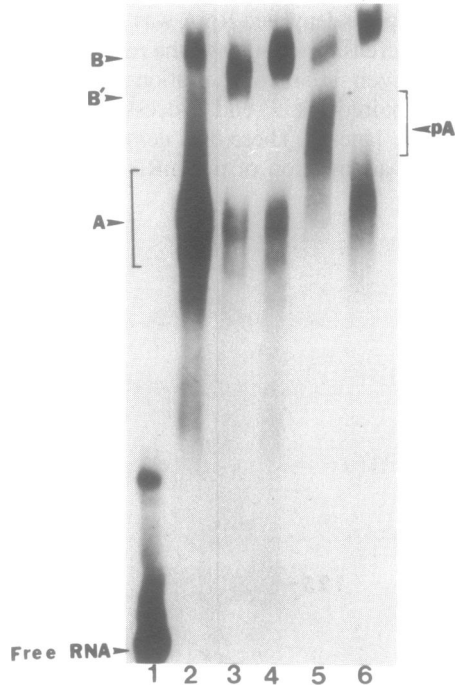

Figure 6. Effect of Ara-ATP on the formation of specific RNA-protein complexes involved in polyadenylation of 'full-length' SV40L pre-mRNA: HeLa nuclear extract (25 μg of protein) was incubated with 1 mM ATP (1 mM) (lanes 3 and 5) or inhibitory concentration (2 mM) of Ara-ATP (lanes 4 and 6) along with other necessary components for 30 minutes to detect 'pre-cleavage' and 'post-cleavage' complexes (lanes 3 and 4) and for 90 minutes to detect 'polyadenylation-specific complex' (lanes 5 and 6). RNA substrate was added to the 0 minute control after heparin addition (lane 2). Lane 1 shows RNA incubated in the absence of HeLa nuclear extract. The reactions were stopped by incubating with heparin (5 mg/ml, final concentration), for 10 minutes on ice and the products were analyzed by 4% native polyacrylamide (acrylamide: N, N' methelene bisacrylamide = 80:1) gel electrophoresis. The positions of distinct complexes indicated on the sides are: A, nonspecific RNA protein complex; B, specific pre-cleavage complex; B', specific post-cleavage complex and pA, polyadenylation-specific complex.

of the faster migrating 'post-cleavage' complex, B' (18). When cleaved RNA is polyadenylated after longer period of incubation, the 'polyadenylation-specific complex' pA, migrating faster than B and B', is formed (19). To investigate whether Ara-ATP inhibits formation of these specific complexes, SV40L 'full-length' pre-mRNA was incubated with HeLa nuclear extract in presence of ATP (1mM) or inhibitory concentration (2mM) of Ara-ATP for different periods of time. At 0 time, the major complex formed was non-specific (A) with a minor amount of pre-cleavage complex (B) (Fig. 6, lane 2). At 30 minutes, both pre-cleavage (B) and post-cleavage (B') complexes were formed in the presence of ATP (Fig. 6, lane 3), whereas only pre-cleavage complex (B) could be detected in the reaction mixture containing Ara-ATP (Fig. 6, lane 4). At 90 minutes, polyadenylation-specific complex (pA) was formed in ATP-containing reaction but not in Ara-ATP-containing reaction (Fig. 6, lane 5 vs. lane 6). Inhibition of specific complex formation by Ara-ATP further proves that this analog is an inhibitor of both cleavage and polyadenylation.

Although previous studies have implicated DNA synthesis as the primary site of action of Ara-A (20, 21), two observations suggest alternate mechanism of action for this compound. First, the inhibitory effects of Ara-A cannot be reverted by deoxyadenosine, but rather by adenosine (22, 23). Second, the adenosine analog can be incorporated into both RNA and DNA (24), which suggests involvement of this compound at some step in RNA synthesis. Further, the relative lack of inhibition of *de novo* RNA synthesis by Ara-A (25) indicates that it affects a post-transcriptional reaction(s) which exclusively utilizes ATP. The present studies have clearly shown that an alternate mechanism of action of Ara-A is via inhibition of cleavage and polyadenylation of pre-mRNAs. Although cordycepin and its nucleotide can inhibit the 3'-end processing reaction *in vivo*, there are a few important differences between this analog and Ara-A and its nucleotide with respect to their mechanisms of action. First, Ara-A or its nucleotide does not inhibit RNA synthesis *in vivo* (25) or *in vitro* (6), whereas cordycepin or its nucleotide could inhibit RNA synthesis *in vivo* (26) or *in vitro* (8) at higher concentrations. Second, cordycepin triphosphate inhibits initial polyadenylation reaction by preventing chain elongation (6). Ara-ATP inhibits the initial polyadenylation *in vitro* by competing with ATP and is not a chain terminator by virtue of the availability of free 3'OH group. Third, cordycepin triphosphate inhibits only the poly(A) addition without affecting the cleavage reaction whereas Ara-ATP inhibits both reactions. In fact, Ara-ATP is the only compound which can inhibit both reactions involved in the 3'-end processing of pre-mRNAs. The present study has not only revealed the exact mechanism of action of Ara-A on the 3'-end processing reaction, but also has provided a useful tool to block cleavage and poly(A) addition.

Although one would like to see restoration of cleavage with purified poly(A) polymerase, extensive purification of this enzyme prevents its ability to function in a reconstitution assay. It appears that such elaborate processing of the enzyme (fractionations on at least 8 different columns) destroys its complete functional integrity probably due to partial degradation of poly(A) polymerase, but retains its non-specific activity in a filter binding assay (K. Chrislip and S. Jacob, unpublished data). Although highly purified calf thymus enzyme has been shown to direct hexamer-dependent polyadenylation of pre-cleaved mRNA (27), such enzyme preparations are unable to direct cleavage-dependent polyadenylation (W. Keller, personal communication). In the absence of a reconstitution assay, the present data at least suggest a direct role of poly(A) polymerase in inhibition of the cleavage reaction by Ara-ATP.

ACKNOWLEDGEMENTS

We thank Drs. M. Wickens and J. Stefano for providing the plasmids used in this study. This work was supported, in part, by a grant (CA 25078) from the National Cancer Institute (to S.T.J.).

REFERENCES

1. Christofori, G. and Keller, W. (1988) *Cell*, **54**, 875–889.
2. Gilmartin, G.M. and Nevins, J. (1990) *Genes and Dev.*, **3**, 2180–2189.
3. Takagaki, Y., Ryner, L.C. and Manley, J.L. (1989) *Genes and Dev.*, **3**, 1711–1724.
4. Moore, C.L. and Sharp, P.A. (1985) *Cell*, **41**, 845–855.
5. Wickens, M. (1990) *Trends Biochem. Sci.*, **15**, 277–281.
6. Rose, K.M. and Jacob, S.T. (1978) *Biochem. Biophys. Res. Commun.*, **81**, 1418–1424.
7. Leonard, T.B. and Jacob, S.T. (1979) *Biochim. Biophys. Acta*, **563**, 150–154.
8. Rose, K.M., Bell, L.E. and Jacob, S.T. (1977) *Nature*, **267**, 178–180.
9. Sheets, M.D., Ogg, S.C. and Wickens, M.P. (1990) *Nucleic Acids Res.*, **18**, 5799–5805.
10. Stefano, J.E. and Adams, D.E. (1988) *Mol. Cell. Biol.*, **8**, 2052–2062.
11. Dignam, J.D., Leboritz, R.M. and Roeder, R.G. (1983) *Nucleic Acids Res.*, **11**, 1475–1488.
12. Takagaki, Y., Ryner, L.C. and Manley, J.L. (1988) *Cell*, **52**, 731–742.
13. Terns, M.P. and Jacob, S.T. (1989) *Mol. Cell. Biol.*, **9**, 1435–1444.
14. Manley, J.L. (1988) *Biochim. Biophys. Acta*, **950**, 1–12.
15. Christofori, G. and Keller, W. (1989) *Mol. Cell. Biol.*, **9**, 193–203.
16. Zhang, F. and Cole. C.N. (1987) *Mol. Cell. Biol.*, **7**, 3277–3286.
17. Skolnik-David, H., Moore, C. and Sharp, P.A. (1987) *Genes and Dev.*, **1**, 672–682.
18. Zarkower, D. and Wickens, M. (1987) *EMBO J.*, **6**, 4185–4192.
19. Bardwell, V.J. and Wickens, M. (1990) *Mol. Cell. Biol.*, **10**, 295–302.
20. Muller, W.E.G., Rolide, H.J., Beyer, R., Maidhoff, A., Lackmann, M. and Taschner, H. (1975) *Cancer Res.*, **35**, 2160–2168.
21. Dicioccio, R.A. and Srivastava, B.I.S. (1977) *Eur. J. Biochem.*, **79**, 411–418.
22. Nichols, W.W. (1964) *Cancer Res.*, **24**, 1502–1504.
23. Brink, J.J. and LePage, G.A. (1965) *Eur. J. Biochem.*, **43**, 1–15.
24. Plunkett, W. and Cohen, S.S. (1975) *Cancer Res.*, **35**, 415–422.
25. Cohen, S.S. (1966) *Prog. Nucl. Acid Res. Mol. Biol.*, **5**, 1–88.
26. Siev, M., Weinberg, R. and Penman, S. (1969) *J. Cell. Biol.*, **41**, 510–519.
27. Wahle, E. (1991) *J. Biol. Chem.*, **266**, 3131–3139.