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Poliovirus-Encoded Protease $2A^{Pro}$ Cleaves the TATA-Binding Protein but Does Not Inhibit Host Cell RNA Polymerase II Transcription In Vitro

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Transient expression of the poliovirus-encoded protease $2A^{Pro}$ in eukaryotic cells results in inhibition of both cellular transcription and translation. The inhibition of transcription observed in cells expressing $2A^{Pro}$ could be due to a primary effect or secondary effect caused by inhibition of translation. Because transcriptional activity of the TATA-binding protein (TBP) is drastically reduced in poliovirus-infected cells, we determined if $2A^{Pro}$ is able to cleave TBP in vitro. We demonstrate here that $2A^{Pro}$ directly cleaves the single tyrosine-glycine bond at position 34 of TBP. This cleavage is also seen in poliovirus-infected HeLa cells. Surprisingly, despite TBP cleavage $2A^{Pro}$ was unable to inhibit RNA polymerase II transcription in vitro. Under similar conditions, however, $2A^{Pro}$ inhibited translation of a capped cellular mRNA in vitro. Thus, cleavage of TBP at position 34 does not alter its transcriptional activity in vitro. These results suggest that inhibition of host cell RNA polymerase II transcription seen in cells transiently transfected with $2A^{Pro}$ is due to host cell translational shutoff.

Poliovirus is the prototype member of the group *Picornaviridae*. The single-stranded, plus polarity RNA genome of poliovirus is translated as a large polyprotein in infected mammalian cells which is then proteolytically processed to produce all viral structural and nonstructural proteins (15, 35). The poliovirus RNA genome encodes three proteases, $3C^{Pro}$, $2A^{Pro}$, and $3CD^{Pro}$, which participate in polyprotein processing. $3C^{Pro}$ and $3CD^{Pro}$ cleave only at glutamine-glycine sites within the polyprotein, while $2A^{Pro}$ cleaves only tyrosine-glycine bonds.

Poliovirus shuts off both host cell transcription and translation. Genetic evidence suggests that $2A^{Pro}$ is involved in host cell translational shutoff (1, 32). This inhibition is thought to be mediated by the cleavage of p220, a component of cap-binding initiation factor eIF-4F (10, 14). However, recent studies showed that cap-dependent translation could still occur in the presence of cleaved p220 (24). It is believed that poliovirus $2A^{Pro}$ does not directly cleave p220, rather it activates a quiescent cellular protease which then cleaves p220 (14, 19, 37, 38). In contrast, rhinovirus or coxsackie virus $2A^{Pro}$ is capable of cleaving p220 directly (16, 18, 31). No cellular protein has been shown to be directly cleaved by poliovirus $2A^{Pro}$ to date. Recent studies suggest $2A^{Pro}$ may play a role in viral replication (22), although an earlier report suggested $2A^{Pro}$ is not required in a minireplicon system (23). $2A^{Pro}$ may also be involved in enhancing translation of mRNAs containing the poliovirus 5′ untranslated region (12).

Studies from our laboratory have shown that poliovirus-encoded protease $3C^{Pro}$ directly inhibits host cell transcription catalyzed by RNA polymerase II (Pol II) and III both in vivo and in vitro (2, 3, 28, 39). The TATA-binding protein (TBP), a component of transcription factor TFIID, is directly cleaved by $3C^{Pro}$, which leads to Pol II transcription shutoff (2). The cleaved TBP is unable to form a complex with the TATA box (38). Likewise, the largest subunit of the Pol III DNA-binding transcription factor TFIIIC is cleaved by $3C^{Pro}$, leading to shut off of Pol III transcription (28). Apparently, cleavage of TBP does not contribute to Pol III transcription shutoff, although the multicomplex Pol III transcription factor TFIIIB contains the TBP polypeptide. $3C^{Pro}$ also catalyzes shut off of Pol I transcription (26, 27). However, the precise nature of the Pol I transcription factor(s) inactivated by $3C^{Pro}$ remains unknown.

Davies et al. demonstrated that transient expression of $2A^{Pro}$ in eukaryotic cells inhibited both cellular translation and transcription (8). The inhibition of transcription seen in cells expressing $2A^{Pro}$ could be due to a primary effect or a secondary effect caused by inhibition of translation. Because TBP contains a cleavable tyrosine-glycine bond, we determined if $2A^{Pro}$ is able to cleave TBP in vitro. We demonstrate here that $2A^{Pro}$ directly cleaves the single tyrosine-glycine bond (at position 34) of TBP. This cleavage is also seen in virus-infected cells. Surprisingly, despite TBP cleavage, $2A^{Pro}$ was unable to inhibit RNA Pol II transcription in vitro. Thus, cleavage of TBP at amino acid 34 does not alter its transcriptional activity in vitro. Under similar conditions, $3C^{Pro}$ totally inhibited Pol II transcription. Thus, inhibition of host cell RNA Pol II transcription seen in cells expressing $2A^{Pro}$ may be due to host cell translational shutoff by $2A^{Pro}$.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were grown in spinner culture containing minimum essential medium, 5% newborn calf serum, and 1 g of glucose and 10^5 U of ampicillin per liter of cells. Cells were infected with poliovirus (Mahoney type 1) at a multiplicity of infection of 25 as previously described (7).

Extract preparation. Nuclear extracts were made from mock- and poliovirus-infected cells as described earlier (9) with slight modifications. Instead of dialysis against buffer D, the extracts were precipitated with ammonium sulfate at 50% saturation. These extracts were centrifuged at 30,000 × *g* for 30 min. The precipitate was dissolved in TMO.1 buffer (50 mM Tris-HCl [pH 7.4], 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 12.5 mM $MgCl_2$, 100 mM KCl) and dialyzed against the same buffer overnight. These extracts were aliquoted and stored at −70°C.

Protein purification. Poliovirus protease $2A^{Pro}$ was subcloned into the pet 15b vector (Novagen) and transformed into *Escherichia coli* BL21 (DE3) cells. The deleted $\Delta 2A^{Pro}$ was generated by cleaving $2A^{Pro}$ at the *Nde*I site. This removes the C-terminal 150 nucleotides which include part of the protease active site. $\Delta 2A^{Pro}$ was also cloned into the pet 15b vector and transformed into BL21 (DE3) cells. 2A BL21 (DE3) or Δ2A BL21 (DE3) cultures at an optical density of 0.7 at 600 nm were induced with 1 mM isopropyl thiogalactoside for 4 h to make $2A^{Pro}$. $2A^{Pro}$ and $\Delta 2A^{Pro}$ were purified from *E. coli* lysates under dena-

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Sequence of human TBP

12 18 34
MDDQNNSLPPYAQGLASPQGAMTPGIPIGSPMMPYGTGLTPQPIQNTNSLSILEEQQR

QQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQAVAAAAVQQSTSQQ

108
AQGGTSGQAPQLFHSQTLTTAPLPGTTPLYPSPMTPMTPITPATPASESSGIVPQLQNI

VSTVNLGCKLDLKTIIALRRRNAEYNPPKRRFFAAVIIMRIREPRTTALIIFFSSGKMVCTG

AKSEEQSRRLAARKYARVVQKLGFPAKFLDFKIQNMVGSSCDVKFPIRLEGLVLTHQQ

FSSYEPELFFPGLIYYRMIRRRIVLLIIFVSGKVVLTGAKVRAEIYEAFENIYPILKGFRKTTZ

FIG. 1. Sequence and potential poliovirus protease cleavage sites in TBP. Predicted amino acid sequence of human TBP is shown. Three glutamine-glycine (QG) sites are boxed, and a tyrosine-glycine (YG) site is circled.

turing conditions by nitriloacetic acid-nickel chromatography. These proteins were renatured for 2 days with gradual dilutions of 4, 2, and 0 M urea containing TMO.1 buffer.

Protease reactions and Western immunoblot analysis. The indicated amounts of $2A^{Pro}$ or $\Delta 2A^{Pro}$ were directly added to in vitro-translated [^{35}S]methionine-labeled TBP, purified TBP, or mock-infected nuclear extracts and incubated at 30°C for 3 h. The reaction was stopped by adding an equal volume of 2× sodium dodecyl sulfate (SDS) gel loading buffer (100 mM Tris [pH 6.8], 200 mM dithiothreitol, 20% glycerol, 4% SDS, 0.002% bromophenol blue) and heating at 95°C for 5 min. These reactions were then separated on an SDS–14% polyacrylamide gel. For Western blotting, the proteins were electrophoretically transferred onto a nitrocellulose membrane (Schleicher and Schuell) and blotted with 10% blotting reagent (Bio-Rad). A rabbit polyclonal TBP antibody (Santa Cruz Biotech, Inc.) was used as the primary antibody. Western blots were developed by using a chemiluminescence detection system (Bio-Rad).

Transcription plasmids and in vitro transcription assays. The plasmids used for the analysis of transcription were a generous gift from Steven Smale, University of California, Los Angeles. These plasmids were identical to those described previously (29, 30). Plasmid 1634 (pl 1634) contains the terminal deoxytransferase initiator (Inr) sequence inserted into pSP72 and simian virus 40 21-bp repeats (Sp1 binding sites). Plasmid 126 (pl 126) contains the adenovirus major late promoter TATA box and Sp1 binding sites inserted into the *Bgl*II site of pSP72. Both plasmids contain 12 Sp1 binding sites.

Transcription reaction mixtures contained 450 μg of template DNA, 150 μg of HeLa nuclear extracts, 2.5 mM ribonucleoside triphosphates, and 100 U of RNAsin, and the reaction volume was made up to 50 μl with TMO.1 buffer. These reaction mixtures were incubated at 30°C for 90 min, and the reactions were terminated by the addition of 90 μl of stop solution (200 mM NaCl, 20 mM EDTA, 1% SDS, 250 μg of tRNA per ml). The RNA was extracted with phenol-chloroform followed by ethanol precipitation. The RNA was annealed with 50,000 cpm of Sp6 primer in 10 μl of annealing buffer (250 mM NaCl, 5 mM Tris [pH 7.4], 1 mM EDTA) for 90 min. This annealed mix was analyzed by the primer extension method. The primer extension reaction mixture contained 10 μl of the above annealed mix, 1 mM dithiothreitol reverse transcriptase buffer (50 mM Tris [pH 8.3], 8 mM $MgCl_2$, 50 mM KCl, 1.5 mM [each] deoxynucleoside triphosphate), and 40 U of avian myeloblastosis virus reverse transcriptase (Life Science Inc.) in a 20-μl reaction volume. These reaction mixtures were incubated at 40°C for 90 min. The reactions were stopped by addition of 15 μl of stop dye (80% formamide, 0.01% xylene cyanol, 0.01% bromophenol blue), loaded onto an 8% acrylamide–8 M urea gel, and subjected to electrophoresis.

In vitro translation assay. HeLa translation extracts were prepared as previously described (6). The extracts were incubated with 10 μl of $2A^{Pro}$ or $\Delta 2A^{Pro}$ for 3 h at 30°C. These extracts were used for translating p53 mRNA as previously described (6). The protein products generated were separated on an SDS–14% polyacrylamide gel electrophoresis (PAGE) gel.

Site-directed mutagenesis of TBP. The parental TBP plasmid clone pKB104 used was as previously described (5). TBP insert from pKB104 was cloned into vector M13mP18 to give pSDTBP (5). The single-amino-acid TBP mutant with the 34th tyrosine mutated to alanine was generated by the protocol established by Amersham. A mutagenic oligonucleotide with the nucleotides corresponding to tyrosine changed to nucleotides corresponding to alanine was annealed to the single-stranded template DNA. The annealed oligonucleotide was extended and ligated with Klenow polymerase and T4 DNA ligase to form the heteroduplex. Deoxythiocytidine triphosphate was used during the extension of the mutant strand which protects it from nicking with the enzyme Nci. The heteroduplex was then treated with Nci to nick the parental strand, which was removed by limited digestion with exonuclease III. The mutant strand was used as a template to form the homoduplex mutant by a repolymerization reaction. The mutant recombinants were then used to transform *E. coli* XL-1 Blue cells.

RESULTS

$2A^{Pro}$ cleaves TBP. The poliovirus-specific $2A^{Pro}$ specifically cleaves tyrosine-glycine bonds in the viral polyprotein (17, 34). To determine whether the single tyrosine-glycine in TBP (Fig. 1) is cleaved by $2A^{Pro}$, in vitro-translated TBP was incubated with purified $2A^{Pro}$. Both the wild-type $2A^{Pro}$ and an inactive deletion mutant ($\Delta 2A^{Pro}$) having no protease activity were expressed in *E. coli* and purified to near homogeneity by using Ni-affinity chromatography. The activity of the purified protease was determined by measuring its ability to cleave a viral precursor protein (data not shown). At the lowest concentration tested, purified $2A^{Pro}$ did not cleave in vitro-translated, [^{35}S]methionine-labeled TBP (Fig. 2, lane 2). However, as the concentration of $2A^{Pro}$ was increased in the reaction, the intensity of full-length TBP decreased with a concomitant increase in a faster-migrating band (Fig. 2). At the highest concentration tested, almost all of the full-length TBP was converted to the faster-migrating product (lane 4). The enzymatically inactive mutant $2A^{Pro}$ was unable to cleave TBP (lane 5).

Cleavage of TBP by $2A^{Pro}$ is direct. Poliovirus $2A^{Pro}$ is known to cleave the p220 component of the cap-binding complex (37, 38). This cleavage, however, does not appear to be direct and is believed to be mediated by a cellular protease following its activation by the viral $2A^{Pro}$. To determine if TBP is cleaved directly by $2A^{Pro}$, both TBP and $2A^{Pro}$ were purified from *E. coli* expressing these proteins. Analysis of these proteins by SDS-PAGE followed by Coomassie blue staining showed a high degree of purity for both proteins (Fig. 3A). The purified TBP was incubated with the purified $2A^{Pro}$, and the products were separated on an SDS–20% PAGE gel and analyzed by Western blotting using a polyclonal anti-TBP anti-

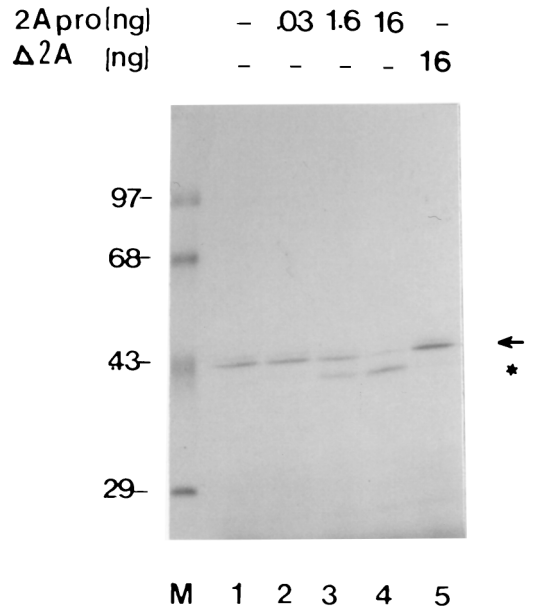

FIG. 2. Cleavage of in vitro-translated TBP with poliovirus protease $2A^{Pro}$. Human TBP cloned into pGEM3 (Promega) was in vitro transcribed with T7 RNA polymerase and translated in vitro in rabbit reticulocyte lysate in the presence of [^{35}S]methionine. One microliter of labeled TBP translation mixture was incubated with the indicated amounts of $2A^{Pro}$ or $\Delta 2A^{Pro}$ ($\Delta 2A$) at 30°C for 4 h. The reaction was stopped by adding equal amounts of 2× SDS-PAGE buffer, and the proteins were separated on an SDS–14% PAGE gel. ^{14}C-labeled molecular weight markers are shown in lane M. Numbers at left are in kilodaltons. The position of full-length TBP is indicated by an arrow and that of the cleaved product is indicated by an asterisk.

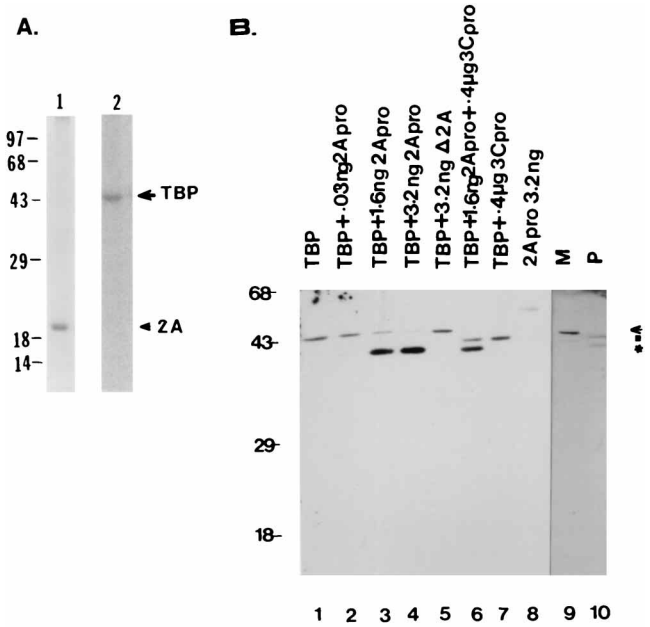

FIG. 3. Western blot analysis of recombinant purified TBP treated with recombinant purified 2A^{Pro} and 3C^{Pro}. (A) One microgram each of recombinant purified 2A^{Pro} (lane 1) and TBP (lane 2) was analyzed by SDS-PAGE followed by Coomassie blue staining. (B) Bacterially expressed and purified TBP (lane 1) was incubated with increasing amounts of 2A^{Pro} (lanes 2 to 4) or with Δ2A^{Pro} (lane 5), 3C^{Pro} (lane 7), or both 2A^{Pro} and 3C^{Pro} (lane 6). 2A^{Pro} alone was also run alongside as a control (lane 8). Mock- and poliovirus-infected HeLa cell extracts (lanes 9 and 10, respectively) were also analyzed. The position of full-length TBP is indicated by an arrow. The positions of 3C^{Pro}- and 2A^{Pro}-cleaved TBP are indicated by a square and an asterisk, respectively. Numbers at the left of both panels are molecular weights in kilodaltons.

body. Again, cleavage of TBP by 2A^{Pro} was apparent in reactions containing various amounts of 2A^{Pro} in the presence of a constant amount of TBP (Fig. 3B, lanes 1 to 4). The cleaved product (38 kDa) was not observed when wild-type 2A^{Pro} was replaced by the inactive, mutant 2A^{Pro} (Fig. 3B, lane 5). Also, no background bands at 43 and 38 kDa were observed when the reaction contained 2A^{Pro} alone (Fig. 3B, lane 8). Incubation of TBP with purified 3C^{Pro} generated a 41-kDa cleaved product as previously observed (2) (Fig. 3B, lane 7). When TBP was incubated with both 2A^{Pro} and 3C^{Pro}, two cleaved products migrating at 41 and 38 kDa were observed, as expected (lane 6). Both the 41- and 38-kDa bands were detected when an extract from poliovirus-infected HeLa cells was analyzed by Western blotting (Fig. 3B, lane 10) compared to mock-infected cell extract (lane 9). The results presented in Fig. 3 suggest that (i) cleavage of TBP by 2A^{Pro} is most probably direct, and (ii) the TBP cleavage products seen in infected cells can be recapitulated by incubating purified TBP with poliovirus-specific proteases 3C^{Pro} and 2A^{Pro}. Thus, it appears that both viral proteases directly cleave TBP in infected cells (2) (Fig. 3).

2A^{Pro} cleaves the tyrosine-glycine bond of TBP at position 34. Both poliovirus 3C^{Pro} and 2A^{Pro} are remarkably specific in cleaving the viral polyprotein to generate mature viral polypeptides. While 3C^{Pro} cleaves at glutamine-glycine pairs, 2A^{Pro} cleaves the viral precursor polypeptide at tyrosine-glycine bonds. Examination of the amino acid sequence of TBP revealed only one tyrosine-glycine pair, which was located at position 34 (Fig. 1). To determine whether 2A^{Pro} cleaves this tyrosine-glycine bond, the tyrosine at position 34 was changed to an alanine moiety by site-directed mutagenesis. Both the cloned wild-type (Y-G) and mutant (A-G) polypeptides were translated in rabbit reticulocyte lysate, and the labeled proteins were incubated with purified 2A^{Pro}. As can be seen in Fig. 4, substituting the tyrosine at position 34 by alanine rendered the mutant TBP almost totally resistant to cleavage by 2A^{Pro} compared to the wild-type TBP which was readily cleaved by 2A^{Pro} (Fig. 4, lanes 1 to 4).

2A^{Pro} does not inhibit RNA Pol II-mediated transcription in vitro. To determine whether 2A^{Pro} is able to inhibit host cell transcription, TATA- and Inr-mediated, Sp1-activated Pol II transcription was examined in the presence and absence of purified, proteolytically active 2A^{Pro}. Nuclear extracts prepared from mock-infected (uninfected) HeLa cells were used for transcription from a plasmid containing the adenovirus major late promoter TATA box and Sp1 binding sites. The RNA transcripts synthesized were analyzed by primer extension (39). Figure 5A indicates the position of the correctly initiated 70-bp transcript. Synthesis of the transcript, as measured by primer extension, was not at all inhibited by prior incubation of the extract with various concentrations of 2A^{Pro} (Fig. 5A). In fact, stimulation of transcription was observed consistently as the amount of 2A^{Pro} was increased in the reaction. Quantitation of the primer-extended product showed an approximately fourfold stimulation over that of the control (Fig. 5A, lanes 1 and 4). When aliquots of the same nuclear extract treated with various contractions of 2A^{Pro} were analyzed by Western blotting, cleavage of TBP was clearly ob-

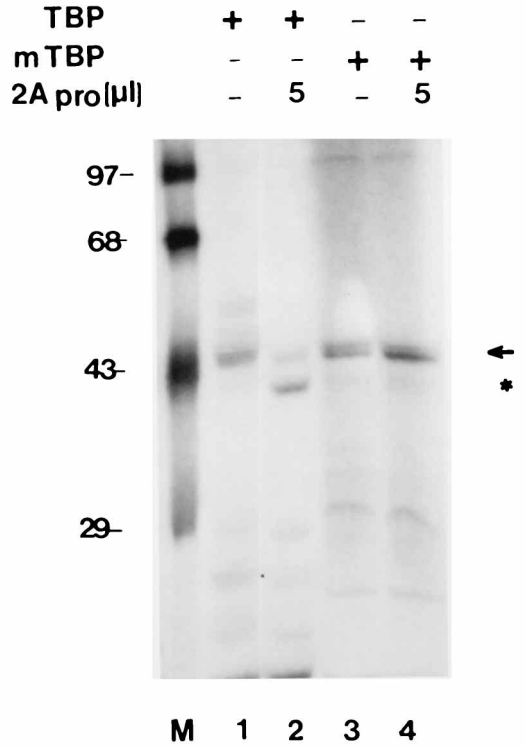

FIG. 4. Identification of 2A^{Pro} cleavage site in human TBP. The lone tyrosine-glycine site in TBP was mutated to an alanine-glycine site by site-directed mutagenesis. Cloned wild-type TBP and mutant TBP (mTBP) were translated in rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Wild-type TBP and mTBP were incubated with 2A^{Pro} (lanes 2 and 4) or buffer alone (lanes 1 and 3). The products were separated on an SDS–14% PAGE gel and visualized by autoradiography. ^{14}C-labeled marker proteins are shown in lane M. Numbers at left are molecular weights in kilodaltons. The positions of full-length TBP and the cleaved product are indicated by an arrow and an asterisk, respectively.

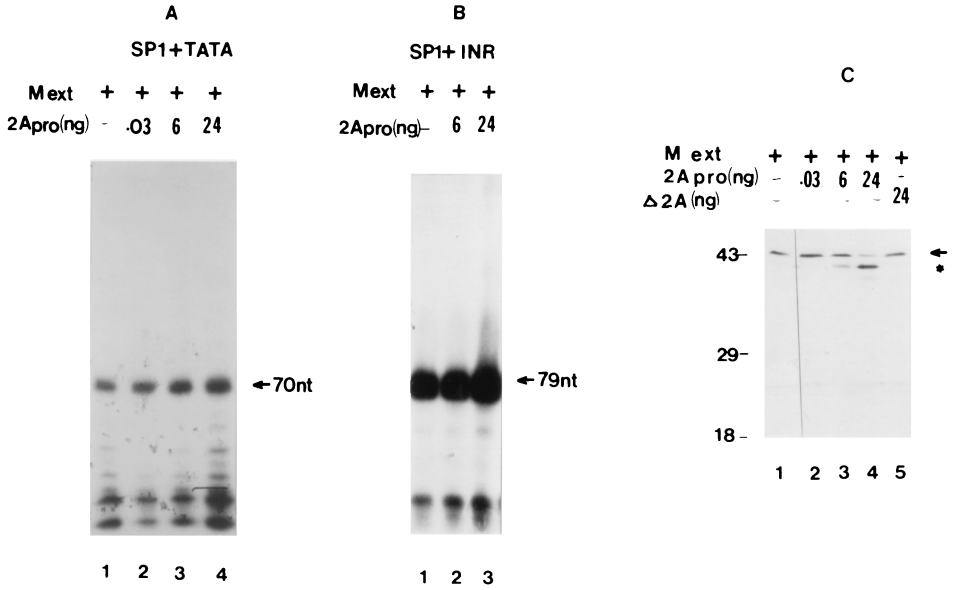

FIG. 5. In vitro transcriptional analysis of nuclear extracts treated with $2A^{Pro}$. Nuclear extracts were treated with increasing amounts of $2A^{Pro}$ for 3 h at 30°C before in vitro transcription analysis. (A) Analysis of transcription from TATA promoter activated by Sp1. Nuclear extracts treated with $2A^{Pro}$ (lanes 2 to 4) were used for in vitro transcription reactions with pl 126. This plasmid has an adenovirus major late promoter TATA box and (Sp1 binding sites). The RNA transcript synthesized was analyzed by primer extension analysis, and the position of the correctly initiated transcript (70 bp) is indicated. (B) Effect of $2A^{Pro}$ on Inr-mediated transcription. Nuclear extracts treated with $2A^{Pro}$ (lanes 2 and 3) were used for in vitro transcription reactions with pl 1634. This plasmid contains the terminal deoxytransferase Inr sequence and Sp1 binding sites. The RNA transcript synthesized was examined by primer extension analysis, and the position of the correctly initiated transcript (79 bp) is indicated. (C) Aliquots of the same nuclear extracts shown in panels A and B were treated with $2A^{Pro}$ or $\Delta 2A^{Pro}$ and analyzed by Western blotting using an anti-TBP antibody. The positions of full-length TBP and cleaved product are indicated by an arrow and an asterisk, respectively. Numbers on the left are molecular weights in kilodaltons. Mext, mock-infected extracts.

served (Fig. 5C, lanes 2 to 4). Thus, although almost all TBP was cleaved at the highest concentration of $2A^{Pro}$, no transcription inhibition was apparent at the same concentration of the protease (compare Fig. 5A, lane 4, with Fig. 5C, lane 4). The precise reason for the stimulation of Pol II transcription in the presence of purified $2A^{Pro}$ is not known and could be due to proteolytic degradation of one or more nonspecific inhibitors of transcription by the protease. A very similar result was obtained when Inr-mediated transcription was examined (Fig. 5B). Again, $2A^{Pro}$ did not inhibit transcription, and a slight stimulation of the intensity of the product was observed at a higher concentration of $2A^{Pro}$ (Fig. 5B, lane 3).

Figure 6A compares the effect of poliovirus infection on host cell transcription with that of $3C^{Pro}$ or $2A^{Pro}$ treatment on transcription. When extracts prepared from poliovirus-infected cells were used for transcription, very little transcription was observed (Fig. 6A, lane 5) compared to that seen in mock-infected cell extracts (lane 1). Transcription in infected extracts was approximately fivefold lower than that observed in mock-infected extracts (Fig. 6A, compare lanes 1 and 5). A similar degree of inhibition of transcription was observed when the mock-infected extracts were treated with $3C^{Pro}$ (lane 4). No significant inhibition of transcription was observed when mock-infected extracts were treated with $2A^{Pro}$ (lane 2). Inhibition of transcription seen in reactions containing both $3C^{Pro}$ and $2A^{Pro}$ (Fig. 6A, lanes 3) was unaltered compared to that containing $3C^{Pro}$ alone (lane 4). These results suggest that host cell transcription shutoff seen in poliovirus-infected cells can be recapitulated in vitro by incubation of cell extracts with $3C^{Pro}$ but not with $2A^{Pro}$.

As a positive control, the effect of $2A^{Pro}$ on capped mRNA translation in HeLa cell extracts was determined. $2A^{Pro}$ is known to inhibit capped, cellular mRNA translation in vitro. Only half of the amount of $2A^{Pro}$ that was used in transcription reactions (Fig. 6A) was sufficient to almost totally inhibit (90%) in vitro translation of a capped mRNA in HeLa cell extracts (Fig. 6B, lanes 1 and 2). This inhibition of translation was specific to $2A^{Pro}$ as the inactive mutant $2A^{Pro}$ had very

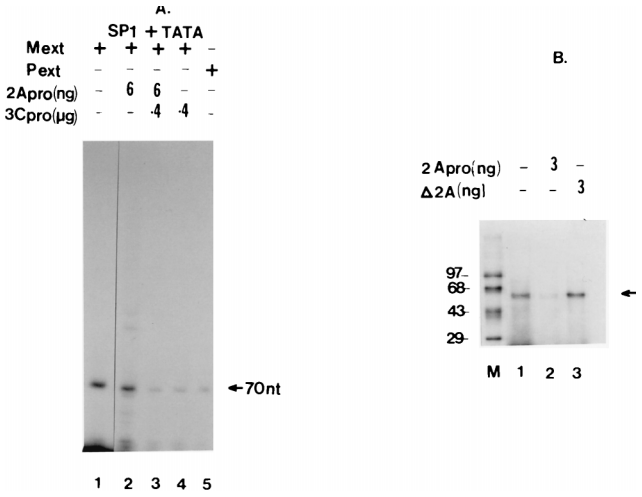

FIG. 6. Effect of $2A^{Pro}$ and $3C^{Pro}$ on cellular translation and transcription in vitro. (A) In vitro transcription was measured from TATA promoter activated by SP1 in mock-infected (Mext, lane 1) and poliovirus-infected (Pext, lane 5) extracts and in mock-infected extracts treated with indicated amounts of $2A^{Pro}$ (lane 2), $3C^{Pro}$ (lane 4), and $2A^{Pro}$ and $3C^{Pro}$ together (lane 3). (B) In vitro translation of a capped cellular mRNA (p53 mRNA) was examined in HeLa cell extract in the absence (lane 1) and presence of the wild type (lane 2) or the enzymatically inactive mutant protease (lane 3). Molecular weight marker proteins are shown in lane M, and numbers at left are molecular weights in kilodaltons.

little effect (2% inhibition) on translation. These results demonstrate that 2A^{Pro} does not inhibit RNA Pol II-mediated transcription in vitro, although it is capable of inhibiting capped mRNA translation under similar conditions.

DISCUSSION

We have shown here that the poliovirus-encoded protease 2A^{Pro} catalyzes cleavage of TBP both in vitro and in HeLa cells infected with poliovirus. The cleavage of TBP by 2A^{Pro} appears to be direct as incubation of purified TBP with purified 2A^{Pro} results in the appearance of the cleaved product (Fig. 3). That a mutant 2A^{Pro} which lacks protease activity is not able to cleave TBP suggests that cleavage of TBP is not due to a contaminating *E. coli* protease. We have also demonstrated that 2A^{Pro}-mediated cleavage of transcription factor TBP occurs between the tyrosine and glycine moieties at position 34. Thus, the specificity of 2A^{Pro} cleavage appears to be identical for both viral precursor proteins and a cellular protein. This is the first example of a cellular protein being cleaved directly by the viral protease 2A^{Pro}. 2A^{Pro}-induced cleavage of the p220 component of eukaryotic initiation factor 4F (eIF-4F) is believed to be mediated by a cellular protease which is activated in the presence of 2A^{Pro} and eukaryotic initiation factor 3 (eIF-3) (37, 38).

The experiments presented here were initiated because of a previous report which showed that transient expression of 2A^{Pro} in COS-1 monkey kidney cells resulted in significant inhibition of both cellular translation and transcription (8). The reduction in transcription seen in 2A^{Pro}-expressing cells could result from direct inhibition of transcription or could be due to inhibition of translation. For example, inhibition of translation of an essential transcription factor with a relatively short half-life would result in inhibition of transcription. Whether cellular translation or transcription is directly affected by 2A^{Pro} can only be addressed by using in vitro translation and transcription systems. Previous results from our laboratory have shown that the poliovirus protease 3C^{Pro} was able to bring about host cell transcription shutoff both in vivo and in vitro in the absence of other viral proteins (2, 3, 39). Results presented in Fig. 6 clearly show that while 2A^{Pro} can inhibit in vitro translation of a capped mRNA, it was totally ineffective in inhibiting cellular transcription by RNA Pol II. This was surprising since 2A^{Pro} cleaved TBP at the tyrosine-glycine bond at position 34 under conditions used for transcription. We, therefore, conclude that removal of the N-terminal 34 amino acids from TBP does not interfere with its transcriptional activity. Indeed, TBP deletion studies have shown that the N-terminal amino acid sequences may not be necessary for TBP transcriptional activity (25, 40). We have recently shown that 3C^{Pro} cleaves TBP at both the 18th and 108th glutamine-glycine sites (references 5 and 39 and unpublished results). It is the 3C^{Pro}-mediated cleavage at the 108th glutamine-glycine bond of TBP which leads to inhibition of transcription (39a).

Why is the tyrosine-glycine bond at position 34 of TBP cleaved by 2A^{Pro}? Clearly, this cleavage does not appear to be necessary for inhibition of host cell Pol II transcription in vitro. There are two possible explanations. The tyrosine-glycine bond at position 34 may be exposed and is fortuitously cleaved by 2A^{Pro}. An alternative hypothesis is that this cleavage plays an important role in the shut off of host cell transcription by RNA Pol I and/or III. Both the Pol III transcription factor TFIIIB and the Pol I transcription factor SL1 contain TBP as one of the components in these multiprotein complexes (4, 13, 20, 33, 36). Previous results from our laboratory have shown that transcription factor fractions containing TFIIIB and SL1 had reduced transcriptional activity when isolated from poliovirus-infected cells compared to those from mock-infected cells (11, 26).

The function of the N-terminal amino acids of TBP is not clear at present. The N-terminal region of TBP does not appear to be needed for Pol II transcription. However, recent studies indicate that the N-terminal region of TBP plays an important role in small nuclear RNA transcription (21). It would be interesting to examine whether 2A^{Pro} is able to inhibit small nuclear RNA transcription in vitro.

Although the poliovirus-encoded protease 2A^{Pro} cleaves TBP both in vitro and in vivo, this cleavage does not appear to bring about host cell RNA Pol II transcription shutoff in vitro. The possibility that 2A^{Pro} inhibits Pol II transcription directly in vivo cannot be completely ruled out. It appears unlikely, however, for the following reasons. Inhibition of host cell translation by 2A^{Pro} has been documented both in vivo and in vitro (15). Likewise, host cell transcription shutoff by 3C^{Pro} has been demonstrated both in vitro and in vivo (2, 3, 11, 39). We, therefore, suggest that the inhibition of host cell transcription observed in cells expressing 2A^{Pro} is due to inhibition of cellular translation.

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