Direct Cleavage of Human TATA-Binding Protein by Poliovirus Protease 3C In Vivo and In Vitro

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Host cell RNA polymerase II (Pol II)-mediated transcription is inhibited by poliovirus infection. This inhibition is correlated to a specific decrease in the activity of a chromatographic fraction which contains the transcription factor TFIID. To investigate the mechanism by which poliovirus infection results in a decrease of TFIID activity, we have analyzed a component of TFIID, the TATA-binding protein (TBP). Using Western immunoblot analysis, we show that TBP is cleaved in poliovirus-infected cells at the same time postinfection as when Pol II transcription is inhibited. Further, we show that one of the cleaved forms of TBP can be reproduced in vitro by incubating TBP with cloned, purified poliovirus encoded protease 3C. Protease 3C is a poliovirus-encoded protease that specifically cleaves glutamine-glycine bonds in the viral polyprotein. The cleavage of TBP by protease 3C occurs directly. Finally, incubation of an uninfected cell-derived TBP-containing fraction (TFIID) with protease 3C results in significant inhibition of Pol II-mediated transcription in vitro. These results demonstrate that a cellular transcription factor can be directly cleaved both in vitro and in vivo by a viral protease and suggest a role of the poliovirus proteinase 3C in host cell Pol II-mediated transcription shutoff.

Infection of HeLa cells with poliovirus leads to dramatic inhibition of all three classes of host cell RNA synthesis (1, 2, 16). Using an in vitro transcription assay, Crawford et al. showed that highly purified RNA polymerase II (Pol II) was unable to restore transcription of a Pol II gene in poliovirus-infected extracts, but a chromatographic fraction containing Pol II transcription factors did restore transcription (8). Recent studies from our laboratory have shown that poliovirus-induced inhibition of transcription in all three polymerase systems is correlated with the inactivation of specific transcription factors (12, 18, 29). While we have proposed mechanisms to explain how poliovirus inactivates host cell RNA Pol III-mediated transcription, mechanisms for the inhibition of Pol I- and Pol II-mediated transcription by poliovirus have not yet been elucidated (4, 5).

A growing number of Pol II transcription factors have been isolated from HeLa cell extracts. To date at least five transcription factors, designated TFIIA, -B, -D, -E, and -F, are required in addition to RNA Pol II for specific transcription of a Pol II gene in a reconstituted system (reviewed in reference 28). Binding of TFIID to the TATA box sequence present in most Pol II promoters is thought to be the first step in the assembly of an active Pol II transcription complex (3, 33). The decrease in Pol II transcription in poliovirus-infected cells is correlated with a specific decrease in the activity of a partially purified fraction which contains TFIID (18). In addition, TFIID and the activity required to specifically restore Pol II transcription in poliovirus-infected cell extracts have been shown to copurify through three columns and to have the same kinetics of heat inactivation (18). However, the mechanism responsible for decreased TFIID activity in poliovirus-infected cells is not known. This question can now be addressed more directly, since the DNA-binding component of human TFIID, the TATA-binding

protein (TBP), has been cloned and characterized in several laboratories (15, 17, 26).

The inhibition of Pol II-mediated transcription by poliovirus infection has many similarities to the inhibition of Pol III transcription by poliovirus. In the Pol III system, the activity of a partially purified fraction which contains the DNA-binding transcription factor TFIIC is reduced in poliovirus-infected cells (12). Recently we have shown that a transcriptionally inactive form of TFIIC found in poliovirus-infected cells can be generated by treating a transcriptionally active form of TFIIC with cloned, purified poliovirus protease 3C (3C^{pro}) (5). Unfortunately, since human TFIIC has not yet been cloned, we do not know whether the cleavage of TFIIC by 3C^{pro} is direct. Poliovirus encodes two proteases which have very specific cleavage sites within the poliovirus polyprotein: 3C^{pro}, which cleaves only at glutamine-glycine bonds, and 2A^{pro}, which cleaves only at tyrosine-glycine bonds (21). In addition, 3CD, a precursor to 3C^{pro}, can also act as a protease and is responsible for maturation of capsid proteins (21). The viral proteases do not cleave every potential cleavage site in the polyprotein, and thus other determinants, such as accessibility and context of the cleavage site, appear to be important. At this time, the only known direct substrate for the viral proteases is the poliovirus polyprotein (20). In this report, we have investigated possible mechanisms for the decrease in TFIID activity in poliovirus-infected cells. We have tested whether a component of TFIID, TBP, is modified in poliovirus-infected cells. We show that poliovirus 3C^{pro}, overexpressed and purified from *Escherichia coli*, does induce cleavage of cloned, purified human TBP in vitro. Further, we show that this cleavage of TBP by poliovirus 3C^{pro} occurs in poliovirus-infected HeLa cells. Thus, the results presented here suggest that poliovirus 3C^{pro} directly proteolyzes TBP in infected cells. This proteolysis could account, at least in part, for the decrease in Pol II-mediated transcription in poliovirus-infected cells, since the purified protease is able to inhibit Pol

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II-mediated transcription in vitro. This is the first example of specific cleavage of a cellular transcription factor by a virus-coded protease both in vivo and in vitro.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were grown in spinner culture with minimal essential media (GIBCO Laboratories) supplemented with 1 g of glucose per liter and 6% newborn calf serum. Cells were infected with poliovirus type 1 (Mahoney) at a multiplicity of infection of 20 as previously described (9). HeLa cell monolayers were grown in Dulbecco's modified Eagle medium (high glucose) (GIBCO) supplemented with 10% fetal calf serum.

Extract preparation and fractionation. Nuclear extracts were prepared from mock- and poliovirus-infected cells as previously described (10) except that extracts were not dialyzed against buffer D. Instead, extracts were precipitated by addition of ammonium sulfate to 50% saturation. Pellets were suspended in a small amount of buffer A (20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.9], 0.2 mM EDTA, 20% glycerol, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 mg of aprotinin [Sigma] per ml) and desalted on a Sephadex G50-150 column (Sigma) equilibrated and eluted with buffer A plus 0.1 M KCl. Nuclear extracts were fractionated by chromatography on phosphocellulose (Whatman P11), using buffer A with increasing step concentrations of KCl (18). The 0.6 to 1 M KCl fraction contained TFIID activity and was designated the PC D fraction. Human TBP cloned into the Bluescript vector (Stratagene) was translated in vitro by using a rabbit reticulocyte lysate system (Promega) (17).

Protease reactions and Western immunoblot analysis. 3C^{pro} and 3C^{pro} mutant 3C C147S were kindly provided by T. Hammerle and E. Wimmer. The proteases were overexpressed and purified as described previously (13, 25). Where indicated, heat treatment of 3C^{pro} was for 10 min at 60°C. The indicated amount of 3C^{pro} was added directly to [³⁵S]Met-labeled in vitro-translated TBP, the PC D fraction, or purified TBP and incubated at 30°C for 2 h, after which the reactions were run on a sodium dodecyl sulfate (SDS)-12.5% polyacrylamide gel. When Western blot analysis was performed, proteins were electrophoretically transferred to nitrocellulose (Bio-Rad) and blocked with 5% BLOTTO. Rabbit anti-TBP polyclonal serum was prepared following immunization with *E. coli*-expressed human TBP purified to homogeneity by preparative SDS-polyacrylamide gel electrophoresis (22, 23). Western blots were developed by using the chemiluminescence detection system (Amersham). Typically less than 1 min was needed to detect TBP.

In vitro transcription assays. Transcription reaction mixtures (25 µl) contained 20 mM HEPES (pH 8.4), 60 mM KCl, 5 mM dithiothreitol, 12% glycerol, 5% polyethylene glycol 8000, 7.5 mM MgCl₂, 20 mM (NH₄)₂SO₄, 0.6 mM each ATP and CTP, 0.025 mM UTP, 6 µCi of [^α-³²P]UTP (3,000 Ci/mmol; Amersham), 0.1 mM 3'-O-methyl-GTP (Pharmacia), 20 U of RNase T₁ (Boehringer Mannheim), 0.4 µg of circular template DNA [pML(C2AT)19], and nuclear extract prepared from uninfected or virus-infected HeLa cells as described previously (18). Reactions were terminated after 45 min of incubation at 30°C by addition of 175 µl of stop buffer (7 M urea, 0.5% SDS, 10 mM EDTA, 100 mM LiCl, 10 mM Tris [pH 7.9]) and extracted with phenol-chloroform. The RNA was ethanol precipitated, suspended in 15 µl of loading buffer (80% formamide-0.01% xylene cyanol-0.01% bromophenol blue in 1× TBE [89 mM Tris-borate, 89 mM

MDQNNLPPYADGLASPDGAMTPGIPIFSPMMRVTGLTPQPIQNTNSLSILEEQQ
RQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQAFAAAVQOSTSQQ
ATDGTSGQAPQLFHSQTLTAPLPGTTPLYPSPMTPTPATPASESSGIVPQL
QNVSTVNLGCKLDLKTIALRARNAYNPKRFAAVIMRIEPTTALIFSSGKMCVCTG
AKSEEQSRLAARKYARVVQKLGFPKFLDFKIQNMVGSQDVKFPRIEGLVLTHQQ
FSSYEPELFPGLIYRMKPRIVLLIFVSGKVLTGAKVRAEYFAFENIYPIKGFRTT

Potential Cleavage at:	Protease	Expected Apparent MW
amino acid 12	3C	39 kD
amino acid 18	3C	38
amino acid 34	2A	36
amino acid 108	3C	28
full length TBP	(37.4)	41

FIG. 1. Sequence and potential poliovirus protease cleavage sites in TBP. (A) Predicted amino acid sequence of human TBP. Three glutamine glycine (QG) sites are boxed, and a tyrosine glycine (YG) site is circled. (B) Predicted size of TBP if it is cleaved by 3C^{pro} at the QG sites or 2A^{pro} at the YG site. The predicted molecular mass of TBP is 37.4 kDa, but the apparent molecular mass in our SDS-gels is 41 kDa.

boric acid, 2 mM EDTA)), and loaded on to a 8% acrylamide-8 M urea gel. Reconstituted transcription reactions were performed with two column-purified general transcription factors derived from HeLa cell nuclear extracts as described previously (34). RNA was synthesized from the pMLP CAT DNA template, which contains the adenovirus type 2 major late promoter (MLP) fused to the chloramphenicol acetyltransferase gene (CAT). RNA was quantitated by primer extension analysis with an oligonucleotide primer complementary to the CAT gene containing the sequence CTCAAAATGTTCTTTACGATGCCATTGGGA.

RESULTS

Human TBP has potential cleavage sites for poliovirus proteinases. Evidence from the Pol III transcription system implicated poliovirus 3C^{pro} in the inactivation of the Pol III transcription factor TFIIC, although cleavage by 3C^{pro} could not be shown directly, since human TFIIC is not yet cloned (5). Since human TBP has recently been cloned, we checked the sequence for any potential poliovirus 3C^{pro} cleavage sites. Three glutamine-glycine sites appear in human TBP at amino acids 12, 18, and 108 (Fig. 1). A cleavage site for the other major poliovirus protease, 2A^{pro}, also occurs at amino acid 34. Two of the potential 3C^{pro} cleavage sites, at amino acids 12 and 18, are surrounded by amino acids that are thought to be important for creating an accessible region for proteolytic cleavage (14). The potential cleavage site at amino acid 108 is also interesting because it occurs after the unusually long polyglutamine stretch of TBP. Glutamine-rich domains have been implicated as transcriptional activating domains in other proteins (7). The expected apparent molecular masses of the different potential TBP cleavage products are also shown in Fig. 1.

3C^{pro} cleaves in vitro-translated TBP. To determine whether TBP could be cleaved by poliovirus 3C^{pro}, [³⁵S]methionine-labeled in vitro translated TBP was incubated with different amounts of 3C^{pro} or with heat-treated 3C^{pro}. The 3C^{pro} was overexpressed in *E. coli* and purified to greater than 98% purity (13). Analysis of 1 to 2 µg of 3C^{pro} by SDS-polyacrylamide gel electrophoresis followed by silver staining reveals a single band at approximately 20 kDa (data not shown). After incubation, the mixtures were separated

FIG. 2. 3C^{pro} cleavage of in vitro-translated TBP. Cloned human TBP was in vitro translated in the presence of [³⁵S]methionine, and 1 µl of the in vitro translation mixture was incubated with the indicated amount of 3C^{pro} or heat-treated 3C^{pro} for 2 h at 30°C. 3C^{pro} was heat treated by heating at 65°C for 10 min. These mixtures were then run on an SDS-12.5% polyacrylamide gel, fixed, and fluorographed with sodium salicylate. Positions of ¹⁴C-labeled protein molecular weight markers are shown on the left in kilodaltons. Positions of full-length TBP and cleavage product a are indicated.

on an SDS-polyacrylamide gel. Incubation of in vitro-translated TBP with the lowest concentration of 3C^{pro} had very little effect on the mobility of TBP (Fig. 2, lane 2). However, at an intermediate concentration of 3C^{pro}, a new labeled species (form a) appeared (lane 3). Finally, at the highest concentration of 3C^{pro} tested, all of the full-length TBP had been cleaved to form a (lane 4). Size markers indicated that form a was about 39 kDa. No cleavage of TBP was seen when heat-treated 3C^{pro} was used (lane 6). Also, no cleavage of TBP was detected when inactive point mutants of 3C^{pro} were used in this assay, but cleavage was seen when active mutants of 3C^{pro} were tested (data not shown). These results show that 3C^{pro} can cause specific cleavage of TBP in vitro.

Western blot analysis of TBP from mock- and poliovirus-infected cell extracts. The in vitro experiment showed that 3C^{pro} can induce cleavage of TBP, but it was important to determine whether this cleavage of TBP occurred in poliovirus-infected cells. To determine whether TBP cleavage occurred in vivo, Western blot analysis of TBP-containing fractions from mock- and poliovirus-infected HeLa cells was performed. Cells were either mock or poliovirus infected for 4 h, after which nuclear extracts were prepared. The extracts were fractionated over phosphocellulose columns, and the 0.6 to 1.0 M KCl step-eluted fractions which contained TFIID activity, and therefore TBP, were separated on an SDS-polyacrylamide gel. After transfer onto nitrocellulose, the blot was probed with a rabbit polyclonal antiserum raised against the whole TBP protein. The predicted molecular mass of TBP is 37 kDa, but the apparent molecular mass in our gel system was about 41 kDa. A 41-kDa protein was recognized by the TBP antibody in the TFIID fraction from mock-infected cells (Fig. 3, lane 1). This was the only protein detected by the antibody, and it exactly comigrated with bacterially expressed human TBP (data not shown). The TFIID fraction from poliovirus-infected cells had a reduced

FIG. 3. Western blot analysis of TBP from mock- and poliovirus-infected cell extracts and 3C^{pro}-cleaved TBP. The 0.6 to 1 M KCl step-eluted phosphocellulose fractions from cells either mock or poliovirus infected for 4 h were run on an SDS-12.5% polyacrylamide gel and transferred onto nitrocellulose. The blot was probed with a rabbit polyclonal antibody prepared against the TBP protein and developed by using the chemiluminescence detection system (Amersham). For lanes 4 to 6, various amounts of cloned, purified 3C^{pro} or 3C C147S (inactive 3C^{pro}) were incubated with the TFIID fraction (PC D) for 2 h at 30°C before being run on the SDS-gel. Positions of full-length TBP and the cleaved a and b forms are shown. Sizes are indicated in kilodaltons.

amount of intact TBP and two new forms of TBP, an upper and a lower form labeled a and b (Fig. 3). The amount of intact TBP in virus-infected cells varied depending on the batch of virus used for infection and the time when the infection was stopped (Fig. 4, lanes 1 and 2). In general, much less intact TBP was left at 4 h postinfection (Fig. 4, lane 2) compared with the amount shown in Fig. 3 (lane 2). Typically, extracts made at 4 h postinfection had three- to fivefold less TFIID activity than did those prepared from 4-h mock-infected cells (18).

Western blot analysis of 3C^{pro}-cleaved TBP. When the TFIID fraction from mock-infected cells is incubated with increasing amounts of 3C^{pro}, the intact form of TBP disappears and the new cleaved form, form a, appears (Fig. 3, lanes 4 and 5). The size of form a corresponds to the size of TBP expected if cleavage occurred at one or both of the N-terminal-most glutamine-glycine sites. Even when the TFIID fraction was incubated with 100 µg of 3C^{pro}, the lower cleaved form b was not detected (data not shown). The size of form b is consistent with cleavage of TBP at amino acid 34 by poliovirus 2A^{pro} (Fig. 1). However, 2A^{pro} is a fairly unstable protein, and our attempts to cleave TBP with 2A^{pro} overexpressed in *E. coli* have shown inconsistent cleavage at this site (data not shown). No cleavage of TBP was seen when the TFIID fraction from mock-infected cells

FIG. 4. Western blot analysis of cloned, purified TBP with 3C^{pro}. TBP which was overexpressed in *E. coli* and highly purified was treated with various amounts of cloned, purified 3C^{pro} or 3C C147S before being run on an SDS-polyacrylamide gel. The Western blot was probed with the antibody used for Fig. 3. Lanes 1 and 2 show Western blot analysis of TBP from mock- and poliovirus-infected phosphocellulose D fractions corresponding to those shown in Fig. 3.

was incubated with the inactive 3C^{pro} point mutant 3C C147S (Fig. 3, lane 6). This mutant protease was overexpressed and purified in the same manner as was the wild-type 3C^{pro} (13). A cleaved product corresponding to cleavage of TBP at the glutamine-glycine bond at amino acid 115 was not detected. It is possible that this cleavage occurs in infected cells as is detected by our TBP antibody. These Western blot analyses of the TFIID fractions from mock- and poliovirus-infected cells show that a modification of TBP seen in poliovirus-infected cells can be reproduced by treating TFIID-containing fractions with cloned, purified 3C^{pro}.

Cleavage of TBP by 3C^{pro} is direct. Since the TFIID fraction used in Fig. 3 was a partially purified fraction obtained from HeLa cells, it was possible that 3C^{pro} induced cleavage of TBP through an intermediary protein present in the TFIID fraction. An intermediary protein could also be present when in vitro-translated TBP in rabbit reticulocyte lysates are used. To determine whether 3C^{pro} could directly cleave TBP to give one of the cleaved forms seen in poliovirus-infected cells, we used TBP which had been overexpressed and highly purified from bacteria. Incubation of the bacterially expressed, purified TBP with bacterially expressed, purified 3C^{pro} resulted in cleavage of TBP to the upper cleaved form, form a, seen in poliovirus-infected HeLa cells (Fig. 4; compare lanes 5 and 2). Treatment of the purified TBP with the 3C^{pro} mutant 3C C147S did not result in cleavage of TBP (lane 6), suggesting that 3C^{pro}, and not a contaminating bacterial protease, was responsible for direct cleavage of TBP.

3C^{pro} inhibits in vitro transcription. Previous studies from our laboratory showed that the TFIID fraction, when isolated from poliovirus-infected cells, was transcriptionally inactive compared with that isolated from mock-infected cells (18). To determine whether 3C^{pro} was involved in Pol II transcriptional shutoff, nuclear extracts isolated from mock-infected cells were incubated with 3C^{pro} under conditions which allow cleavage of TBP; their ability to catalyze

FIG. 5. In vitro transcriptional analysis of nuclear extract or TFIID fraction treated directly with 3C^{pro}. Nuclear extract was prepared from uninfected HeLa cells as previously described (18) and was not treated (lane 1), treated with 2 µg of wild-type purified 3C^{pro} (lane 2), or treated with 2 µg of mutant 3C^{pro} (3C C147S) (lane 3) before the in vitro transcription reaction. TFIID was isolated from uninfected HeLa cells as previously described (34) and was treated with buffer (lane 4), 2 µg of 3C^{pro} (lane 5), or 2 µg of mutant 3C C147S (lane 6) before use in a reconstituted transcription assay as previously described (34). Arrows indicate positions of transcripts resulting from transcription from the MLP. The arrowhead indicates the position of the primer-extended product. Lanes 7 and 8 show transcription from the MLP by mock-infected and poliovirus (PV)-infected cell extracts, respectively.

transcription was then assayed by using the MLP. As shown in Fig. 5, incubation of extract with the purified wild-type protease (3C^{pro}) significantly inhibited transcription from the MLP compared with that treated with the purified mutant protease (3C C147S) (lanes 2 and 3). Slight inhibition was also detected with the mutant protease compared with the untreated extract (lane 1). This could be due to nonspecific inhibition by salt, as the proteases are kept in high salt in order to maintain their activity. The effect of the protease on in vitro transcription was also determined in a reconstituted system using a primer-extension assay (lanes 4 to 6). In this experiment, transcription from the MLP was reconstituted in partially purified transcription factor fractions isolated from uninfected HeLa cells (18). Preincubation of the TBP-containing fraction with the wild-type protease before addition to the reconstituted reaction completely abolished transcription from the MLP (lane 5). However, incubation of this fraction with an equivalent amount of the mutant protease did not affect transcription significantly compared with the control (compare lanes 4 and 6). These results suggest that the viral protease is capable of inhibiting transcription from a Pol II promoter in an in vitro system. We have tried using protease inhibitors which inhibit 3C^{pro} to show that the proteinase does not cleave other components of the transcriptional machinery, but unfortunately these inhibitors themselves inhibit transcription nonspecifically (data not shown).

DISCUSSION

We report here that a viral protease can directly and specifically cleave a cellular transcription factor. We have shown that TBP from mock-infected cells can be converted to a form of TBP found in poliovirus-infected cells by treatment with cloned, purified 3C^{pro}. In vitro-translated TBP was also cleaved when treated with 3C^{pro}. Further, we

have shown that the cleavage of TBP by poliovirus 3C^{pro} is direct. We also show that the wild-type, purified 3C^{pro} but not a mutant protease (3C C147S) was able to inhibit *in vitro* transcription from the MLP. We postulate that cleavage of TBP by 3C^{pro} may reduce TBP transcriptional activity or ability to interact with upstream transcriptional activator proteins and thus result in the decrease of TFIID activity seen in poliovirus-infected cells. This decrease in TFIID activity has previously been correlated with the inhibition of Pol II-mediated transcription seen in infected cells (18).

It has been shown previously that Pol II-mediated transcription is reduced three- to fivefold in poliovirus-infected HeLa cells (18); thus, it is not surprising that we see only a portion of full-length TBP cleaved in poliovirus-infected cells (Fig. 4, lane 2). It was also known from DNase I footprinting experiments that the binding of the TFIID fraction to the TATA box sequence is the same when the TFIID fraction from mock- or poliovirus-infected cells is used (19). Our results are consistent with this observation, since we predict that the cleavage of TBP is in the most N-terminal region of the protein, which is not required for the DNA-binding activity of TBP (28).

Although poliovirus encodes at least two mature proteinases, poliovirus infection does not result in large-scale or random proteolysis of viral or cellular proteins. When two-dimensional gel analysis is performed on mock- and poliovirus-infected cell extracts, fewer than 10 proteins are shown to be altered by poliovirus infection (32). We do not know whether the modification of TBP seen in our Western blot analysis would have been detected in the two-dimensional gel analysis, since TBP is not an abundant protein in the cell. However, the lack of large-scale modification of cellular proteins in poliovirus-infected cells argues that the proteolysis of TBP by 3C^{pro} is a specific event and is not due to generalized proteolysis in the infected cell. Also, a viral protease responsible for production of mature viral proteins from the polyprotein precursor is not expected to cleave cellular proteins at random, as this would most probably interfere with viral growth and replication. The two-dimensional gel analysis shows that 3C^{pro} does not cleave many cellular proteins, although many proteins are predicted to have the glutamine-glycine cleavage site. This may be because other determinants, such as accessibility of the cleavage site or subcellular localization of the proteins, are also necessary for cleavage. Immunohistochemical studies have shown that a precursor of 3C^{pro}, 3CD, migrates to the nucleus after infection of cells with poliovirus (11). Thus, TBP and 3C^{pro} could interact in the nucleus of the infected cell (11). We have tested other proteins which contain glutamine-glycine bonds as substrates for 3C^{pro}. Neither *in vitro*-translated UBF-1, a human Pol I DNA-binding transcription factor, nor the bacterial CAT gene is affected by 3C^{pro} treatment (30 and data not shown).

Recently TBP has been shown to be involved in transcription mediated by RNA Pol I and III as well as RNA Pol II (6, 24, 31). This finding is interesting since transcription mediated by all three polymerases is inhibited by poliovirus infection. When extracts from cells infected for 1 to 7 h were used in the Western blot analysis, cleavage of TBP first appeared at 4 h postinfection (data not shown). This is 1 to 2 h after the inhibition of Pol I transcription is first observed in infected cells; thus, other events must be responsible for the inhibition of Pol I transcription as well. However, 4 h postinfection correlates to the time when Pol II-mediated transcription is first inhibited. Pol III-mediated transcription is inhibited at 5 h postinfection, and thus the cleavage of TBP

could contribute to the inhibition of Pol III-mediated transcription. It is postulated that TBP may be associated with TFIIB, which shows a threefold reduction in activity in poliovirus-infected cells (12). However, the inhibition of Pol III transcription in infected cells is probably more affected by the complete inactivation of TFIIC that occurs in poliovirus-infected cells (5).

When mock-infected cell-derived nuclear extracts or a TBP-containing fraction (TFIID) were incubated with 3C^{pro}, inhibition of transcription from a Pol II-promoter was evident (Fig. 5). The degree of inhibition seen in these assays correlates well with that seen in poliovirus-infected cell extracts (Fig. 5, lanes 7 and 8) (18). However, since 3C^{pro} could not be specifically inactivated with protease inhibitors (without nonspecifically inhibiting the transcription assay), it was not certain whether the inhibition of transcription was due to cleavage of TBP or other proteins in the TFIID fraction. Thus, it is possible that other proteins (TBP-associated factors) which are necessary for induced levels of transcription are also affected by poliovirus infection (27). We believe that other activities in the TFIID fraction could also be inactivated by poliovirus infection, since yeast TBP or bacterially expressed human TBP only partially restored Pol II-mediated transcription in poliovirus-infected cell extracts, whereas the phosphocellulose-purified TFIID fraction completely restored transcription (17a). Of course, there may be more than one mechanism for inhibition of transcription in poliovirus-infected cells. Previous results from our laboratory have shown that dephosphorylation of the Pol II promoter-specific factor CREB occurs in poliovirus-infected cell extracts (19). Also, dephosphorylation could be part of the mechanism for decrease in Pol III-mediated transcription in poliovirus-infected cells (4).

Future studies will be aimed at delineating the role of cleaved TBP in the inhibition of Pol II transcription. These studies will require a fully reconstituted transcription system in which TBP and TBP-associated factors can be incubated individually with 3C^{pro} and added back to the reconstituted system for analysis of activity of these proteins. Furthermore, a specific inhibitor of 3C^{pro} which does not inhibit transcription nonspecifically will be necessary to understand the precise mode of action by which the viral protease inhibits host cell Pol II transcription.

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