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# Eukaryotic initiation factor 3 is required for poliovirus 2A protease-induced cleavage of the p220 component of eukaryotic initiation factor 4F

(picornavirus/translation)

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**ABSTRACT** **After cultured cells are infected with poliovirus, cellular mRNA fails to bind to ribosomes, and synthesis of the majority of cellular proteins ceases. The defective step has been localized to the cap-dependent activity of the eukaryotic translation initiation factor 4F. Inactivation of this factor correlates with the cleavage of its largest subunit, p220, into characteristic products observed in infected cells. This cleavage is mediated by the poliovirus protease $2A^{pro}$. Previous work suggests that $2A^{pro}$ does not catalyze the reaction directly, suggesting that one or more cellular proteins is required for the degradation of p220. To identify such a protein, we have developed an assay in which cleavage of a p220 substrate in the presence of poliovirus $2A^{pro}$ is dependent upon the addition of HeLa cell proteins. By using this assay, we show that another factor, eukaryotic translation initiation factor 3, is required for $2A^{pro}$-dependent cleavage of p220.**

Infection of cells with many different viruses results in specific inhibition of host cell protein synthesis (1). One of the best studied examples of this type of virus–host cell interaction is the early and marked shut-off of host cell translation that occurs in cells infected with most, but not all, members of the family Picornaviridae (2). In poliovirus-infected cells, this early shut-off of host protein synthesis correlates with the cleavage of the p220 component of eukaryotic translation initiation factor 4F (eIF-4F) (3, 4). eIF-4F consists of three polypeptides, which include the p25 cap binding protein, p220, and eIF-4A. Although the biochemical function of the p220 subunit in the complex is not known, eIF-4F binds the 7-methylguanosine 5′-triphosphate cap group on the mRNA and appears to mediate its binding to the 40S ribosomal subunit (5, 6). Cleavage of p220 is thought to inactive this function, thus inhibiting the translation of capped mRNAs. The 5′ end of poliovirus RNA is uncapped (7, 8), and translation initiates by a cap-independent mechanism (9, 10) that does not require the eIF-4F cap-recognition system.

p220 isolated from uninfected cells is visualized on SDS/polyacrylamide gels as four closely spaced bands with mobilities corresponding to an average molecular weight of 220,000. After poliovirus infection, p220 is cleaved to several antigenically related polypeptides with molecular weights of 100,000–130,000 (3). It has been shown that extracts prepared from poliovirus-infected cells contain an activity that catalyzes p220 cleavage *in vitro* (3). This p220 protease activity separates during purification from both of the poliovirus-encoded proteases, $3C^{pro}$ (11, 12) and $2A^{pro}$ (13). $2A^{pro}$ is a small cysteine protease that cleaves the poliovirus polyprotein to separate the capsid protein precursor from the nonstructural proteins (14). Although $2A^{pro}$ does not itself appear to catalyze the cleavage of p220, several lines of evidence show that functional $2A^{pro}$ is required for p220 cleavage. Genetically engineered poliovirus mutants that synthesize an altered $2A^{pro}$ neither inhibit host protein synthesis nor induce p220 cleavage (15, 16). Translation of poliovirus transcripts *in vitro* will promote the cleavage of p220 only when the $2A^{pro}$ coding region is intact (2, 17), and expression of $2A^{pro}$ from cloned cDNAs in HeLa cells causes both p220 cleavage and an inhibition of protein synthesis (18). These results have led to a model in which $2A^{pro}$ activates a cellular protease, which then cleaves p220. A p220-specific protease has been partially purified from extracts of poliovirus-infected HeLa cells (11), but this protease is highly unstable during purification, and the level of purification that is required to identify this enzyme has not been achieved.

We are interested in learning how the combined action of cellular and viral proteins leads to p220 cleavage. As a first step toward characterizing the role of cellular proteins, we developed an assay to identify proteins in extracts of uninfected HeLa cells that are required for $2A^{pro}$-dependent cleavage of p220. The rationale for this approach is that when material from uninfected cells is mixed with $2A^{pro}$ and a p220 substrate, $2A^{pro}$ will induce p220 cleavage only if all the cellular factors required for p220 cleavage are present. By using this assay, we show that eukaryotic initiation factor 3 (eIF-3) is required for $2A^{pro}$-induced cleavage of p220.

## MATERIALS AND METHODS

**Plasmid Construction and Preparation of Bacterial Extracts.** The construction of pATH-2A and pATH-2Arev will be described in detail elsewhere. To construct pATH-2A, a *Xho* II restriction fragment containing the C-terminal five codons of VP1 and the entire *2A* gene (poliovirus nucleotides 3370–3832) was inserted into the *Bam*HI site of pATH-22 (19), to create an in-frame fusion with the *Escherichia coli trpE* gene. This *Xho* II fragment was derived from a clone in which an oligonucleotide had been used to insert a UAG translation termination codon immediately after the glutamine codon at the 3′ end of the *2A* gene. pATH-2Arev was constructed in exactly the same manner, except that the *Xho* II fragment was inserted into the pATH vector in the opposite orientation. *E. coli* cell extracts were prepared as follows: Overnight cultures of bacterial strain DH5α containing either pATH-2A or pATH-2Arev were diluted into M9 minimal medium and grown at 30°C to an $OD_{595}$ of 0.3. The tryptophan operon inducer 3β-indoleacrylic acid was added to a final concentration of 10 μg/ml, and cells were grown an additional 2.5 hr at 30°C. Cells were harvested by centrifugation and resuspended in 20 mM Hepes, pH 7.4/1 mM EDTA/1 mM

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Abbreviations: eIF-3, eukaryotic initiation factor 3; eIF-4F, eukaryotic initiation factor 4F; RSW, ribosomal salt wash.

EGTA/7 mM 2-mercaptoethanol. Cells were lysed by sonication using four 30-sec bursts at 60 W with a Heat Systems/Ultrasonics sonicator, and cell debris was removed by centrifugation for 15 min at 10,000 rpm in a Beckman JS-13 rotor.

**Assays for p220 Cleavage.** Two types of assays for p220 cleavage activity were used in this study. In early experiments, p220 in uninfected HeLa cell extracts was not separated from other proteins required for cleavage activity. In these assays, each 10-μl reaction mixture contained 20 mM Hepes (pH 7.4), 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 10 mM KCl, 4 μl of HeLa cell post-mitochondrial supernatant, and 2 μl of *E. coli* extract or buffer control, as indicated. In later assays, the p220 substrate was separated from other activities. Each of these 10-μl assay mixtures contained 20 mM Hepes (pH 7.4), 5 mM $MgCl_2$, 0.5 mM dithiothreitol, 2 μl of partially purified p220 (described below), 1–5 μl of column fraction or other sample, as indicated, and 2 μl of *E. coli* extract or buffer control.

**Preparation of HeLa Cell Extracts and p220 Substrate.** HeLa $S_3$ cells were grown in suspension in Joklik's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum. The cells were lysed in a Dounce homogenizer and a post-mitochondrial supernatant was prepared as described (20). To make the p220 substrate, a ribosomal salt wash (RSW) was prepared, and p220 was precipitated with 40% saturated ammonium sulfate (cut A; RSW-A) (20). After ammonium sulfate precipitation, samples were dialyzed into buffer H (20 mM Hepes, pH 7.4/1.5 mM $MgCl_2$/7 mM 2-mercaptoethanol/0.02% $NaN_3$) containing 100 mM KCl and then loaded into a Mono Q 5/5 prepared column (Pharmacia) that had been equilibrated in buffer H containing 100 mM KCl. Bound protein was eluted in a 100 mM–500 mM KCl gradient, and p220 was eluted at approximately 360 mM KCl, as detected by immunoblot analysis using an antiserum raised to p220 polypeptide from poliovirus-infected cells (4). p220-containing fractions were dialyzed against buffer H containing 100 mM KCl.

**Purification of eIF-3.** The RSW-A cut was prepared as described above and dialyzed against buffer H with 100 mM KCl. This material was loaded on a Sephacryl S-200 column (Pharmacia) equilibrated in the same buffer, and eIF-3 was eluted with the excluded proteins. Pooled fractions were loaded on a Mono Q 5/5 prepacked column (Pharmacia) equilibrated in buffer H with 100 mM KCl and bound protein was eluted in a 100–500 mM KCl gradient. eIF-3 was eluted at approximately 440 mM KCl. Pooled fractions were diluted 1:4 with buffer H and loaded on a Mono S 5/5 prepacked column (Pharmacia) that had been equilibrated in buffer H containing 100 mM KCl. Bound protein was eluted in a 100–500 mM KCl gradient. eIF-3 was eluted at approximately 250 mM KCl. eIF-3 was detected throughout purification by immunoblot analysis using an antiserum raised to rabbit reticulocyte eIF-3 (21).

Other preparations of eIF-3 from HeLa cells or from rabbit reticulocytes (22, 23) as well as the preparation of antiserum to rabbit reticulocyte eIF-3 (21) have been described.

## RESULTS

**Expression of $2A^{pro}$ in *E. coli* and Induction of p220 Cleavage in HeLa Extracts.** Purification of cellular proteins required for p220 cleavage activity requires a source of poliovirus $2A^{pro}$ free of these proteins. To obtain this, clones were constructed that express the $2A^{pro}$ in *E. coli*. In infected cells, $2A^{pro}$ efficiently cleaves at its N terminus in the poliovirus polyprotein (14, 24). This property of $2A^{pro}$ was used to create plasmids that generate mature $2A^{pro}$ by cleavage of a hybrid protein. pATH-2A encodes a hybrid protein with the poliovirus VP1/$2A^{pro}$ cleavage site and the complete $2A^{pro}$ coding region fused in frame to the 3′ end of the first 969 nucleotides of the *E. coli trpE* gene in the cloning vector pATH-22 (19). Induction of the tryptophan operon results in the synthesis of a fusion protein, and immunoblots indicate that more than 90% of these molecules are cleaved, resulting in separation of the mature $2A^{pro}$ from TrpE and VP1 protein sequences (data not shown). The control plasmid pATH-2Arev was constructed in the same manner, except that the *2A* gene was inserted into the pATH-22 vector in the reverse orientation, and it produces a protein containing predominantly TrpE sequences. The observed cleavage of the TrpE–$2A^{pro}$ fusion protein in bacteria transformed with pATH-2A demonstrates that these $2A^{pro}$ sequences are autocatalytically active.

To determine whether the $2A^{pro}$ produced in *E. coli* was active in trans for the induction of p220 cleavage activity, extracts were prepared from cultures of *E. coli* containing these plasmids and incubated with extracts from uninfected HeLa cells. Fig. 1 shows an immunoblot of the HeLa p220 remaining after incubation of the cytoplasmic extract with buffer or with bacterial extracts, at several temperatures. When the HeLa extract was incubated with either buffer (Fig. 1, lane a) or the *E. coli* extract prepared from the strain containing pATH-2Arev (Fig. 1, lanes b–d), p220 was quite stable, and no characteristic cleavage products were formed. A trace amount of immunoreactive products were formed that have mobilities similar, but not identical, to the cleavage products observed in poliovirus-infected cells. These products appear to result from nonspecific cleavage of p220, perhaps in a protease-sensitive region, by *E. coli* proteases. In contrast, when HeLa cell extract was incubated with the $2A^{pro}$-containing extract, p220 was efficiently cleaved, yielding the characteristic cleavage products (Fig. 1 lanes e–g). The induction of p220 cleavage in an extract of uninfected HeLa cells by the $2A^{pro}$ present in an *E. coli* extract is consistent with the previous observation that $2A^{pro}$ is the only poliovirus-encoded protein required for p220 cleavage (17, 18).

The low molecular weight immunoreactive proteins seen in all lanes containing *E. coli* extract are bacterial proteins recognized by the anti-p220 serum used in this immunoblot.

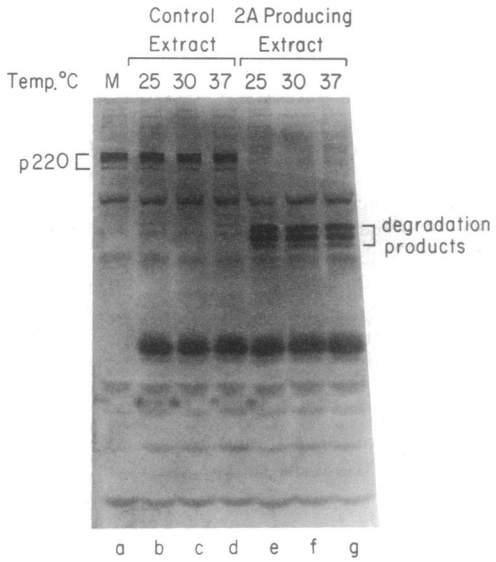


FIG. 1. $2A^{pro}$ expressed in *E. coli* induces p220 cleavage in HeLa cell extracts. A cytoplasmic extract from uninfected HeLa cells was incubated with buffer (lane a) or with an extract prepared from *E. coli* containing the control plasmid pATH-2Arev (lanes b–d) or the 2A-producing plasmid pATH-2A (lanes e–g). Incubations were for 90 min at the indicated temperature. The reaction mixtures were electrophoresed on a 6% polyacrylamide gel containing SDS and p220 was visualized in immunoblots with an anti-p220 serum (4). The location of p220 and the degradation products are indicated.

The major immunoreactive protein migrating between p220 and the degradation products is the p170 subunit of eIF-3, as indicated by the strong reaction of this antiserum with the p170 subunit in purified eIF-3 (see below). The antiserum used in this blot was raised against p220 cleavage products from poliovirus-infected HeLa cells (4), and similar preparations of p220 cleavage products have been shown to contain p170 (25).

**Partial Purification of p220 Substrate.** p220 was partially purified to separate it from proteins required for p220 cleaving activity. Since p220 is known to be located primarily in the RSW and to precipitate in 40% saturated ammonium sulfate (3), the initial purification was by subcellular fractionation and ammonium sulfate precipitation. When the RSW-A cut was incubated with buffer alone or with the control *E. coli* extract (Fig. 2, lanes a and b), p220 was not efficiently cleaved. Addition of the $2A^{pro}$-containing extract to the RSW-A cut resulted in p220 cleavage (Fig. 2, lane c). Thus, this partial purification did not separate p220 from cellular proteins required for p220 cleavage, and all of the required cellular proteins are present in the RSW-A cut. As expected, the post-mitochondrial supernatant from poliovirus-infected HeLa cells induced efficient cleavage of the p220 in the RSW-A cut (Fig. 2, lane d).

p220 was further purified by chromatography on Mono Q. When pooled Mono Q fractions containing p220 were incubated with buffer alone or the control *E. coli* extract (Fig. 2, lanes e and f) only a low level of p220 degradation was observed. When the p220 substrate was incubated with the $2A^{pro}$-producing extract (Fig. 2, lane g), most of the p220 remained intact, although a very low level of p220 cleavage products was formed. The extent of cleavage product formation varied in different preparations of the substrate and may be due to incomplete separation of the p220 substrate from other required cellular proteins. The failure of p220 to be efficiently cleaved by the $2A^{pro}$ present in the *E. coli* extract shows that $2A^{pro}$ alone is not sufficient for p220 cleavage, consistent with previous experiments suggesting that $2A^{pro}$ does not cleave p220 directly (13, 17). This p220 substrate was cleaved by a post-mitochondrial supernatant from poliovirus-infected cells, indicating that this p220 is still a substrate for the poliovirus-induced cleavage reaction (Fig. 2, lane h). When the p220 substrate was mixed with the post-mitochondrial supernatant from uninfected cells, there was little p220 cleavage after the addition of buffer (Fig. 2, lane i) or the control *E. coli* extract (Fig. 2, lane j). However, the addition of the $2A^{pro}$-containing extract induced cleavage of the p220 (Fig. 2, lane k). This indicates that some component in the post-mitochondrial supernatant could be added to the partially purified p220 to allow its cleavage in the presence of $2A^{pro}$. The cleavage products induced by the addition of $2A^{pro}$ to this extract comigrate with the cleavage products induced by the extract from poliovirus-infected cells (Fig. 2, lanes h and k).

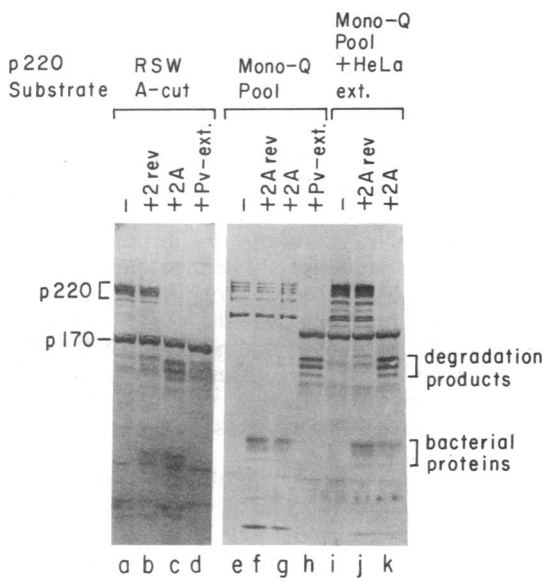


FIG. 2. Preparation of a p220 substrate. p220 degrading activity was assayed with 4 μl of RSW-A cut (lanes a–d), 4 μl of pooled Mono Q fractions (lanes e–h), or 4 μl of pooled Mono Q fractions plus 3 μl of post-mitochondrial supernatant from uninfected HeLa cells (lanes i–k) as the p220 substrate. Added to the indicated substrate was buffer control (lanes a, e, and i), 2 μl of bacterial extract from *E. coli* containing the control plasmid pATH-2Arev (lanes b, f, and j), 2 μl of extract from *E. coli* containing pATH-2A (lanes c, g, and k), or 2 μl of post-mitochondrial supernatant from poliovirus-infected HeLa cells (lanes d and h). Reactions were incubated either at 37°C for 90 min (lanes a–d) or 30°C for 90 min (lanes e–k). p220 was detected by immunoblot analysis, as described in Fig. 1.

**Purification and Identification of a Protein Required for p220 Cleavage.** To identify the components present in the post-mitochondrial supernatant that are required for p220 cleavage, HeLa cell extracts were fractionated by various methods and portions were mixed with the p220 substrate and the $2A^{pro}$-containing *E. coli* extract. As shown above, during subcellular fractionation the activity required for efficient p220 cleavage was found in the RSW-A cut. The activity was further purified by chromatography on Mono Q and Mono S. It bound tightly to both columns and was eluted with about 440 mM KCl from Mono Q and 250 mM KCl from Mono S. A remarkable feature of the active fractions from both columns was that they contained eIF-3. This was observed fortuitously during the assays of column eluates, because our anti-p220 serum reacted with the p170 subunit of eIF-3. Immunoblots using a serum raised to eIF-3 (21) indicated that these active fractions also contained the other eIF-3 subunits in addition to p170 (data not shown).

To test directly whether eIF-3 is required for p220 cleavage, eIF-3 was purified in a series of chromatographic steps, using immunoblots to detect eIF-3 (21). This eIF-3 preparation was tested for $2A^{pro}$-dependent cleavage of p220 (Fig. 3). Control reactions show that the p220 substrate was not efficiently cleaved even in the presence of $2A^{pro}$ (Fig. 3, lanes a–c), although, as seen previously, some cleavage products are detected, presumably due to incomplete purification of the substrate (lane c). However, when eIF-3 was added to the reaction, there was significant cleavage of p220 in the presence of the $2A^{pro}$ (Fig. 3, lane e), but not the control *E. coli* extract (Fig. 3, lane d). Preparations of eIF-3 that had been purified by standard procedures (22) and were active for eIF-3 function in protein synthesis were also tested for $2A^{pro}$-dependent cleavage of p220. As observed above, in the absence of added eIF-3, p220 was not efficiently cleaved (Fig. 3, lanes f–h). In this case, a different p220 substrate preparation was used, and no cleavage products were detected (lane h). However, addition of eIF-3 prepared from HeLa cells (Fig. 3, lanes i and j) or from a rabbit reticulocyte lysate (Fig. 3, lanes k and l) induced efficient, $2A^{pro}$-dependent cleavage of p220. The eIF-3 preparations from HeLa cells (lane m) and rabbit reticulocytes (lane n) incubated alone, in the absence of substrate and bacterial extracts, show the reactivity of this antiserum with the p170 subunit. The p170 in this rabbit reticulocyte preparation is present in a cleaved form.

eIF-3 is a large complex with a molecular weight of about 500,000 (23). HeLa eIF-3 contains at least seven antigenically distinct polypeptide subunits p170, p115, p66, p47, p44, p40, and p35 (26). Several of the minor components present in the purified factor appear to be proteolytically derived from larger subunits (26, 27). Most of the polypeptide bands in the

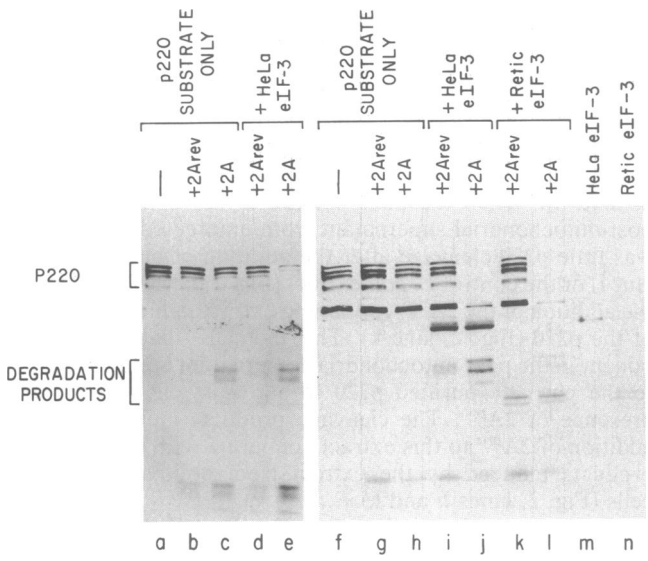


FIG. 3. eIF-3 is required for p220 cleavage. p220 cleavage activity was assayed, using pooled Mono Q column fractions as p220 substrate. Added to this substrate was (*i*) buffer control (lanes a and f), (*ii*) 2 μl (lanes b and d) or 1.5 μl (lanes g, i, and k) of control *E. coli* extract, or (*iii*) 2 μl (lanes c and e) or 1.5 μl (lanes h, j, and l) of $2A^{pro}$-containing extract. Reaction mixtures contained approximately 0.05 μg of purified HeLa eIF-3 (lanes d and e), 0.4 μg of HeLa eIF-3 (lanes i, j, and m), or 1.5 μg of rabbit reticulocyte eIF-3 (lanes k, l, and n) purified by published procedures (22, 23). Reactions were incubated for 90 min at either 25°C (lanes a–e) or 30°C (lanes f–j). Immunoblot analysis was performed as in Fig. 1.

purified eIF-3 (Fig. 4*A*, lane 1) comigrate with the polypeptides observed in both HeLa eIF-3 (lane 2) and rabbit reticulocyte eIF-3 (lane 3) that were prepared by standard procedures (22). All seven of the characteristic eIF-3 peptides were visualized in the silver-stained HeLa protein, although the amount of intact p170 subunit was low. There are a few minor differences between the polypeptide patterns observed in these preparations. Similar variations in the protein bands observed on stained gels are often seen among different eIF-3 preparations, and these may be the result of limited proteolysis occurring during purification (26). A number of these eIF-3 polypeptides were detected in immunoblots using an antiserum raised against rabbit reticulocyte eIF-3 (21) (Fig. 4*B*).

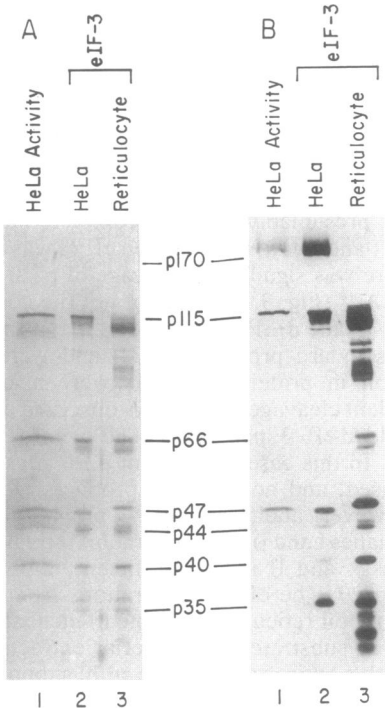


FIG. 4. Subunit structure of purified eIF-3. Each lane contained approximately 200 ng of HeLa purified eIF-3 (lane 1) or eIF-3 purified from HeLa cells (lane 2) or from rabbit reticulocytes (lane 3) (22, 23). Samples were electrophoresed on a 7.5% polyacrylamide gel containing SDS and protein was visualized by silver staining (*A*) or blotting and probing with an antiserum raised to rabbit reticulocyte eIF-3 (21) (*B*).

Since three preparations of highly purified eIF-3 are active in inducing p220 cleavage in a $2A^{pro}$-dependent reaction, eIF-3 is likely to be the component of these preparations that is required for p220 cleavage. The precise polypeptide composition and structure of eIF-3 required for its activities in protein synthesis are not known; indeed, the current standard of eIF-3 is the preparations utilized in these experiments. For this reason we define the activity we have purified as eIF-3, rather than as an eIF-3-associated protein, with the knowledge that our understanding of what eIF-3 is may change with increased understanding of the structure and function of eIF-3. Anti-eIF-3 serum inhibits the cleavage reaction (data not shown), although this antiserum also contains anti-p220 antibodies (3, 21), and thus the specificity of the antibody inhibition is uncertain. Inhibition by anti-$2A^{pro}$ serum is also seen when the anti-$2A^{pro}$ serum is incubated with the $2A^{pro}$-containing *E. coli* extract prior to addition of post-mitochondrial supernatant. The $2A^{pro}$ dependence of the reaction plus the formation of the characteristic cleavage products indicate that the eIF-3-dependent p220 cleavage is the same reaction that occurs in poliovirus-infected cells.

## DISCUSSION

We have shown that $2A^{pro}$ expressed in *E. coli* is active in inducing p220 cleavage but that $2A^{pro}$ is not sufficient for this cleavage. Previous work had presented evidence that cellular proteins were required for the p220 cleavage activity (13, 17). In this work we show directly that cellular proteins are necessary for this reaction and identify eIF-3 as a required cellular protein.

Initiation of translation in eukaryotic cells is a complex process and many aspects are incompletely understood (for reviews see refs. 28 and 29). Although eIF-3 is required for efficient initiation of translation *in vitro*, its precise role in this process has not been completely defined. Several activities have been described for this large protein, and there are likely to be others. eIF-3 binds tightly to the 40S ribosomal subunit (30). It stimulates binding of the initiator tRNA to the 40S ribosome in the presence of eIF-2 and is required for binding of the 43S preinitiation complex to mRNA (31). It also promotes dissociation of 80S ribosomes into 40S and 60S subunits (32).

In this report, we present data that suggest a functional interaction between eIF-3 and eIF-4F. Several previous observations have indicated a physical interaction between these two protein complexes. Cap binding proteins have been observed in purified eIF-3 (33), and antibody that specifically reacts with p220 was affinity purified from an antiserum raised to purified eIF-3 (3, 21). The two proteins cosediment in sucrose gradients of extracts derived from uninfected HeLa cells (34, 35). In poliovirus-infected cells, both the p25 cap binding protein (34) and the p220 cleavage products (25, 35) are dissociated from eIF-3, possibly as a result of p220 cleavage. Finally, the p25 cap binding protein and an eIF-4F-associated activity bind to eIF-3-Sepharose (36).

Our data demonstrate that eIF-3 is required for efficient $2A^{pro}$-induced p220 cleavage, but its exact role in the cleavage reaction is unknown. The protein that functions as the

protease that cleaves p220 has not been identified. Although an attractive model is that an eIF-3–eIF-4F complex presents p220 in a proper conformation to be a substrate for cleavage by the $2A^{pro}$, previous experiments indicated that $2A^{pro}$ is unlikely to catalyze this reaction (13, 17). Since the p220 substrate used in these experiments was not completely purified, additional cellular proteins could be involved. This suggests a variation of the above model in which the eIF-3–eIF-4F complex presents p220 to an as yet unidentified cellular protease that is activated by $2A^{pro}$. An alternative model is that eIF-3 is the protease that cleaves p220 after activation by $2A^{pro}$. To our knowledge, eIF-3 has not previously been reported to be a protease, and it is not clear how a protease activity would relate to its activities in protein synthesis. Preliminary evidence suggests that the p220 cleavage activity in extracts from poliovirus-infected cells does not copurify with eIF-3, and no cleavage of eIF-3 subunits has been observed in infected cell extracts (unpublished observations). Furthermore, extracts from poliovirus-infected cells are known to contain active eIF-3 (37). However, little is known about the role of individual eIF-3 polypeptides in the activities reported for the complex, and their sequences have not been determined. Given that a change in a small fraction of eIF-3 molecules could be difficult to detect and that our understanding of eIF-3 structure and function is incomplete, it is possible that eIF-3 contains the p220 cleavage catalytic site.

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