High level expression in *Escherichia coli* cells and purification of poliovirus protein 2A\(^{pro}\)

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The poliovirus protease 2A\(^{pro}\) has been produced to high levels in *Escherichia coli* using the inducible system that utilizes T7 RNA polymerase. The protease coding sequences that contained an additional AUG to start translation were cloned in pET vectors. Synthesis of 2A\(^{pro}\) was induced by IPTG or IPTG plus rifampicin, the levels of the protein made being higher when IPTG alone was used. The expression of the protein is not toxic for *E. coli* cells and can be readily visualized by Coomassie blue staining of total bacterial protein extracts separated in polyacrylamide gels. Centrifugation of the broken bacterial cells sediments more than 95% of the 2A\(^{pro}\) synthesized at a 95% purity level after sarkosyl treatment. Antibodies raised against 2A\(^{pro}\) in *E. coli* recognize a 16K protein in poliovirus-infected cells. In addition, 2A\(^{pro}\) shows activity in trans as measured by the cleavage of p220 in HeLa cell extracts and by cleavage of a poliovirus protein substrate that contains the junction between the P1 and P2 polypeptides.

### Introduction

Poliovirus contains a 7.5 kb ssRNA of positive polarity that encodes a polyprotein from which the mature viral proteins are generated by proteolytic cleavages (Kräusslich & Wimmer, 1988). These cleavages are accomplished by the two virus-encoded proteases known as 3C\(^{pro}\) and 2A\(^{pro}\) (Kräusslich & Wimmer, 1988; Hellen & Wimmer, 1992). 3C\(^{pro}\) is responsible for most of the cleavages that generate the mature viral proteins. This protease cleaves the dipeptide Q-G and leads to the formation of a number of precursors and mature polypeptides (Kräusslich & Wimmer, 1988; Hellen & Wimmer, 1992). Not all Q-G dipeptides present in the polyprotein are cleaved by 3C\(^{pro}\), suggesting that other constraints determine the specificity of 3C\(^{pro}\) activity (Kräusslich & Wimmer, 1988). The precursor of 3C\(^{pro}\), 3CD has been implicated as the actual protease responsible for the maturation of the capsid proteins present in the P1 polypeptide (Jore et al., 1988; Ypma Wong et al., 1988). The other viral protease, 2A\(^{pro}\), cleaves the dipeptide Y-G present at the junction between P1 and P2 (Toyoda et al., 1986). A second site recognized by 2A\(^{pro}\) lies within 3C\(^{pro}\) giving rise to an alternative cleavage of 3CD, generating 3C’ and 3D’. This second cleavage and the products formed are not required for efficient growth of poliovirus in cultured cells (Lee & Wimmer, 1988). The requirements in P1 and P2 for recognition and cleavage of Y-G at the junction site by 2A\(^{pro}\) have been studied in detail (Hellen et al., 1992). The P2 and P1’ positions are important in determining the cis cleavage at this site (Hellen et al., 1992). Studies with protease inhibitors suggested that poliovirus and rhinovirus protease 2A\(^{pro}\) belong to the sulphhydryl protease group (Konig & Rosenwirth, 1988; Sommergruber et al., 1989). However, analysis of the sequences of 2A\(^{pro}\) and 3C\(^{pro}\) indicated that they are related to the trypsin-like serine proteases (Sommergruber et al., 1989; Bazan & Fletterick, 1988; Gorbalenya et al., 1989), despite the fact that 2A\(^{pro}\) has a cysteine residue instead of serine in the catalytic triad of this enzyme (Yu & Lloyd, 1991). Recent results suggest that 2A\(^{pro}\) belongs to a new class of cysteine proteases (Sommergruber et al., 1992). Site-directed mutagenesis studies helped in attempts to delineate the sites in 2A\(^{pro}\) involved in the catalytic triad and to define some of the residues in this protease involved in substrate recognition in trans (Hellen et al., 1991; Yu & Lloyd, 1991). His20, Asp38 and Cys109 residues may form the catalytic triad of 2A protease (Hellen et al., 1991; Yu & Lloyd, 1991). Apart from the poliovirus polyprotein a number of cellular proteins are selectively cleaved during poliovirus infection (Urzainqui & Carrasco, 1989). One of the best studied of these cellular proteins is a component of the translation initiation factor eIF4F known as p220 (Kräusslich & Wimmer, 1988). Degradation of p220 is not directly accomplished by 2A\(^{pro}\), but most probably by a cellular protease that is activated by 2A\(^{pro}\), perhaps in a cascade fashion (Kräusslich et al., 1987; Lloyd et al., 1986; Wyckoff et al., 1992). Very soon after poliovirus...
infection, p220 is cleaved to two peptides of approximately 110K (Etchison et al., 1982). This degradation precedes the inhibition of host protein synthesis induced by poliovirus (Bonneau & Sonenberg, 1987; Pérez & Carrasco, 1992) and it is still debatable whether p220 cleavage is involved in the poliovirus shut-off of host translation (Sonenberg, 1990; Carrasco & Castrillo, 1987).

Several attempts have been made to clone and purify poliovirus 2Apro. The protease 2Apro has been purified from infected HeLa cells, but was very unstable in extracts and a rapid assay was necessary to follow its activity (Konig & Rosenwirth, 1988). Poliovirus protein 2Apro has been expressed in an inducible manner in HeLa cells. The principal effect of 2Apro expression was on transcription mediated by RNA polymerase II, whereas there was only a partial reduction of translation or DNA replication after 42 h of transfection (Davies et al., 1991). Attempts to make vaccinia recombinant viruses containing the intact 2Apro sequence have been unsuccessful thus far (Jewell et al., 1990; Turner et al., 1989), indicating that vaccinia virus growth cannot tolerate the expression of 2Apro. The protease 2Apro gene has been cloned and expressed in Escherichia coli as a fusion protein containing part of the bacterial TrpE protein (Sun & Baltimore, 1989). Transient expression of 2Apro in COS cells leads to about 40% cleavage of p220, corresponding to the percentage of transfected cells (Davies et al., 1991). The principal effect of 2Apro expression was on transcription mediated by RNA polymerase II, whereas there was only a partial reduction of translation or DNA replication after 42 h of transfection (Davies et al., 1991).

Methods

Plasmid construction. To construct pT72A, two primers were designed to hybridize with the 5' and 3' ends of the 2Apro gene of poliovirus type 1 cDNA. The resulting PCR product of 446 bp was partially digested with NdeI and totally with BclI. The fragment was purified using GeneClean and subcloned in pET3a (Studier et al., 1990) digested with NdeI and BamHI. Two positive colonies were isolated and assayed for recombinant 2Apro protein (2Apro) expression. Clone 2.1 was sequenced by the dideoxynucleotide method (Smith et al., 1980) to check that no alterations had been introduced in the 2Apro sequence during amplification.

Expression and purification of 2Apro in E. coli. Plasmid pT72A was introduced into E. coli BL21(DE3)(pLysS) cells. To induce expression, single colonies were grown overnight in LB medium in the presence of 100 μg/ml ampicillin and 34 μg/ml chloramphenicol. Then cultures were diluted 100- to 200-fold in M9 medium (Maniatis et al., 1982) supplemented with 0.2% glucose and antibiotics. Induction was carried out by adding IPTG when the cultures reached 0.6 to 0.8 A600. The cultures were centrifuged at 138000 kPa and centrifuged twice at 20000 g for 5 min to remove the unbroken cells. The supernatant was centrifuged for 20 min at 15000 g at 4°C to separate the pellet and the supernatant. Ninety-five percent of the 2Apro protein was found in the pellet. The pellet was washed three times with 1% sarkosyl in lysis buffer to remove the rest of the proteins. 2Apro protein constitutes 95% of the total protein in this pellet. Protein 2Apro was finally solubilized in lysis buffer containing 6 M-urea and dialysed stepwise into 2 M-urea. The yield was 20 mg of 2Apro from 10 g (wet weight) E. coli cells.

Production of rabbit antiserum against 2A. Polycyclamide gel slices containing protein 2A were resuspended in PBS and mixed 1:1 (v/v) with complete Freund's adjuvant. Immunization of rabbits was carried out as described elsewhere (Harlow & Lane, 1988). The serum was tested by Western blotting, and the background was diminished by preabsorbing the antiserum with acetone powders of BL21(DE3)(pLysS) host cells.

Immunoblot analysis. Immunoblot analysis of protein 2Apro was performed using the ECL Western blotting detection reagent kit (Amersham) following the protocol supplied by the manufacturer. Briefly, extracts were harvested, electrophoresed and transferred to a nitrocellulose membrane (Harlow & Lane, 1988). The nitrocellulose sheet was blocked with 5% non-fat dry milk, 20 mM-Tris-HCl pH 7.4 and 500 mM-NaCl for 2 h. After blocking, the specific antiserum was added at a dilution of 1:1000 in a solution of PBS with 1% dry milk plus 0.01% sodium azide. The blot was incubated with the diluted antiserum for 18 h and then washed three times with PBS containing 0.05% Tween 20. A second incubation with biotinylated anti-rabbit Ig antiserum (1:10000) in PBS containing 0.05% Tween 20 was carried out before washing the blot again three times with the same buffer. A third incubation of 1 h was done with streptavidin–peroxidase conjugate (1:20000). After four washes the blot was subjected to the luminescence reaction: one volume of solution A (100 mM-Tris-HCl pH 8, 5 mM-H2O2) was mixed with one volume of solution B (2.5 mM-luminol and 78 μM-luciferin) and added to the blot for 1 min. The blot was dried on a Whatman 3MM paper and exposed to X-ray film for 10 to 30 s. The p220 blots were carried out as indicated (Perez & Carrasco, 1992).

In vitro p220 cleavage assay. The ability of purified 2A protein to induce p220 cleavage was assayed on post-mitochondrial HeLa cell extracts (S10) that were obtained as previously described (Brown & Ehrenfeld, 1979). The S10 extract was incubated with purified 2Apro protein (5 μg) for 30 min at 37°C. The incubation was stopped by adding one volume of 2x sample buffer (320 mM-Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 200 mM-DTT, 2 mM-PMSF and 0.066%
bromophenol blue). The samples were analysed by Western blotting with a monoclonal antibody against p220 (kindly provided by Dr D. Etchison, Kansas University, Kansas City, Kans., U.S.A.).

In vitro transcription, translation and cleavage. [35S]Methionine-labelled substrate was produced by in vitro translation of RNA transcripts of linearized plasmid pT7101D2AB (Lama et al., 1992) with PhMI. After 90 min of translation, protein synthesis was stopped by the addition of RNase (10 μg/ml), unlabelled methionine (1 mM) and cycloheximid (0.5 mg/ml). The major protein observed had an approximate M_r of 57.8K; this is the predicted size of a 1C*D2A* hybrid protein that contains 11 amino acids of the T~ terminator from the gene 10 protein of T7 phage, 126 amino acids of 1C, 302 amino acids of 1D and 80 amino acids of 2A. To a portion of this extract a volume of purified 2A pr° was added and the mixture was incubated at 30 °C for 120 min. The reaction was stopped by adding one volume of 2 x sample buffer.

Results

Molecular cloning of poliovirus 2A pr°

2A pr° was cloned from the poliovirus genome by PCR techniques, using the oligonucleotides depicted in Fig. 1. Thus, 2A pr° contains an additional methionine codon to start translation. The protein made in E. coli would contain this methionine before the first amino acid of 2A pr°, which is glycine, and may be removed as is known to occur for most proteins with N-terminal Met–Gly (Maniatis et al., 1982).

The system we have used to express 2A pr° has been described in detail (Studier et al., 1990). The cloning vector contains a promoter recognized by the T7 RNA polymerase. The gene of this polymerase is integrated into the bacterial genome under a u.v. light-inducible lac promoter. The T7 RNA polymerase is counteracted by the expression of the T7 lysozyme which is a natural inhibitor of T7 RNA polymerase. The T7 lysozyme is expressed at low levels from a compatible plasmid present in the BL21(DE3)-(pLysS) E. coli strain (Studier et al., 1990; Dubendorff & Studier, 1991).

The use of this system has permitted cloning and expression of all the poliovirus non-structural proteins at high levels (Nicklin et al., 1988; Lama et al., 1992; Lama & Carrasco, 1992; Plotch et al., 1989). Only the expression of proteins 2B and 3A was highly toxic for E. coli (Lama et al., 1992; Lama & Carrasco, 1992).

Inducible expression of 2A pr°

Cells were transformed with the plasmid pT72A and induced to express 2A pr° with 1 mM-IPTG. Fig. 2(a) shows the Coomassie blue staining of protein extracts from bacteria harvested at different times after induction. There is a clear induction of a protein, with an apparent molecular mass of 16K, which coincides with the expected size of 2A pr° (16-6K). Maximal accumulation of this protein occurs after 2 to 3 h of induction. To analyse the synthesis of proteins at different times after induction, cells were labelled with [35S]methionine for 15 min, and the proteins were analysed by SDS–PAGE (Fig. 2b). Induction with 1 mM-IPTG gives rise to the labelling of a prominent protein of 16K, plus a number of polypeptides expressed in lower amounts. The induction with both IPTG and rifampicin inhibited the synthesis of the majority of these proteins and allowed the virtually exclusive synthesis of the putative 2A pr° (Fig. 2b). Evidence that the 16K band corresponded to poliovirus 2A pr° came from the immunoblot analysis shown in Fig. 2(c). The 16K protein induced in bacteria reacted with antibodies against 2A pr° generously provided by Dr P. Sarnow (University of Colorado, Denver, Colo., U.S.A.). Finally, the growth of bacteria expressing 2A pr° after IPTG induction was analysed. In contrast to the expression of other poliovirus non-structural proteins such as 2B and 3A (Lama et al., 1992; Lama & Carrasco, 1992), 2A pr° was not toxic for the bacteria which were not lysed and even grew during 2A pr° expression.

In order to grow bacteria expressing 2A pr° in larger quantities to allow the purification of this poliovirus protease, we analysed the conditions for its expression.
Significant induction of 2A pr° was observed only with 0.1 mM IPTG in the presence of rifampicin after 3 h (results not shown). No differences were found when using rifampicin from different sources (results not shown). However, optimal induction of 2A pr° was found with IPTG alone in the absence of rifampicin. Although there are no significant differences in the labelling of 2A pr° plus or minus rifampicin, the amount of transcripts found in the presence of rifampicin was lower (Lama et al., 1992), influencing the final amount of protein made in this system.

**Purification of 2A pr°**

For the purification of 2A pr° we routinely started with 10 g of bacteria that had been induced with 0.5 mM IPTG for 3 h. The bacteria were lysed in a French press and the lysate was centrifuged as shown in Fig. 3(b). The 2A pr° sediments in the insoluble fraction (P1), but it is not in the form of inclusion bodies as shown by analysis using phase-contrast microscopy (results not shown). Only about 2% of 2A pr° remains in the soluble fraction; this protein was discarded in our purification protocol. Washing the insoluble pellet three times with buffer containing 1% sarkosyl renders a preparation of 2A pr° (P2) of approximately 95% purity. The presence of a smaller component related to 2A pr° is clear at this stage because the two proteins copurify. We believe that this smaller protein is related to 2A pr° because it also reacts with the anti-2A pr° antibodies (results not shown); it may arise by premature termination during translation of 2A pr°. The precipitate is readily soluble in 6 M-urea which can be lowered to 2 M-urea by gradual dialysis. Removal of the insoluble proteins by low-speed centrifugation gives a preparation of 2A pr° of high purity (Fig. 3b) with a yield of 20 mg of protein per 10 g (wet weight) of starting bacteria.

This protein band was used to raise antibodies in

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**Fig. 2. Expression of 2A protease in E. coli.** BL21(DE3)(pLysS) cells containing pET3xa (control) or pT72A plasmids were grown as described in Methods. Cells were induced with 1 mM IPTG at zero time and harvested at the times indicated, h post-induction (p.i.). (a) Coomassie blue staining of total protein extracts obtained at the h p.i. indicated and fractionated by SDS-PAGE. M, markers are shown (M). (b) Labelling recombinant 2A protein. At 0 (lanes 1, 3) or 1 (lanes 2, 4) h p.i. 1 ml of culture was labelled with 2 × 10⁶ cpm/ml [35S]methionine (10 min) in the presence (+; lanes 1, 2) or in the absence (−; lanes 3, 4) of 150 μg/ml of rifampicin. Labelled proteins from poliovirus-infected cells were used as markers (M). (c) Immunoblot analysis of 2A protein. Blots containing extracts from control cells that lack the 2A insert (pET3xa vector (1)), and from two clones [1.1 (2) and 2.1 (3)] of E. coli cells expressing 2A were analysed with a specific anti-2A peptide rabbit antiserum (kindly given to us by Dr P. Sarnow). The position of 2A protein is indicated by an arrow. (d) Time course of growth of BL21(DE3)(pLysS) cells expressing 2A protein. Control cells (pET3xa vector) (○), clone 1.1 (■) and clone 2.1 (▲).
Poliovirus protein 2A<sup>pro</sup> expression

![Image](image.png)

(a) S2

P1 1 2 3 P2/S3 S4

2A

(b) (a) P1 11K 20 min Sarkosyl 1% (3x)

S1

P2 11K 20 min

Urea 6 M

S2

11K 20 min

Stepwise dialysis until 2 M-urea

S3

P3 11K 20 min

Urea 6 M

S4

Fig. 3. Purification of 2A expressed in E. coli cells. BL21(DE3)(pLysS) cells containing the plasmid pT72A were induced with 0.5 mM IPTG and the cells were harvested 3 h later and frozen at -70 °C. We then proceeded as indicated in Methods. (a) Samples at different stages of purification were analysed by SDS-PAGE and stained with Coomassie blue. The position of 2A is indicated with an arrow. (b) The steps used for purification are shown. 11K, Centrifugation at 11000 r.p.m. Fractions containing 2A protein are enclosed with a circle.

rabbits, and the antibodies were tested by immunoblot analysis of poliovirus-infected cells. Fig. 4 shows that a band of 16K specifically reacted with these antibodies in poliovirus-infected cells, but not in control HeLa cells. These results further confirm that the 16K protein made in E. coli corresponds to 2A<sup>pro</sup>.

![Image](image.png)

(a) b

(b) (a)

0 1 2 0 1 2 1 2 3

66K

45K

31K

21.5K

14.4K

Fig. 4. Immunoblot analysis of 2A. Immunoblot analysis was done with rabbit antiserum obtained as indicated in Methods. (a) Extracts of E. coli cells containing the plasmid pT72A induced as indicated in Fig. 2 were immunoreacted either with antiserum against 2A<sup>pro</sup> (a) or with the same antiserum pre-adsorbed with acetone powders of BL21(DE3)(pLysS) cells (b). (b) Lysate (120 µg) from poliovirus-infected (lane 3) or non-infected (lane 2) or the same lysate from non-infected cells plus 100 ng of recombinant 2A (lane 1) were immunoreacted with the same antiserum against recombinant 2A. The positions of Mr markers and 2A<sup>pro</sup> protein are shown.

Activity of the 2A<sup>pro</sup> made in E. coli

To assay the activity of the 2A<sup>pro</sup> made in E. coli and purified as indicated above we tested the cleavage of polypeptide p220 that is a component of eIF4F. Addition of 5 µg of our preparation of 2A<sup>pro</sup> resulted in a partial cleavage of the p220 present in uninfected HeLa cell extracts (Fig. 5a), suggesting that the 2A<sup>pro</sup> obtained was active. However, the specific activity of this 2A<sup>pro</sup> is not high because, as is apparent in Fig. 5(a), about half of the p220 molecules remain intact even in the presence of 5 µg of 2A<sup>pro</sup>. Finally, the proteolytic activity of 2A<sup>pro</sup> was assayed using part of the protein P1(1C*D) and a portion of 2A<sup>pro</sup> protein (IC*2DA*) as a substrate. Fig. 5(b) shows that addition of 2A<sup>pro</sup> cleaves the IC*2DA* giving rise to a polypeptide of 48.5K (1C*D); the truncated Δ2A (8.8K) is not observed in the gel.

Discussion

The expression of poliovirus proteins relies on the proteolytic cleavage of a polypeptide precursor (Hellen et al., 1989; Kräusslich & Wimmer, 1988). Since the
Fig. 5. Activity of the 2A<sup>pro</sup> in vitro. (a) Cleavage of p220 in HeLa cell extracts. A cytoplasmic extract of HeLa cells was incubated for 30 min at 37 °C with either buffer (lane 3) or 5 μg of 2A<sup>pro</sup> (lane 4), purified as described in the text. Proteins were separated by SDS-PAGE (7.5 % polyacrylamide) with markers from non-infected (lane 1) and infected HeLa cells as controls (lane 2). The p200 was detected with a monoclonal antibody kindly provided by D. Etchison. cp, Cleavage products. (b) Cleavage of the P1/P2 junction in trans by 2A<sup>pro</sup>. pT7101D2AB was linearized with PflMI and transcribed and translated as described in Methods. After in vitro translation, samples of the extracts were incubated for 2 h at 30 °C with buffer only (lane 1), 2 μg (lane 3) of 2A<sup>pro</sup> purified as indicated in the text. The locations of the 1C*D2A* and 1C*D hybrid proteins are indicated. [35S]Methionine-labelled proteins from poliovirus-infected cells are shown as marker proteins (lane M).

cytoplasm of mammalian cells is devoid of known proteases with appropriate specificity and because the cleavages of the precursor polypeptide are highly specific, poliovirus, like other animal viruses, encodes the proteases required to accomplish these cleavages (Hellen et al., 1989; Kräusslich & Wimmer, 1988). The two known poliovirus proteases 3C<sup>pro</sup> and 2A<sup>pro</sup> are thus active in cis and trans (Hellen & Wimmer, 1992). The first of these cleavages is carried out in cis by the 2A<sup>pro</sup>, while it is still on the nascent polypeptide chain in polysomes (Hellen & Wimmer, 1992). This cleavage releases the capsid protein precursor P1 from the rest of the polyprotein, P2 + P3. In order to obtain poliovirus 2A<sup>pro</sup> in sufficient quantities for undertaking biochemical and structural studies, an efficient system to synthesize 2A<sup>pro</sup> is needed. Initial attempts to purify this protein from poliovirus-infected HeLa cells indicated that the protease was very unstable in crude extracts (Konig & Rosenwirth, 1988). These studies described a number of purification steps that can be used to isolate active 2A<sup>pro</sup>, but the yields of protease obtained were very low and the procedures were too cumbersome to be used routinely (Konig & Rosenwirth, 1988). Further progress towards the production of 2A<sup>pro</sup> in higher quantities and its purification was recently described by the cloning and expression of this protein in E. coli cells (Alvey et al., 1991). The protease made by E. coli was active particularly in cis, rendering the genuine poliovirus protein 2A<sup>pro</sup> (Alvey et al., 1991). The 2A<sup>pro</sup> present in the supernatant was used to assay for these activities; this soluble protease is presumably thus in its native form and hence is more active than the insoluble 2A<sup>pro</sup> that we have obtained. The system used to express 2A<sup>pro</sup> in E. coli was still not very efficient, as compared to other systems available: it did not accumulate in E. coli and was not visualized by staining the bacterial extracts (Alvey et al., 1991). Our present results indicate that 2A<sup>pro</sup> can be expressed to high levels in E. coli by using the inducible system described by Studier et al. (1990). In this system 2A<sup>pro</sup> is synthesized as one of the major proteins, is not toxic for the bacteria and accumulates in cells, representing more than 5 % of the total E. coli protein. This represents a double advantage, because the synthesis and purification of the protein can be rapidly and easily followed by gel staining.

Since the 2A<sup>pro</sup> is present in the insoluble fraction, centrifugation of the ruptured bacteria gives a 2A<sup>pro</sup> preparation 95 % pure after washing the pellet with 1 % Sarkosyl. This fraction can be used in a number of studies, for example to raise antibodies. Affinity columns made with these antibodies will allow further purification of 2A<sup>pro</sup> to enable structural studies of this protein.

Perhaps one of the major pitfalls with the purified 2A<sup>pro</sup> is the low efficiency in trans-cleavage reactions. This low efficiency was previously noted for the 2A<sup>pro</sup> present in poliovirus-infected HeLa cells (Nicklin et al., 1987) and also for the protease made in E. coli (Alvey et al., 1991), although these preparations are more active in trans than our 2A<sup>pro</sup> is. It seems that 2A<sup>pro</sup> is rather efficient in cleaving itself from the P1 precursor, whereas its effects on exogenous substrates might not always be complete and occur after long incubation periods in the
presence of high quantities of 2Apro. Our results are in agreement with the idea that the efficiency of different 2Apro preparations on exogenous substrates is not high. This result could be attributed to the conditions used in the cell-free system not faithfully reflecting those present in intact cells or to the intrinsic enzymatic characteristics of the 2Apro obtained. Experiments on the action of 2Apro injected into whole cells might help to clarify this point.

Apart from the proteolytic tailoring of the poliovirus polyprotein a number of cellular proteins are degraded during poliovirus infection (Urzáinzki & Carrasco, 1989). The best studied of these proteins is a component of the eIF4F, known as p220 (Etchison et al., 1982). Protease 2A is involved in p220 cleavage, perhaps in a cascade-fashion mechanism (Kräusslich et al., 1987; Lloyd et al., 1986; Wyckoff et al., 1992). The implications that p220 cleavage has for cellular metabolism have not yet been fully evaluated (Sonenberg, 1990; Carrasco & Castrillo, 1987). Thus, degradation of p220 by the action of 2Apro was implicated in the shut-off of host translation induced by poliovirus (Etchison et al., 1982; Sonenberg, 1990). However, there is no correlation between p220 cleavage and the inhibition of cellular protein synthesis in poliovirus-infected cells (Bonneau & Sonenberg, 1987; Pérez & Carrasco, 1992). Inducible expression of cloned 2Apro in mammalian cells reduces the synthesis of chloramphenicol acetyltransferase from a reporter gene (Davies et al., 1991). The action of 2Apro on cellular metabolism. These strategies can rely upon the introduction of the 2Apro into cells, either by microinjection or by permeabilization procedures.


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