Baculovirus P35 interacts with a subunit of human RNA polymerase II and can enhance promoter activity in human cells

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The early protein P35 from the baculovirus Autographa californica nucleopolyhedrovirus is a direct inhibitor of caspases and can block apoptosis in a wide variety of systems. In addition, it has been linked to the regulation of viral gene expression, shut-down of protein synthesis in infected insect cells and malignant transformation of mouse fibroblasts. By yeast-two-hybrid screening we identified the RPB11a subunit of human RNA polymerase II as an interaction partner of P35. Specificity of the interaction was confirmed by affinity blotting. By immunocytology, P35 was in part found in the nucleus of transfected cells. Homology searches further revealed that P35 has structural similarity with RPB3, the subunit of RNA polymerase II that has been demonstrated to interact directly with RPB11a. When transfected into human colon carcinoma cells, P35 was able to enhance the activity of E-cadherin and β-actin promoters by about a factor of two as measured by luciferase reporter assay. P35 and hRPB11a together enhanced the E-cadherin activity about three- to fourfold. These data suggest an additional role for P35 in the regulation of cellular transcription.

INTRODUCTION

Cell death by apoptosis in metazoans occurs when a specialized intracellular signal transduction pathway is activated. This pathway involves a series of protein–protein interaction and proteolytic events, and apoptosis is finally executed by the cleavage of cellular protein substrates by caspases. Caspases are cysteine proteases and members of this family have functions in apoptosis either by activating further caspases or by targeting cellular proteins whose cleavage brings about the features of apoptosis such as DNA degradation and changes in cellular shape (Earnshaw et al., 1999).

Interference with the host cell’s apoptosis system is a common feature of virus infection (for example, see reviews by Barry & McFadden, 1998; Everett & McFadden, 1999). The fact that a number of viruses carry genes whose products function to inhibit cell death strongly suggests that apoptosis in virus infection is favourable to the host. This ‘strategy’ to target the apoptotic pathway is found in viruses from various organisms and classes. In baculoviruses, the mechanism of apoptosis inhibition has been worked out in some detail. A number of baculoviruses carry genes for anti-apoptosis proteins, which fall into two classes: inhibitor of apoptosis proteins (IAP) and P35 proteins (Clem & Miller, 1994; Miller, 1997). Both types of proteins can function as inhibitors of apoptosis: IAP by inhibiting the insect pro-apoptotic proteins HID, Grim and Reaper or by directly inhibiting caspases (Clem, 2001), and P35 probably largely by inhibiting cellular effector caspases (LaCount et al., 2000; Vier et al., 2000). Two very similar P35 proteins and one structurally related protein, termed P49, from different baculoviruses are known (Clem et al., 1991; Du et al., 1999; Kamita et al., 1993). P35 from Autographa californica nucleopolyhedrovirus (AcP35; AcNPV) inhibits apoptosis in virus-infected cells as shown by the finding that infection with a p35-deficient mutant virus induces apoptosis (Clem & Miller, 1993). However, P35 from Bombyx mori NPV (BmP35) appears to play a far less important role in this respect: a virus deficient in BmP35 displays almost normal infectious behaviour (Kamita et al., 1993) and BmP35 is comparatively poor at inhibiting caspase activity (Morishima et al., 1998; Vier et al., 2000). This could
mean that P35 has additional functions during virus infection and indeed other molecular roles have been proposed. In one study, P35 has been found to have a direct anti-oxidant function (Sah et al., 1999). Furthermore, a regulatory function of P35 has been implied in two reports. Studies employing a series of virus mutants and analysing protein synthesis during AcNPV infection suggest that AcP35 is involved in inducing protein synthesis shut-down (Du & Thiem, 1997). The AcP35 was found to promote transformation of mouse fibroblasts, an effect for which mere inhibition of apoptosis was insufficient (Resnoff et al., 1998). It is therefore not unlikely that viral P35 proteins act in roles other than apoptosis inhibition during baculovirus infection.

A cellular homologue of viral P35 has still not been found. We noticed that AcP35 was able to dimerize by yeast-two-hybrid assay. Pursuing the idea that AcP35 might also form dimers with a hypothetical cellular P35, we performed a yeast-two-hybrid screen with AcP35 as a bait in human cDNA libraries. We did not find the sought-after protein but isolated multiple clones of the hRPB11a subunit of the human RNA-polymerase II as an interactor with AcP35. Affinity blotting, homology searches and luciferase-reporter assays were performed to validate this interaction.

**METHODS**

**Constructs and libraries.** The bait plasmids pLexA/p35, pLexA/C-p35 and pLexA/N-p35, and the prey plasmids pG4-5/p35 and pG4-5/N-p35 which encode the bait fusion proteins LexA/p35, LexA/C-p35 and LexA/N-p35 and the prey fusion proteins B42/P35 and B42/N-P35 were generated by subcloning the respective DNA sequences of the P35 protein of baculovirus AcNPV (p35, full-length P35-sequence; C-p35, encodes amino acids 132–299; N-p35, encodes amino acids 1–147) into the multiple cloning site of the vectors pLexA202 and pG4-5 (both gifts from B. Holzmann, Technical University Munich; the plasmids pLexA202 and pG4-5 code for the bacterial full-length DNA-binding LexA protein and the activating domain B42, respectively; Gyuris et al., 1993). The bait plasmid pLexA/hRPB11a, which encodes the fusion protein LexA/hRPB11a, was generated by subcloning the sequence encoding hRPB11a protein into pLexA202. The construct pEF-hRPB11a for expression of hRPB11a in human cells was generated by subcloning the full-length cDNA of hRPB11a into a derivative of the pEF-BOs vector [derived by inserting a puromycin-resistance cassette into the pEF-BOs vector (Mizushima & Nagat, 1990); the vector was provided by D. Huang, Walter and Eliza Hall Institute for Medical Research, Melbourne]. pCMV-FLAG-hRPB11a was generated by subcloning the full-length coding sequence of hRPB11a into a commercially available vector [which already contains the FLAG-coding sequence under a CMV-promoter (pFLAG-CMV2, Sigma)]. The resulting protein contains an N-terminal FLAG-tag directly fused to the first methionine from hRPB11a.

Libraries used for yeast-two-hybrid screening were purchased from Invitrogen. The libraries used were constructed from Jurkat (human T cell leukaemia) or human placenta cDNA in the prey vector pYESTrip (yielding expressed cDNAs as fusion proteins with B42).

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electroporation in a Bio-Rad gene pulser (about $5 \times 10^6$ cells per sample, 230 or 260 V, 960 $\mu$F, 400 $\mu$l complete medium). The reporter plasmids used were pGL2-Ecad-Luc (a gift from E. R. Fearon, University of Michigan) and pGL3-β-actin-Luc (a gift from R. M. Vabulas, Technical University Munich). In these constructs, the firefly luciferase (Luc) gene is placed under the control of the constitutively active promoters from the human E-cadherin and β-actin genes. Two $\mu$g of pGL3-β-actin-Luc or 8 $\mu$g of pGL2-Ecad-Luc were co-transfected with various concentrations of pEF-p35 and or pEF-hRPB11a. In some experiments, the plasmid pEF-bcl-2 (a gift from D. Huang, WEHI, Melbourne) encoding human Bcl-2 protein was used as an additional control. After transfection cells were cultured for 24 or 48 h under normal culture conditions. Cell extracts were then prepared and luciferase activity was measured in a LB9907 luminometer (EG & G Berthold) using substrate and buffers from Promega.

RESULTS

Dimerization of AcP35

While testing a number of interactions in a yeast-two-hybrid-screen, we noticed that P35 was able to interact with itself: when P35 was expressed in yeast as a fusion protein with the LexA DNA-binding protein together with a fusion protein of P35 and the B42 transactivating peptide, a strong activity of the reporters was seen, resulting in growth on selective media and expression of β-galactosidase activity (Fig. 1). The capacity of P35 to form homodimers has also recently been described by others (Zoog et al., 1999). A further two truncation mutants were used in this system, an N-terminal part of P35 (P351–147; N-P35) and a C-terminal part (P35132–299; C-P35). As shown in Fig. 1, both mutants were able to interact with full-length P35 as shown by β-galactosidase reporter activity; a weaker reporter activity was seen when yeast were co-transformed with N-P35 and C-P35. Interaction between LexA-C-P35 and B42-full-length-P35 was also weaker; the interaction between the two N-P35 constructs and full-length P35 was somewhat variable (the interaction between LexA-N-P35 and B42-P35 always appeared stronger than in the case of LexA-P35 and B42-N-P35). No interaction was seen between LexA-N-P35 and B42-N-P35; a likely possibility is that the interaction of the two monomers occurred in a head-to-tail fashion, i.e. that the N terminus of each molecule interacts with the C terminus of the other. To demonstrate the breadth of the observed variation, two plating experiments are shown (Figs 1 and 3).

When P35 was expressed by transfection into mammalian cells, not only the monomer but also larger complexes were detected by immunoblotting (Fig. 2). The sizes of the complexes indicate that one form could be an SDS-resistant P35-dimer; further possibilities are complexes with mammalian proteins. The only known mammalian interaction partners of P35 are the members of the caspase family. However, P35 interacts only with active (i.e. proteolytically processed) caspases. The apparent molecular mass of these complexes on SDS-PAGE is around 35–40 kDa (Bump et al., 1995; Xu et al., 2003), suggesting that the bands we observed were not AcP35-caspase-complexes but complexes with other cellular interaction partners.

It is not readily conceivable why P35 must dimerize in order to be bound and cleaved by a caspase. Therefore, we hypothesized that the dimerization serves some

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Fig. 1. Homodimerization of AcP35. Yeast were transformed with the indicated pair of plasmids (in each case, one plasmid carried the LexA-DNA-Binding domain, the other the B42 transactivating domain fused onto the indicated partner). Cells were selected for presence of the plasmids by growth on media deficient in histidine and tryptophan. To test for interaction, colonies were then streaked onto solid growth medium deficient in histidine, tryptophan and leucine containing X-Gal. Cells were cultured for 3 days at 30 °C and photographed. β-galactosidase activity indicative of interaction is seen as a blue coloration of the colonies.

Fig. 2. Detection of higher molecular mass bands by anti-AcP35 immunoblotting. COS7 cells were transfected with either the empty expression vector or the same vector carrying an insert encoding full-length AcP35. After 24 h, cells were collected and detergent-lysates were analysed by Western blotting for AcP35 using a polyclonal rabbit anti-AcP35 antiserum. Closed arrow, AcP35; open arrow, higher molecular mass complexes reactive with anti-AcP35 antiserum. *, this background band was always detected by this antiserum.
other purpose, perhaps conserved from the cellular P35-precursor. In this case, AcP35 might also be able to interact with the hypothetical cellular P35. Furthermore, we reasoned that the observed high molecular mass bands could indicate the interaction of AcP35 with an unknown cellular protein. To search for these hypothetical interaction partners, yeast-two-hybrid screens were performed using LexA-AcP35 as a bait in human cDNA libraries.

Isolation of hRPB11a as an interaction partner of P35

A number of screens (five separate transformations, eight screens) were performed with libraries from Jurkat (human T cell line) or human placenta cDNA. A large number of lacZ-positive clones was isolated and further characterized. PCR screening of about 200 yeast clones gave one predominant size for the interactor insert (PCR products from over 80% of the clones had the same size of about 500 bp). Interactor plasmids were recovered from seven of these clones and re-transformed into LexA-P35-expressing yeast; in all cases, strong reporter activity signifying interaction was seen as a blue coloration of the colonies.

To obtain further independent evidence for the specificity of this interaction, hRPB11a binding to P35 was investigated by far-Western (affinity) blotting. P35 and a truncated version of human FADD as a negative control (both with the same N-terminal tag, see Methods) were expressed in bacteria. Total bacterial cell preparations were run on SDS-polyacrylamide gels and Coomassie staining showed the presence of the two proteins (Fig. 4a, left panel, arrows). Bacterial extracts were then blotted onto nitrocellulose membranes and probed with cytosol from COS7 cells transfected with an expression vector for FLAG-hRPB11a (Fig. 4, right panel) or with cytosol from control (vector)-transfected COS7 cells (middle panel); membrane-bound hRPB11a was consecutively visualized by anti-FLAG immunodetection. As shown in Fig. 4a, FLAG-hRPB11a was detected by this assay when cytosol from COS7 cells transfected with this expression construct was loaded directly (middle and right panels, first lane, open arrows); as a control, cytosol from control-transfected cells was loaded (middle and right panels, second lane). A number of background bands were visible in the lanes where the bacteria had been loaded and a strong FLAG-positive band was seen in the position where bacterially expressed AcP35 ran (Fig. 4a, right panel, closed arrow); this band was absent when the membrane was probed with cytosol not containing FLAG-hRPB11a (middle panel), indicating that hRPB11a had been specifically retained on the membrane by AcP35. To confirm the interaction between hRPB11a and AcP35 in solution, pull-down experiments were performed. Recombinant (baculovirus-expressed) GST–hRPB11a was co-incubated with extracts from 293T cells transfected to express FLAG-AcP35. When GST–hRPB11a was collected onto glutathione beads, FLAG-AcP35 was efficiently co-precipitated (Fig. 4b). These experiments provide independent evidence for the interaction between AcP35 and hRPB11a, confirming the yeast-two-hybrid results.

Subcellular localization of P35 in human cells

Since transcription takes place in the nucleus and hence hRPB11a must also function in this location, P35 would probably have to be able to enter the nucleus for a meaningful interaction with hRPB11a. To investigate the intracellular localization of P35, mammalian cells were transfected with an expression vector for P35-FLAG either...
alone or together with a construct encoding hRPB11a-GFP. Cells were stained for FLAG-expression and analysed by confocal laser scanning microscopy. As expected, hRPB11a was predominantly localized in the nucleus of the cell but also to a varying extent in the cytoplasm (which may have been the result of overexpression, Fig. 5). P35 was predominantly found in the cytoplasm as expected but it was also clearly identifiable in the nucleus of the transfected cell (Fig. 5). Expression in the nucleus was not seen in all cells but in approximately 50% of transfected cells, suggesting that further cellular factors play a role (data not shown). The observed localization pattern, however, suggests that P35 indeed also plays a role in the cell nucleus.

Structural similarity between P35 and RPB3

RNA polymerase II is a complex of 12 subunits that assembles through specific interactions between subunits. Extensive studies of the pairwise interactions has been performed previously in various organisms (Ishiguro et al., 1998; Schaller et al., 1999). In particular, the isolated hRPB3 and hRPB11 subunits can interact (Acker et al., 1997; Grandemange et al., 2001). As hRPB11a can bind to both hRPB3 and P35, we performed homology searches between P35 and the RPB3 subunits from a range of organisms. Two parts of the proteins were found where the two known P35 proteins (from AcNPV and BmNPV) showed a high degree of similarity to hRPB3 proteins (Fig. 6), indicating that P35 is able to compete with hRPB3 for hRPB11a binding. The caspase-cleavage site in P35 known to be required for the anti-apoptotic function of P35 was not conserved: among the RPB3 proteins, only hRPB3 contains a caspase-cleavage

![Image of Fig. 4](http://vir.sgmjournals.org)

**Fig. 4.** Interaction of AcP35 with hRPB11a detected by affinity blotting. (a) Left panel: E. coli bacteria carrying expression plasmids for either a truncated version of human Fas-associated protein with death domain (FADD, control) or AcP35 were grown in liquid culture, induced with IPTG and extracts analysed by SDS-PAGE (equivalents of 30 μl of culture). The gel was stained with Coomassie blue to visualize the expressed proteins (arrows). Middle and right panels: COS7 cells were transfected with either an expression construct for FLAG-hRPB11a or the empty vector and cytosolic extracts were prepared by lysis in hypotonic buffer. Aliquots of these preparations were analysed by SDS-PAGE alongside lysates from E. coli expressing recombinant proteins as in the left panel. The proteins were blotted onto nitrocellulose membranes and the membranes probed with aliquots from the same cytosolic COS7 cell preparations: middle panel, control (vector) transfected COS7 cells; right panel, COS7 cells transfected with the FLAG-hRPB11a expression construct. Membranes were washed, and retained hRPB11a protein was detected by anti-FLAG immunodetection. The directly loaded FLAG-hRPB11a protein (detected by the anti-FLAG antibody, open arrows) is visible and only in the right panel hRPB11a retained by the AcP35 protein and detected by the anti-FLAG antibody. (b) Extracts of cells transfected with a FLAG-P35 expression plasmid or a control plasmid were incubated with 100 μg extract containing GST–hRPB11a as indicated. The pull-down was performed using glutathione beads, and the resulting pellets were subjected to Western blotting with anti-FLAG (upper panel) or anti-GST antibody (lower panel) on the same blot. FLAG-P35 and control extracts (100 μg each) were run on SDS-PAGE along with GST–hRPB11a.

![Image of Fig. 5](http://vir.sgmjournals.org)

**Fig. 5.** Subcellular localization of AcP35 and hRPB11a. HeLa epitheloid cells were co-transfected with expression constructs of AcP35-FLAG and hRPB11a-GFP. After 24 h, cells were fixed, stained with mouse anti-FLAG followed by Cy3-labelled anti-mouse antibodies and analysed by confocal laser scanning microscopy. Top left, anti-FLAG staining; top right, GFP fluorescence; bottom left, transmitted light; bottom right, merged anti-FLAG and GFP fluencescences.
consensus site at this position [DIVD79 for the consensus sequence see Thornberry et al. (1997); Fig. 6]. However, recombinant hRPB3 protein was not cleaved by recombinant human caspase-3 in vitro (data not shown).

Influence of P35 on cellular transcription

The physiological role of RNA polymerase II is the nuclear transcription of mRNA. To validate the significance of the interaction between P35 and hRPB11a, we asked whether P35 was able to alter transcription in human cells. The approach we took was to measure the impact of P35 on the transcription of constitutively active promoters by luciferase-reporter assay. Little is known about the importance of individual RNA polymerase subunits. We therefore chose a system where hRPB11a had been shown previously to play a role in transcription: the levels of this subunit were found to affect expression of the human E-cadherin protein in LoVo colon carcinoma cells (Bruno et al., 1998) (with the only difference being that in that study a LoVo subclone selected for doxorubicin resistance was used).

P35 was found to enhance the activity of both the human β-actin promoter and the E-cadherin promoter in this assay. In a series of 12 experiments using various amounts of the pEF-p35 expression construct, the range of enhancement was two- to fivefold (Fig. 7a and data not shown); similar enhancement was seen when using a construct encoding P35 with a C-terminal FLAG-epitope (together with the E-cadherin-promoter, data not shown). Thus, the activity of constitutively active promoters can be enhanced by a factor of about two to five; this increase in already strong promoters might well be expected to affect a cell. Similar enhancing results were also seen with a reporter construct where luciferase was under the control of the elongation factor 1α (data not shown). To control for the possibility that the anti-apoptotic activity of P35 contributed to the enhanced luciferase expression, cells were control-transfected and incubated with the caspase-inhibitor z-VAD-fmk (which, like P35, blocks apoptosis at the level of caspase-activity). This inhibitor did not have any enhancing effect on promoter activity (Fig. 7b). Also, co-transfection of cells with reporter and an expression plasmid of human Bcl-2 (which blocks apoptosis upstream of caspase-activation) had no effect on promoter activity (data not shown). These results indicate that it was not the anti-apoptotic function of P35 that produced the observed effect.

HRPB11a, when transfected under the same conditions, had also an enhancing effect but significantly larger amounts of plasmid DNA were required (Fig. 7c and data not shown). Under these conditions, the effect of P35 reached saturation at about 12 μg of transfected plasmid DNA; no additional enhancement was seen at higher amounts (not shown). However, co-transfection of hRPB11a together with a saturating amount of the P35-expression construct led to a stronger enhancement of promoter activity than either construct alone (Fig. 7c). It thus appears that P35 is not only able to bind to hRPB11a but also to affect cellular gene expression, a function which is distinct from its role as an anti-apoptotic protein.

**DISCUSSION**

In this study, we found that P35 was able to interact not only with itself but also with the hRPB11a subunit of human RNA polymerase II. It is suggested that a similarity between P35 and hRPB3 forms the structural basis of this interaction. Further, P35 was shown to have the capacity to increase the activity of constitutively active human promoters.

RNA polymerase II is part of the large (2 MDa) so-called pre-initiation complex that binds to the promoter regions.
Fig. 7. Effect of AcP35 on the activity of two constitutively active human promoters. LoVo human colon carcinoma cells were transfected with reporter plasmids where the firefly luciferase gene was under the control of the human E-cadherin promoter (a) and (c) or the human β-actin promoter (b), together with the expression plasmids encoding AcP35 or hRPB11a or an empty vector control. After transfection cells were cultured under normal culture conditions, lysed and luciferase activity was measured. (a) Reporter construct (8 μg per transfection for E-cadherin, 2 μg for β-actin) was transfected together with AcP35 expression plasmid (12 or 20 μg for co-transfections with E-cadherin, 18 μg for co-transfections with β-actin reporter) or the same amount of empty vector. A total of five independent experiments comprised of three (in one case, two) replicates each were performed (total transfections, \( n = 14 \)). In each experiment, luciferase activity upon vector transfection was set to equal 1, and the relative reporter activity of the co-transfections was calculated. Data are presented as mean/standard deviation of the means of the five experiments. (b) β-actin reporter construct (2 μg) was transfected with 18 μg of AcP35 expression construct or a vector control. AcP35-transfected aliquots were processed as above. Vector transfections were then incubated either in the absence or the presence of 50 μM of the caspase-inhibitor z-VAD-fmk. The cells were incubated for 48 h, lysed and luciferase activity was measured. Each bar equals three experiments with three independent transfections each (\( n = 9 \)). (c) E-cadherin reporter (8 μg) was transfected together with 12 μg of AcP35 expression construct, 40 μg of hRPB11a construct or both constructs (12 μg AcP35/40 μg hRPB11a) together. Total amount of transfected DNA was 60 μg. After 24 h, luciferase activity was measured in cell extracts. Each independent transfection is depicted as a filled circle; means of the two transfections are shown as bars.
of a gene to start transcription (for a recent model see (Langelier et al., 2001). The components of this enzyme have been well conserved throughout evolution. The structure of the complex and the function of the subunits have been studied especially well in the yeast Saccharomyces cerevisiae and are believed to be similar in all eukaryotes (Cramer et al., 2000). The specific functions of the individual subunits are not well characterized. While some of them form the core of the enzyme, others are associated at the periphery of the complex and might play regulatory roles. In this respect, integration of RPB4 into the complex appears to contribute to stress resistance in yeast (Choder & Young, 1993). Moreover, the RPB3 subunit (which exhibits a structural similarity to eubacterial RNA polymerase α subunit) has been shown to be involved in transcriptional activation, as has its eubacterial homologue (Tan et al., 2000).

We found by yeast-two-hybrid analysis that hRPB11a can form homodimers. This homodimer may form under certain conditions (perhaps cellular stress which can change yeast RNA polymerase II composition as mentioned), and this may modify the availability of this subunit for RNA polymerase II complex formation. In addition, a physiological role for partial RNA polymerase subcomplexes cannot be excluded. An example of a situation where a viral protein binds to a subunit of human RNA polymerase II is provided by hepatitis B virus infection. The HBx protein from this virus binds to hRPB5 and likely uses this interaction to activate transcription (Cheong et al., 1995). The ‘strategy’ of AcNPV in targeting a subunit of human RNA polymerase may serve a similar purpose.

One important function of AcP35 is undoubtedly the inhibition of apoptosis in the cytosol of the cell, where it can inhibit caspases. However, it has also been found to some extent in the nuclear fraction of infected cells (Hershberger et al., 1994), and studies with mutant viruses have suggested that AcP35 is involved in the protein synthesis shut-down during virus infection: the DNA synthesis inhibitor aphidicolin blocked protein synthesis shut-down in Ld652Y insect cells infected with AcNPV lacking p35, but not in cells infected with wild-type virus (Du & Thiem, 1997). Although we have investigated the effect of P35 only on transcription in human cells, it is likely (given the high level of conservation of the RNA polymerase subunits) that P35 can also affect transcription in insect cells.

When SF21 cells are infected with mutant virus lacking P35, they undergo apoptosis (Clem & Miller, 1993). In the same study, it was noted that the transcription of early viral genes was delayed in the absence of P35, a finding that would fit well with a role of P35 in transcription. Eventually, transcription of cellular mRNA is shut off during infection with AcNPV (Ooi & Miller, 1988). A role for P35 in activating cellular transcription would therefore have to be confined to the early stages of infection; alternatively, P35 might also be capable of activating viral transcription. In this context, it should also be remembered that BmNPV carries a p35 gene whose product is a very poor inhibitor of caspases (Kamita et al., 1993; Morishima et al., 1998).

In mouse fibroblasts, P35 has been found to promote malignant transformation: cells transfected with p35 formed colonies in soft agar and tumours in nude mice (Resnicoff et al., 1998). Although the molecular circumstances are undefined, this could be the result of an activating effect on transcription of cellular promoters, perhaps of oncogenes such as myc and bcl-2. We have consistently been unable to express AcP35 stably in mammalian cells whereas, at least in our hands, it is exceedingly easy to generate cells lines overexpressing Bcl-2 (unpublished observations). It is possible that the basis of this difference is the interference of AcP35 with cellular transcription. Despite this, a number of P35-expressing cell lines have been described in the literature, such as in the above mentioned study about transformation. One could speculate that P35 interferes with gene expression in a way which is not compatible with normal cell division. For a cell to express P35 stably in spite of this, further mutations are perhaps necessary, and such mutations may affect other regulatory systems of the cell such as control of cell division. If this was the case, the interpretation could be that P35 does not actually promote malignant transformation but works to select for cells which already have further abnormalities.

The available evidence suggests that inhibition of apoptosis is a very important function of AcP35, at least in the tested model systems. However, as detailed above, there is evidence that P35 is involved in further biological processes both during infection of insect cells and when experimentally expressed in mammalian cells. We believe that the binding of P35 to hRPB11a could account for some of these additional functions.

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