Disruption of the Interaction of Mammalian Protein Synthesis Eukaryotic Initiation Factor 4B with the Poly(A)-binding Protein by Caspase- and Viral Protease-mediated Cleavages*  

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Eukaryotic initiation factor (eIF) 4B interacts with several components of the initiation pathway and is targeted for cleavage during apoptosis. In a cell-free system, cleavage of eIF4B by caspase-3 coincides with a general inhibition of protein synthetic activity. Affinity chromatography demonstrates that mammalian eIF4B interacts with the poly(A)-binding protein and that a region consisting of the N-terminal 80 amino acids of eIF4B is both necessary and sufficient for such binding. This interaction is lost when eIF4B is cleaved by caspase-3, which removes the N-terminal 45 amino acids. Similarly, the association of eIF4B with the poly(A)-binding protein in vivo is reduced when cells are induced to undergo apoptosis. Cleavage of the poly(A)-binding protein itself, using human rhinovirus 3C protease, also eliminates the interaction with eIF4B. Thus, disruption of the association between mammalian eIF4B and the poly(A)-binding protein can occur during both apoptosis and picornaviral infection and is likely to contribute to the inhibition of translation observed under these conditions.

Binding of mRNA to eukaryotic ribosomes involves numerous protein-RNA and protein-protein interactions at both ends of the mRNA (reviewed in Refs. 1–4). The 40 S ribosome and associated initiation factors (5–8) bind to eIF4G, which acts as a scaffold molecule (1, 3, 9–11). eIF4G associates with the mRNA cap structure via eIF4E (3, 12, 13), as well as with eIF4A (3) and the poly(A)-binding protein (PABP) linked to the poly(A) tail (2, 14–18). This complex facilitates the ability of the 40 S ribosomal subunit to “scan” along the mRNA until it reaches a suitable initiation codon, whereupon the 60 S subunit joins to form the 80 S initiation complex (3, 19). eIF4B, which can also interact with the cap (20, 21), stimulates the RNA helicase activity of eIF4A (13, 22–25) and is required to mediate mRNA binding to ribosomes (26–28). It possesses three potential regulatory domains: an RNA binding domain (RNA recognition motif), a hydrophobic region (DRYG) that mediates its binding to eIF3p170 (29), and a serine-rich region at the C terminus (22).

Both the induction of apoptosis and viral infection result in a rapid but incomplete inhibition of protein synthesis, accompanied by the proteolytic cleavage of certain initiation factors (reviewed in Refs. 3, 4, and 30). During apoptosis, degradation of both forms of eIF4G (eIF4GI and eIF4GII) occurs in a variety of cell types (31–38). Selective cleavages of eIF4B, eIF3p35, a population of eIF2a, and the eIF4E-binding protein 4E-BP1 have also been observed (33, 35, 36, 39–41).

In plants, eIF4B can interact with PABP, and this leads to enhancement both of the RNA binding activity of PABP (42) and of the RNA helicase activity of the [eIF4A-eIF4B] complex (43). It has remained unclear whether such an interaction occurs in mammalian cells and, if so, whether it might be affected by the cleavage of eIF4B that occurs in apoptotic cells. PABP itself can also be a target for proteolytic cleavage, notably in picornavirus-infected cells, in which inhibition of host protein synthesis and the selective translation of viral mRNAs correlate with the cleavage of both eIF4GII and PABP (30, 44–48). The cleavage of PABP by the picornavirus-encoded 2A or 3C proteases separates a large N-terminal fragment from a C-terminal homodimerization domain (47, 48), and this may contribute to the inhibition of protein synthesis.

Here, we provide evidence for a direct interaction between mammalian eIF4B and PABP. We demonstrate that recombinant eIF4B can bind to PABP via the N terminus of eIF4B and that this interaction is ablated when eIF4B is cleaved by caspase-3 in vitro or when cells are induced to undergo apoptosis. Furthermore, the cleavage of PABP with human rhinovirus (HRV) 3C protease eliminates the ability of PABP to bind to eIF4B. These data suggest that in addition to the cleavage of eIF4G that occurs during apoptosis and picornavirus infection, the association between mammalian eIF4B and PABP is targeted under these conditions by the cleavage of eIF4B and PABP, respectively.

EXPERIMENTAL PROCEDURES  

Purification of Recombinant Proteins—The vector encoding full-length human His-eIF4B (in pET15b) was a gift from Dr. C. Hellen (State University of New York Health Center, Brooklyn, NY) and that encoding human glutathione S-transferase (GST)-PABP (in pGEX2T) was provided by Dr. J. Hensold (Case Western Reserve University). Vectors encoding truncated forms of human His-eIF4B were provided by Dr. J. W. B. Hershey (University of California-Davis). His-tagged
forms of full-length and truncated eIF4B, 4E-BP1, and the C-terminal region of eIF4GI were expressed in BL21 cells and purified on nickel-NTA-agarose (Qiagen) (49). GST-PABP was expressed in baculovirus-infected SF9 insect cells and purified on glutathione-Septharose (Amersham Pharmacia Biotech), as per the manufacturer’s instructions. Purified, recombinant proteins were dialyzed against Buffer A (20 mM MOPS-K, pH 7.2, 25 mM NaCl, 10 mM KCl, 7 mM 2-mercaptoethanol, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride) and stored in aliquots at −70 °C. The baculovirus vector encoding untagged Xenopus PABP was provided by Dr. D. Schoenberg (Ohio State University). Seventy-two hours after baculovirus infection, insect cell extracts were assayed for the ability to translate endogenous globin mRNA and added capped or uncapped polyadenylated luciferase mRNAs (ClucA+ and UlucA+, respectively) in the presence of [35S]methionine for 60 min at 30 °C. The translation products indicated were analyzed by SDS-PAGE and autoradiography of 2-µl samples (left panel).

**Immunoblotting—**Antisera to eIF4B and PABP were gifts from Drs. J. W. B. Hershey and D. Schoenberg, respectively. Antiserum specific for the C-terminal domain of eIF4G was as described previously (36). Using the Ambion Message Machine system as per the manufacturer’s instructions. Samples were subsequently resolved by SDS-PAGE. Translation of the luciferase reporter mRNA was quantified by analysis on a PhosphorImager (Molecular Dynamics Storm 860) using ImageQuant software.

**Co-isolation of Recombinant Proteins—**Following incubation together in PBS-TB, His-eIF4B or GST-PABP and their associated proteins were recovered on nickel-NTA-agarose or glutathione-Sepharose, respectively. All resins were washed five times each (with 1 ml of PBS-TB), and the recovered proteins were eluted in SDS-PAGE sample buffer prior to analysis by SDS-PAGE and immunoblotting.

**Induction of Apoptosis and Analysis of Cell Extracts—**Jurkat cells were incubated in the absence or presence of 250 ng/ml anti-Fas antiserum for 2 h at 37 °C. Extracts from control and apoptotic cells were prepared as described previously (33).

**RESULTS**

**eIF4B Cleavage Coincides with Inhibition of Protein Synthesis by Caspase-3—**Initiation factor eIF4B is cleaved in apoptotic cells to yield a smaller fragment (ΔeIF4B). In addition, other factors, including eIF4GI, eIF4GII, eIF3p35, and eIF2α are cleaved under the same conditions (31–40). However, the relative contribution of each of these events to the overall down-regulation of protein synthesis during apoptosis is not yet known. Using a cell-free system, we have investigated the time at which cleavage of eIF4B occurs relative to that of other caspase targets in the protein synthetic machinery upon addition of purified caspase-3. Fig. 1A (right panel) shows that in the reticulocyte lysate system, eIF4B is cleaved within 10 min of caspase-3 treatment, whereas eIF4G remains intact (as
judged by the appearance of the specific cleavage products p120 and M-FAG (35)) until at least 20 min of incubation. At similarly early times following caspase-3-treatment, eIF3p35 and eIF2α are also not significantly cleaved (data not shown). The early cleavage of eIF4B coincides with the start of caspase-induced loss of protein synthetic activity, which affects both endogenous globin translation (Fig. 1A, left panel, and Fig. 1B, left panel, lanes 2, 5, and 8) and the translation of exogenous capped or uncapped polyadenylated luciferase mRNA (Fig. 1B, left panel, lanes 5 and 8, respectively). The caspase-mediated cleavage is prevented by inclusion of zVAD-FMK in the incubation (Fig. 1B, right panel), as is the inhibition of protein synthesis (Fig. 1, A and B).

eIF4B Interacts with PABP in a Caspase-sensitive Manner—
Cleavage of eIF4B by caspase-3 removes an N-terminal frag-

cent of 45 amino acids (36). As eIF4B interacts with PABP in plants to modulate the activity of the cap-binding complex (42, 43), we have examined, using affinity chromatography, whether eIF4B and PABP interact directly in the mammalian system and whether caspase-3 cleavage of eIF4B affects this binding. Fig. 2A shows that whereas mammalian His-eIF4B did not itself bind to glutathione-Sepharose (lane 3), there was substantial recovery of the factor on these beads following incubation with GST-tagged PABP (lane 5). Conversely, PABP could be isolated from a reticulocyte lysate by chromatography on nickel-agarose in the presence but not in the absence of His-eIF4B that was bound to the beads (Fig. 2B). An irrelevant His-tagged protein (the eIF4E-binding protein 4E-BP1) did not bind PABP in this system, ruling out the possibility of nonspecific interactions between PABP and nickel-agarose or the His tag. Similarly, purified His-eIF4B associated with GST-PABP could be recovered with the latter protein on glutathione-Sepharose, whereas another His-tagged protein (the C-terminal part of eIF4GI (49)) did not bind under the same conditions (Fig. 2C). This eliminates the possibility of nonspecific interactions between the His tag and PABP, GST, or glutathione-Sepharose itself. The interaction between eIF4B and PABP was not prevented by incubation with RNase A (Fig. 2A, lane 6), suggesting that it is not mediated by RNA bridging between the two proteins. However, we cannot entirely discount a role for a fragment of RNA that is protected from RNase action by one or both proteins.

to delineate the region of eIF4B that interacts with PABP, we incubated truncated versions of His-eIF4B with untagged PABP. Fig. 3 shows that whereas PABP alone was not recovered on nickel-agarose (lane 1), the N-terminal region of eIF4B (amino acids 1–150) permitted the co-isolation of PABP (lane 5). Further delineation of this region showed that amino acids 81–180 of eIF4B did not interact with PABP (lane 3), but amino acids 1–80 (lane 8) were sufficient to allow the interaction.

To determine whether the caspase-mediated truncation of eIF4B at the N terminus disrupts the interaction of the factor with PABP, we incubated full-length and caspase-cleaved forms of eIF4B with GST-PABP. Protein complexes were subsequently isolated on glutathione-Sepharose. Fig. 4A shows that neither full-length His-eIF4B (lane 2) nor the caspase-truncated ΔeIF4B (lane 3) alone was recovered on the resin. However, in the presence of GST-PABP, full-length His-eIF4B was co-isolated by this procedure (lane 5). In contrast, ΔeIF4B did not associate with GST-PABP (lane 6). Thus, loss of the N-terminal 45 amino acids, as a result of cleavage of eIF4B by
PABP Interacts with eIF4B in a Protease-sensitive Manner—Mammalian PABP is cleaved during picornavirus infection by the virally encoded proteases 2A and 3C, and the sites of cleavage have been mapped to near the C terminus (47, 48). We have used HRV protease 3C to determine whether such cleavage interferes with the ability to interact with eIF4B. Protease 3C was able to utilize both GST-PABP (Fig. 4, lane 3) and untagged PABP (Fig. 4C) as substrates but did not degrade eIF4B (Fig. 4C). Following cleavage of GST-PABP, there was a reduction in the co-isolation of full-length eIF4B with PABP (Fig. 4 B, lane 3 versus lane 2). Protease 3C cleavage also prevented the association of untagged PABP with the His-tagged N terminus of eIF4B (Fig. 4C, lane 5 versus lane 4). These data suggest that the highly conserved C-terminal domain of PABP (53) is required for the interaction with the N-terminal region of eIF4B.

We have investigated the consequences for protein synthesis of the cleavage of PABP in the reticulocyte lysate. In contrast to the reported effect of foot-and-mouth disease virus protease 3C in vivo (54), HRV protease 3C, at the concentration and times used here, did not degrade eIF4G or eIF4A in vitro but caused cleavage of ~50% of the PABP in the lysate (Fig. 5A). Consequently, it was possible to examine the effect of this enzyme specifically on PABP cleavage and protein synthesis in parallel. In a non-nuclease-treated lysate, to which was added either poly(A)+ or poly(A)− luciferase mRNA, HRV protease 3C had a small inhibitory effect on the translation of both the endogenous globin mRNA (data not shown) and the exogenous poly(A)+ RNA (Fig. 5B, top panel). In contrast, the protease treatment resulted in a substantial increase in the ability to translate the poly(A)− luciferase mRNA (Fig. 5B, bottom panel). These data suggest that either the PABP cleavage product shows a gain of function toward translation of poly(A)− mRNA or, more likely, the latter acquires a translational advantage in competition with the endogenous globin mRNA. These results also indicate that exposure to HRV protease 3C does not impair the function of other initiation factors required for the translation of both poly(A)+ and poly(A)− mRNAs.

The Interaction between PABP and eIF4B Is Diminished in Apoptotic Cells—A prediction that can be made on the basis of our results is that in cells induced to undergo apoptosis, where eIF4B is cleared (36), the association of PABP with eIF4B...
should be disrupted. To address this, extracts were prepared from control and apoptotic Jurkat cells. The integrity of eIF4G, PABP, eIF4B, and eIF4E and the association of these proteins with each other were monitored by immunoblotting. In agreement with published data (31, 36), Fig. 6A shows that eIF4G (lane 2 versus lane 1) and eIF4B (lane 4 versus lane 3), but not PABP or eIF4E, were cleaved during apoptosis. PABP and associated proteins were isolated by affinity chromatography on poly(A)-Sepharose (Fig. 6B). Although similar levels of PABP were recovered from apoptotic and control cell extracts, the amount of eIF4E associated with PABP was substantially reduced in apoptotic extracts (lane 2 versus lane 1). In addition, the amount of eIF4E recovered was greatly diminished (lane 2 versus lane 1), as would be predicted from the cleavage of eIF4G, which results in the separation of the eIF4E binding site from the PABP binding site (35). We have also used

FIG. 6. Induction of apoptosis reduces the association of eIF4B with poly(A)-interacting proteins in vivo. A, Jurkat cells were incubated for 2 h in the absence (control (C) (lanes 1 and 3) or presence (Fas) (lanes 2 and 4) of an agonistic antibody to the Fas receptor, and extracts were prepared. Equal amounts of protein were analyzed by immunoblotting for eIF4G (and its cleavage product M-FAG), PABP, eIF4B/eIF4G, and eIF4E, as indicated. B, PABP and associated proteins were isolated from control (lane 1) or apoptotic (lane 2) cell extracts using affinity chromatography on poly(A)-Sepharose. Non-specific binding of proteins was minimized by the inclusion of poly(C) (1 mg/ml) in the binding buffer. The resin was washed and the bound proteins analyzed as in A. C, eIF4E and associated proteins were isolated from control (lane 1) or apoptotic (lane 2) cell extracts using affinity chromatography on m'GTP-Sepharose. The samples were processed and analyzed as in B.

FIG. 7. A, schematic representation of the interaction of eIF4B and PABP. The domain structures of human PABP and eIF4B and the sites of protein-protein interaction and cleavage by picornaviral proteases and caspase-3 are shown. For eIF4B, the RNA recognition motif (RRM), the region containing DRYG repeats, and the basic domain (BD) are indicated (22, 25). For PABP, the four RNA recognition motifs and the proline-rich domain are shown (22, 24, 47, 48). The region of eIF4B required for PABP binding contains a sequence (residues 6–20) with similarity to amino acid sequences 133–145 and 135–147 within the PABP-binding domains of human eIF4GI and eIF4GII, respectively (17, 60). Homologous regions in the three sequences are boxed, with identical residues indicated in italics. B, disruption of the initiation complex by proteolytic cleavages of initiation factors. The known interactions between eIF3, eIF4A, eIF4B, eIF4E, eIF4G, and PABP are shown on the left, and the sites at which these factors are cleaved by caspase-3 and by the picornavirus-encoded 2A, 3C, and L proteases are indicated. In apoptotic cells, caspase-mediated cleavages result in the appearance of three fragments of eIF4G and a truncated form of eIF4B (top right). The cleavages separate the N-terminal region of eIF4G that binds PABP from the central region that binds eIF4E, eIF4A, and the [eIF3[eIF4B] complex (4, 35) and also disrupt the interaction of eIF4B with PABP. In cells infected with poliovirus, HRV, or foot-and-mouth disease virus, viral protease-mediated cleavage results in the appearance of two fragments of eIF4G and a truncated form of PABP (bottom right). The fragmentation of eIF4G again separates the regions that bind PABP and the [eIF3[eIF4B] complex (9, 63, 64). In both apoptotic and infected cells the links between the 5' end of an mRNA (via recognition of the cap by eIF4E) and the 3' end (via recognition of the poly(A) tail by PABP) or between the mRNA and the ribosome (via binding of eIF3 to the latter) are broken, potentially reducing the efficiency with which reinitiation of protein synthesis on capped poly(A)+ mRNA can take place.
mGTP-Sepharose chromatography to investigate the association of PABP with eIF4F (Fig. 6C). Following induction of apoptosis, there was a decrease in the level of PABP recovered with eIF4E (lane 2 versus lane 1). However, in this instance, it is not possible to discern whether this effect is attributable to the loss of integrity of eIF4E or to the caspase-mediated cleavage of eIF4G (31, 35, 37), because both these proteins bind PABP independently.

**Discussion**

The direct interaction between mammalian eIF4B and PABP, in conjunction with the binding of eIF4G to PABP, may facilitate the functional association of the 5' and 3' ends of mRNA (1–3, 55–57). In wheat germ, the association of eIF4B with PABP increases the efficiency of reinitiation of protein synthesis (58), a process that is also enhanced by the interaction between eIF4G and PABP (17, 43). As depicted in Fig. 7A, the loss of the N terminus of eIF4B (by caspase-mediated cleavage) or of the C terminus of PABP (by picornavirus protease-mediated cleavage) disrupts complex formation between the two proteins. Such an effect would be predicted to inhibit reinitiation. Indeed, cleavages of eIF4B (Fig. 1 and Ref. 36) and PABP (Fig. 5 and Refs. 47 and 48) are associated with inhibi-

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