Identification of Caspase 3-mediated Cleavage and Functional Alteration of Eukaryotic Initiation Factor 2α in Apoptosis*

(Received for publication, August 18, 1999, and in revised form, December 10, 1999)

Wilfred E. Marissen‡, Yanwen Guo§, Adri A. M. Thomas¶, Robert L. Matts§, and Richard E. Lloyd**

From the ‡Department of Microbiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190, the §Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma 74078, the ¶Department of Developmental Biology, University of Utrecht, 3584 CH Utrecht, The Netherlands, and the **Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas 77030

Induction of apoptosis in a variety of cell types leads to inhibition of protein synthesis. Recently, the cleavage of eukaryotic translation initiation factor 4G (eIF4G) by caspase 3 was described as a possible event contributing to translation inhibition. Here, we report the cleavage of another initiation factor in apoptotic cells, eIF2α, that could contribute to regulation of translation during apoptosis. This cleavage event could be completely inhibited by pretreatment of HeLa cells with Z-VAD-fmk. In vitro analysis using purified eIF2 and purified caspases showed cleavage of eIF2α by caspase 3, 6, 8, and 10 but not 9. Caspase 3 most efficiently cleaved eIF2α and this could be inhibited by addition of Ac-DEVD-CHO in vitro. Comparison of cleavage of phosphorylated versus non-phosphorylated eIF2α revealed a modest preference of the caspases for the nonphosphorylated form. When eIF2B complex was used as substrate, only caspase 3 was capable of eIF2α cleavage, which was not affected by phosphorylation of the α subunit. The eIF2B-globular complex was cleaved much less efficiently by caspase 3. Sequence analysis of the cleavage fragment suggested that the cleavage site is located in the C-terminal portion of the protein. Analysis showed that after caspase cleavage, exchange of GDP bound to eIF2 was very rapid and no longer dependent upon eIF2B. Furthermore, in vitro translation experiments indicated that cleavage of eIF2α results in functional alteration of the eIF2 complex, which no longer stimulated upstream AUG selection on a mRNA containing a viral internal ribosome entry site and was no longer capable of stimulating overall translation. In conclusion, we describe here the cleavage of a translation initiation factor, eIF2α that could contribute to inhibition or alteration of protein synthesis during the late stages of apoptosis.

Initiation of protein synthesis in mammalian cells is a highly regulated process that requires multiple translation initiation factors. The eukaryotic translation initiation factor 2 (eIF2) plays a key role in the initiation of translation. eIF2 is a heterotrimeric protein consisting of three subunits α, β, and γ, which forms a so-called ternary complex with GTP and the initiator Met-tRNA. The ternary complex interacts with the 40 S ribosomal subunit thereby forming a 43 S preinitiation complex that is capable of recognizing the proper start codon of the mRNA. It has been shown in yeast that eIF2 itself is involved in this process of start codon selection (1) indicating its importance in translation initiation. Upon joining of the 60 S ribosomal subunit to the 43 S preinitiation complex, the GTP moiety is hydrolyzed and an eIF2-GDP complex is released from the ribosome (2). Participation of the eIF2-GDP complex in a new round of translation requires the exchange of the GDP moiety for a new GTP molecule, a process carried out by the guanine nucleotide exchange factor, eIF2B.

The global rate of protein synthesis in eukaryotes is mainly regulated by the specific phosphorylation of Ser-51 of the eIF2α subunit. eIF2α is known to be phosphorylated by kinases such as PKR (double-stranded RNA dependent eIF2 kinase) in response to viral infection, and stress conditions (3), HRI (heme-regulated inhibitor) in response to heme availability and a host of environmental stress conditions (4–6) and GCN2 from the yeast S. cerevisiae in response to amino acid starvation (7). Phosphorylated eIF2 binds eIF2B tightly, forming a poorly dissociable eIF2αP•eIF2B complex. Since eIF2B exists in relatively low molar quantities with respect to eIF2 in the cytoplasm in many systems, phosphorylation of as little as 25% of eIF2α can be sufficient to sequester virtually all the available eIF2B, thereby blocking the eIF2B catalyzed recycling of the eIF2-GDP and subsequently inhibiting protein synthesis completely (8).

It has been well established that protein synthesis is inhibited during apoptosis or programmed cell death but the mechanism of translation inhibition has remained unclear until recently. We and others have recently shown that eukaryotic translation initiation factor 4GI (eIF4GI), which is required for binding of capped mRNA to ribosomal 43 S subunits, was cleaved by caspase 3 during apoptosis (9, 10). Similar cleavage of eIF4GI is one of the major causes of translation inhibition during enterovirus infection of cells (11). Likewise, the cleavage of eIF4GI correlates with the observed inhibition of protein synthesis in apoptotic cells (9). Other reports indicate that phosphorylation of eIF2α could represent another mechanism by which translation is inhibited during apoptosis. For instance, heme-regulated eIF2 kinase; Ac-DEVD-CHO, acetyl-DEVD-aldehyde; Z-VAD-FMK, benzylxocarbonyl-VAD-fluoromethylketone; pNA, para-nitroaniline; TNP-α, tumor necrosis factor-α; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PARP, poly(ADP-ribose) polymerase; IRES, internal ribosome entry site.

* This work was supported by National Institutes of Health Grants AI27914 and GM59803 (to R. E. L.), Oklahoma Agricultural Experiment Station project number 1757, and NIAMS, National Institutes of Health Grant E5 042299 (to R. L. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This paper is available on line at http://www.jbc.org

** To whom correspondence should be addressed. Tel.: 713-788-8993; Fax: 713-788-5075; E-mail: rilloy@bcm.tmc.edu.

† The abbreviations used are: eIF, eukaryotic translation initiation factor; ORF, open reading frame; 5′-UTR, 5′-untranslated region; FMDV, foot and mouth disease virus; CAT, chloramphenicol acetyltransferase; PKR, double-stranded RNA-dependent eIF2 kinase; HRI, heme-regulated eIF2 kinase; Ac-DEVD-CHO, acetyl-DEVD-aldehyde; Z-VAD-FMK, benzylxocarbonyl-VAD-fluoromethylketone; pNA, para-nitroaniline; TNP-α, tumor necrosis factor-α; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PARP, poly(ADP-ribose) polymerase; IRES, internal ribosome entry site.
stance, overexpression of PKR in transfected cells resulted in elevated levels of phosphorylated eIF2α concomitant with reduced protein synthesis and induction of apoptosis in these cells (12). In addition, it has been reported that PKR is a tumor suppressor protein as it was shown that overexpression of mutant PKR incapable of phosphorylating eIF2α resulted in tumor formation (13, 14). Similarly, others have described that lowering the level of the eIF2 associated protein, p67, resulted in increased levels of phosphorylated eIF2α concomitant with induction of apoptosis in these cells (15). In both cases, the decrease in the overall rate of protein synthesis was ascribed to the phosphorylation of eIF2α. Here, we report the cleavage of eIF2α by caspase 3 during the late stages of the execution phase of apoptosis in HeLa cells, K562 cells, and Jurkat T cells. The cleavage of the 38-kDa α subunit resulted into a 36-kDa cleavage product as observed by both Coomassie stain and immunoblots analysis. In vitro experiments using purified eIF2 and purified caspases showed that eIF2α could be a substrate for caspases 3, 6, 9, and 10 but not caspase 8. Although cleavage by caspase 3 appeared to be most efficient we do not rule out the possibility that other caspases might be responsible for eIF2α cleavage in vivo. In vitro experiments using both nonphosphorylated and phosphorylated eIF2α as a substrate showed that all caspases had a preference for the nonphosphorylated form of eIF2α. Interestingly, when eIF2α-B complex was used as substrate no preference was observed between nonphosphorylated and phosphorylated eIF2α. The cleavage site appears to be located in the C-terminal region of the protein as determined by N-terminal amino acid sequencing of the 36-kDa cleavage product. Close examination of this region revealed two putative caspase 3 cleavage sites which would lead to the release of a 14-or 11-amino acid peptide. Interestingly, results indicate that cleavage of eIF2α does inactivate the eIF2 complex as determined by in vitro translation assays.

EXPERIMENTAL PROCEDURES

Cells—HeLa S3 cells were grown as described (9). K562 cells were grown at 37 °C in RPMI (Irvine Scientific) supplemented with 10% bovine calf serum, 0.5% fetal calf serum (Summit Biotechnology), 100 units of penicillin, and 100 μg of streptomycin/ml (Sigma) in a humidified chamber containing 5% CO2. For induction of apoptosis, stock solutions of cisplatin, etoposide, cycloheximide (Sigma), or TNF-α (Peptide) were diluted with medium and then incubated with cells at 37 °C for the time indicated in each figure.

Preparation of Cell Lysates and Immunoblot Analysis of Fractions—Cell extracts were prepared as described (9). Briefly, HeLa cells were washed with phosphate-buffered saline, resuspended in CHAPS lysis buffer (20 mM Tris pH 7.2, 0.1 mM NaCl, 10 mM dithiothreitol, 0.5% CHAPS, 10% sucrose) and incubated on ice for 30 min. Cell lysates were then centrifuged for 10 min at 10,000 × g at 4 °C, supernatants were collected and stored at −80 °C. Cell pellets were resuspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading sample buffer, boiled for 10 min, and subjected to SDS-PAGE. Proteins were labeled with 35S]methionine (ICN), and incubated for 10 min at 37 °C in the presence of 30 μM GDP. To “freeze” the GDP on the eIF2 and stabilize the conformation of eIF2, MgCl2 was added to a final concentration of 1 mM. For each 20-μl aliquot of nucleotide exchange assay mixture, ~1.5 pmol of eIF2 was incubated in the presence or absence of 18 units of caspase 3 (Upstate Biotechnology) in buffer containing 20 mM Tris (pH 7.4), 100 mM NaCl, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1% CHAPS, and 10% polyethylene glycol for 3 h at 37 °C. [3H]GDP (5 μM) was then added and after 10 min of additional incubation, 3 mM Mg(OAc)2 was added to stabilize the eIF2-ligand-[3H]GDP complex. After a further 5-min incubation, 100 μM unlabeled GDP was added and the amount of eIF2-[3H]GDP complex remaining was determined after various times of incubation by filtration of a 29-μl aliquot of each assay mixture through nitrocellulose filters as described previously (17).

Isoelectric Focusing—To determine the phosphorylation status of eIF2α in vivo, 5 × 106 cells from apoptotic or control cultures were taken and resuspended in 200 μl of V50EF buffer (9.5 mM urea, 5% CHAPS, 50 mM sodium fluoride, 5% β-mercaptoethanol). Samples were then clarified and subjected to isoelectric focusing on vertical isoelectric focusing gels by a slightly modified protocol of the method of Mauritides et al. (21) as described previously (22).

Plasmid Constructs—TTTS plasmid (gift of P. A. Krieg) contains a multiple cloning site upstream of the Xenopus β-globin-3'-UTR and a track of 30 adenyl residues which allows generation of poly[A] mRNAs. The region encoding the 5'- untranslated region (5'-UTR) of FMDV coupled to the reporter gene chloramphenicol acetyltransferase (CAT) as described (23) was excised from FMDV-CAT with EcoRV and BamHI and subsequently cloned into blunt-ended HindIII-BglII-digested pTTTS plasmid to generate plasmid pTTTSFMDVCAT. pTTTSglobinCAT was generated by insertion of a HindIII-Smal globin-CAT fragment into a HindIII-EcoRV-digested pTTTS plasmid. Plasmid DNAs were linearized with XbaI, purified by phenol/chloroform extraction, and concentrated by alcohol precipitation for use in transcription reactions (see below).

Transcription and Translation—Transcription reactions for FMDV-CAT using T7 RNA polymerase (Promega) were carried out according to manufacturer’s recommendations. For capped globin-CAT, transcription was performed using the Mmessage mMachine T7 kit from Ambion. In both cases, the resulting RNAs were purified using spin columns (Invitrogen). Translation reactions were performed in rabbit reticulocyte lysate (Promega) according to the manufacturer’s recommendations in a 20-μl reaction volume. Addition of buffer control, caspase inhibitor Ac-DEVD-CHO, eIF2, caspase 3-treated eIF2 plus Ac-DEVD-CHO were added (2 μl) to the translation reaction prior to the addition of [35S]methionine (ICN), and incubated for 10 min at 30 °C. After addition of the label, reactions were incubated for another 90 min.
Migration of molecular weight markers, eIF2α, and eIF4GI cleavage products are indicated on the left and right, respectively. B, cell pellets from cisplatin-treated HeLa cells derived from the experiment described in panel A were resuspended in SDS-PAGE sample buffer, and analyzed by immunoblot using a monoclonal anti-PARP antibody. PARP and cleaved PARP (arrow) are indicated on the right. Migration of molecular weight markers are indicated on the left. C, proteins in lysates were separated by SDS-PAGE and immunoblotted with antisem specific for eIF2α cleavage fragment. Migration of eIF4GI and its N-terminal cleavage products (eIF4GIcpN) are indicated on the right. D, after treatment of the cells with 100 μM cisplatin for the indicated time periods, cells were labeled with [35S]methionine for 30 min at 37 °C. The cells were then lysed, and analyzed by SDS-PAGE and autoradiography.

The loss of approximately 2 kDa in molecular mass implies that only a small portion of either the N or C terminus of eIF2α is removed, which raises the question whether this cleavage event results in inactivation of the eIF2 protein complex and could contribute to inhibition of protein synthesis or may modulate eIF2 activity in another way. Therefore, we further examined whether the cleavage of eIF2α correlated with translation inhibition in apoptotic cells. Cisplatin-treated HeLa cells were pulse labeled at the time points indicated for 30 min, subsequently lysed and analyzed by SDS-PAGE. Fig. 1D shows that between 3 and 5 h post-induction the incorporation of [35S]methionine into protein decreased significantly, followed by an abrupt further decline to near zero between 6 and 7 h. Much of this translation decrease is likely due to rapid cleavage of eIF4GI or other factors, however, the latter abrupt decline may correlate with the appearance of eIF2α cleavage products.

Cisplatin treatment of HeLa cells produces apoptosis, eIF4GI cleavage, and translation shutoff faster than most other inducer/cell combinations we have tested. Thus, in order to reveal possible correlations between eIF2α cleavage and translation shutoff, we explored other apoptotic systems in which eIF4GI cleavage was not so rapid. Fig. 2 summarizes data taken from HeLa cells or K562 erythroblastoid cells treated with etoposide and compares those data taken from cisplatin-treated HeLa cells. Etoposide treatment of HeLa or K562 cells resulted in slower induction of apoptosis and eIF4GI cleavage, yet the kinetics of cleavage of eIF2α were nearly equivalent. In both cases, translation rates declined as cleavage of eIF2α proceeded. In particular, 80% of eIF4GI was still intact in K562 cells after 16 h treatment, yet translation rates had declined to 50%. Since studies with poliovirus-infected cells have shown that translation rates can still proceed at near 70–80% of intact eIF2α, this implies that additional events are occurring in apoptosis to inhibit translation. At this same 16-h time point, eIF2α was 32% cleaved, suggesting a possible contribution to the translation inhibition phenotype. Interestingly, by 24 h, translation was still occurring in etoposide-treated cells even though 70–80% of eIF2α had been processed. This may reflect the

RESULTS

Cleavage of eIF2α during Apoptosis—In order to identify possible target proteins that could contribute to translation inhibition during cell death, apoptosis was induced in multiple cell lines using a variety of apoptosis-inducing agents. We started with a system in which we have previously extensively characterized cleavage of eIF4GI to determine if other factors are also modified. Thus, HeLa cells were treated with 100 μM cisplatin and aliquots were harvested over a 24-h time period as indicated (Fig. 1A). Fig. 1A shows the eIF2α protein at 0 h with a molecular mass of approximately 38 kDa, at which time cisplatin (100 μM) was added to the HeLa cell culture. Subsequent lanes show that cisplatin treatment of HeLa cells led to cleavage of eIF2α starting between 6 and 7 h post-induction, near registered completion at 24 h and generated a cleavage fragment of about 36 kDa in size. The cleavage of eIF2α appears to initiate later than cleavage of the well defined caspase 3 substrate PARP as shown inFig. 1B and eIF4GI as shown in Fig. 1C. Generation of the characteristic 85-kDa caspase 3-derived PARP cleavage product and eIF4GI cleavage products can be detected as early as 4 h post-induction. We noted that the eIF2α cleavage fragment is barely detectable at 24 h post-induction. Two factors are likely to contribute to the poor detection of the eIF2α cleavage product: (i) we have noted that the anti-eIF2α monoclonal antibody has a lower affinity for the cleavage fragment of eIF2α as compared with intact eIF2α; and (ii) the stability of the cleaved eIF2α subunit appears to be lower than the intact protein in vivo. In addition, treatment of HeLa cells with etoposide, cycloheximide, or tumor necrosis factor-α (TNF-α), K562 cells with cisplatin, etoposide, cycloheximide, or MG132, or Jurkat T cells with cisplatin, etoposide, cycloheximide, MG123, or TNF-α all resulted in the generation of a similar 36-kDa cleavage product (data not shown), indicating that this type of response can be generated in a wide variety of cell types using different inducers of apoptosis.
observation (see Fig. 9 below) that eIF2 complex containing cleaved eIF2α rapidly exchanges GDP, unlike its unprocessed precursor. Despite testing several combinations of cell types and apoptosis inducers, we have not discovered conditions where eIF2α cleavage precedes or occurs independently of eIF4GI cleavage. In summary, these data reveal that cleavage of eIF4GI and eIF2α often occur simultaneously, cleavage rates are variable depending upon the apoptosis inducer and cell type used, and that the mechanism of translation inhibition may be multifactorial, involving simultaneous modification of more than one translation factor.

Inhibition of eIF2α Cleavage by Z-VAD-fmk—To explore whether caspase activation is required for the cleavage of eIF2α, we performed in vivo inhibition experiments using the broad spectrum cell-permeable caspase inhibitor Z-VAD-fmk. Cell cultures were preincubated with 75 μM Z-VAD-fmk, followed by treatment with apoptosis inducers for 16 h. Analysis of cell lysates by immunoblot (Fig. 3A) show that eIF2α cleavage was completely inhibited in HeLa cells pretreated with Z-VAD-fmk regardless of the inducing agent used. The observed inhibition of cleavage was concomitant with a reduction in overall caspase activation as measured by the decrease in release of pNA from the caspase substrate Ac-DEVD-pNA (Fig. 3B). Release of pNA was analyzed by optical density at 405 nm, and caspase activity is displayed as nanomoles of pNA released per hour per total milligram of protein. C, control; CP, cisplatin; Etop, etoposide.

FIG. 2. Cleavage of eIF2α in etoposide-treated HeLa and K562 cells. HeLa or K562 cells were treated with 50 μM etoposide and incubated for the indicated time periods followed by a 30-min pulse label using [35S]methionine at 37°C. A, to determine protein synthesis levels, cell lysates were analyzed by autoradiography and actin protein bands were quantitated for each time point by densitometry. The amount of protein synthesis was plotted as percentage of control levels. B, the amount of eIF4GI cleavage was determined by quantification of intact eIF4GI protein from immunoblots for each time point, with time 0 set at 100%. C, the amount of eIF2α cleavage was assessed by quantification of intact eIF2α protein on immunoblots for each time point, with time point 0 set as 100%. Quantification of protein bands was performed using NIH image software. Triangle, cisplatin-treated HeLa cells (as described in Fig. 1); square, etoposide-treated HeLa cells; circle, etoposide-treated K562 cells.

FIG. 3. In vivo inhibition of eIF2α cleavage by Z-VAD-fmk. A, inhibition of eIF2α cleavage in HeLa cells. HeLa cells were preincubated with 75 μM Z-VAD-fmk (indicated by +) for 1 h at 37°C before addition of cisplatin (100 μM), etoposide (50 μM), TNF-α (40 ng/ml) plus cycloheximide (10 μg/ml). Cells were treated for 15 h, lysed, and analyzed by immunoblot as described for Fig. 1. Migration of molecular weight marker protein, and eIF2α and eIF2α cleavage products are indicated on the left and right, respectively. B, in vivo inhibition of caspase activity. Cell lysates derived from the experiment described in panel A were incubated in the presence of 0.2 mM Ac-DEVD-pNA for 2 h at 37°C. Release of pNA was analyzed by optical density at 405 nm, and caspase activity is displayed as nanomoles of pNA released per hour per total milligram of protein. C, control; CP, cisplatin; Etop, etoposide.
rectly involved in the induction or catalysis of eIF2α cleavage.

**In Vitro Cleavage of eIF2α by Caspases**—To identify caspases that could be responsible for the observed eIF2α cleavage *in vivo*, we screened a panel of purified recombinant human caspases for their ability to cleave eIF2α *in vitro*. All purified caspases were pre-standardized for equivalent levels of enzymatic activity before incubation with protein substrates. Fig. 4A shows cleavage of eIF2α occurred when HeLa cell ribosomal salt wash (crude initiation factor preparation containing eIF2) was incubated with caspase 3 and 6, and to a lesser extent with caspase 8 and 10, but not with caspase 9. Comparison of these results with *in vivo* studies shown in Figs. 1 and 3 reveals that the caspases generate an identical eIF2α cleavage fragment, further supporting our hypothesis that caspases are indeed responsible for eIF2α cleavage. In this experiment caspases 3, 6, or 10 could indirectly induce eIF2α cleavage via activation of other proteases present in the HeLa ribosomal salt wash, which subsequently could use eIF2α as a substrate. To address this question, we incubated highly purified rabbit eIF2 with purified caspases to see if the observed cleavage in Fig. 4A could be generated directly by caspases. Purified eIF2α was cleaved by caspase 3 (Fig. 4B) whereas incubation with caspase 6, 8, and 10 resulted in only minor or undetectable cleavage of eIF2α. These results might indicate that cleavage of eIF2α by caspases 6 and 10 in panel A could have been enhanced by activation of other caspases (e.g. caspase 3) in the lysate. Levels of cleavage obtained with caspase 3 over several experiments were significantly greater than the low, somewhat variable cleavage obtained with other caspases, implying that eIF2α is a better substrate for caspase 3 than caspases 6 and 10. However, it is possible that purified eIF2 might not be an appropriate substrate for caspases 6 and 10, instead may function as a more efficient substrate when it is bound within a higher order translation complex or require other unknown cofactors for efficient cleavage of eIF2α. Furthermore, in this initial experiment, low amounts (2 units) of caspase units were used to cleave eIF2α, which also may explain the low level of eIF2α cleavage. However, further experiments, in which higher levels (17 units) of caspases were used in cleavage assays, indicated that the amount of cleavage increased greatly only with caspase 3 but not with the other caspases tested (see Fig. 7 below). Overall, these results indicate that eIF2α can serve as a substrate for caspases and is cleaved by the effector caspase 3 more efficiently than initiator caspases (caspases 8, 9, and 10).

**Direct Cleavage of eIF2α by Caspase 3**—Based on the results from Fig. 4 we further explored the caspase 3-induced cleavage of eIF2α *in vitro*. First, we performed a time course to analyze the kinetics of the eIF2α cleavage reaction. Therefore, purified caspase 3 was incubated with purified eIF2α at 37 °C, aliquots were taken at the indicated time points, and analyzed by immunoblot (Fig. 5A). Significant cleavage of eIF2α occurred within the first hour followed by complete cleavage of eIF2α between 5 and 18 h of incubation. To rule out the possibility that other contaminant proteases in the purified preparations could be responsible for the eIF2α cleavage, purified eIF2α and purified caspase 3 were incubated in the presence of specific caspase 3 inhibitor Ac-DEVD-CHO (Fig. 5B). Increasing concentrations of the inhibitor coincided with a decrease in the amount of eIF2α cleavage product and as little as 1 μM of the inhibitor was sufficient to completely block the cleavage of eIF2α. Overall, these results indicate that caspase 3 is responsible for the observed cleavage of eIF2α.

**Characterization of the Caspase 3 Cleavage Site on eIF2α**—Next we wished to determine the cleavage site on eIF2α utilized by caspase 3. Therefore, we performed an *in vitro* cleavage assay as described for Fig. 5A, transferred the proteins to polyvinylidene difluoride, and subjected the 36-kDa fragment to amino acid sequencing. The sequencing results showed that the N-terminal sequence of the 36-kDa fragment matched 9 of 10 amino acids in the authentic N-terminal amino acid sequence of intact eIF2α (Fig. 6A), strongly suggesting that the caspase 3 cleavage site is located in the C-terminal portion of the eIF2α protein. Close examination of the amino acid se-
Caspase 3-mediated Cleavage of eIF2α during Apoptosis

Fig. 6. Amino acid sequencing of the eIF2α cleavage product. A, comparison of eIF2α amino acid sequences (residues 1–10) and sequences derived from microsequencing of the eIF2αcp. Vertical bars indicate identical residues. Microsequencing was performed at the Protein Chemistry Core Facility, Baylor College of Medicine. B, proposed caspase 3 cleavage sites on eIF2α. The C-terminal sequence of the eIF2α protein is shown. Classic caspase 3 cleavage site (DXXD) and an alternate site are indicated in bold; arrows indicate putative cleavage sites.

Fig. 7. Comparison of cleavage of nonphosphorylated versus phosphorylated eIF2α by caspases. Purified caspases (17 units) were incubated with either purified eIF2A (A) or purified eIF2αB (B) for 18 h at 37 °C. Both eIF2α and eIF2αB were phosphorylated by preincubation with HRI as described under “Experimental Procedures” and are indicated by P on the top of the figure. Samples were analyzed as described in the legend to Fig. 4. Molecular weight markers, eIF2α and eIF2αB cleavage products are indicated on the left and right, respectively.

Fig. 8. Effect of phosphorylation and GDP on the cleavage of eIF2α in the eIF2 versus the eIF2αB complex. Unphosphorylated purified eIF2 (A), partially phosphorylated purified eIF2α (B), or eIF2αB (C) complex were incubated in the presence (+) or absence (−) of 50 μM GDP for 10 min at 37 °C, followed by a further incubation for 5 min after the addition of 3 mM Mg(OAc)2. Samples, treated (+) or untreated (−) with caspase 3 (18 units) were then incubated for 3 h at 37 °C under the conditions described for cleavage of eIF2 for guanine nucleotide exchange assays under “Experimental Procedures.” Samples were then separated by SDS-PAGE (A) or on slab gels by vertical isoelectric focusing (B and C) and eIF2α was detected by immunoblotting. Migration of unphosphorylated eIF2α (eIF2α), phosphorylated eIF2α (eIF2α(P)), unphosphorylated eIF2α cleavage product (eIF2αcp), and phosphorylated eIF2α cleavage product (eIF2αPcp) are indicated on the right. A second phosphorylated form of eIF2α that has previously been reported to exist by Maurides et al. (21) is also visible in part B of the figure.

Mg2+ in vitro, eIF2 can assume the conformation from which bound GDP freely dissociates in the absence of eIF2B, indicating that Mg2+ is required to freeze the conformation of the eIF2-GDP complex.

To determine whether the conformational state of eIF2 affects the ability of eIF2 to serve as a substrate for caspase 3, we incubated purified eIF2 with GDP for 10 min at 30 °C followed by addition of magnesium to “lock” the eIF2-GDP in its inactive conformation. Surprisingly, when this substrate was tested, cleavage of eIF2α by caspase 3 was found to be severely inhibited compared with cleavage of eIF2α (Fig. 8A). This observation could not be ascribed to a possible inhibitory effect of magnesium on caspase 3 activity since we found no such effect on Ac-DEVD-pNA cleavage activity by caspase 3 under similar conditions with the same concentration of magnesium (data not shown). Also, addition of magnesium to eIF2 and eIF2αB had
The ability of caspase 3 to cleave eIF2\(\alpha\) affects the ability of caspase 3 to cleave eIF2\(\alpha\). In contrast, the cleavage observed upon eIF2\(\alpha\) phosphorylation in Fig. 7A is most likely due to the fact that GDP remains bound to a small fraction of purified eIF2\(\alpha\) (26). As such, the Mg\(^{2+}\) added to the kinase reaction would stabilize the binding of this GDP to eIF2 and inhibit its cleavage by caspases.

To determine whether the phosphorylation state of the \(\alpha\)-subunit of eIF2 affects the ability of caspase 3 to cleave eIF2 containing bound GDP, purified fractions of eIF2 or eIF2\(\alpha\) complex whose \(\alpha\)-subunit was 40–50% phosphorylated were preincubated in the presence or absence of GDP (Fig. 8, B and C). The ability of caspase 3 to cleave eIF2\(\alpha\) was then examined after addition of Mg\(^{2+}\). Samples were separated on slab gels by vertical isoelectric focusing to separate full-length and cleaved fragments of unphosphorylated and phosphorylated eIF2\(\alpha\). Immunoblot analysis indicated that the presence of eIF2-bound GDP markedly inhibited the cleavage of both phosphorylated and unphosphorylated eIF2\(\alpha\) by caspase 3. In contrast, the presence of GDP only marginally inhibited the cleavage of phosphorylated or unphosphorylated eIF2\(\alpha\) present in the eIF2\(2B\)-2B complex.

**Effect of Caspase 3-induced Cleavage of eIF2\(\alpha\) on eIF2 Activity**—To initially address the effect of eIF2\(\alpha\) cleavage on the function of eIF2, we determined the effect of caspase 3 on the ability of eIF2 to bind and exchange GDP. eIF2 was incubated in the presence or absence of caspase 3 for 3 h at 37 °C. Samples were then incubated with \(^{3}H\)GDP for 10 min followed by the addition of 1 mM Mg\(^{2+}\). Binding of an aliquot of each sample to nitrocellulose filters indicated that cleavage of eIF2\(\alpha\) by caspase 3 had no effect on the ability of eIF2 to bind GDP (data not shown). Excess unlabeled GDP was then added to the samples and the extent of guanine nucleotide exchange was measured at the times indicated in Fig. 9. SDS-PAGE and immunoblot analysis of aliquots of each sample taken at the end of the incubation verified that the eIF2\(\alpha\) had been quantitatively cleaved by caspase 3 (data not shown). As previously reported, bound \(^{3}H\)GDP was chased from eIF2 at slow rate. In contrast, after cleavage of eIF2\(\alpha\) by caspase 3 over 90% of the eIF2-bound \(^{3}H\)GDP was chased in 15 min. Thus, exchange of eIF2 bound GDP was no longer dependent upon eIF2B after cleavage of eIF2\(\alpha\) by caspase 3.

To further address the effect of eIF2\(\alpha\) cleavage on the activity of the eIF2 complex we took advantage of a well defined characteristic of the 5'-UTR of FMDV RNA. FMDV 5'-UTR initiates translation via a cap-independent mechanism mediated by a large RNA structure called the internal ribosome entry site (IRES). The 3' margin of this structure possesses two initiation codons in tandem which lead to initiation of two forms of the leader protease of the virus (27). The selection of the initiation codon in mRNAs containing the FMDV 5'-UTR has been shown to be influenced by the activity of eIF2 and the amount of ternary complex eIF2Met-tRNA\_GTP (23, 28) in a mechanism that may involve direct binding of eIF2 to the viral IRES RNA element (29). In previous studies, it was shown that the addition of eIF2 to in vitro translation lysates programmed with reporter mRNAs containing the FMDV 5'-UTR resulted in an increase in initiation from the upstream AUG (23).

Here, we used the same system to test whether caspase-cleaved eIF2\(\alpha\) could still function in modulating initiation site selection. We used mRNA transcripts containing the CAT gene downstream of either the Xenopus globin 5'-UTR (globin-CAT) or the FMDV 5'-UTR (FMDV-CAT), which was fused at the second, downstream AUG to CAT. Untreated, intact eIF2, and caspase 3-treated eIF2 were added to in vitro translation assays programmed with these transcripts and their effects on translation were assessed by SDS-PAGE autoradiography (Fig. 10A). As previously reported (23), translation of FMDV-CAT RNA yielded the normal size CAT protein (which comigrated with CAT produced using the globin 5'-UTR, Fig. 10, lane c) which initiated from the downstream AUG, and a larger product (preCAT), which initiated from the upstream AUG (lane a).

Addition of intact eIF2 resulted in a slight stimulation in overall translation as expected (Fig. 10B) and a significant increase in translation (34%, \(p < 0.006\)) from the upstream AUG which was evidenced by the increase in the ratio of preCAT:CAT (lane f); also in good agreement with previous studies (23). In contrast, addition of caspase 3-treated eIF2 did not stimulate translation in general or specifically increase translation from the upstream AUG (lane e). Immunoblot analysis of caspase 3-treated eIF2 showed that eIF2 was completely cleaved before addition to translation lysates (data not shown). This loss of eIF2 function in IRES-AUG selection was completely attributed to the addition of cleaved eIF2\(\alpha\) rather than caspase 3 itself since caspase 3 activity (lane d) was completely abolished by the caspase inhibitor Ac-DEVDFCHO (lane c) under the conditions used in this assay. Last, addition of 2-aminopurine, an inhibitor of eIF2\(\alpha\) kinases (30), which prevents the phosphorylation of eIF2, caused an expected enhancement of the activity of endogenous eIF2 and translation of pre-CAT (lane g) in a similar fashion to addition of intact eIF2 (lane f). Fig. 10B shows averaged translation levels of pre-CAT and CAT generated by the FMDV IRES as well as CAT translated using a globin 5'-UTR. These data reveal that addition of eIF2 modestly and reproducibly stimulated translation of CAT from both the FMDV IRES (10.8%, \(p < 0.02\)) and the globin 5'-UTR (10.3%, \(p < 0.01\)) when compared with buffer controls. A similar, slightly larger stimulation in translation was observed when 2-aminopurine was added as a positive control. Larger levels of translation stimulation from addition of eIF2 or
2-aminopurine were not anticipated since the translation lysate contained active endogenous eIF2. In contrast, eIF2 which had been cleaved with caspase reproducibly failed to stimulate translation of either mRNA used in this assay. Furthermore, the cleaved eIF2 did not inhibit translation levels, suggesting that this form of eIF2 does not function in a dominant negative manner in this assay. Importantly, however, both cap-dependent (globin-CAT) and cap-independent (FMDV-CAT) translation reactions were carried out as described under “Experimental Procedures” using FMDV-CAT (lanes a-g) or globin-CAT (lane h). Components added to the reactions are as follows: lanes a and h, buffer control; lane b, Ac-DEVD-CHO (10 μM); lane c, caspase 3 plus Ac-DEVD-CHO (10 μM); lane d, caspase 3; lane e, 200 ng of eIF2; lane f, caspase 3-treated eIF2 (200 ng) plus Ac-DEVD-CHO (10 μM); lane g, 2-aminopurine (5 mM). Migration of CAT and pre-CAT proteins are indicated on the left side and the ratio of pre-CAT to CAT as determined by PhosphorImager analysis is shown below lanes a-g. B, caspase-treated eIF2 does not stimulate cap-dependent or cap-independent translation. Results from four independent experiments similar to panel A were quantified, then results were averaged and standard deviations (error bars) determined. Relative translation levels are represented as arbitrary units (AU). Treatment groups correspond to those in panel A, lanes a–g. Abbreviations: I, caspase inhibitor (AcDEVD-CHO); C, caspase 3; 2AP, 2-aminopurine.

**DISCUSSION**

In this report, we demonstrate that the α-subunit of eIF2 is a target for cleavage by caspases upon induction of apoptosis in cultured cells. The cleavage of eIF2α was induced in HeLa and K562 cells, but also Jurkat T cells (data not shown) by cisplatin, etoposide, TNF-α, or MG132 (data not shown). Thus, the cleavage of eIF2α does occur generally in apoptotic cells, however, the possible contribution of eIF2α cleavage to translation inhibition during apoptosis may vary dependent upon the cell type and apoptotic stimulus utilized. For instance, eIF2α cleavage may play a more important role in translation inhibition or modulation in etoposide-treated cells than cisplatin-treated HeLa cells. In fact, we expect that the drastic inhibition of translation in apoptosis results from a multifactorial process involving modification of several initiation factors. To this end, we have also documented cleavage of eIF4GII and poly(A)-binding protein in apoptotic cells, also with variable kinetics. Further experiments with cleavage-resistant mutants of eIF4G1 and eIF2α and other factors will need to be performed to clarify the role and impact of each cleavage event in the process of translation inhibition.

Although eIF2α could be cleaved in vitro by several of the caspasas tested, caspase 3 appears to be the prime candidate for the observed cleavage in vivo. Compared with caspase 3, cleavage of eIF2α by caspase 6, 8, and 10 was inefficient. In addition, only caspase 3 cleaved eIF2α that was present in the eIF2-2B complex. Since eIF2 is a heterotrimeric complex we tested cleavage of other subunits of eIF2. Although preliminary experiments show possible cleavage of eIF2β (but not eIF2γ) in vitro, we do not have evidence that this occurs in vivo.

The data suggest that the eIF2 present in the eIF2-2B complex is the preferred target for caspase 3 cleavage in vivo. Caspase 3 efficiently cleaved the α-subunit of eIF2 in vitro only when eIF2 was in a conformation from which GDP could readily dissociate (i.e., eIF2 in the absence of Mg2+; or eIF2α present in the eIF2-2B complex). In the absence of Mg2+, phosphorylation of the eIF2α present in eIF2 or the eIF2-2B complex had little or no effect on its ability to be cleaved by caspase 3. However, in the presence of physiological Mg2+ concentrations, which locks the conformation of eIF2 complexes containing GDP, cleavage of eIF2α present in eIF2-GDP and eIF2α-GTP/GDP complexes by caspase 3 is severely, if not totally inhibited in vitro. Therefore, the eIF2-GDP complex generated at the last step of initiation of translation is not likely to be a physiologically relevant substrate for caspase 3 in vivo. On the other hand, the presence of Mg2+ and GDP had relatively little affect on the ability of caspase 3 to cleave either unphosphorylated or phosphorylated eIF2α present in the eIF2-2B complex. Thus, while we cannot currently rule out eIF2α present in the eIF2-GTP-Met-tRNAi complex as a potential target for cleavage by caspase 3, our data firmly establishes the eIF2α present in the eIF2-2B complex as a target for caspase cleavage in vivo.

eIF2α is the second initiation factor we have identified to be cleaved during apoptosis and that potentially contributes to alterations in translation or inhibition of translation in apoptotic cells. eIF2α is a G-protein, which is known to be a critical factor in translation initiation. In its GTP-bound conformation, eIF2α binds Met-tRNAi to the 40 S ribosomal subunit to form a 43 S preinitiation complex and aids in the recognition of the initiation codon (1). In its GDP-bound conformation, eIF2α is inactive. Recycling of eIF2-GDP requires its interaction with the guanine nucleotide exchange factor, eIF2B. As discussed in the Introduction, the activity of eIF2 and eIF2B is regulated by phosphorylation of the α-subunit of eIF2 by several kinases (e.g., PKR and HR1) in response to certain stimuli which leads to global inhibition of translation. Our data indicate that cleavage of eIF2α alters the activity of the eIF2 complex. Cleavage of eIF2α: (i) generated an eIF2 complex from which GDP can...
dissociate independent of eIF2B; (ii) eliminated the ability of eIF2 to stimulate translation from upstream AUG codons; and (iii) inhibited the ability of the eIF2 complex to stimulate translation in vitro. Even though our data indicate that eIF2a cleavage followed or was coincident with eIF4GI cleavage in cultured cells, it is possible that the alterations in eIF2a activity induced by eIF2a cleavage contribute significantly to the inhibition of protein synthesis at later stages in apoptotic cells. Furthermore, it is known that phosphorylation of as little as 25% of all eIF2 is sufficient to cause inhibition of protein synthesis through the ability of phosphorylated eIF2a to sequester eIF2B. Since the eIF2a present in the eIF2a-B complex has been identified as the likely target for cleavage in vivo, it is possible that cleavage of only a small portion of eIF2a suffices to alter translation.

Based on the results presented in this report, we postulate that caspase 3-induced removal of the C-terminal portion of eIF2a could contribute to the regulation of translation in apoptotic cells through three potential mechanisms which may combine to provide a complex phenotype. (i) The cleaved form of eIF2a which exchanges GDP may partly counteract PKR-driven translation inhibition after partial caspase activation occurs. (ii) Large levels of cleaved eIF2a in apoptotic cells might mimic the effect of eIF2a phosphorylation and contribute to a general block in translation. (iii) Cleavage of eIF2a by caspase 3 might result in an eIF2a complex similar to that described for yeast, a complex capable of inhibiting protein synthesis, and perhaps, causing a specific up or down-regulation of certain apoptosis-related genes containing ORFs in their 5′-UTR. Those yeast studies indicated that removal of the short, highly acidic C-terminal region of eIF2a had the same regulatory effect on GCN4 mRNA translation as phosphorylation of eIF2a (31). Phosphorylation of eIF2a in yeast stimulates the translation of GCN4 mRNA by slowing the recycling of eIF2-GDP by eIF2B. Reduction in the rate of eIF2 regulates translation initiation of translation of GCN4 mRNA at the authentic downstream AUG start codon by eliminating the inhibitory effect of upstream ORFs in the 5′-UTR of GCN4 mRNA (32).

Could cleavage of eIF2a contribute to regulation of apoptosis? Conceivably, cleavage of eIF2a could result in translation inhibition that potentially contributes to the induction of apoptosis by suppressing anti-apoptotic genes that normally block apoptosis. Indeed, several reports have shown that treatment of cells with the protein synthesis inhibitor cycloheximide does induce apoptosis by itself, indicating that blocking protein synthesis can lead directly to cell death (33–35). However, several other publications showed that cycloheximide prevented apoptosis, so the necessity of protein synthesis appears to be dependent on cell type and trigger of apoptosis (36–38). While extensive phosphorylation of eIF2α by an eIF2α kinase, such as PKR, could lead to a complete global arrest of translation which induces apoptosis in many cell types, cleavage of eIF2a by caspase 3 would yield eIF2a from which GDP can dissociate independent of active eIF2B and potentially relieve global arrest during early stages. Alternatively, cleaved eIF2a might function similar to the yeast GCN4 system (32) to enhance the translation of mRNAs coding for pro-apoptotic proteins that contain upstream ORFs in their 5′-UTRs as postulated above, thus contributing to the completion of the apoptotic process.

PKR itself has been shown to induce apoptosis in several systems (39–41) whereas mutant forms of PKR have been implicated in malignant transformation (13). Srivastava et al. (12) showed that during TNF-induced cell death levels of PKR activity were increased and concomitantly levels of phosphorylated eIF2α were increased as well. Expression of a non-phosphorylatable form of eIF2α, S51A, partially protected against TNF-induced apoptosis, suggesting that PKR is required for and mediates stress-induced apoptosis through eIF2α phosphorylation. Other studies showed that increased PKR activity leads to transcriptional and translational up-regulation of Fas and Bax, two pro-apoptotic proteins (42). The mRNAs for both Fas and Bax have upstream ORFs in their 5′-UTRs which could account for translational up-regulation through a eIF2α phosphorylation-mediated mechanism similar to the regulation of yeast GCN4 mRNA (31, 32). Finally, while this article was in review, another group (43) has also shown that eIF2α is cleaved in apoptosis, generating results similar to those herein. In that study it was noted that cleaved eIF2α was able to repress PKR-mediated suppression of translation, but no mechanistic explanation for that observation was provided (43). It is likely that the enhanced ability of cleaved eIF2α to exchange GDP reported here helps counteract this effect of PKR. Much more investigation is required to unravel these complexities and to fully discern the role of eIF2a cleavage in apoptosis.

Acknowledgment—We thank Alike van der Velde for construction of plasmids.

REFERENCES

1. Donahue, T. F., Cigan, A. M., Pabich, E. K., and Valavicius, B. C. (1988) Cell 54, 621–632.
2. Pain, V. M. (1996) Eur. J. Biochem. 236, 747–771.
3. Meurs, E. F., Chong, K., Galabru, J., Thomas, N. S., Kerr, I. M., Williams, B. R., and Hovanessian, A. G. (1990) Cell 62, 379–390.
4. Samuel, C. E. (1993) J. Biol. Chem. 268, 7663–7666.
5. Chen, J. J., and London, I. M. (1995) Trends Biochem. Sci. 20, 105–108.
6. Chen, J.-J. (1993) in Translational Regulation of Gene Expression 2 (Han, J., ed) pp. 349–372, Plenum Press, New York.
7. Dever, T. E., Feng, L., Wek, R. C., Cigan, A. M., Donahue, T. F., and Hinnebusch, A. G. (1992) Cell 68, 555–596.
8. Merrick, W. C., and Hershey, J. W. B. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 31–69, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
9. Marisens, W. E., and Lloyd, R. E. (1986) Mol. Cell Biol. 18, 7565–7574.
10. Clemens, M. J., Bushell, M., and Murray, S. J. (1998) Oncogene 17, 2921–2931.
11. Etchison, D., Hansen, J., Ehrenfeld, E., Edery, L., Sonenberg, N., Milburn, S., and Hershey, J. W. B. (1984) J. Virol. 51, 832–837.
12. Srivastava, S. P., Kumar, R. U., and Kaufman, R. J. (1998) J. Biol. Chem. 273, 2416–2423.
13. Koromilas, A. E., Roy, S., Barber, G. N., Katz, M. G., and Sonenberg, N. (1992) Science 257, 1665–1669.
14. Meurs, E. F., Galabru, J., Barber, G. N., Katz, M. G., and Hovanessian, A. G. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 232–236.
15. Datta, B., and Datta, R. (1999) Exp. Cell Res. 246, 376–383.
16. Andrews, N. C., Levin, D., and Baltimore, D. (1985) J. Biol. Chem. 260, 7628–7635.
17. Matts, R. L., Levin, D. H., and London, I. M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2559–2563.
18. Trachsel, H., Ranu, R. S., and London, I. M. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3654–3658.
19. Oldfield, S., and Proud, G. C. (1992) Eur. J. Biochem. 208, 73–81.
20. Scorrano, K. A., Panniers, R., Howland, A. G., and Henschel, E. C. (1987) J. Biol. Chem. 262, 14538–14543.
21. Maurides, P. A., Akkaraju, G. R., and Jagus, R. (1989) Anal. Biochem. 183, 154–151.
22. Benton, P. A., Barrett, D. J., Matts, R. L., and Lloyd, R. E. (1996) J. Virol. 70, 5525–5532.
23. Thomas, A. M., Rijnbrand, R., and Voorma, H. O. (1996) J. Gen. Virol. 77, 265–272.
24. Benton, P. A., Murphy, J. W., and Lloyd, R. E. (1995) Virology 213, 7–18.
25. Siekiera, J., Manne, V., and Ochoa, S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 352–356.
26. Siekiera, J., Manne, V., Mauser, L., and Ochoa, S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1225–1235.
27. Belshaw, G. J. (1992) EMBO J. 11, 1105–1110.
28. Dasso, M. C., Milburn, S. C., Hershey, J. W., and Jackson, R. J. (1999) Eur. J. Biochem. 267, 371–378.
29. He, A. W., and Cory, J. G. (1999) Anticancer Res. 19, 421–428.
30. Tang, D., Lahti, J. M., Grenet, J., and Kidd, V. J. (1999) J. Biol. Chem. 274, 7245–7252.
31. Abo, Y. H., Kim, Y. H., Hong, S. H., and Koh, Y. J. (1998) Exp. Neurol. 154, 32.
Caspase 3-mediated Cleavage of eIF2α during Apoptosis

47–56
37. Coxon, F. P., Benford, H. L., Russell, R. G., and Rogers, M. J. (1998) Mol. Pharmacol. 54, 631–638
38. Goering, P. L., Thomas, D., Rojko, J. L., and Lucas, A. D. (1999) Toxicol. Lett. 105, 183–195
39. Der, S. D., Yang, Y., Weissman, C., and Williams, B. R. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3279–3283
40. Lee, S. B., Rodriguez, D., Rodriguez, J. R., and Esteban, M. (1997) Virology 231, 81–88
41. Lee, S. B., and Esteban, M. (1994) Virology 199, 491–496
42. Balachandran, S., Kim, C. N., Yeh, W. C., Mak, T. W., Bhalla, K., and Barber, G. N. (1998) EMBO J. 17, 6888–6902
43. Satoh, S., Hijikata, M., Handa, H., and Shimotohno, K. (1999) Biochem. J. 342, 65–70
Identification of Caspase 3-mediated Cleavage and Functional Alteration of Eukaryotic Initiation Factor 2 α in Apoptosis
Wilfred E. Marissen, Yanwen Guo, Adri A. M. Thomas, Robert L. Matts and Richard E. Lloyd

J. Biol. Chem. 2000, 275:9314-9323.
doi: 10.1074/jbc.275.13.9314

Access the most updated version of this article at http://www.jbc.org/content/275/13/9314

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 20 of which can be accessed free at http://www.jbc.org/content/275/13/9314.full.html#ref-list-1