Inhibition of Protein Synthesis in Apoptosis: Differential Requirements by the Tumor Necrosis Factor α Family and a DNA-damaging Agent for Caspasases and the Double-stranded RNA-dependent Protein Kinase

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ABSTRACT

Exposure of mammalian cells to agents that induce apoptosis results in a rapid and substantial inhibition of protein synthesis. In MCF-7 breast cancer cells, tumor necrosis factor α (TNFα) and TNF-related apoptosis-inducing ligand inhibit overall translation by a mechanism that requires caspase (but not necessarily caspase-3) activity. This inhibition is associated with the increased phosphorylation of eukaryotic initiation factor (eIF2) α, increased association of eIF4E with the inhibitory eIF4E-binding protein (4E-BP1), and specific cleavages of eIF4B and eIF2α. All of these changes require caspase activity. The cleavage of eIF4GI, which specifically needs caspase-3 activity, is dispensable for the inhibition of translation in MCF-7 cells. Similar experiments with embryonic fibroblasts from control mice and animals defective for expression of the double-stranded RNA-regulated protein kinase (PKR) reveal requirements for both caspase activity and PKR for inhibition of protein synthesis in response to TNFα. In contrast, treatment of cells with the DNA-damaging agent etoposide inhibits protein synthesis equally well in the presence of a pan-specific caspase inhibitor and in the presence or absence of PKR. Surprisingly, the ability of etoposide to cause increased association of eIF4E with 4E-BP1 does require PKR activity. However, our data suggest that neither increased phosphorylation of eIF2α nor increased [eIF4E.4E-BP1] complex formation is essential for the inhibition of overall translation by the DNA-damaging agent.

INTRODUCTION

Protein synthesis in mammalian cells is subject to rapid regulation after exposure of cells to a wide variety of growth-promoting, growth-inhibitory, and stress-inducing conditions. Ablant control of translation can result in cell transformation, and changes in the expression of key initiation factors or the signaling pathways that regulate them are often observed in tumors (reviewed in Refs. 1 and 2). The initiation of protein synthesis is controlled at a number of levels in cells that are induced to undergo apoptosis after serum deprivation (3) or after treatment with TNFα (4), agonistic Fas antibodies (5), DNA-damaging agents (6, 7), or staurosporine (4, 7). Changes in the protein synthetic machinery associated with early stages of apoptosis (reviewed in Ref. 8) include increased phosphorylation of initiation factor eIF2α (5), decreased phosphorylation of 4E-BP1 and increased association of the latter with eIF4E (6, 7, 9), caspase-mediated cleavages of certain initiation factors (eIF4GI, eIF4GII, eIF4B, the j subunit of eIF3, and the α subunit of eIF2α; Refs. 3 and 9–12), and specific cleavage of the 28S rRNA component of the larger ribosomal subunit (13). However, the signal transduction pathways by which these events are activated and the relative contributions of each of these changes to the overall inhibition of translation remain to be established.

Members of the TNF family, including TNFα, TRAIL, and Fas ligand, inhibit growth and induce programmed cell death in a wide variety of target cells. Association of these ligands with their specific cell surface receptors (14) initiates a sequence of intracellular events that results in the recruitment of adapter proteins, such as TNF receptor-associated death domain protein and FADD, to form a DISC (15). TNF receptor-associated death domain protein and FADD in turn recruit procaspase-8, the proteolytic activation of which leads to the activation of effector caspases (14, 16) and is essential for subsequent apoptosis (17, 18).

In contrast to cytokine-mediated apoptosis, ionizing radiation and DNA-damaging agents, such as etoposide, exert their effects through the activation of downstream factors, such as the DNA-dependent protein kinase; the protein kinases c-Ab1, ATM, and ATR; and the tumor suppressor protein p53 (19, 20). DNA damage-dependent or p53-induced apoptosis may also involve TNF family receptors and/or components of the DISC complex (21–23), as well as changes in mitochondrial function, and the patterns of caspase activation and protein cleavages are very similar to those seen after exposure of cells to members of the TNF family (21).

The requirement for caspase activation in the process of cell death varies with different stimuli, and caspase-independent mechanisms can also cause loss of viability (24–26). Consistent with this, the inhibition of protein synthesis resulting from exposure of cells to inducers of apoptosis is prevented by caspase inhibitors in some cases but not others (8).

Recent studies have identified some of the signaling pathways by which protein synthesis may be regulated by cellular stresses and conditions that induce cell death. The cleavage of initiation factor eIF4GI shows a specific requirement for caspase-3 (4). In addition, important roles have been suggested for the protein kinase PKR, which phosphorylates polypeptide chain initiation factor eIF2α (27, 28), for the rapamycin-sensitive protein kinase mTOR, which is involved in the phosphorylation of the 4E-BPs (29), and for stress-activated protein kinases, which activate the phosphorylation of eIF4E itself (29). Previous work has shown that cells that are deficient in PKR expression or that contain a dominant-negative form of PKR are more resistant than control cells to antiapoptotic effects of TNFα and TNF-related apoptosis-inducing ligand (20). However, the regulation of protein synthesis itself has not been investigated previously in these systems. Changes in the activity of mTOR rather than PKR have been suggested to be important for the inhibition of transla-
tion in response to DNA-damaging agents (6). Thus, although we know a great deal both about the mechanisms of induction of apoptosis and the changes in the translational machinery associated with the early stages of programmed cell death, little is understood concerning how the two series of events are linked mechanistically. With this in mind, we have begun to dissect the requirements for the various events associated with translational down-regulation in cells induced to undergo apoptosis by a variety of treatments. We have used genetically well-characterized MCF-7 breast cancer cell lines (33) and MEFs (34) to determine the roles of caspases and PKR in the response of protein synthesis to treatment of cells with TNFα, TRAIL, and etoposide.

MATERIALS AND METHODS

Reagents. Antiserum against the caspase cleavage product of PARP was from Promega (Southampton, United Kingdom). Monoclonal antibody to eIF2α and rabbit antiserum to eIF4G (raised against the COOH-terminal fragment of eIF4GI expressed in bacteria), eIF4E (raised against a COOH-terminal peptide), and phosphorylated eIF2α were as described previously (3, 5). Immobilon polyvinylidene difluoride was from Millipore, and human TNFα and TRAIL were from PeproTech EC Ltd. Etoposide and z-VAD.FMK were from Calbiochem, m7GTP-Sepharose was from Pharmacia-LKB, and [35S]methionine was from New England Nuclear.

Tissue Culture and Treatment with Inducers of Apoptosis. MCF-7 cells (caspase-3 deficient) and MCF-7.3.28 cells (stably transfected to express caspase-3; Ref. 33) were kindly provided by Dr. R. Jänicke (University of Singapore) and cultured as described previously (4, 33, 35). MEFs from PKR knockout (PKR−/−) and control (PKR+/+) mice were a gift from Professor C. Weissmann and were maintained in DMEM with 10% FCS and 0.1 mM 2-mercaptoethanol (34). The cell lines were treated with TNFα, TRAIL, or etoposide at concentrations of 5 ng/ml, 0.5 μg/ml, and 100 μg/ml, respectively. Cell viability was determined by trypan blue exclusion.

Measurement of Protein Synthesis. Overall protein synthesis in intact cells was measured by the incorporation of [35S]methionine into trichloroacetic acid-insoluble material. After pulse labeling with 10 μCi/ml of the radioactive amino acid (in methionine-free DMEM with 10% dialyzed FCS), cells were washed in cold PBS and dissolved in 0.3 M NaOH. The protein content was determined, and total proteins were then precipitated, washed, and analyzed by scintillation counting. Rates of protein synthesis were calculated as counts per min of radioactivity incorporated per μg of total protein.

Preparation of Cell Extracts and Analysis by Immunoblotting. Approximately 2–3 × 10⁶ cells/6-cm² dish were harvested. The medium containing any free cells was retained, and monolayers were scraped into this with 20 ml of PBS. The cells were washed in PBS twice by centrifugation in the cold, and resuspended in 200 μl of buffer A [50 mM 3-(N-morpholino)propanesulfonic acid (pH 7.4), 50 mM NaCl, 2 mM EDTA, 50 mM β-glycerophosphate, 1 mM microcystin, 2 mM benzamidine, 2 mM Na vanadate, 5 mM p-nitrophenylphosphate, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM GTP, 50 mM Na fluoride, and 7 mM 2-mercaptoethanol]. Cells were lysed by adding NP40 and Triton X-100 to final concentrations of 1.2 and 2.4% (volume for volume), respectively, and centrifuged at 10,000 × g for 10 min at 4°C. For the isolation of eIF4E, cell extracts of equal protein concentration were subjected to m7GTP-Sepharose chromatography as described previously (3, 9).

Samples containing equal amounts of protein were subjected to electrophoresis on SDS polyacrylamide gels, and the proteins were analyzed by immunoblotting using alkaline phosphatase-linked secondary antibodies with nitroblue tetrazolium as substrate (3, 9). Blots of total and phosphorylated eIF2α were analyzed by scanning densitometry using an AlphaImager (Alpha Innotech Corp.), and the relative extent of phosphorylation of the factor was calculated from the ratio between the values obtained for each pair of samples.

Analysis of Caspase-8 Activation. The extent of activation of caspase-8 in MEFs was analyzed by immunoblotting for the active (p20) fragment of this enzyme and by assay of the cleavage of the caspase-8 substrate acetyl-isoxyethyl-glutamyl-threonyl-aspartyl-p-nitroanilide by cell extracts (36).

RESULTS

TNFα- and TRAIL-induced Down-Regulation of Protein Synthesis and Concurrent Initiation Factor Modifications Require Caspase Activity. MCF-7 cells are sensitive to a number of apoptotic inducers despite being deficient in caspase-3 activity (33, 35, 37). As shown in Fig. 1A, treatment of MCF-7 cells with TNFα results in a progressive inhibition of protein synthesis, culminating in an 80–90% decrease after 24 h. No greater down-regulation is observed in MCF-7.3.28 cells stably transfected with the caspase-3 gene (33), which express pro-caspase-3 to high levels (data not shown). Treatment of the two cell lines with TRAIL has a much more rapid effect, such that by 4 h, protein synthesis is inhibited by 70–75% (Fig. 1B). Again, caspase-3 is not essential for the effect, but the presence of caspase-3 in the MCF-7.3.28 cells does accelerate the response to TRAIL. Nonetheless, it is clear from these results that the inhibition of protein synthesis by TNFα or TRAIL is not dependent on caspase-3 activity.

To determine whether other caspas may be involved, cells were exposed to TNFα or TRAIL in the presence of the broad spectrum caspase inhibitor z-VAD.FMK. Fig. 1, C and D show that z-VAD.FMK largely prevents the down-regulation of protein synthesis that is otherwise observed after 16-h treatment with TNFα or 8-h treatment with TRAIL. A similar effect was also observed with another caspase inhibitor, N-[tert-butoxycarbonyl]-Asp-fluoromethylketone (data not shown). Other proteases may also be inhibited by z-VAD.FMK, and cathepsin-B, in particular, is a target for this compound (38). However, benzoyloxycarbonyl-Phe-Ala-fluoromethylketone, which inhibits cathepsin-B but not caspases, was unable to rescue cells from the inhibitory effect of TRAIL (data not shown). Collectively, these data suggest that the inhibition of general protein synthesis brought about by proapoptotic members of the TNF family requires active caspas but not caspase-3.
specifically. The early down-regulation of protein synthesis by TNFα or TRAIL is not a consequence of a possible loss of cell viability associated with apoptosis, because more than half of the cells treated with TNFα and >90% of the cells treated with TRAIL are viable (as judged by trypan blue exclusion) at 16 and 4 h, respectively (Table 1), a time when protein synthesis had been inhibited by ≥75%.

Fig. 2A shows that there is partial cleavage of eIF4B in MCF-7 cells treated with TNFα for 16 h, whereas eIF4G remains intact, as reported previously (4). The well-characterized caspase substrate PARP is also cleaved to give rise to an M r 89,000 fragment (Fig. 2A). The eIF4B and PARP cleavages are blocked when z-VAD.FMK is included in the culture medium. Because MCF-7 cells are deficient in caspase-3, we can conclude that neither PARP cleavage nor eIF4B cleavage is dependent on this enzyme. In extracts from TNFα-treated MCF-7.3.28 cells, the eIF4GI cleavage product M-FAG was clearly visible (Fig. 2A), confirming our earlier observations (4).

We have shown previously that, in Jurkat cells, stimulation of the Fas receptor results in increased phosphorylation of the α subunit of eIF2 (5). This blocks the ability of the factor to participate in successive rounds of initiation complex formation, leading to a decrease in the rate of global protein synthesis (39). A similar phenomenon occurs in response to TNFα treatment, the level of phosphorylated eIF2α being increased by 50–80% in both MCF-7 and MCF-7.3.28 cells (Fig. 2, A and B). Interestingly, this increase is abolished when z-VAD.FMK is present. In apoptotic cells, a small fraction of eIF2α is cleaved by one or more caspases (5, 9, 10, 12), and we have also observed this, particularly for phosphorylated eIF2α, after TNFα treatment (Fig. 2A). Thus, both the phosphorylation and cleavage of eIF2α occur by mechanisms that require caspase (but not specifically caspase-3) activity under these conditions.

Protein synthesis may also be inhibited as a result of dephosphorylation of 4E-BP1, causing sequestration of eIF4E away from initiation factors and impairment of cap-dependent initiation of translation (29). Phosphorylation of eIF2a, causing sequestration of eIF4E away from the initiation complex, leading to a decrease in the rate of global protein synthesis (39). A similar change is prevented by z-VAD.FMK.

Fig. 1. Effects of TNFα and TRAIL on viability of cell lines

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<th>A. Time of treatment with TNFα (h)</th>
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<th>B. Time of treatment with TRAIL (h)</th>
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<th>MCF-7</th>
<th>MCF-7.3.28</th>
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Fig. 2. Caspase-dependent initiation factor modifications after TNFα treatment of MCF-7 cells. In A, exponentially growing MCF-7 and MCF-7.3.28 (C3+) cells (see Fig. 1) were incubated in the absence or presence of TNFα for 16 h, without or with z-VAD.FMK as indicated. Total cytoplasmic extracts were prepared as described in “Materials and Methods” and analyzed by immunoblotting for eIF4G, the Mr 89,000 caspase cleavage product of PARP, eIF4B, total eIF2α, and phosphorylated eIF2α as indicated. Bottom two panels, eIF4G and associated proteins were isolated by m7GTP-Sepharose chromatography, and the recovered eIF4G and bound 4E-BP1 were visualized by immunoblotting. In B, the levels of total eIF2α and phosphorylated eIF2α were measured by scanning densitometry, and the relative extent of phosphorylation of the initiation factor (in arbitrary units) was calculated from the ratio between the two values for each condition.
are deficient in the protein kinase. The TNFα effect in control MEFs was again sensitive to the caspase inhibitor z-VAD.FMK, suggesting roles for both PKR and caspase activity in the translational response to the cytokine. There is no significant loss of cell viability in the MEFs under these conditions of TNFα treatment (Table 1).

To determine whether the loss of sensitivity of PKR−/− cells to TNFα-induced inhibition of translation is accompanied by a lack of response of these cells to other TNF-induced signaling events, we assayed the state of phosphorylation of the stress-activated protein kinases c-Jun-NH2-terminal kinase and p38, enzymes that are known to be activated by TNFα in fibroblasts (14). As has been reported by others (41), activation of c-Jun-NH2-terminal kinase and p38 by TNFα treatment was defective in PKR−/− cells (data not shown). We also investigated whether the apical caspase, caspase-8, was activated in these cells. This enzyme associates with the DISC complex and is activated rapidly by autocatalytic cleavage in cells treated with members of the TNFα family (15). Fig. 4B shows that PKR−/− cells respond to TNFα with appearance of the active, cleaved form of caspase-8 (p20) within 15 min, although the effect is transient. A substantial but transient increase in caspase-8 activity can also be detected in extracts of these cells over a similar period of time (Fig. 4C). In contrast, neither caspase-8 cleavage nor caspase-8 activity could be detected in TNFα-treated PKR−/− cells. These data suggest that PKR is required for many of the cellular responses to activation of the TNFα receptor.

The DNA-damaging Agent Etoposide Inhibits Protein Synthesis by a PKR- and Caspase-independent Mechanism. Agents and conditions that cause DNA damage are well known as inducers of apoptosis (42), and, although there are conflicting reports as to whether TNF family receptors are involved (23, 43), the apoptotic response activates downstream pathways similar to those stimulated by TNFα and TRAIL (42). Protein synthesis is inhibited rapidly in response to exposure of cells to the DNA topoisomerase II inhibitor etoposide and other DNA-damaging agents (5, 6). Similarly, etoposide is a potent inhibitor of protein synthesis in MCF-7 cells, irrespective of whether caspase-3 is expressed in the cells (Fig. 5). Again, this is not because of any extensive loss of cell viability (data not shown). In contrast to the situation with
Etoposide-induced Association of elf4E with 4E-BP1 Requires PKR. In further contrast to the effects of TNFα and TRAIL, etoposide does not cause any consistent increase in the extent of phosphorylation of elf2α in MCF-7 cells (Fig. 6, A and C). However, MEFs do respond to etoposide with an increase in elf2α phosphorylation, and this requires the presence of PKR (Fig. 6, B and D). These findings suggest that the ability of DNA-damaging agents to activate PKR may be cell-type specific. Regardless of this, etoposide is clearly capable of acting by other mechanisms also because there is a lack of requirement for PKR for the overall inhibition of protein synthesis.

Etoposide also causes a dramatic increase in the association of elf4E with 4E-BP1 after 20 h in both MCF-7 and MCF-7.3.28 cells, with no change in the levels of extractable elf4E, and this is accompanied by a decrease in the association of elf4G with elf4E (Fig. 6A). However, [elf4E:4E-BP1] complex formation is only increased marginally at 4 h, in agreement with data reported for Swiss 3T3 cells (6). A similar late increase in binding of 4E-BP1 to elf4E occurs in etoposide-treated PKR+/− MEFs (Fig. 6B). Strikingly, however, 20 h of etoposide treatment completely fails to stimulate 4E-BP1 binding to elf4E in PKR−/− MEFs. This finding suggests a novel requirement for PKR activity in the regulation of elf4E function.

DISCUSSION

Members of the TNF family are well-known inducers of cellular growth inhibition and apoptosis in many systems. However, in comparison with several other cytokines and hormones, their effects on overall protein synthesis have received relatively little attention. TNFα inhibits translation in skeletal muscle (44), and stimulation of the Fas receptor down-regulates translation in Jurkat cells (5), but little is known of the mechanisms by which these effects are mediated.

Previously, we reported that elf4GI cleavage in response to TNFα and other proapoptotic agents in MCF-7 cells requires caspase-3 (4). However, apoptosis itself can occur in MCF-7 cells in the absence of caspase-3 (33, 37), and other caspases have been implicated in TNFα effects on cells (22). Here we have shown that protein synthesis is inhibited in both caspase-3-positive and -negative cells in response to TNFα or TRAIL. Thus, mechanisms other than elf4GI cleavage must be sufficient for the inhibition. Nevertheless, translational down-regulation is likely to be a caspase-dependent process in this system because it is prevented by z-VAD.FMK. It is possible that the cleavages of elf4G and other caspase-dependent phosphorylation of elf2α and increased association of 4E-BP1 with elf4E may be involved. There could also be a role for caspase-dependent 28S rRNA cleavage, which occurs in response to apoptotic stimuli (13). In 293 cells, cleavage of elf4GI by an inducible poliovirus 2A protease is sufficient to cause apoptosis (47).

Numerous studies have implicated PKR in both the inhibition of protein synthesis and the induction of stress responses and apoptosis (28, 31, 32, 41, 48, 49). One report (50) indicates that PKR can be activated by ceramide, a lipid second messenger generated during cellular stress and associated with apoptotic responses (51, 52). PKR is also involved in the activation of transcription factors of the NFκB family, although the kinase is not essential for the activation of NFκB by either TNFα or double-stranded RNA (32, 53–57). Both elf2α phosphorylation and NFκB activation are necessary for PKR-induced apoptosis, and translational inhibition alone is not sufficient (32, 48). Taken in conjunction with these...
Fig. 5. Lack of requirement for caspases or PKR for etoposide-induced inhibition of protein synthesis. In A, MCF-7 cell lines (MCF-7, caspase-3 deficient and MCF-7.3.28, expressing stably transfected caspase-3) were incubated in the absence or presence of etoposide (100 μg/ml) for 4 or 20 h. Protein synthesis was measured as described for Fig. 1D. The data are expressed as a percentage of untreated control values and are the means ±SE of six determinations. In B, MCF-7 cells were incubated with etoposide in the absence or presence of z-VAD.FMK (10 μM) for the times indicated, and protein synthesis was measured as in A. In C, MEFs from PKR knockout (PKR−/−) mice and control (PKR+/+) mice were incubated in the absence or presence of etoposide (100 μg/ml) for 4 or 20 h. Protein synthesis was measured as described for Fig. 1D. The data are expressed as a percentage of untreated control values and are the means ±SE of six determinations.

Fig. 6. Effects of etoposide treatment on phosphorylation of eIF2α and association of eIF4E with 4E-BP1 in MCF-7 cells and MEFs. In A, MCF-7 and MCF-7.3.28 (C3+) cells (see Fig. 1) were incubated with etoposide (100 μg/ml) for the times shown. Total cytoplasmic extracts were prepared as described in "Materials and Methods" and analyzed by immunoblotting for total eIF2α and phosphorylated eIF2α. In addition, eIF4E and associated proteins were isolated by m7GTP-Sepharose chromatography, and the recovered eIF4E, eIF4G, and 4E-BP1 were visualized by immunoblotting. In B, MEFs from PKR knockout (PKR−−) and control (PKR+/+) mice were incubated with etoposide for the indicated times, and extracts were analyzed as in A. In C, the relative extent of phosphorylation of eIF2α in the MCF-7 cells was determined by densitometry as described in Fig. 2. In D, the relative extent of phosphorylation of eIF2α in the MEFs was determined by densitometry as described in Fig. 2.
earlier observations, our present findings with PKR−/− MEFs imply that TNFα-induced activation of NFκB alone is not sufficient to inhibit translation.

In the case of MCF-7 cells, PKR is present at relatively high levels and is active (58). Our observations of substantial phosphorylation of eIF2α in the exponentially growing cells are in agreement with this. MCF-7 cells seem to tolerate significant phosphorylation of eIF2α, perhaps because they contain high eIF2B activity (58), and this may be a property that contributes to their malignant phenotype. Although it is not clear whether the enhanced phosphorylation of eIF2α seen after TNFα or TRAIL treatment (Figs. 2 and 3 and Ref. 32) is sufficient to affect overall protein synthetic rates in MCF-7 cells, both the TNFα-stimulated phosphorylation of eIF2α and the inhibition of protein synthesis show a common requirement for caspase activity.

It is possible that the effects of TNFα on apoptosis in the MCF-7 cell system involve the tumor suppressor protein p53 (59). Cell lines (including MCF-7 cell variants) that contain mutant or inhibited p53 show resistance to TNFα-induced cell death, and this can be reversed by the expression of wild-type p53 (22, 59). TNFα is also able to induce p53 expression (60, 61). We have found recently that activation of a temperature-sensitive form of p53 leads to a rapid inhibition of overall translation. However there are some differences between the actions of TNFα or TRAIL and the effects of p53 activation.5

Taken together, our data are compatible with a model in which PKR is essential for the expression or activation of caspase-8 and/or other components of a signaling pathway that leads from TNF family receptors to the translational machinery (Fig. 7). This conclusion is consistent with work showing that the receptor-associated adapter protein FADD is required for the apoptotic response to PKR activation and is down-regulated in cells expressing dominant-negative PKR (62). Our observation that caspase-8 is not activated by TNFα treatment in PKR−/− cells places the kinase (or a PKR-dependent event) upstream of FADD and caspase-8. Interestingly, PKR can activate caspase-8 by a FADD-mediated mechanism downstream of TNFα receptors (63) and can itself be activated after cleavage by effector caspses (64). This suggests the existence of a positive feedback loop in the regulation of apoptosis by the kinase. Our data on the effects of etoposide, on the other hand, suggest that DNA damage regulates PKR more distally, at a point beyond that requiring any caspase activity (Fig. 7).

One of the most striking changes in the protein synthetic machinery in cells treated with either TNF family receptors or etoposide is the increased association of 4E-BP1 with eIF4E. This is a common response to conditions that cause cell growth inhibition or induce stress responses (29), and it is not associated exclusively with apoptosis. Nevertheless, the z-VAD-FMK sensitivity of [eIF4E.4E-BP1] complex formation in cells treated with TNFα or TRAIL indicates a caspase dependency that links the effect with apoptosis. In contrast, Tee and Proud (6) found that z-VAD-FMK was unable to block the effect of etoposide on 4E-BP1 binding to eIF4E. This is consistent with our observation of a lack of effect of the caspase inhibitor on the inhibition of protein synthesis by this DNA-damaging agent. The requirement for PKR for the association of 4E-BP1 with eIF4E after etoposide treatment (Fig. 6) indicates that the protein kinase is involved in the regulation of 4E-BP1 phosphorylation downstream of the DNA damage pathway (Fig. 7). This may reflect the activation of protein phosphatase PP2A as a result of the phosphorylation of its regulatory subunit B56α by PKR (65). Thus, although DNA damage can inhibit the activity of the 4E-BP1 protein kinase mTOR (66), PKR-mediated activation of PP2A may also bring about the dephosphorylation of 4E-BP1 (67).

Finally, the differential requirements for PKR for increased eIF2α phosphorylation and 4E-BP1 association with eIF4E, versus the inhibition of methionine incorporation after etoposide treatment, suggest that inhibition of the availability of eIF2 and eIF4E cannot alone account for the effect of the DNA-damaging agent on overall translation. Therefore, it is likely that etoposide has additional mechanisms of action on the protein synthetic machinery, the nature of which remain to be determined.

REFERENCES


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REGULATION OF TRANSLATION BY CASPASES AND PKR


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