Cleavage of translation initiation factor 4G (eIF4G) during anti-Fas IgM-induced apoptosis does not require signalling through the p38 mitogen-activated protein (MAP) kinase

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Abstract Initiation factor (eIF) 4G plays a key role in the regulation of translation, acting as a bridge between eIF4E and eIF3, to allow an mRNA molecule to associate with the 40S ribosomal subunit. In this study, we show that activation of the Fas/CD95 receptor complex in Jurkat cells induces the degradation of eIF4G, the inhibition of total protein synthesis and cell death. These responses were prevented by the caspase inhibitors, zVAD.FMK and zDEVD.FMK. We also show that, in contrast to *Saccharomyces cerevisiae*, although rapamycin caused a modest inhibition of protein synthesis it did not induce apoptosis or the cleavage of eIF4G. Studies with the specific inhibitor, SB203580, have shown that signalling through the p38 MAP kinase pathway is not required for either the Fas/CD95-induced cleavage of eIF4G or cell death. These data suggest that the cleavage of eIF4G and the inhibition of translation play an integral role in Fas/CD95-induced cell death in Jurkat cells.

Key words: Initiation factor;
Translation initiation factor 4G; Apoptosis

1. Introduction

Apoptosis, a major form of cell death characterised by a series of distinct morphological and biochemical changes [1], is an important process in a wide variety of biological systems [2], with inappropriate activation implicated in human disease [3]. The regulation of this process has been proposed to be mediated via a fine balance between the activation of protein synthesis-independent cytotoxic mechanisms and maintenance of protein synthesis-dependent protective effects [1,2,4].

Fas (Apo-1/CD95) encodes a transmembrane receptor belonging to the tumour necrosis factor (TNF) receptor family. Interaction of the receptor with its cognate ligand or cross-linking with anti-Fas IgM has been employed as a model system for apoptosis, as occupation of this receptor results in rapid programmed cell death in Jurkat cells [5,6]. The essential signalling events coupling Fas/CD95 receptor to apoptosis have been studied intensively. It has been shown that the intracellular domain of Fas/CD95 contains a highly conserved 'death domain', which is required for the induction of apoptosis [1,2,7]. Stimulation of Fas/CD95 results in aggregation of its death domains, leading to the recruitment of at least two key signalling proteins that together with the receptor, form the death-inducing signalling complex (DISC) [2,8]. FADD/MORT-1, an adaptor protein [9,10], couples via its C-terminal death domain to the aggregated Fas/CD95 receptor and recruits caspase 8 (FLICE/MACHα1/Mch5), a cysteine protease, through its N-terminal death effector domain to the DISC [11,12]. Recruitment of caspase 8 to the Fas/CD95 DISC results in its autocatalytic cleavage at internal Asp residues, with the release of active subunits (p18 and p10) into the cytosol [1,2,13]. In Jurkat cells, the inhibition of caspase 8 activity is sufficient to prevent anti-Fas IgM-induced apoptosis [14–17]. Downstream of the DISC, the Fas/CD95 signalling pathway has been shown to involve additional caspases (such as caspases 1, 3, 6–10), loss of mitochondrial integrity, the release of cytochrome c and activation of caspase 9 (and subsequently caspase 3) via its interaction with Apaf-1 (reviewed in [2]). Caspase 3 is believed to play the role of the main executioner downstream in the apoptotic pathways as it is normally activated in cells by a variety of death stimuli [1,2], although it may not be required for all of the morphological and biochemical changes observed during this process [18].

The role of the mitogen-activated protein kinase (MAPKs) signalling pathways in apoptosis is more controversial. Although activation of the ERK MAPK has been generally associated with cell survival [19], the stress-activated MAPKs, such as p38 MAPK and JNK [20–22], have been implicated as possible mediators of the apoptotic process [23,24] as well as regulating cell survival in response to TNFα [25]. Fas/CD95 receptor activation has been demonstrated to increase the activity of p38 MAPK and JNK, in a caspase-dependent manner, events which can be temporally correlated with the onset of apoptosis [26].

Central to the process of apoptosis is the finding that caspase-dependent cleavage of target proteins can lead to their inactivation (e.g. poly(ADP-ribose) polymerase (PARP), lamin, focal adhesion kinase (FAK)) or in some cases to their activation (e.g. MEKK1, p21-activated kinase 2 (PAK2), protein kinase C-δ, phosphatase 2A and gelsolin ([1,2,27] and references therein). Recently, Widmann et al. have catalogued the caspase-dependent cleavage of signalling proteins during apoptosis and shown that cleavage of Raf-1 and Akt-1 inhibits their kinase activity [27]. This has been proposed to lead to the inhibition of cell survival pathways [28,29], in part, by preventing the ability of Akt-1 to phosphorylate and inactivate BAD, a pro-apoptotic member of the Bcl family of proteins [1,2]. In other physiological systems, inhibition of signalling via the Akt-1 pathway has been associated with decreased phosphorylation of ribosomal protein S6 and initiation factor (eIF) 4E binding protein 1 (4E-BP1) (reviewed in [30]); this results in a decrease in the translation of a class of mRNAs containing a 5′ polypyrimidine tract [31], and the sequestration of eIF4E from eIF4G producing a general inhibition of translation, respectively [30,32].

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Previously, we have shown that starvation of BJAB cells for serum results in decreased rates of translation, the induction of apoptosis and the cleavage of eIF4G [33]. We now show that activation of the Fas/CD95 receptor by anti-Fas IgM results in an inhibition of total protein synthesis in Jurkat cells. In addition, Fas/CD95 signalling impinges directly on the translational apparatus by mediating the cleavage of eIF4G, which is temporally correlated with the inhibition of protein synthesis. The degradation of eIF4G is dependent upon caspase 3 activity but does not require signalling through the p38 MAPK pathway. These data suggest that the cleavage of eIF4G and the inhibition of translation play an integral role in cell death following activation of the Fas/CD95 signalling pathway in Jurkat cells.

2. Materials and methods

2.1. Chemicals and biochemicals

Materials for tissue culture were from Gibco Life Technologies, Immobilon PVDF was from Millipore, anti-Fas IgM (clone CH-11) was from TCS Biologicals, zVAD.FMK and SB203580 were from Alexis Corporation, zDEVD.FMK was from Calbiochem, m^7GTP-Sepharose was from Pharmacia-LKB and all other chemicals were from Sigma. Rabbit antisera to eIF4G (raised against the C-terminal fragment expressed in bacteria) and eIF4E (raised against a C-terminal peptide) were as described previously [33,34], antiserum to PARP was from Boehringer Mannheim and antiserum specific for p38 and phospho-p38 were from New England Biolabs. Rapamycin was a kind gift from Dr. J. Kay (Sussex, UK).

2.2. Tissue culture

Human Jurkat T cells were grown in RPMI 1640 with Glutamax supplemented with 10% foetal calf serum (FCS) as described previously [34]. Cell cultures, in the mid-log phase of growth, were incubated for 1 h with vehicle alone, zVAD.FMK, zDEVD.FMK or SB203580, as described in individual figure legends, prior to stimulation.

2.3. Preparation of cell extracts

Following treatment, cells were isolated by centrifugation in a cooled microfuge, the medium removed and cells resuspended in 500 μl ice cold PBS supplemented with 40 mM β-glycerophosphate and 2 mM benzamidine. Following a wash in the same buffer, cells were resuspended and lysed by vortexing in 0.2 ml (per 10 ml of original culture) buffer A (50 mM MOPS-KOH, pH 7.4, 2.5 mM EGTA, 1 mM EDTA, 40 mM β-glycerophosphate, 1 μM microcystin, 120 mM NaCl, 7 mM 2-mercaptoethanol, 2 mM benzamidine, 1 mM phenylmethylsulphonyl fluoride (PMSF), 0.1 mM GTP, 2 mM Na_3VO_4, 1% (by vol.) of each of Nonidet P40 and Triton X-100). Cell debris was removed by centrifugation in a microfuge for 5 min at 4°C and the resultant supernatant was frozen in liquid N_2.

2.4. Polyacrylamide gel electrophoresis (SDS-PAGE), vertical slab iso-electric focusing (VS.IEF) and immunoblotting

One-dimensional polyacrylamide gels and vertical slab iso-electric focusing gels were run as described [34]. Proteins transferred to PVDF and eIFE, eIF4G, PARP, p38 and phosphorylated p38 MAPKs were detected with specific rabbit anti-peptide antisera as described in the individual figure legends.

2.5. m^7GTP-Sepharose chromatography

For the isolation of eIF4E, cell extracts of equal protein concentration were subjected to m^7GTP-Sepharose chromatography as described [33,34]. The beads were washed three times in buffer A and bound protein eluted with VS.IEF sample buffer, as indicated.

2.6. p38 MAPK immunocomplex kinase assays

Jurkat cells were treated with anti-Fas IgM for the times indicated in the figure legends, extracts prepared and immunocomplex kinase assays performed using anti-p38 MAPK polyclonal antiserum and GST-ATF2 (Santa Cruz) as substrate as described [24]. The activity of the immune complex was assayed at 30°C for 30 min in 20 μl of kinase buffer (50 mM MOPS-KOH, pH 7.4, 2.5 mM EGTA, 0.1 mM EDTA, 10 mM $MgCl_2$, 5 mM *p*-nitrophenylphosphate, 7 mM 2-mercaptoethanol, 2 mM benzamidine, 1 mM PMSF, 0.2 mM Na_3VO_4), in the presence of 10 μM ATP and 10 μCi of [γ-^{32}P]ATP. Reactions were terminated with SDS-PAGE sample buffer and substrates separated on SDS-PAGE followed by quantification by a phosphoimager.

3. Results and discussion

3.1. Protein synthesis inhibition is an early event during Fas-induced apoptosis in Jurkat cells

We have used the anti-Fas IgM antiserum to study how activation of the CD95 signalling pathway impinges upon the translational machinery during the process of apoptosis. As shown in Fig. 1A, protein synthesis in Jurkat cells, measured by incorporation of [^{35}S]methionine into acid-insoluble material, is sensitive to anti-Fas IgM or etoposide, both of which induce cell death (Fig. 1B). With either treatment, the rate of translation declined to about 35–40% of control rates within 6 h (Fig. 1C) and polysomes were disaggregated (data not shown). The effect of anti-Fas IgM on protein synthesis and cell death was prevented by pre-treatment of cells with the general caspase inhibitor, zVAD.FMK, which also maintained polysome integrity in the presence of anti-Fas IgM (data not shown). In contrast, in etoposide-treated cells, cell death but not the inhibition of protein synthesis, was prevented by zVAD.FMK (Fig. 1A–C).

We have also used the immunosuppressant, rapamycin, which, in mammalian cells, partially inhibits cap-dependent initiation [35,36] by stabilising the interaction between eIF4E and 4E-BP1 and preventing the association of eIF4E with eIF4G [30,32]. Rapamycin has been shown to antagonise the increase in protein synthesis brought about by insulin and prevent the translation of the class of mRNAs containing a 5′ polypyrimidine tract [31]. In the yeast *Saccharomyces cerevisiae*, rapamycin also inhibits the basal rate of protein synthesis [37]. In Jurkat cells, rapamycin does not affect early rates of translation following mitogenic stimulation [34,38] and had a less potent inhibitory effect than anti-Fas IgM, reducing protein synthesis by only 40% after 24 h. Under these assay conditions, rapamycin did not alter the phosphorylation of 4E-BP1 or the binding of 4E-BP1 to eIF4E at early times of incubation (data not shown). To determine whether the observed inhibition of translation was a general effect or was specific to certain classes of mRNA, cells were pulse-labelled with [^{35}S]methionine at 6 h following various treatments (in the presence of 1 mM unlabelled methionine to reduce pool effects), extracts prepared and aliquots containing equal amounts of [^{35}S]methionine-labelled protein resolved by SDS-PAGE. As shown in Fig. 1A, although anti-Fas IgM or etoposide decreased the overall rate of translation, this appears to be a general affect on total protein synthesis with no gross qualitative changes in the translation of specific proteins observed (Fig. 1D). Under these assay conditions, rapamycin alone had no detectable effect on the synthesis of specific proteins (lane 5) and pre-treatment of cells with rapamycin prior to anti-Fas IgM did not further decrease the rate of translation over that of anti-Fas IgM alone (Fig. 1C, lane 6 vs 2), alter the spectrum of proteins synthesised (Fig. 1D, lane 6 vs 2) or influence anti-Fas IgM-induced cell death (data not shown). Two-dimensional gel electrophoresis would be needed to detect more subtle changes in the syn-

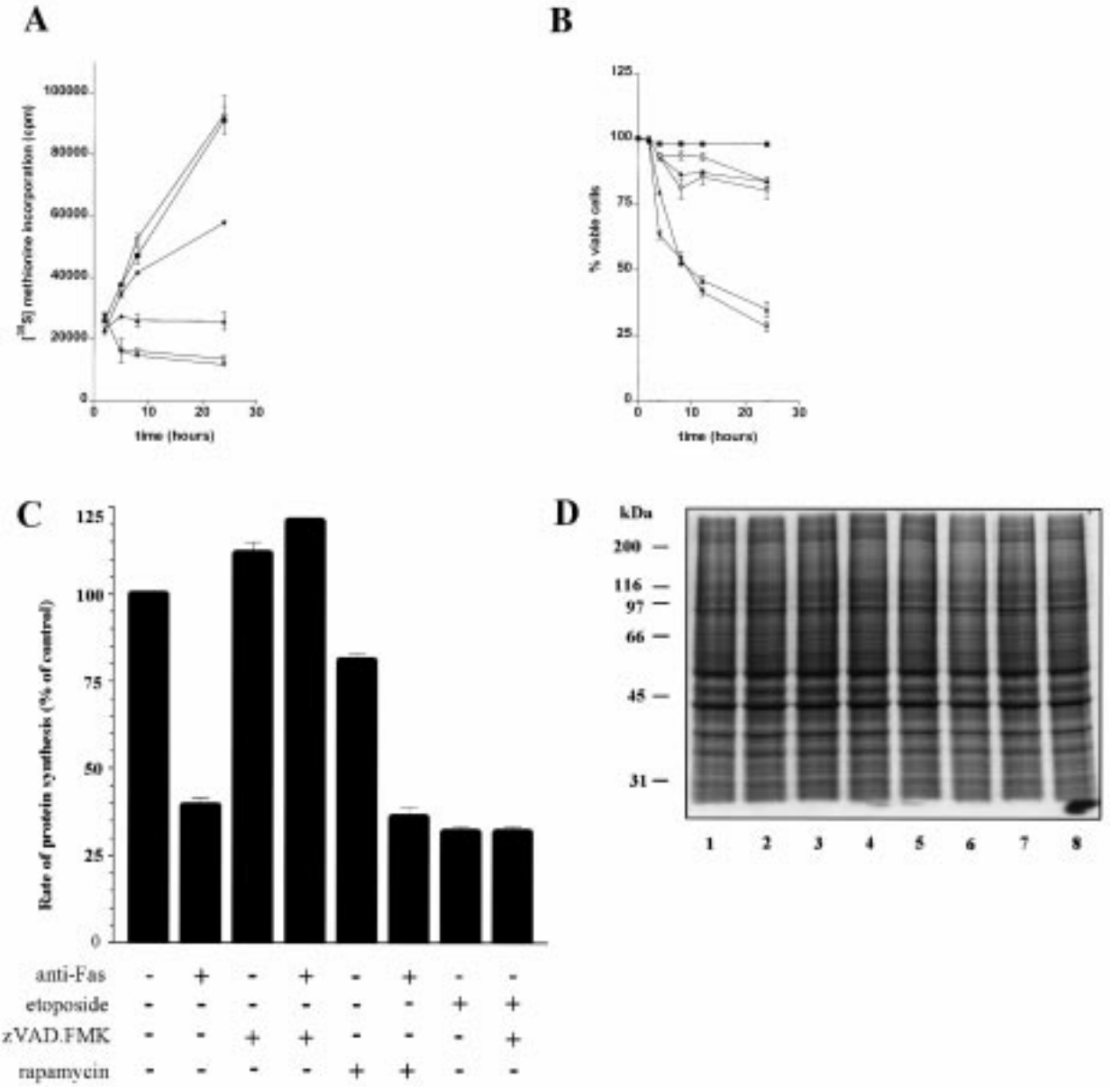

Fig. 1. Anti-Fas IgM or etoposide treatment of Jurkat cells causes a general inhibition of translation and decreased cell viability. A: Jurkat cells (10^6 cells in 1 ml, in triplicate) were preincubated for 60 min in the absence (■◆▲▼) or presence (◇▽) of 50 μM zVAD.FMK prior to a further incubation in the absence (■) or presence of 250 ng/ml anti-Fas IgM (▲◇), 100 μg/ml etoposide (▼▽) or 10 nM rapamycin (◆). The rate of protein synthesis was estimated by pulse-labelling cells for 30 min with 10 μCi [^{35}S]methionine before harvesting at the times indicated. B: Parallel cultures of Jurkat cells were used to determine cell viability, as estimated by trypan blue exclusion. C: Jurkat cells (10^6 cells in 1 ml, in triplicate) were preincubated for 60 min in the absence or presence of 50 μM zVAD.FMK, or 10 nM rapamycin, prior to a further incubation for 6 h with vehicle alone, 250 ng/ml anti-Fas IgM, or 100 μg/ml etoposide. The rate of translation was measured as in A, except that pulse-labelling was for 15 min. Rates of protein synthesis are expressed as % of that obtained with vehicle alone. D: Parallel cultures of cells (10^7 cells in 10 ml) as in C were used to prepare cell extracts, as described in Section 2. Aliquots containing equal cpm of radioactivity were resolved by SDS-PAGE and the resulting autoradiograph is presented.

thesis of specific proteins. Recently, Zhou et al. have reported a severe inhibition of translation by anti-Fas IgM in Jurkat cells which was not reversed by zVAD.FMK [39]. The reasons for these differences are unclear but may reflect the fact that Zhou et al. starved their cells prior to activation and measured translation rates with reduced levels of unlabelled methionine in the medium. Recently, such a regime has been shown to result in the rapid deactivation of $p70^{S6K}$ and dephosphorylation of 4E-BP-1, rendering the cells unresponsive to all agonists [40].

3.2. Anti-Fas IgM induces the acute degradation of eIF4G

Previously, we have reported that serum-starvation of BJAB cells induces apoptosis with concomitant cleavage of initiation factor eIF4G [33]. It is now established that the large polypeptide chain initiation factor eIF4G plays a crucial role in translation initiation by acting as a bridge between other components such as the mRNA cap-binding protein eIF4E and the multi-subunit eIF3 complex, which allows the mRNA molecule to associate with the 40S ribosomal subunit (reviewed in [41,42]). The association of the N-terminal

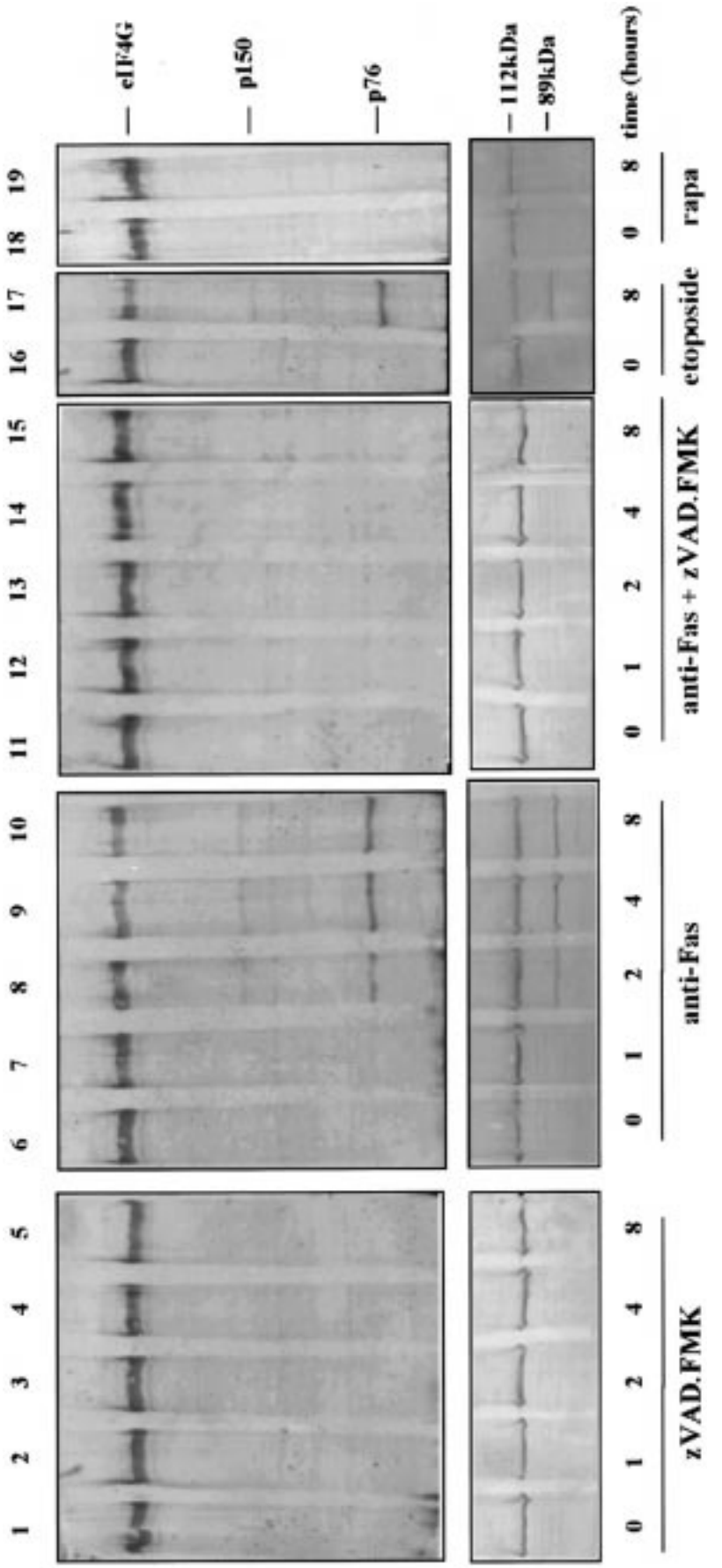
— eIF4G
— p150
— p76
— 112kDa
— 89kDa
time (hours)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
0 1 2 4 8 0 1 2 4 8 0 1 2 4 8 0 8 0 8
zVAD.FMK
anti-Fas
anti-Fas + zVAD.FMK
etoposide
rapa

Fig. 2. Inhibition of translation following the induction of apoptosis is correlated with the rapid cleavage of eIF4G and PARP. Jurkat cells (2 ml) were preincubated for 60 min in the absence or presence of 50 μM zVAD.FMK before the addition of 250 ng/ml anti-Fas IgM, 100 μg/ml etoposide or 10 nM rapamycin (rapa), as indicated. At the times shown, cells were harvested, extracts prepared and equal amounts of protein (7.5 μg) were resolved by SDS-PAGE. eIF4G and PARP were visualised by immunoblotting and the migration of the p150 and p76 cleavage products of eIF4G, and that of the 89 kDa cleavage product of PARP, are indicated. Results are representative of at least 10 experiments. ←

portion of eIF4G with eIF4E strongly enhances the binding of the latter to 5′ mRNA cap structures [43]. eIF4G possesses domains for the binding of eIF3 in the central part of its sequence, and also has binding sites for eIF4A [44–46] and (at least in yeast) for the poly(A) binding protein (PABP) [47]. Interaction of PABP with eIF4G may allow functional association of the 3′ end of an mRNA with the 5′ end, thus effectively causing 'circularisation' of the mRNA during protein synthesis [48]. In this present study, we have investigated the extent to which the decrease in translational activity in Jurkat cells is associated with the loss of the key initiation factor, eIF4G. Cells were incubated in the absence or presence of zVAD.FMK before exposure to anti-Fas IgM for the times indicated. Fig. 2 shows that within 2 h of treatment with anti-Fas IgM there is a rapid degradation of eIF4G into its characteristic cleavage products (p150, p76) and the appearance of a specific 89 kDa cleavage product of PARP [1,2]. At early times of incubation with anti-Fas IgM, the integrity of eIF4E, eIF2α, eIF4A, PABP and eIF4B were unaffected (data not shown). As with the effects on translation, apoptosis-induced cleavage of eIF4G and PARP was prevented by preincubating the cells with zVAD.FMK (Fig. 2, lanes 13 vs 8), suggesting that these proteins are targeted during programmed cell death. In addition, extracts prepared from cells incubated with anti-Fas IgM retained the ability to cleave exogenously added eIF4F into the characteristic products, a process also prevented by zVAD.FMK (S.J. Morley and L. McKendrick, unpublished data). As seen with anti-Fas IgM, etoposide also promoted the cleavage of eIF4G and PARP (Fig. 2, lane 17 vs 16), and this was again prevented by zVAD.FMK (data not shown). In all instances, the cleavage of eIF4G was unaffected by the calpain inhibitor, calpeptin, but accelerated by proteosome inhibitors such as MG132 (data not shown), consistent with the ability of this compound to induce apoptosis in its own right [59].

In an effort to gain insight into which caspase(s) are important in the cleavage of eIF4G, we have extended these studies to include the caspase 3-specific cell permeable inhibitor, zDEVD.FMK. Fig. 3A shows that like zVAD.FMK (lanes 4 vs 3), zDEVD.FMK (lanes 5 vs 3) also prevented the anti-Fas IgM-induced cleavage of eIF4G observed at 4 h following stimulation, and prevented cell death (Fig. 3B). The fact that several disparate inducers of apoptosis, mediated by the activation of one or more 'executioner' caspases and possibly via different mechanisms (reviewed in [1,2]), all lead to the cleavage of eIF4G indicates that the pathways activated by these agents converge on eIF4G. This is consistent with the ability of the caspase inhibitors (zVAD.FMK and zDEVD.FMK) to block the disappearance of eIF4G regardless of the apoptosis-inducing stimulus. However, etoposide must affect other processes in Jurkat cells as these caspase inhibitors do not reverse the inhibition of translation observed in these cells. It is noted that these data do not establish that caspase 3 is the physiological catalyst of eIF4G cleavage, but indicate that this caspase activity is necessary for the cleavage of eIF4G. In addition, studies in vitro with recombinant caspase 3 and in vivo with MCF-7 cells which naturally lack caspase 3 activity have indicated that it is both necessary and sufficient to cleave eIF4G (M. Bushell, L. McKendrick, R. Janicke, and S.J. Morley, manuscript in preparation). At this time, we do not know whether cleavage of eIF4G is the cause or consequence of the inhibition of protein synthesis

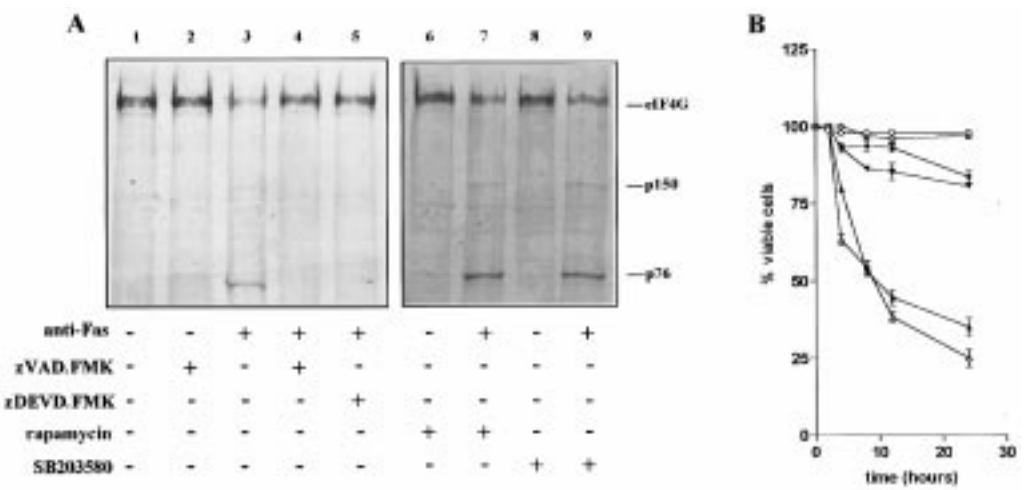

Fig. 3. Cleavage of eIF4G is prevented by zDEVD.FMK but is not affected by SB203580. A: Jurkat cells (10^7 cells in 10 ml) were preincubated for 60 min in the absence or presence of 50 μM zVAD.FMK, 50 μM zDEVD.FMK, 10 nM rapamycin or 50 μM SB203580 before further incubation for 4 h with or without 250 ng/ml anti-Fas IgM, as indicated. Cell extracts were prepared and eIF4G was visualised as in Fig. 2. Results are representative of six experiments. B: Parallel cultures of Jurkat cells were used to determine cell viability, as estimated by trypan blue exclusion. Vehicle alone (○); zVAD.FMK or SB203580 alone (◇); anti-Fas IgM (▲); anti-Fas IgM with zDEVD.FMK (▼); rapamycin (◆); anti-Fas IgM with SB203580 (△).

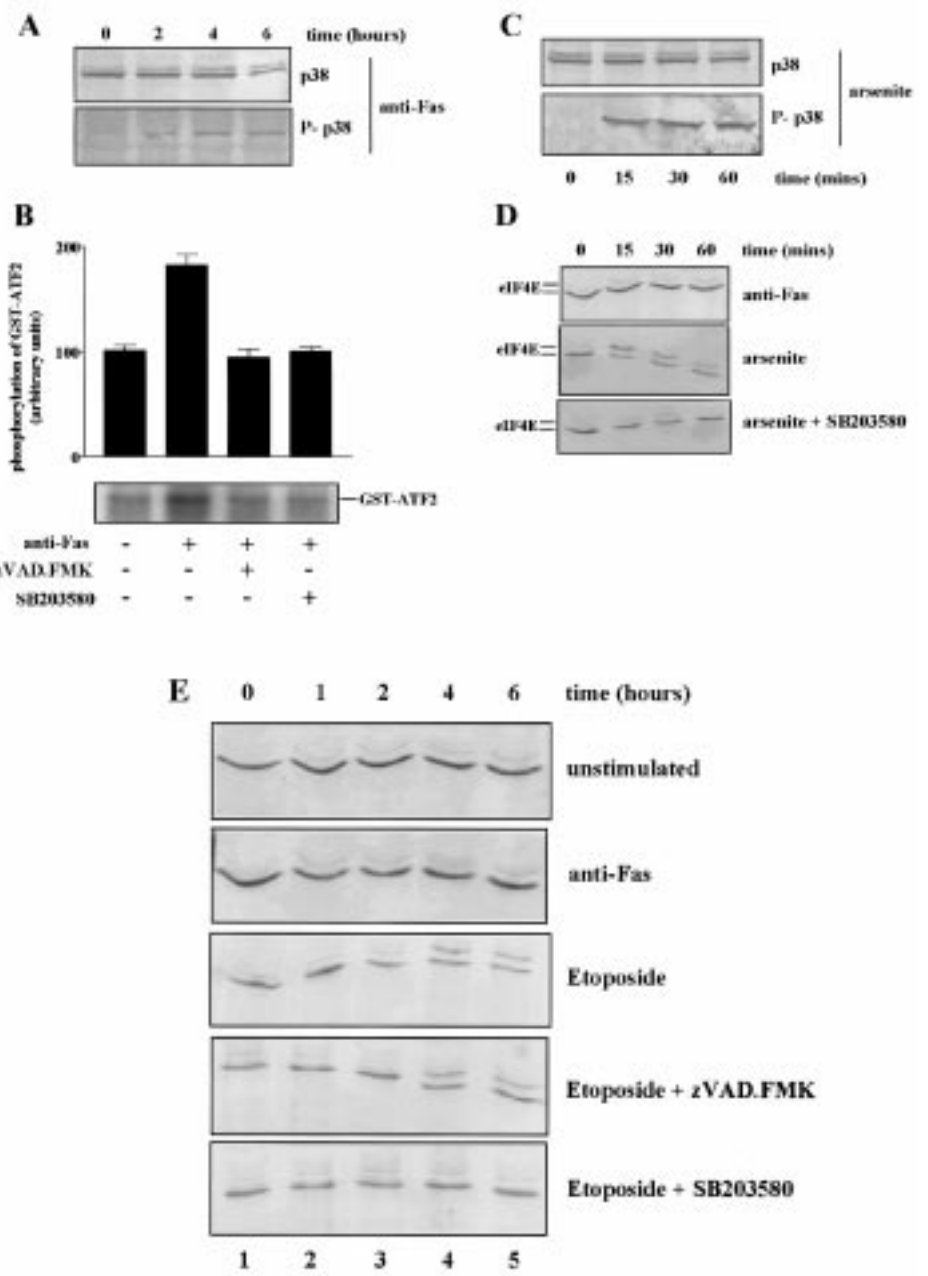

Fig. 4. Arsenite, but not anti-Fas IgM, enhances the phosphorylation of eIF4E in Jurkat cells. A: Jurkat cells (10^7 cells in 10 ml) were incubated with 250 ng/ml anti-Fas IgM for the times indicated, cell extracts prepared and equal amounts of protein (7.5 μg) resolved by SDS-PAGE. p38 and p38 phosphorylated on threonine 180/tyrosine 182 (P-38) were visualised by immunoblotting. B: Jurkat cells (10^7 cells in 10 ml) were incubated with 250 ng/ml anti-Fas IgM for 4 h, extracts prepared and aliquots containing equal amounts of protein (30 μg) were used to measure p38 MAPK activity in an immunocomplex kinase assay with GST-ATF2 as substrate, as described in Section 2. Presented data are the means and S.D. (bars) of three separate experiments whilst the autoradiograph is from one such experiment. C: Jurkat cells (10^7 cells in 10 ml) were incubated with 5 mM arsenite for the times indicated and the activation of p38 MAPK monitored as in A. D: Jurkat cells (10^7 cells in 10 ml) were incubated with or without 50 μM SB203580 before incubation with 250 ng/ml anti-Fas IgM or 5 mM arsenite for the times indicated. Cell extracts were prepared and eIF4E was isolated and analysed by VS.IEF and immunoblotting, as described in Section 2. Results are representative of five experiments. E: Jurkat cells (10^7 cells in 10 ml) were incubated for 60 min with or without 50 μM zVAD.FMK or SB203580, prior to the addition of vehicle alone (unstimulated), 250 ng/ml anti-Fas IgM or 10 μg/ml etoposide for the times indicated. The phosphorylation of eIF4E was analysed as in C. Results are representative of three experiments.

reported here. Preliminary work has suggested that caspase 3-induced cleavage of eIF4G results in the inhibition of translation in the reticulocyte lysate (S.J. Morley and W. Wood, unpublished data). Further work will be required to determine the sites of cleavage of eIF4G in vivo and how the p76 fragment influences translation of mRNA.

3.3. *Rapamycin does not induce the cleavage of eIF4G in Jurkat cells*

In *S. cerevisiae*, nutrient deprivation or interruption of a rapamycin-sensitive signalling pathway induces the cleavage of eIF4G [49] and inhibition of translation initiation [37]. We therefore examined the effect of rapamycin on the integ-

rity of eIF4G in Jurkat cells. As shown in Fig. 2 (lanes 18 and 19) and Fig. 3 (lanes 6 vs 1), incubation with rapamycin alone for 8 h or 4 h, respectively, did not induce the degradation of eIF4G. In addition, rapamycin had no effect of the ability of anti-Fas IgM to inhibit translation rates (Fig. 1C), to induce the cleavage of eIF4G (Fig. 3, lane 7) or influence cell death (data not shown). This is in contrast to a finding that rapamycin can potentiate dexamethasone-induced apoptosis in S49 cells [50].

3.4. Cleavage of eIF4G does not require signalling through p38 MAPK

A variety of extracellular stimuli produce cellular responses via activation of MAPKs (reviewed in [21,22,51]). The p38 subfamily of MAPKs consists of p38α, p38β, p38γ and p38δ, and regulates gene expression in response to stimuli such as TNFα and interleukin-1 [51]. p38 is activated by dual phosphorylation on threonine and tyrosine and in turn, stimulates the activity of downstream transcription factors and other protein kinases, such as MAPKAPK2, MAPKAPK3 and Mnk1 [51–54]. The latter has been identified as a potential physiological kinase for translation initiation factor, eIF4E [53,54]. The p38 MAPK pathway has been implicated as playing a critical role in apoptosis in several types of cells [23,24,26], with the forced over-expression of p38α competent to induce apoptosis, whilst that of p38β inhibits apoptosis in Jurkat cells [24]. Studies with various Jurkat cell clones have shown that anti-Fas IgM induces the activation of endogenous p38 MAPK, which correlates well with the induction of apoptosis [26]. In order to address whether p38 MAPK activity was required for cleavage of eIF4G, we have used SB203580, which inhibits the α and β forms of p38 MAPK [51]. Incubation of cells with SB203580 alone did not induce the cleavage of eIF4G (Fig. 3A, lane 8) or promote cell death to any degree (Fig. 3B). In contrast to caspase inhibitors, pre-incubation of cells with SB203580 before exposure to anti-Fas IgM (or etoposide; data not shown) did not prevent apoptosis-induced eIF4G cleavage (Fig. 3A, lane 9) and actually slightly potentiated cell death (Fig. 3B). Under these assay conditions, inhibition of ERK MAPK by PD98059 did not result in cell death or prevent the anti-Fas IgM-induced cleavage of eIF4G (data not shown). Therefore, these data show that signalling through p38 MAPK is not required for anti-Fas IgM-induced apoptosis or the cleavage of eIF4G.

3.5. Anti-Fas IgM activates p38 MAPK but does not increase the phosphorylation of eIF4E

Initiation factor eIF4E plays a key role in mRNA translation and the regulation of this process by growth factors and hormones. It is a phosphoprotein, with its phosphorylation generally enhanced by agents which activate translation and decreased in response to treatments which inhibit translation (reviewed in [30,32]). However, it is still not clear how phosphorylation of eIF4E modulates its activity. Whilst there may be a direct effect of phosphorylation on cap structure recognition in vitro [55], phosphorylation of eIF4E in vivo can also be correlated with its enhanced interaction with eIF4G [30,38,56,57]. Both effects may be important in the activation of protein synthesis under conditions that enhance eIF4E phosphorylation. Studies from this lab [34,58] and others [54] have shown that phorbol esters and cellular stress can increase the phosphorylation of eIF4E. In Jurkat cells this can occur via the p38 MAPK signalling pathway [34], possibly mediated by activation of Mnk1 [53].

Consistent with published data [26], Fig. 4A shows that anti-Fas IgM treatment of cells causes a modest increase the phosphorylation (and hence activity) of p38 MAPK within 2 h, peaking at 4 h after addition. Immunoprecipitation and in vitro kinase assays using GST-ATF2 as substrate showed that although the twofold increase in the activity of p38 MAPK was dependent on caspase activity and was sensitive to SB203580 (Fig. 4B), anti-Fas IgM treatment did not increase the phosphorylation of eIF4E (Fig. 4D). Under the same assay conditions, arsenite caused a more pronounced activation of p38 MAPK (fivefold, peaking at 30–60 min; Fig. 4C) and increased the level of eIF4E phosphorylation within 15 min of addition (Fig. 4D). Consistent with signalling through the p38 MAPK pathway, the effects of arsenite on eIF4E phosphorylation were sensitive to SB203580 (Fig. 4D), but insensitive to zVAD.FMK (data not shown). Fig. 4E shows the effect of anti-Fas IgM and etoposide on the phosphorylation of eIF4E over an extended time course. Although anti-Fas IgM increased p38 MAPK activity, it did not increase eIF4E phosphorylation above control levels. Etoposide however, increased the phosphorylation of eIF4E within 2 h (peaking at 4 h). Moreover, this increase was insensitive to zVAD.FMK but sensitive to SB203580, consistent with a model whereby etoposide activates multiple pathways involved in activation of p38 MAPK and cell death [26].

The reasons why anti-Fas IgM does not increase the phosphorylation of eIF4E, in spite of the activation of p38 MAPK and MAPKAPK2 (data not shown) are unclear. Previous studies with this cell line have shown that phosphorylation of eIF4E is modulated primarily at the level of protein phosphatase activity, with little evidence for changes in eIF4E kinase activity [34]. Recently, Santoro et al. have shown that phosphatase 2A activity is increased by 4.5-fold at 6 h in anti-Fas IgM-treated Jurkat cells [59]. The importance of this response in the modulation of eIF4E phosphorylation is currently under investigation.

In conclusion, these data show that the anti-Fas IgM-induced inhibition of protein synthesis in Jurkat cells is temporally correlated with the degradation of eIF4G. It is possible that, in conjunction with the cleavage of eIF4G, maintenance of eIF4E in the less phosphorylated form potentiates the inhibition of translation required for the progression of apoptosis in response to activation of the Fas receptor.

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