The caspase-cleaved DAP5 protein supports internal ribosome entry site-mediated translation of death proteins

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Communicated by Leo Sachs, Weizmann Institute of Science, Rehovot, Israel, February 21, 2002 (received for review October 22, 2001)

Apoptosis is characterized by a translation switch from cap-dependent to internal ribosome entry site (IRES)-mediated protein translation. During apoptosis, several members of the eukaryotic initiation factor (eIF)4G family are cleaved specifically by caspases. Here we investigated which of the caspase-cleaved eIF4G family members could support cap-independent translation through IRES elements that retain activity in the dying cell. We focused on two major fragments arising from the cleavage of eIF4GI and death-associated protein 5 (DAP5) proteins (eIF4GI M-FAG/p76 and DAP5/p86, respectively), because they are the only potential candidates to preserve the minimal scaffold function needed to mediate translation. Transfection-based experiments in cell cultures indicated that expression of DAP5/p86 in cells stimulated protein translation from the IRESs of c-Myc, Apaf-1, DAP5, and XIAP. In contrast, these IRESs were refractory to the ectopically expressed eIF4GI M-FAG/p76. Furthermore, our study provides *in vivo* evidence that the caspase-mediated removal of the C-terminal tail of DAP5/p97 relieves an inhibitory effect on the protein's ability to support cap-independent translation through the DAP5 IRES. Altogether, the data suggest that DAP5 is a caspase-activated translation factor that mediates translation through a repertoire of IRES elements, supporting the translation of apoptosis-related proteins.

Apoptosis is an intrinsic self-elimination mechanism for the removal of unwanted cells. Apoptosis involves posttranslation regulation of preexisting proteins such as phosphorylation alterations, proteolytic cleavage, and intracellular translocations (1, 2). *De novo* synthesis of proteins also contributes to the apoptotic process, because various apoptotic events require ongoing translation for their proper execution (3, 4). Although the identity of the proteins whose sustained translation is required in these death systems has not been revealed yet, recent findings point to a series of events that impinge on translation control as a conserved apoptosis hallmark as detailed below.

Apoptosis induction in several cell lines and by numerous triggers has been associated with a rapid and substantial reduction in protein translation rate (4, 5) primarily because of reduced translation initiation. This reduced translation has been correlated with caspase-mediated activation of the dsRNA-activated protein kinase (PKR), which inhibits initiation of protein translation by phosphorylating eukaryotic initiation factor (eIF)2α (6) as well as with the caspase-mediated inactivation of several translation initiation factors including eIF4B, eIF3/p35, eIF2α, and proteins of the eIF4G family (5, 7). It was correlated also with alterations in the phosphorylation state of eIF4E, 4E-BP1, and eIF2α (8). Importantly, $\approx$30% of the normal level of translation events persist, at least in some apoptotic circumstances (9). It seems that the translation rate is not inhibited in a global and uniform fashion. Rather, the translation of a subset of mRNAs prevails in the dying cells. These mRNAs include those of the proapoptotic proteins death-associated protein 5 (DAP5), c-Myc, and Apaf-1 and the antiapoptotic protein XIAP (9–13). A common feature of these mRNAs is their translation via an alternative mode named cap-independent translation, mediated by internal ribosome entry site (IRES) elements in their 5′ untranslated regions (UTRs). Furthermore, each of these IRES elements suffices to maintain the translation of a reporter gene in dying cells, whereas the cap-dependent translation mode is abrogated severely. These IRESs are referred to in this work as "death IRESs," because they maintain their translation rate in the dying cell.

What molecular mechanisms underlie the switch from cap-dependent to IRES-mediated translation in dying cells? Normally eIF4G proteins coordinate the assembly of the translation initiation complex, bridging between the translation machinery (via eIF3) and the capped mRNA (via eIF4E). The eIF4G family includes eIF4GI, eIF4GII, PAIPI, and DAP5 proteins, all sharing homology in a segment that enables their interaction with eIF4A and eIF3 (14). eIF4GI and eIF4GII are the family prototypes and normally support cap-dependent translation. PAIPI is a stimulatory translation coactivator (15). DAP5 (also named NAT1 and p97) lacks an interaction with the mRNA cap and thus cannot support cap-dependent translation. Findings based on overexpression experiments suggested that DAP5/p97 might function as a translation inhibitor (16, 17). However, another work based on translation assays in a cell-free system suggested that DAP5 could act as a positive mediator of cap-independent translation, at least through its own IRES (9). The critical role of eIF4G proteins in translation initiation marks them as potential targets for regulation of translation initiation in apoptosis. Strikingly, at least three eIF4G proteins are cleaved by caspases after apoptosis induction, altering their potential to support translation initiation (7, 9, 18). In this work we show that the caspase-cleaved DAP5/p86 is capable of supporting translation through the death IRESs of DAP5, c-Myc, XIAP, and Apaf-1. In addition, we provide *in vivo* evidence showing that the conversion of DAP5/p97 to DAP5/p86 by caspase cleavage is an important regulatory step that releases an inhibition on the ability of DAP5 protein to support death IRES-mediated translation.

Materials and Methods

DNA Constructs. Green fluorescent protein (GFP)-fusion proteins were expressed from pEGFP-C vectors (CLONTECH). pEGFP-DAP5/p97 and p86 were generated by inserting the pECE-DAP5/p97 or p86 *Sal*I-*Eco*RI fragments into *Xho*I-*Eco*RI sites of pEGFP-C1. An eIF4GI-M-FAG/p76 cDNA was obtained by PCR using BS-eIF4GI as template with primers encompassing a *Hin*dIII restriction site at the 5′ (5′-AAGCTTGCCTTCAAGGAGGCGAACC-3′) and a stop codon at the 3′ (5′-ATCCTCAATCAAGCCGGTCCCCACG-3′), mimicking the expected caspase cleavage sites. The PCR product was inserted

Abbreviations: eIF, eukaryotic initiation factor; DAP5, death-associated protein 5; IRES, internal ribosome entry site; UTR, untranslated region; GFP, green fluorescent protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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 www.pnas.org/cgi/doi/10.1073/pnas.082102499

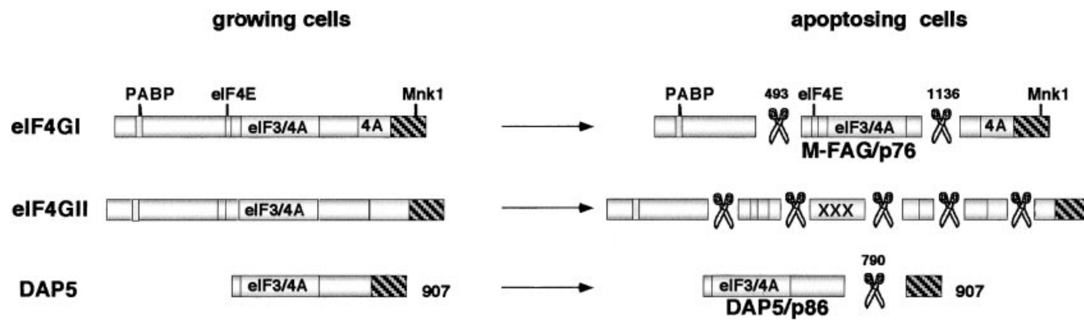

Fig. 1. A diagram to scale highlighting conserved protein-binding regions of mammalian eIF4G homologs aligned through the eIF3/eIF4A-binding region. Regions binding PABP, eIF4E, and Mnk-1 are marked. (*Left* and *Right*) Status of eIF4G protein family in growing and dying cells, respectively. eIF4GI numbering is based on the extended sequence (GenBank accession no. AF104913). Names of the apoptosis-associated eIF4G forms conserving an integral eIF3/eIF4A-binding region that arise after caspase cleavage are indicated below. The disintegrated eIF3/eIF4A-binding region is marked by XXX. Scissors mark caspase cleavage sites based on refs. 7, 9, and 18.

into the pGEM-EASY vector (Promega), and a *Hin*dIII-*Eco*RI fragment was subcloned into the corresponding sites in the pEGFP-C3 vector, giving rise to pEGFP-M-FAG/p76. The LL plasmid (dual luciferase bicistronic vector) is a pcDNA3 construct encoding for *Renilla* luciferase in the first cistron, firefly luciferase in the second cistron, and a multiple cloning site in between. The 5′ UTR of DAP5 (306 bp) was obtained by PCR with primers encompassing *Not*I restriction sites (5′-GGGGCG-GCCGCCAGTGAGTCGGAGCTCTATGG-3′ and 5′-GGG-GCGGCCGCTTTGGCGGCTTGACAACGAAGAATC-3′). c-Myc 5′ UTR (396 bp) and Apaf-1 5′ UTR (578 bp) were obtained by PCR using a human cDNA library as template (5′-GGGCTCGAGTAATTCCAGCGAGAGGCAGA-3′ and 5′-CCCCCATGGTCGCGGGAGGCTGCTGGTTTTCC-3′ for c-Myc IRES and 5′-GGGCTCGAGAAGAAGAGGTAGC-GAGTGGACG-3′ and 5′-CCCCCATGGGCTTCCCTCA-GATCTTTCTCTCTC-3′ for Apaf-1 IRES). The PCR products were inserted into the pGEM-EASY vector, and *Not*I fragments were subcloned into the LL vector *Not*I site, giving rise to LL-DAP5, LL-Myc, and LL-Apaf. A *Not*I-*Xho*I XIAP 5′-UTR fragment (162 bp) excised from the pβgal/5′-(162)/CAT plasmid (12) was subcloned into the corresponding sites of the pBluescript KS vector, and a *Not*I-*Kpn*I fragment was subcloned into the same sites of the LL vector, giving rise to LL-XIAP. The luciferase-SeAP bicistronic (LS) vector and the LS-DAP5 plasmid have been described (9). Sequences and orientations were verified by sequencing. All the subcloned 5′ UTRs have been shown previously to function as IRES elements in the context of bicistronic vectors (9–13).

Cell Lysates. PBS-washed cells were lysed in buffer B as described (9). Cell extract (100 μg of protein) were resolved by 7.5% SDS/PAGE. For immunoprecipitations, 1.5 mg of protein extract was precleared and processed further as detailed (9).

Antibodies. Anti-DAP5 rabbit polyclonal antibodies generated against amino acids 488–742 (19) and anti-eIF4GI rabbit polyclonal generated against amino acids 934-1390 (20) were used at 1:350 dilution for Western blotting. Anti-GFP monoclonal antibodies for Western blotting and immunoprecipitations were purchased from Babco (Richmond, CA). The anti-eIF4A and anti-eIF3/p116 antibodies used in these experiments were described previously (9). 7-Methyl GTP-Sepharose beads (Amersham Pharmacia) were used for affinity binding of eIF4E.

Reporter Assays. 293 cells were transfected with 1.5 μg of bicistronic plasmid and 10 μg of GFP-fusion plasmid per 9-cm plate by calcium phosphate and harvested 16 h posttransfection. Luciferase activity was assessed by the commercial luciferase or dual luciferase assay systems (Promega) for the LS and LL vectors. Light emission was quantified with a Lumac/3M BIO-COUNTER M2010 luminometer. SeAP activity was determined as described (9).

Reporter activity was normalized to protein concentration measured by the Bradford procedure and to transfection efficiency determined in a translation-independent manner by Northern blotting. Total cellular RNA was isolated by Tri-Reagent (Molecular Center, Cincinnati), DNase I (Promega)-treated or poly(A)-selected on Dynabeads, and separated on 1% gel. Northern blotting was carried out as described (9). The quantity of loaded RNA per sample was assessed by using a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe, and the amount of bicistronic transcript was determined by using a reporter probe; the ratio between the two signals represents the transfection efficiency. The intensity of the bands was determined by using a BAS-2000 phosphorimager (Fuji).

Results

Removal of DAP5's C-Terminal Tail Activates Its Ability to Mediate Translation via DAP5 IRES *in Vivo*. DAP5/p86 is the predominant DAP5 form in several apoptotic systems. It is a truncated form of DAP5/p97 that arises naturally by caspase cleavage, which removes its C-terminal tail (Fig. 1; ref. 9). Here, we assessed the effects of DAP5/p86 on cap-dependent and DAP5 IRES-mediated translation *in vivo* and compared them to the effects of the full-length noncleaved DAP5/p97. Our focus on DAP5 IRES stemmed from the fact that it represents the first identified IRES element that functions as a target for DAP5-mediated translation in the *in vitro* translation assay (9). To this end, we used the previously described LS-DAP5 bicistronic vector (Fig. 2*A*; ref. 9). Its first cistron, translated in a cap-dependent manner, encodes luciferase, whereas the second cistron, encoding SeAP, can be translated only in a cap-independent manner via the DAP5 IRES that lies immediately upstream. Transcription of this vector leads to the production of a single ≈4,000-bp mRNA corresponding to the expected size of the bicistronic transcript, as observed by Northern blotting (Fig. 2*C*).

To test the effect of DAP5/p97 or DAP5/p86 on cap-dependent and DAP5 IRES-mediated translation (represented by luciferase and SeAP activities, respectively), the LS-DAP5 vector was cotransfected into 293 cells with GFP, GFP-DAP5/p97, or GFP-DAP5/p86. Expression of DAP5/p97 or DAP5/p86 enhanced the SeAP/luciferase ratio by 1.6- or 2.8-fold, respectively ($P < 0.01$ according to Student's t test), indicating that the DAP5 proteins affected the ratio between cap-dependent and DAP5 IRES-mediated translation (Fig. 2*B Left*).

The elevated SeAP/luciferase ratio obtained in the presence of the DAP5 proteins could result from reduced cap-dependent

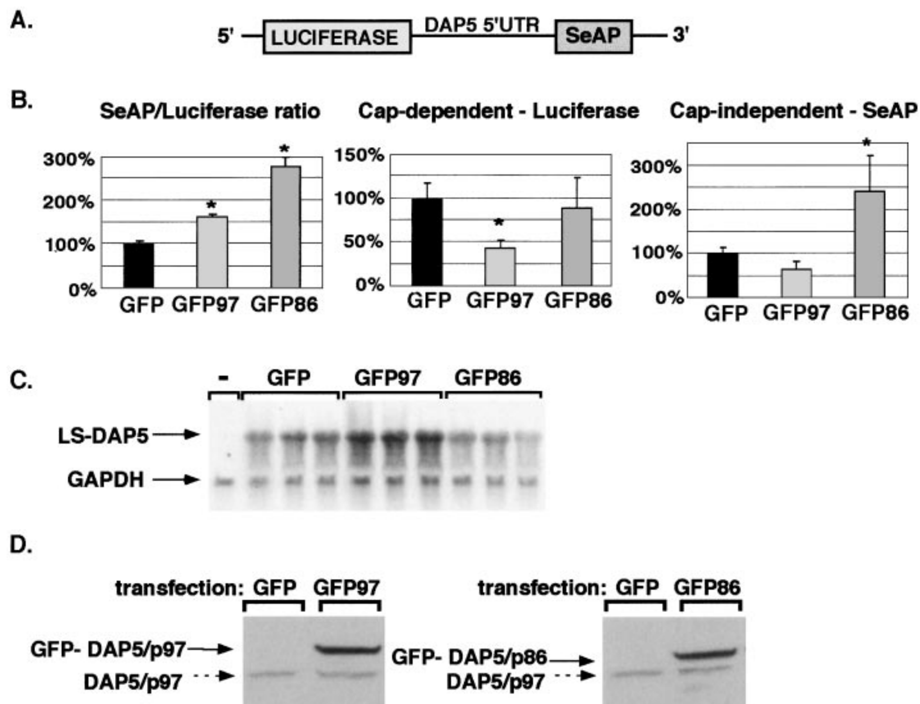

Fig. 2. (*A*) Schematic representation of LS-DAP5 bicistronic transcript. (*B*) 293 cells were cotransfected with GFP, GFP-DAP5/p97, or GFP-DAP5/p86 and LS-DAP5. Reporter levels and the ratio of translation via DAP5 IRES and cap-dependent translation were determined. The reporter ratio obtained by the GFP vector was set at 100%, and the relative fold increase in the SeAP/luciferase ratio was calculated accordingly (*Left*). The effects of the GFP-DAP5 forms on each reporter independently, normalized to protein level and transfection efficiency, and relative to the reporter activity obtained by the GFP vector are presented (*Middle* and *Right*, respectively). The results represent the average of three independent experiments. Asterisks mark statistically significant results. (*C*) Total RNA samples of the experimental points were Northern-blotted and reacted with probes raised against SeAP cDNA for detection of the bicistronic transcript and GAPDH cDNA for determination of total amount of loaded RNA. The ratio between the bicistronic and GAPDH signals represents the transfection efficiency. The arrows on the left indicate the positions of the bicistronic and GAPDH transcripts. (*D*) Protein extract samples of representative experimental points were immunoblotted with anti-DAP5 antibodies. The dashed arrow on the left indicates endogenous DAP5/p97. The full arrow indicates exogenous GFP-DAP5/p97 and GFP-DAP5/p86.

translation or elevated DAP5 IRES-mediated translation. To differentiate between these two options, we assessed the effect of DAP5 on each reporter separately. This separation was achieved by normalizing the activity of each reporter to protein levels and transfection efficiencies in each sample (Fig. 2*C*). This type of analysis revealed that the two DAP5 forms differed substantially from each other. The moderate elevation in the ratio of the reporters caused by GFP-DAP5/p97 resulted from a reduction in cap-dependent translation ($P < 0.01$), whereas no effects were detected on the DAP5 IRES-mediated translation (Fig. 2*B*). Conversely and most importantly, the pronounced elevation in the ratio of reporters caused by the GFP-DAP5/p86 form resulted from elevation of the DAP5 IRES-driven reporter ($P < 0.02$), whereas no detectable effects could be seen at the level of the cap-dependent translation. Western blot analysis showed that GFP-DAP5/p97 and p86 were expressed to a similar extent (4–5-fold over the endogenous levels; Fig. 2*D*). This result confirmed that the different effects of DAP5/p97 and DAP5/p86 on cap-dependent and DAP5 IRES-mediated translation were conferred by the mere removal of the C-terminal tail and were not caused by different expression levels. The inhibition by DAP5/p97 of cap-dependent translation is consistent with previous reports (16, 17) and may result from the sequestration of rate-limiting translation initiation cofactors away from the active translation system (21). Interestingly, we find here that the presence of DAP5's C-terminal tail also is essential for getting this reduction in cap-dependent translation. Yet, the relevance of this inhibitory effect to the mode of action of the endogenous DAP5 is not clear, as detailed in *Discussion*. The other most important outcome of the C-terminal truncation is the activation of DAP5's ability to promote DAP5 IRES-dependent translation, an intriguing property that was analyzed further in detail in the next experiments.

The Caspase Cleavage Product of DAP5 but Not That of eIF4GI Supports Death IRES-Mediated Translation *in Vivo*. It has been suggested that during apoptosis mRNAs harboring death IRESs are preferentially translated, whereas the cap-dependent translation of the majority of mRNAs is abrogated severely (13). These alterations in translation have been associated with caspase cleavage of several members of the eIF4G translation initiation factor family. Determination of the caspase cleavage sites of the eIF4G proteins indicates that although eIF4GII is disintegrated functionally (7), DAP5/p86 and eIF4GI middle fragment M-FAG/p76 are the only caspase-cleaved eIF4G products that harbor an intact minimal core region required for their scaffold function in translation (Fig. 1; refs. 7, 9, and 18). This domain is considered essential for translation initiation support, as has been demonstrated in ribosome binding experiments (22–24). We therefore examined how these two caspase-cleaved eIF4G proteins that arise after apoptosis induction affect translation in the cell.

To do so, we constructed a set of bicistronic vectors (LL, LL-DAP5, LL-Myc, LL-Apaf-1, and LL-XIAP) that encode *Renilla* luciferase in the first cistron, firefly luciferase in the second cistron, and the corresponding IRES elements in between. The presence of each of these IRESs gives rise to enhanced firefly/*Renilla* luciferase ratios of varying degrees when expressed in 293 cells in comparison with a vector lacking an IRES element (Fig. 3*A*).

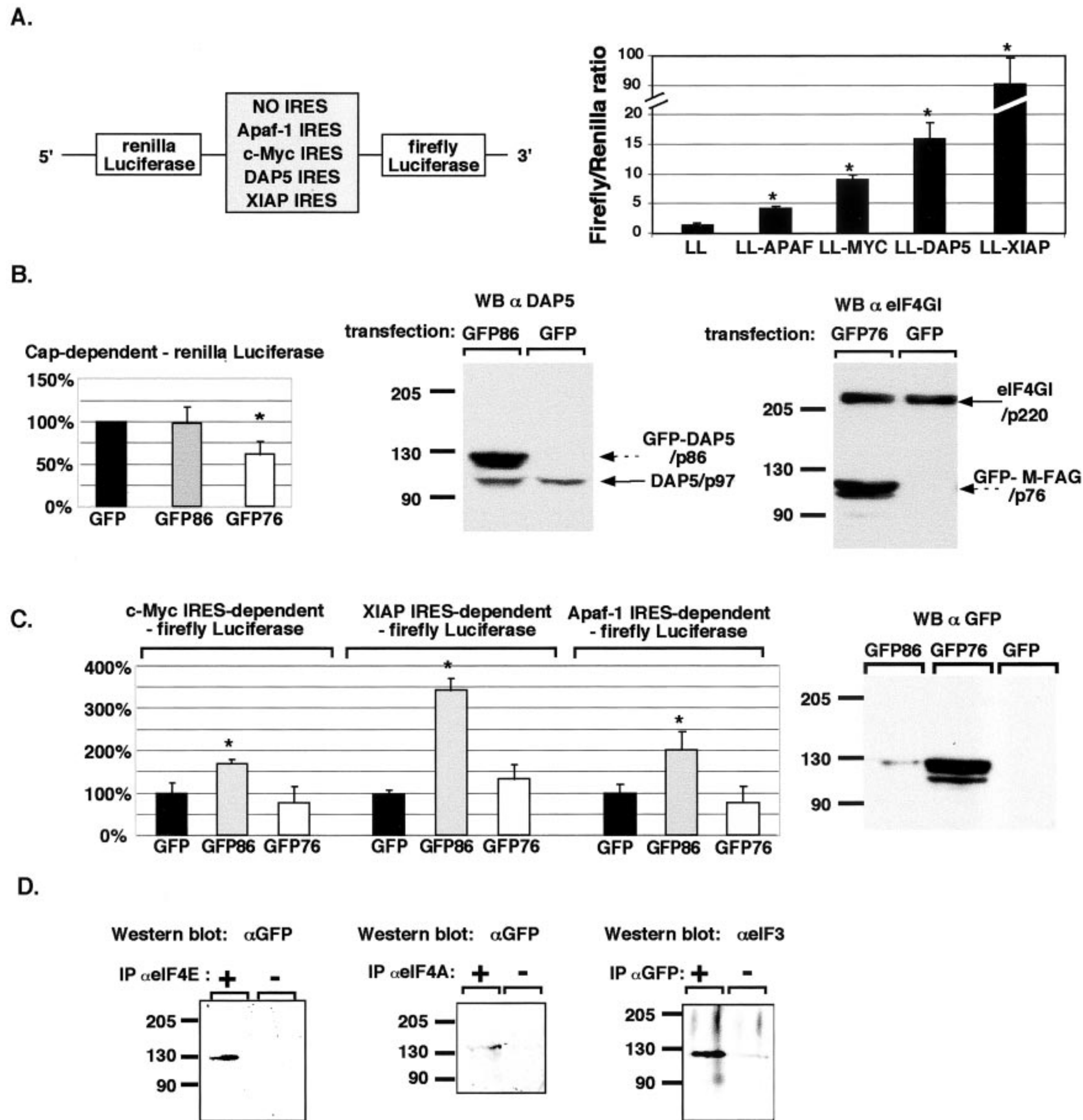

Fig. 3. (*A*) Schematic representation of LL bicistronic transcripts (*Left*). 293 cells were cotransfected with GFP and each LL-based bicistronic vector. Reporter levels were assessed, and the firefly/*Renilla* luciferase ratio was calculated. The ratio obtained by the empty LL vector was set at 100%, and the relative fold increase of the ratio in the presence of each IRES was set accordingly (*Right*). The results represent the average of three independent experiments. Asterisks mark statistically significant results. (*B*) 293 cells were cotransfected with GFP, GFP-DAP5/p86, or GFP-M-FAG/p76 and LL-based bicistronic vectors. *Renilla* luciferase activity was assessed and normalized to protein and transfection level. The activity obtained by the GFP vector was set at 100%, and the relative activity with GFP-DAP5/p86(GFP86) and GFP-M-FAG/p76(GFP76) was calculated accordingly (*Left*). The results represent the average of three independent experiments. Protein extract samples of representative experiments were immunoblotted with anti-DAP5 or anti-eIF4GI antibodies (*Middle* and *Right*, respectively). The full arrows indicate endogenous DAP5/p97 or eIF4GI. The dashed arrows mark exogenous GFP-DAP5/p86 and GFP-M-FAG/p76. WB, Western blot. (*C*) 293 cells were cotransfected with GFP, GFP-DAP5/p86, or GFP-M-FAG/p76 and LL-based bicistronic vectors LL-Myc, LL-Apaf-1, and LL-XIAP. The levels of the firefly luciferase were assessed and normalized to protein and transfection levels. For each bicistronic vector, the firefly luciferase activity obtained by the GFP vector was set at 100% (black bar), and relative activities of the GFP-DAP5/p86 (GFP86) and GFP-M-FAG/p76 (GFP76) were calculated accordingly (gray and white bars, respectively). The results of each IRES represent the average of at least three independent experiments. Protein extract samples of representative experiments were immunoblotted with anti-GFP antibodies (*Right*). (*D*) 293 cells were transiently transfected with GFP-4GM/p76. Twenty-four hours posttransfection the cells were extracted gently in B buffer. Extract (1.5 mg) was incubated with naked beads as a control, with 7-methyl GTP beads (to trap eIF4E protein, *Left*) or anti-eIF4A antibodies conjugated to agarose beads (*Middle*) and washed extensively. Coimmunoprecipitation of GFP-4GM/p76 was assessed by Western blotting the immunoprecipitates with antibodies against the GFP epitope. Alternatively, the ectopically expressed GFP-4GM/p76 was immunoprecipitated (IP) with anti-GFP-conjugated antibodies or naked beads as a control. After resolving the immunoprecipitates on gel, coimmunoprecipitation of endogenous eIF3 was assessed by Western blotting with antibodies against the eIF3/p116 subunit (*Right*).

To study the effects of DAP5/p86 and eIF4GI-M-FAG/p76 on cap-dependent translation, the LL vectors were transfected into 293 cells together with GFP, GFP-M-FAG/p76, or GFP-DAP5/p86. By analyzing and normalizing the activity of the *Renilla* luciferase, the effects on cap-dependent translation were determined. Western blot analysis indicated that GFP-M-FAG/p76 was expressed 4-fold over the endogenous eIF4GI level, similar to the overexpression level of GFP-DAP5/p86 relative to endogenous DAP5/p97 (Fig. 3*B*). As observed already, overexpression of GFP-DAP5/p86 had no significant effect on cap-

dependent translation. In contrast, overexpression of GFP-M-FAG/p76 repressed cap-dependent translation by nearly half ($P < 0.01$). Pull-down experiments indicated that the GFP-M-FAG/p76 fused protein was capable of binding to the endogenous eIF4E, eIF4A, and eIF3/p116 translation initiation factors, suggesting that it retained the overall properties of a scaffold protein (Fig. 3*D*). M-FAG/p76 contains only one of the two eIF4A binding sites present in full-length eIF4GI/II. This property may reduce its efficiency as a translation initiation factor (21), and as a consequence high expression levels of this form should compute with the more effective full-length eIF4GI, resulting in the observed repression of cap-dependent translation.

Finally, we assessed which caspase-cleaved eIF4G protein might be capable of supporting translation through death IRESs. To this end the LL vectors harboring the IRES elements of c-Myc, Apaf-1, and XIAP were cotransfected into 293 cells with GFP, GFP-M-FAG/p76, or GFP-DAP5/p86. By normalizing the activity of the firefly luciferase reporter to protein and transfection levels, the effects on translation mediated by each IRES was determined. Although overexpression of GFP-M-FAG/p76 had no statistically significant effect on c-Myc, Apaf-1, or XIAP IRES-mediated translation, overexpression of GFP-DAP5/p86 significantly enhanced translation via these death IRESs (Fig. 3*C*). Overexpression of GFP-DAP5/p86 had no significant effect on the translation of the second cistron when assayed on the empty LL vector, devoid of an IRES element (data not shown). Western blot analysis against the common GFP epitope confirmed that the lack of effects of GFP-M-FAG/p76 on death IRES-mediated translation was not caused by insufficient expression, because it was expressed in excess over GFP-DAP5/p86, which was effective (Fig. 3*C*). Thus, we conclude that in this experimental system, overexpression of DAP5/p86 but not eIF4GI M-FAG/p76 enhances translation through death IRESs and marks these IRESs as translation targets of DAP5/p86.

Discussion

Apoptosis is an active process, characterized by a distinct set of ordered morphological and biochemical changes including translation alterations. These changes involve cap-dependent translation shutdown as well as selective death IRES ongoing translation. These changes are correlated tightly with caspase cleavage of a variety of translation initiation factors. Our present work investigated the consequences of the caspase cleavage of eIF4G proteins and their ability to support death IRES-mediated translation.

Besides the prototypic eIF4GI/II proteins, DAP5, a less characterized eIF4G protein, is an appealing candidate to support death IRES translation. This suggestion is based on previous work marking DAP5 as an indispensable, nonredundant, positive mediator of apoptosis induced by IFN-γ (19). Specifically, it was found that interference with DAP5 function (using a dominant negative DAP5 fragment) conveyed some resistance to IFN-γ-induced apoptosis. Because several redundant mechanisms for apoptosis-associated shutdown of cap-dependent translation are activated simultaneously, we explored whether the rate-limiting function of DAP5 in apoptosis could be associated with a rather unique ability to support death IRES translation. To this end we examined the ability of the caspase-cleaved eIF4G proteins DAP5/p86 and eIF4GI M-FAG/p76 to support translation through IRESs of death genes, the translation of which is maintained during apoptosis. We found that DAP5/p86 stimulated translation through the death IRESs of c-Myc, XIAP, and Apaf-1, whereas eIF4GI M-FAG/p76 failed to do so. Thus, the caspase-cleaved DAP5/p86 possesses the specific properties required for driving death IRES-mediated translation, properties that are not shared by other caspase-cleaved eIF4G products. Furthermore, taking into account that the caspase-cleaved eIF4G products are naturally present only in cells undergoing apoptosis, we reason that the caspase-cleaved DAP5 is the most probable mediator of death IRES-dependent translation in the dying cell.

In addition, we studied the impact of the caspase-mediated removal of DAP5's tail. To this end we compared the consequences of overexpressing DAP5/p97 (normally present in growing cells) or DAP5/p86 (normally present in dying cells) on cap-dependent and DAP5 IRES-mediated translation in an *in vivo* translation assay in growing cells. We found that conversion of DAP5/p97 to DAP5/p86 regulates its function translation-wise in at least two modes. First, the removal of the C-terminal tail activates its ability to mediate translation via DAP5 IRES *in vivo*. Second, it abolished the inhibitory effect of the overexpressed DAP5 protein on cap-dependent translation. These findings assign an important regulatory role to the protein's C-terminal tail (60 aa) that is absent in DAP5/p86 and provide a tight link between caspase activation and IRES-mediated translation. Furthermore, the experimental settings of expressing a caspase-cleaved protein form in growing cells allow us to conclude that the caspase cleavage of DAP5 protein alone is enough to activate DAP5's ability to support death IRES translation and does not require any accompanying apoptosis-associated events. Interestingly, although overexpression of DAP5/p97 in cells does not enhance translation through DAP5 IRES, it does support DAP5 IRES-mediated translation to some extent after purification and addition to an *in vitro* translation system (9), which indicates that the mere presence of the C-terminal tail does not suffice to restrain DAP5/p97 function completely and that additional factors must contribute to the repression of DAP5/p97. Such factors might bind to the C-terminal tail and evoke a reversible posttranslation modification that restrains its ability to drive IRES-mediated translation. One such candidate factor is Mnk1, which binds DAP5/p97 through its C-terminal tail (25). Its possible involvement in repressing the translation by DAP5/p97 should be addressed in the future.

As for cap-dependent translation, we found that it is inhibited by overexpression of M-FAG/p76 or of DAP5/p97. In both cases the inhibition of translation probably is attributed to competition and titration of translation cofactors by the over-

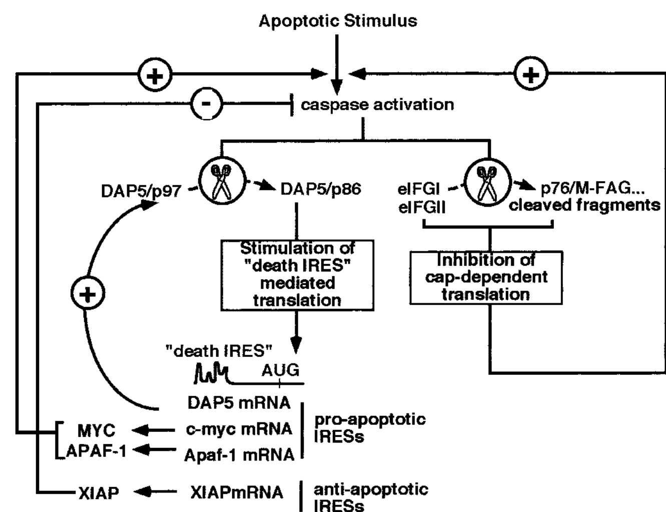

Fig. 4. A model scheme illustrating the contribution of eIF4G proteins in general and DAP5 protein specifically to the fine balance between cell death and viability in the presence of an apoptotic trigger. Positive and negative feedback loops are marked by plus and minus signs, respectively. Caspase cleavage events are marked by scissors.

expressed forms as discussed above. However, it is most probable that both DAP5/p97 and M-FAG/p76 were found to act as translation inhibitors because they were overexpressed beyond their physiological levels, thus pushing the translation system out of balance in a physiologically irrelevant manner. This notion is supported by the fact that DAP5 knockout embryonic stem cells do not show enhanced translation as would be expected under conditions where a translation repressor is lost (26). As for M-FAG/p76, although some reports show that it may accumulate to similar levels as did the eIF4GI levels in growing cells (5), other studies marked it as an extremely unstable protein in dying cells, making its role as a translation competitor much less probable in these systems (9, 27).

It is suggested that DAP5 is a caspase-activated translation initiation factor, with the potential to support translation at least through the death IRESs of DAP5, c-Myc, XIAP, and Apaf-1 during apoptosis. By doing so, not only does DAP5/p86 set up a local positive feedback loop feeding more DAP5 protein into the system, but it also contributes to wider feedback loops, influencing the apoptotic process (Fig. 4). DAP5/p86 contributes to negative feedback loops, counteracting the apoptotic process by feeding antiapoptotic proteins such as XIAP, the natural antagonist of caspases 3, 7, and 9 (28), into the dying cell. DAP5/p86 also contributes to positive feedback loops, reinforcing the apoptotic process by feeding more proapoptotic proteins into the system. Examples of such proteins are Apaf-1, which mediates cytochrome *c*-dependent activation of procaspase-9 (29), and c-Myc, which sensitizes cells to a variety of apoptotic triggers through cytochrome *c* release and collaboration with other apoptotic signals such as Fas and p53 (30, 31). In death systems such as the original IFN-γ/HeLa cells from which the *DAP5* gene was rescued as a rate-limiting death-promoting factor (19), the proapoptotic targets of DAP5 protein dominated. Because overexpression of DAP5/p86 does not suffice to induce apoptosis (unpublished results), it is clear that DAP5-independent proapoptotic events have to take place as well. These events may include the mere shutdown of cap-dependent translation that normally supports cell growth (32), at least partially mediated by the cleavage of translation initiation factors such as the eIF4GI and eIF4GII proteins, and also other proapoptotic events that do not involve translation regulation at all.

Today it is clear that apoptosis is an active process involving a wide variety of genes, some of which remain to be uncovered. A challenge in the apoptosis field is to identify genes that enable the cell to initiate and carry out the execution of the apoptotic program. The unique characterization of DAP5/p86 as a translation factor with selectivity toward mRNAs harboring death IRESs over mRNAs translated in the conventional cap-dependent mode marks it as a promising future tool to point out additional yet-unidentified death IRES-harboring genes.

We thank Dr. Chaim Kahana for the anti-eIF4GI antibodies and the dual luciferase bicistronic vector, Dr. Nahum Sonenberg for the BS-eIF4GI plasmid, and Dr. Martin Holcik for the pβgal/5′-(162)/CAT plasmid. We thank Dr. Shani Bialik for critically reading the manuscript. This work was supported by the Deutsches Krebsforschungszentrum (DKFZ) and Israel's Ministry of Science (MOS). A.K. is the incumbent of Helena Rubinstein Chair of Cancer Research.

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