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The 5′ untranslated region of Apaf-1 mRNA directs translation under apoptosis conditions via a 5′ end-dependent scanning mechanism

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ABSTRACT

We have previously shown that translation driven by the 5′ UTR of Apaf-1 mRNA is relatively efficient in the absence of m7G-cap, but no IRES is involved. Nevertheless, it may be speculated that a "silent" IRES is activated under apoptosis conditions. Here, we show that translation of the mRNA with the Apaf-1 5′ UTR is relatively resistant to apoptosis induced by etoposide when eIF4E is sequestered by 4E-BP and eIF4G is partially cleaved. However, translation under these conditions remains governed by 5′ end-dependent scanning. We hypothesize that the observed phenomenon is based on the intrinsic low cap-dependence of the Apaf-1 5′ UTR.

1. Introduction

Regulation of translation in most cases occurs at the level of initiation by modulating the activity of key proteins involved either in recruitment of mRNA onto ribosomes (eukaryotic translation initiation factors eIF4E, eIF4G) or Met-tRNA$_i^{Met}$ delivery (eIF2). The signal transduction pathways by which the activities of these translation initiation factors are repressed or activated is now under intense investigation [1–6]. The translation of individual mRNAs is affected by environmental changes or stresses to a different extent and these individual responses mostly depend on the length and structure of their untranslated regions.

The availability of eIF4E, and therefore formation of eIF4F, is regulated by a family of translation repressors, the eIF4E-binding proteins (4E-BPs) [7–9], which sequester eIF4E from eIF4F. The capacity of the 4E-BP1 to bind eIF4E is determined by phosphorylation status of the former. Several stress conditions (including serum deprivation, hypoxia, heat shock, viral infection and apoptosis) block mTOR kinase pathway and cause dephosphorylation of 4E-BP1 which in turn sequesters eIF4E away from eIF4G and inhibits cap-dependent translation [10]. In particular, this occurs during the treatment of cells with etoposide, a DNA damaging pro-apoptotic agent used in this study [11].

It is conceivable that the expression of some specific proteins should be maintained or, at least, affected less dramatically under unfavorable conditions when cap-dependent translation is compromised. For instance, during apoptosis the synthesis of pro- and anti-apoptotic proteins can be expected to still occur in a preferential manner. One of them, apoptotic peptidase activating factor 1 (Apaf-1), which is a component of apoptosome, behaves this way [12]. Specific features of the 5′ UTR of Apaf-1 mRNA have been subject of several studies [13–15] and this paper is also concerned with the mechanism of translation of this mRNA.

To explain the preferential synthesis of some proteins under stress conditions, or even to explain the efficient translation of mRNAs with long and structured 5′ UTRs, the concept of cellular IRESs was put forward nearly two decades ago [16] and still dominates. According to this hypothesis, some cellular mRNAs harbor IRESes and can be translated by the 5′ end dependent scanning and/or by internal ribosome entry mechanisms, but under stress

Abbreviations: IRES, internal ribosome entry site; CITE, cap-independent translation enhancer; Apaf-1, apoptotic peptidase activating factor 1; eIF, eukaryotic initiation factor; ITAF, IRES trans-activating factor; 5′ UTR, 5′ untranslated region; nt, nucleotide

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the internal initiation mode becomes predominant (obviously, it is logical because their resistance to stress resembles the behavior of well established viral IRESes) [17]. However, the real contribution of the IRES-dependent mechanism *vs*. the 5′ end-dependent one to the translation of some individual mRNA has never been assessed either under normal conditions or under stress. Usually, only the internal initiation mode is believed to confer a resistance of some mammalian mRNAs to abnormal conditions [18–22].

An alternative explanation for differential translation of mRNAs under stress conditions emerged from our recent experiments. We found that the contribution of the m^7G-cap to translational efficiency under normal conditions varies for selected individual 5′ UTRs, though none of them had an IRES: they could not support the expression of the second reporter in bicistronic mRNAs to any significant extent, even when compared to monocistronic mRNAs lacking m^7G-cap [23]. Notably, contrary to conventional wisdom, there was no correlation between the magnitude of the stimulation by the 5′-cap (cap-dependence) and the length of the 5′ UTR or overall stability of its secondary structure. Among tested 5′ UTRs the 5′ UTR of Apaf-1 mRNA had the lowest cap-dependence. We hypothesized that this reduced cap-dependence of some 5′ UTRs, rather than IRESs, might confer a relative resistance of translation of the corresponding mRNAs under conditions inactivating eIF4E.

However, it is believed that "special" IRESes exist that are "silent" under normal conditions but become active under stress conditions. Here we show that under hard apoptotic conditions induced by etoposide the Apaf-1 5′ UTR is still scanned by ribosomes from the 5′ end and no internal ribosome entry is involved. Moreover, in line with our hypothesis, the translation driven by 5′ UTR of Apaf-1 is more resistant to apoptotic stress conditions than that directed by many other 5′ UTRs.

2. Materials and methods

2.1. Plasmids

All dicistronic DNA constructs were based on the pGL3R vector [24]. pGL3R-Apaf-1, and pGL3R-β-globin constructs were described in [23,25]. pGL-Apaf-1 Fluc was prepared by removing the Rluc sequence to obtain a monocistronic construct. pGL-Apaf-1 Rluc was prepared by replacing the Fluc sequence with Rluc sequence by digestion with NcoI and XbaI of the corresponding vector and the PCR product of Rluc with the NcoI site incorporated from a primer (NcoI is absent in the pRluc construct). The pRluc plasmid was described in [26]. To obtain constructs with upstream AUG codons, we made corresponding insertions between nts 45 and 46 of Apaf-1 5′ UTR in pGL-Apaf-1 Fluc; insertion of the CCATGGATAAA sequence resulted in formation of a 20 codon long uORF, whereas insertion of the CCATGGATAAAA resulted in a 130 codon long uORF.

2.2. mRNA preparation

mRNAs were prepared exactly as in [26]. Briefly, we prepared PCR products with the universal reverse primer 5′-$(T)_{50}$-AACTTGTTTATTGCAGCTTATAATGG-3′ and either 5′ UTR-specific primers containing the T7-promotor, or the universal forward primer 5′-CTAGCAAAATAGGCTGTCCC-3′ for those constructs that already have the T7 promoter. The PCR products were used then as templates for in vitro RNA transcription using a T7 RiboMAX Large Scale RNA Production kit (Promega). For preparation of m^7G- or A-capped transcripts, the 3′-O-Me-m^7GpppG or ApppG (NEB) was added to the transcription mix in a proportion of 10:1 to GTP. The resulting RNAs were purified by LiCl precipitation and checked for integrity by denaturing PAGE.

2.3. Cell culture and transfection procedures

HEK293T cells were cultivated in DMEM supplemented with 10% FBS as described [26]. The day before transfection, exponentially growing cells were replated to 24-well plates. After 12–16 h of growth, when the cell density reached 60–80% the transfection was performed using Unifectin-56 (RusBioLink) [26]. The protocol was slightly modified to obtain the maximal yield of transfection. For a typical RNA transfection, a mixture of 0.2 μg of reporter mRNA and 0.01 μg of a reference reporter mRNA (m^7G-capped Rluc-poly(A) or m^7G-capped β-globin Fluc-poly(A)) was incubated with 0.42 μl of Unifectin in 125 μl DMEM for 15 min and then added to the growth medium. Two hours later, cells were harvested and luciferase activities were analyzed with the Dual Luciferase Assay kit (Promega). All the transfections were repeated several times in different cell passages. Several most important experiments were repeated using Lipofectamin 2000 (Invitrogen) and produced the same results. For experiments with etoposide, cells were replated to 24-well plates in two sets with different densities, the second set being ~2.5-fold more diluted than the first one. 6 h after replating, the more dense set was treated with 100 μM etoposide. After 38 h both sets attained approximately the same density and then the transfection was performed as described above.

2.4. Western blotting

The same cell lysates as for luciferase measurements were used for Western blotting. As primary antibodies, we used Rb X eIF4E Binding Protein (Chemicon International, AB3251) and rabbit anti-GAPDH (Proteintech Group INC, PTG10494-1-AP); rabbit anti-eIF4GI antibodies were a kind gift by Dr. R. Rhoads.

3. Results

3.1. Intrinsic reduced cap-dependence of the Apaf-1 5′ UTR may account for a higher resistance of the corresponding mRNA to apoptosis conditions

Translation of some specific cellular mRNAs, including the Apaf-1 mRNA, is known to be relatively resistant to various types of cell stress. The continuous synthesis, even at a lower level, of specific proteins encoded by such mRNAs may be required to determine the fate of cells (i.e. survival or death) exposed to stress conditions. We have recently proposed a hypothesis that such a relative resistance to stress conditions may be explained by structural features of 5′ UTRs of mammalian mRNAs that determine the requirement for the m^7G cap [23]. Here we decided to check whether the reduced cap-dependence of the Apaf-1 5′ UTR reflects the property of the mRNA to be translated under conditions of apoptosis where the Apaf-1 protein should play an important functional role – activation of caspases. For these experiments, we selected etoposide, the drug that exerts its anticancer effects by inhibiting the topoisomerase II which ultimately leads to rapid accumulation of un-repaired breaks in cellular DNA. It has been demonstrated that etoposide treatment inhibits cap-dependent translation by activating (*via* dephosphorylation) the eIF4E repressor, 4E-BP1, and by inducing cleavage of the key initiation factor eIF4G [11]. Since cap-dependence itself is somewhat artificial parameter (there are no A-capped mRNAs in cells), we decided to first check whether the low cap-dependence reflects the ability of m^7G-capped mRNA to be resistant to stress conditions. We compared the effect of the drug on the translation of m^7G-capped mRNAs carrying standard 5′ UTRs (from β-globin mRNA or non-specific vector sequence) and those possessing the entire Apaf-1 5′ UTR. To this end, HEK293T cells were treated with 100 μM etoposide for 38 h, then transfected

with mRNAs, and 2 h later the Fluc and Rluc activities were determined. Fig. 1A confirms that the treatment with etoposide did lead to accumulation of hypophosphorylated 4E-BP1 and cleavage of eIF4G. As clear from Fig. 1B, the ratio of β-globin Fluc/Apaf-1 Rluc significantly reduced on exposure of cells to the drug, the reverse effect was found for the Apaf-1 Fluc mRNA and Rluc mRNA pair, whereas the ratio of Fluc/Rluc did not change when the two different coding sequences carried the same Apaf-1 leader (control). It should be noted that in all experiments with etoposide treatment the activity of standard capped reporter mRNA (i.e. standard Rluc mRNA reporter) was inhibited 7–8 times which reflects the degree of protein synthesis inhibition in cells.

3.2. An upstream AUG codon inserted in the 5′ proximal part of Apaf-1 5′ UTR inhibits mRNA translation even under conditions of apoptosis, and irrespectively of whether the mRNA is m^{7}G capped or not

The question arises what translation initiation mode is involved in the selection of the AUG initiation codon in the Apaf-1 mRNA under conditions of apoptosis? Is it possible that under severe inactivation of eIF4F in living cells mammalian ribosomes are still capable of binding to the 5′ end of mRNAs and scanning highly structured 5′ UTRs? To gain an answer to this question, we introduced an upstream AUG codon near the 5′ end, at position 45 of the 577 nt-long Apaf-1 5′ UTR, i.e. very far from position of the putative Apaf-1 IRES. Two constructs were obtained, one – with the uORF encoding 20 amino acids (aa) and another encoding the uORF of 130 aa. Each of the mRNAs was prepared in the m^{7}G-capped and in the uncapped (A-capped) forms and tested in the presence or absence of etoposide. The results of these transfections are presented in Fig. 2A and B for the m^{7}G-capped and A-capped mRNAs, respectively. Clearly, the insertion of uORFs severely inhibited the translation, irrespectively of whether the mRNA was m^{7}G-capped or not, and also dramatically inhibited translation under stress induced by etoposide. Notably, the increased etoposide resistance of translation for mRNAs with the Apaf-1 5′ UTR described above was maintained in all cases. It should be emphasized that exposure to etoposide did not stimulate the background translational activity of the Apaf-1 5′ UTR placed in the intercistronic position of a dicistronic mRNA. This residual activity even slightly decreased (Fig. 3), arguing against involvement of an internal ribosome entry. In Fig. 3 we present the absolute Fluc activities (though expressed in arbitrary units) to draw attention to the fact that the translational efficiency of the second cistron in these cases is extremely low and close to the background determined in samples with no RNA transfected (data not shown). The corresponding

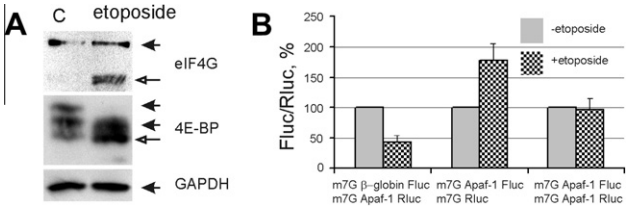

Fig. 1. Reduced cap-dependence of the mRNA with the Apaf-1 5′ UTR accounts for the relative resistance of its translation to apoptosis. (A) Inactivation of eIF4G and eIF4E on exposure of cells to etoposide. Arrows indicate the intact eIF4G and its cleavage product as well as conversion of the translation repressor 4E-BP1 into its inhibitory hypophosphorylated form, GAPDH serves as a loading control; (B) Translation of co-transfected pairs of mRNAs under normal conditions (grey bars) and after treatment of cells with etoposide (hatched bars). The Fluc/Rluc ratio under normal conditions was set to 100%. It should be noted that in all cases with etoposide treatment the activity of standard capped reporter mRNA was inhibited 7–8 times.

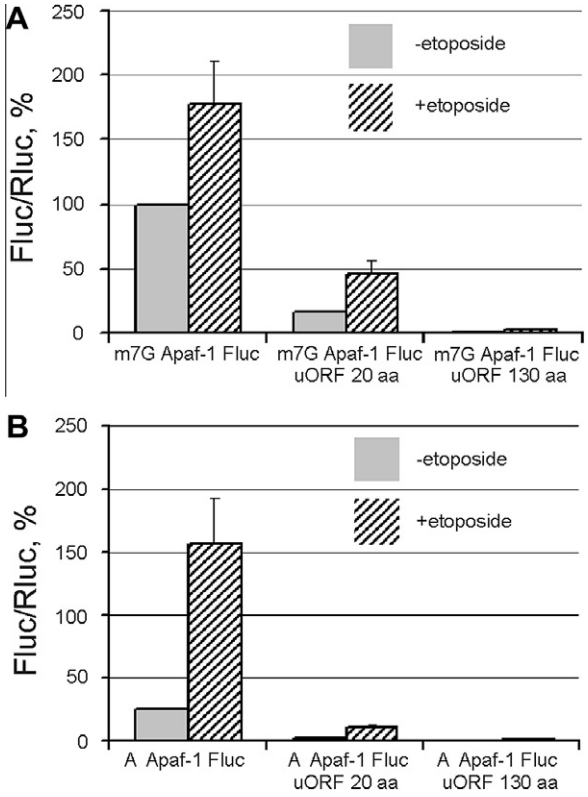

Fig. 2. Insertion of an upstream AUG codon dramatically inhibits translation of mRNAs carrying the Apaf-1 5′ UTR under normal and stress conditions, irrespective of whether they are capped or not. (A) Translation of capped mRNAs under normal conditions (grey bars) and after treatment of cells with etoposide (hatched bars). (B) The same as in (A) but for uncapped (A-capped) mRNAs. The transcripts with the inserted uAUG codons were co-transfected with the control m^{7}G Rluc mRNA used for normalization and the ratio of Fluc/Rluc was determined 2 h later.

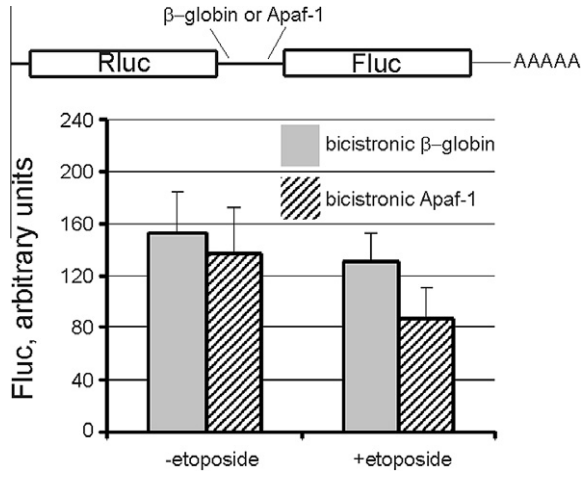

Fig. 3. The translation of the second cistron (Fluc) directed by the Apaf-1 5′ UTR in a dicistronic mRNA is not activated upon etoposide treatment. The constructs with the β-globin and Apaf-1 5′ UTRs in the intercistronic position are shown above the diagram. The Fluc activities were normalized to Rluc values determined in the same samples. It should be noted that the translational efficiency of the second cistron is extremely low, whereas under the same conditions even an A-capped monocistronic Apaf-1 Fluc mRNA reveals a two order higher Fluc activity (data not shown).

values for the Apaf-1 5′ UTR in monocistronic constructs, even in uncapped (A-capped) form, are tens of thousand units.

4. Discussion

The currently dominating concept claims that any secondary structure within 5′ UTRs of eukaryotic mRNAs causes a negative effect on the translation of mRNAs unless the corresponding stem-loop structures form an IRES. The well-known inhibitory effect of a stable stem-loop placed at the very 5′ end of an mRNA is often automatically extended to natural stem-loop structures found in internal positions of 5′ UTRs. As shown in our previous studies [23,26,27] and in this paper, the real situation is far more complex. A specific secondary structure within a 5′ UTR can play a positive role even in the case of 5′ end dependent scanning mechanism of translation. Moreover, under some circumstances, e.g. unfavorable conditions, it may enhance rather than decrease the efficiency of translation. Our data also suggest that the widespread recognition of the cap-independent mechanism as the IRES-directed mode of translation initiation is not justified: the scanning mechanism can operate in living cells with a reasonable efficiency even for mRNAs that have no functional caps at the 5′ end. Although the level of translation of the Apaf-1 mRNA is substantially decreased in a complete absence of the eIF4E-cap interaction (i.e. for A-capped mRNA), it is not negligible and makes up 15–20% of the level under normal conditions, whereas the translation efficiency of mRNAs with standard 5′ UTRs drops to 2–3%.

The Apaf-1 5′ UTR inherently has a relaxed cap-dependence as compared to standard 5′ UTRs (e.g. from β-actin, β-globin mRNAs), though the translation initiation it directs is strictly 5′ end-dependent. Thus, the reduced cap-dependence (rather than internal ribosomal entry) may play an important role under conditions of apoptosis to maintain the synthesis of Apaf-1 protein. Our preliminary data suggest that this phenomenon is accounted for by specific structural elements within the Apaf-1 leader (manuscript in preparation). Again, these elements do not constitute an IRES, as they do not allow the internal ribosome entry in the case when the 5′ UTR is placed into intercistronic position. Moreover, in the course of translation initiation they are "inspected" by the scanning apparatus presumably in the same way as standard 5′ UTRs. On the other hand as discussed below, they might promote recruitment of the translational machinery onto the Apaf-1 5′ end in eIF4E-independent manner.

Our case may be supported by some published reports, though they are not numerous. The availability of the free 5′ end of mRNA has been demonstrated to be strictly necessary to provide loading the preinitiation complex onto regular (IRES-less) mRNAs regardless whether mRNAs are capped or not. Indeed, eIF4G fragments lacking eIF4E binding site can support in vitro the 5′ end-dependent translation and availability of a free 5′ end is indispensable [28,29]. Moreover, it has been shown that uncapped mRNAs generated in vivo by transcription with RNA polymerase III are nevertheless translated by a 5′ end-dependent scanning mechanism [30].

Which features of mRNAs, especially of their 5′ UTRs, can mediate cap- and IRES-independent accommodation of ribosomes? We speculate that some components of translation apparatus, for example eIF4G, eIF3 (or their analogues with a similar function), are capable to be directly or indirectly recruited onto 5′ UTRs of some mRNAs via RNA–protein interactions which are independent of the 5′-terminal cap, with concomitant recruitment of other components of the scanning apparatus. To attract these components, a 5′ UTR should possess corresponding binding sites playing the role of Cap-Independent Translational Enhancers (CITEs) [31], though their affinity to various initiation factors and ITAFs may be modest. It is logical to assume that such binding sites are easier to organize within the 5′ UTR with a complex secondary structure. In this way, the 5′ UTR of an mRNA creates in its vicinity an elevated concentration of translational components. This also helps to overcome the competition for factors from other cellular mRNAs. We propose that whether this recruitment takes place at the very 5′ end or at some distance from it, the only point of the ribosome entry where the scanning can be initiated is the 5′ terminus of mRNA. This hypothesis was described in detail in [31], and the feasibility of such a model has been recently demonstrated by direct experiments in our lab (Terenin et al., submitted).

Recent genome-wide studies of effects of mTOR inhibitors uncover intriguing features concerning the differential effect of eIF4E inactivation on translation of cellular mRNAs. It has been shown that a special class of mRNAs bearing common features in their 5′ UTRs exists which is extremely sensitive to mTOR inhibitors, whereas the majority of cellular mRNAs are affected much less significantly. Intriguingly, Apaf-1 has been found among mRNAs with the highest resistance to eIF4E inhibition [32,33].

To summarize, we show here that the Apaf-1 5′ UTR can mediate an m^7G-cap independent but 5′ end-dependent scanning even under apoptosis. As a consequence, this leads to the relatively preferential translation of Apaf-1 mRNA under these stress conditions. We hypothesize that this may also be true for other cellular mRNAs with a low cap-dependence.

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References

[1] Cully, M. and Downward, J. (2009) Translational responses to growth factors and stress. Biochem. Soc. Trans. 37, 284–288.
[2] Polunovsky, V.A. and Bitterman, P.B. (2006) The cap-dependent translation apparatus integrates and amplifies cancer pathways. RNA Biol. 3, 10–17.
[3] Averous, J. and Proud, C.G. (2006) When translation meets transformation: the mTOR story. Oncogene 25, 6423–6435.
[4] Robert, F. and Pelletier, J. (2009) Translation initiation: a critical signalling node in cancer. Expert Opin Ther Targets 13, 1279–1293.
[5] Silvera, D., Formenti, S.C. and Schneider, R.J. (2010) Translational control in cancer. Nat. Rev. Cancer 10, 254–266.
[6] Proud, C.G. (2009) MTORC1 signalling and mRNA translation. Biochem. Soc. Trans. 37, 227–231.
[7] Lin, T.A., Kong, X., Haystead, T.A., Pause, A., Belsham, G., Sonenberg, N. and Lawrence Jr., J.C. (1994) PHAS-I as a link between mitogen-activated protein kinase and translation initiation. Science 266, 653–656.
[8] Pause, A., Belsham, G.J., Gingras, A.C., Donze, O., Lin, T.A., Lawrence Jr., J.C. and Sonenberg, N. (1994) Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. Nature 371, 762–767.
[9] Poulin, F., Gingras, A.C., Olsen, H., Chevalier, S. and Sonenberg, N. (1998) 4E-BP3, a new member of the eukaryotic initiation factor 4E-binding protein family. J. Biol. Chem. 273, 14002–14007.
[10] Reiling, J.H. and Sabatini, D.M. (2006) Stress and mTORture signaling. Oncogene 25, 6373–6383.
[11] Morley, S.J., Coldwell, M.J. and Clemens, M.J. (2005) Initiation factor modifications in the preapoptotic phase. Cell Death Differ. 12, 571–584.
[12] Schafer, Z.T. and Kornbluth, S. (2006) The apoptosome: physiological, developmental, and pathological modes of regulation. Dev. Cell 10, 549–561.
[13] Coldwell, M.J., Mitchell, S.A., Stoneley, M., MacFarlane, M. and Willis, A.E. (2000) Initiation of Apaf-1 translation by internal ribosome entry. Oncogene 19, 899–905.
[14] Mitchell, S.A., Spriggs, K.A., Coldwell, M.J., Jackson, R.J. and Willis, A.E. (2003) The Apaf-1 internal ribosome entry segment attains the correct structural conformation for function via interactions with PTB and unr. Mol. Cell 11, 757–771.
[15] Ungureanu, N.H., Cloutier, M., Lewis, S.M., de Silva, N., Blais, J.D., Bell, J.C. and Holcik, M. (2006) Internal ribosome entry site-mediated translation of Apaf-1, but not XIAP, is regulated during UV-induced cell death. J. Biol. Chem. 281, 15155–15163.
[16] Macejak, D.G. and Sarnow, P. (1991) Internal initiation of translation mediated by the 5′ leader of a cellular mRNA. Nature 353, 90–94.
[17] Pestova, T.V., Kolupaeva, V.G., Lomakin, I.B., Pilipenko, E.V., Shatsky, I.N., Agol, V.I. and Hellen, C.U. (2001) Molecular mechanisms of translation initiation in eukaryotes. Proc. Natl. Acad. Sci. USA 98, 7029–7036.
[18] Holcik, M. and Sonenberg, N. (2005) Translational control in stress and apoptosis. Nat. Rev. Mol. Cell Biol. 6, 318–327.
[19] Komar, A.A. and Hatzoglou, M. (2005) Internal ribosome entry sites in cellular mRNAs: mystery of their existence. J. Biol. Chem. 280, 23425–23428.

[20] Graber, T.E. and Holcik, M. (2007) Cap-independent regulation of gene expression in apoptosis. Mol. BioSyst. 3, 825–834.
[21] Spriggs, K.A., Stoneley, M., Bushell, M. and Willis, A.E. (2008) Re-programming of translation following cell stress allows IRES-mediated translation to predominate. Biol. Cell 100, 27–38.
[22] Komar, A.A. and Hatzoglou, M. (2011) Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. Cell Cycle 10, 229–240.
[23] Andreev, D.E., Dmitriev, S.E., Terenin, I.M., Prassolov, V.S., Merrick, W.C. and Shatsky, I.N. (2009) Differential contribution of the m7G-cap to the 5′ end-dependent translation initiation of mammalian mRNAs. Nucleic Acids Res. 37, 6135–6147.
[24] Stoneley, M., Paulin, F.E., Le Quesne, J.P., Chappell, S.A. and Willis, A.E. (1998) C-Myc 5′ untranslated region contains an internal ribosome entry segment. Oncogene 16, 423–428.
[25] Dmitriev, S.E., Andreev, D.E., Adyanova, Z.V., Terenin, I.M. and Shatsky, I.N. (2009) Efficient cap-dependent translation of mammalian mRNAs with long and highly structured 5′-untranslated regions in vitro and in vivo. Mol. Biol. (Mosk) 43, 108–113.
[26] Dmitriev, S.E., Andreev, D.E., Terenin, I.M., Olovnikov, I.A., Prassolov, V.S., Merrick, W.C. and Shatsky, I.N. (2007) Efficient translation initiation directed by the 900-nucleotide-long and GC-rich 5′ untranslated region of the human retrotransposon LINE-1 mRNA is strictly cap dependent rather than internal ribosome entry site mediated. Mol. Cell. Biol. 27, 4685–4697.
[27] Vassilenko, K.S., Alekhina, O.M., Dmitriev, S.E., Shatsky, I.N. and Spirin, A.S. (2011) Unidirectional constant rate motion of the ribosomal scanning particle during eukaryotic translation initiation. Nucleic Acids Res. 39, 5555–5567.
[28] De Gregorio, E., Preiss, T. and Hentze, M.W. (1998) Translational activation of uncapped mRNAs by the central part of human eIF4G is 5′ end-dependent. RNA 4, 828–836.
[29] Ali, I.K., McKendrick, L., Morley, S.J. and Jackson, R.J. (2001) Truncated initiation factor eIF4G lacking an eIF4E binding site can support capped mRNA translation. EMBO J. 20, 4233–4242.
[30] Gunnery, S., Maivali, U. and Mathews, M.B. (1997) Translation of an uncapped mRNA involves scanning. J. Biol. Chem. 272, 21642–21646.
[31] Shatsky, I.N., Dmitriev, S.E., Terenin, I.M. and Andreev, D.E. (2010) Cap- and IRES-independent scanning mechanism of translation initiation as an alternative to the concept of cellular IRESs. Mol. Cells 30, 285–293.
[32] Thoreen, C.C., Chantranupong, L., Keys, H.R., Wang, T., Gray, N.S. and Sabatini, D.M. (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 485, 109–113.
[33] Hsieh, A.C. et al. (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature 485, 55–61.