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TRANSLATION CONTROL IN APOPTOSIS

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Regulation of protein synthesis, although known for many decades, has only recently begun to be recognized as a critical control mechanism for the maintenance of cellular homeostasis and cellular stress response. One of the key advantages of translational control is the ability of cells to rapidly reprogram the protein output in response to internal or external triggers. This is particularly important during cellular response to stress that may lead to apoptosis by providing cells with a fine tuning mechanism that tips the balance between cell survival or apoptosis. In the following review we highlight several distinct mechanisms of translation control and provide specific examples of translational control during apoptosis. This article is part of a Special Issue entitled "Apoptosis: Four Decades Later".
***Key Words*: selective translation, IRES, IAP, Bcl-x_L, p53, miRNA.**

MECHANISMS OF TRANSLATION INITIATION

The regulation of gene expression occurs at many levels including the transcriptional and translational steps. In order for a cell to quickly respond to its changing environment, control of gene expression at the translational level is ideal since it allows for rapid and immediate changes in protein levels required to respond to the particular stress. Protein translation can be separated into three main steps including initiation, elongation, and termination. Translation initiation is often regarded as the rate-limiting step and thus it is highly regulated by several mechanisms including modifications of the initiation factors involved in the process as well as regulation by microRNAs.

The principal method of translation initiation occurs by means of a cap-dependent scanning mode, which is the primary source of *de novo* synthesized cellular proteins under normal growth conditions (Fig. 1).

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Abbreviations used: 4E-BP – eIF4E binding protein; ALL – acute lymphoblastic leukemia; ARE – AU-rich sequence; CAT-1 – cationic amino acid transporter 1; CLL – chronic lymphocytic leukaemia; CrPV – cricket paralysis virus; eIF – eukaryotic initiation factor; ER – endoplasmic reticulum; FAG – fragment of apoptotic cleavage of eIF4G; FGF – fibroblast growth factor; GCN2 – general control non-derepressible-2; HCV – hepatitis C virus; hnRNP – heterogeneous nuclear ribonucleoprotein; HRI – haem-regulated inhibitor; IAP – inhibitor of apoptosis; IR – ionizing radiation; IRES – internal ribosome entry site; ITAF – IRES-*trans* acting factors; mRNA – messenger RNA; NF-κB – nuclear factor-κB; OIS – oncogene-induced senescence; PABP – poly(A)-binding protein; PDCD4 – programmed cell death 4; PERK – PKR-like endoplasmic reticulum kinase; PKC – protein kinase C; PKR – protein kinase activated by double-stranded RNA; S6K2 – S6 kinase 2; SCLC – small-cell lung cancer; TNFα – tumour necrosis factor α; TRAIL – TNFα-related apoptosis-inducing ligand; uORF – upstream open reading frames; UPR – unfolded protein response; UTR – untranslated region; UV – ultraviolet irradiation; X-DC – X-linked dyskeratosis congenita.

This process requires the involvement of many eukaryotic initiation factors (eIFs), which themselves can be regulated to control rates of protein synthesis, as will be discussed below. In brief, the cap-dependent process involves the recognition of the 5' m^7G cap structure, invariably present on all mature cellular messenger RNAs (mRNAs), by the eIF4F complex, comprised of the cap binding protein eIF4E, the scaffold protein eIF4G, and the RNA helicase eIF4A. Separately, the formation of the 43S pre-initiation complex occurs through the association of the 40S ribosomal subunit with eIF3, eIF1A, and eIF2 bound to the initiator methionyl transfer RNA (Met-tRNA; Met). The 43S pre-initiation complex is then recruited to the mRNA through the interaction between eIF3 and eIF4G and is believed to subsequently scan the mRNA until it locates the initiation codon, typically AUG, in an appropriate context. Subsequently, joining of the 60S ribosomal subunit occurs, which forms the translationally competent 80S ribosome, while the eIFs are released and recycled for the next round of initiation. Furthermore, poly(A)-binding protein (PABP) associates with the poly(A) tail on the 3' terminus of mRNA and is thought to interact with eIF4G causing circularization of the mRNA to enhance translation as well as to protect the mRNA from degradation (see Fig. 1 for details, reviewed in [1]).

The process of translation consumes a significant amount of cellular energy (estimated to be as much as 50%, depending on the organism [2]). It is therefore not surprising that exposure of cells to majority of environmental stressors such as hypoxia, irradiation, or nutrient deprivation leads to modifications of the eIFs involved in the regulation of cap-dependent translation, ultimately resulting in attenuation of global protein synthesis. In addition to saving cellular energy, the attenuation of translation prevents synthesis of unwanted proteins that could obstruct the cellular stress response. Under these conditions, cells are able to cope with the stress or, if the damage to the cell is beyond repair, to initiate apoptosis. To facilitate the

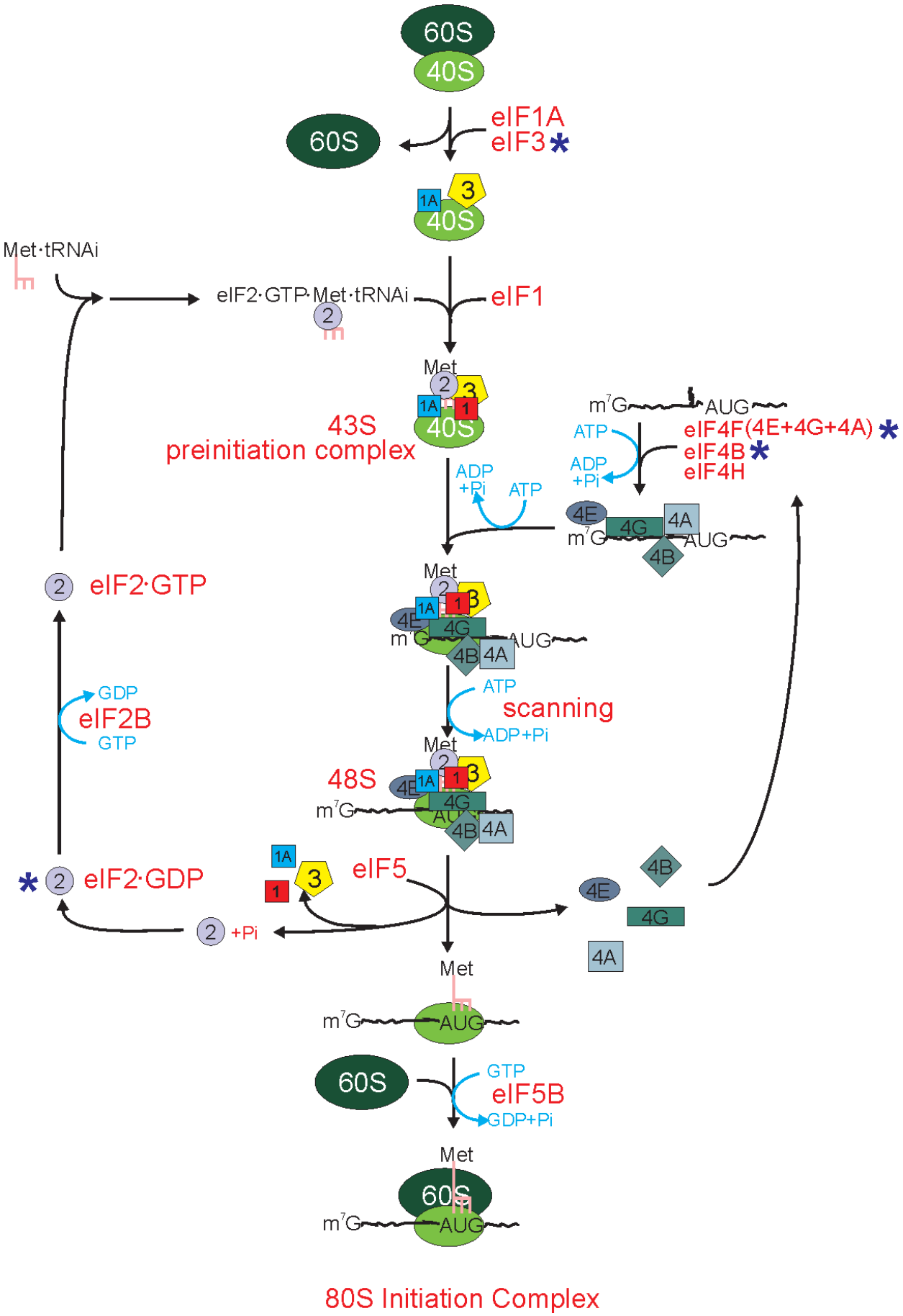

Fig. 1. Schematic diagram outlining the key points of regulation during translation initiation. For simplicity, not all initiation factors are shown. Initiation factors that are described in this review are indicated with asterisks. (Adapted from [123])

decision making process, some proteins, in particular those required for the stress response, are selectively translated even though cap-dependent translation is attenuated. It is the relative levels of these pro- and anti-apoptotic proteins that are important in tipping the balance in favour of survival or cell death. The question of how a cell is able to translate proteins when the required eIFs for cap-dependent translation are not available is at the centre of investigations in many laboratories, and a subject of this review.

One important mechanism that has acquired recent attention is the internal ribosome entry site (IRES) mediated translation initiation process that utilizes specialized RNA elements to selectively recruit ribosomes to mRNA without a need for the cap structure [3]. IRES elements are found in the 5' untranslated region (UTR) of mRNAs and were initially discovered in RNAs of picornaviruses [4]. Although the RNAs of these viruses do not contain a m^7G cap, they are still effectively translated. In addition, many viruses encode proteases that cleave several canonical eIFs in order to block translation of host proteins. For example, upon infection of cells with polio virus, the virus-encoded protease 2A specifically cleaves eIF4G thus inactivating the eIF4F complex and effectively preventing ribosome recruitment to capped cellular mRNAs. This ensures that the host cell's translational machinery is now available for virus protein translation [5]. Importantly, the polio virus IRES element is able to utilize the cleaved eIF4F complex and recruit the ribosome for efficient translation of its own proteins. In other viruses, such as the hepatitis C virus (HCV), the presence of eIF4F is not required at all and the IRES is able to recruit the ribosome in its absence [6]. Thus, even with the loss of some eIFs, the viral IRES elements are able to recruit the ribosome for efficient translation. These observations led researchers to study cellular mRNAs to determine if a similar mechanism(s) exists. In recent years, it has been proposed that an estimated 10% of all cellular mRNAs may contain IRES elements. Interestingly, many of these mRNAs encode proteins involved in processes such as cell proliferation and apoptosis, and are critical in determining the survival of a cell under physiological and pathophysiological stress conditions [3]. For example, IRESs have been identified in mRNAs encoding XIAP, cIAP1, Bcl-x_L, Bcl-2, Bag-1, Apaf-1, p53, c-myc, DAP5, all proteins that are critically involved in the regulation of cell survival.

Although the mechanism of IRES-mediated translation is still poorly understood, it has become evident that not all cellular IRES elements act in a similar manner. That is, most cellular IRES elements require binding of some of the canonical initiation factors as well as for other protein factors termed ITAFs (IRES-*trans* acting factors) that modulate the IRES activity [3]. Most of the ITAFs identified thus far are RNA binding proteins that fulfill a variety of functions including involvement in mRNA splicing (for a review on splicing in apoptosis see [7]), export, stress granule formation, as well as having important roles in translation initiation. The binding of ITAFs can either enhance or repress IRES activity; it is thought that the positive regulators act either as RNA chaperons that aid in the formation of the proper IRES structure, or directly recruit the ribosome. The precise mechanism of how the repressive ITAFs function is not clear. Interestingly, many ITAFs shuttle between the nucleus and cytoplasm and this shuttling is regulated by posttranslational modifications such as phosphorylation in response to a variety of triggers. Therefore, the cytoplasmic availability of positive or negative regulators can determine the extent of IRES translation (see below).

Table. Select eukaryotic translation initiation factors that are modified during apoptosis induced by different triggers. The type of modifications and their consequence for translation and cell survival, along with key references are shown on the right

Translation initiation factors	Modifications	Effects	Apoptotic triggers	References
eIF2	Phosphorylation of eIF2α subunit at Ser51	Inhibition of GDP to GTP exchange on eIF2: • inhibition of global translation and apoptosis • translation of specific transcripts and cell survival	Iron deficiency, heavy metals, osmotic or oxidative stress, heat shock, double stranded RNA, amino acid starvation, unfolded protein response (UPR)	[1, 11]
eIF4E	De-phosphorylation	Global translation inhibition.	Stimuli that activate protein phosphatase 2A	[20]
4E-BPs	De-phosphorylation	Competition with eIF4G on eIF4E. Global translation inhibition and apoptosis	DNA damage, TRAIL, staurosporine, rapamycin	[12, 16, 21, 22]
4E-BPs	Cleavage by caspases at Asp-24	Cleaved form strongly binding eIF4E and inhibition of cap-dependent translation	Staurosporine, etoposide, p53 activation	[21, 22]
eIF4GI and eIF4GII	Cleavage by caspase 3 at Asp-532 and Asp-1176 of eIF4GI. Cleavage by caspase 3 at Asp-560, 851, 978, 1162 and 1407 of eIF4GII.	Cleaved forms (except for M-FAG from eIF4GI) cannot bridge eIF4E, 4A and eIF3 together. Global translation and attenuation of anti-apoptotic response	TNFα, TRAIL, cisplatin, etoposide, cycloheximide, MG132, serum deprivation, Fas receptor activation	[3, 9, 16]
p97/DAP5/NAT1	Cleavage by caspase 3 at Asp-790	p86 fragment with eIF4A and eIF3 binding sites but no eIF4E site. Inhibition of cap-dependent translation but stimulation of specific IRES-dependent translation	UPR, Fas receptor activation	[18, 26–28]
eIF4B	Cleavage by caspase 3 at Asp-45	Fragment is still able to interact with eIF4F and eIF3 but lacks the region mediating PABP binding	Cycloheximide, Fas receptor activation	[16, 31–33]
eIF3j (p35)	Cleavage by caspase 3 at Asp-242	Reduced affinity of the eIF3 complex for the 40S ribosomal subunit. Inhibition of global protein translation	Cycloheximide, Fas receptor activation	[16, 29]
eIF3f (p47)	Phosphorylation	Enhanced association with the core subunits of eIF3. Inhibition of global protein translation	Staurosporine	[30]

GLOBAL TRANSLATION REGULATION DURING APOPTOSIS: MODIFICATIONS OF TRANSLATION INITIATION FACTORS

Induction of apoptosis is accompanied by a pronounced down-regulation of protein synthesis [8]. This inhibition in global translation rates is characterized by a decrease in polysome chains, suggesting that at least some regulation occurs at the translation initiation step [9]. Indeed, there is extensive evidence that apoptosis triggered by different stimuli leads to modifications in a defined set of canonical initiation factors that ultimately results in the inhibition of translation initiation (reviewed in [8, 10]). These modifications generally consist of changes in phosphorylation status (e.g. eIF2α, eIF4E, eIF3, eIF4E binding proteins (4E-BPs)) or protein cleavage by caspases or viral proteases (e.g. eIF4G, eIF4B, eIF3 (Table; Fig. 2)).

eIF2α

eIF2 plays a central role in translation initiation by bringing the initiator Met-tRNA to the 40S ribosomal subunit for the formation of the 43S pre-initiation complex. eIF2 is composed of three subunits (α, β and γ), of which the γ subunit is bound by GTP that is later hydrolyzed during translation initiation [1]. GDP to GTP exchange is necessary for regenerating active eIF2 and this process is catalyzed by eIF2B (Fig. 1). However, in response to different stress stimuli, the α subunit of eIF2 is phosphorylated at serine 51 (Ser51), thus increasing its affinity for eIF2B and trapping the two in an inactive complex [11]. As a result, pre-initiation complex formation and global mRNA translation are inhibited. eIF2α phosphorylation is mediated by four different kinases that are activated by various stress triggers (reviewed in [1]): HRI (haem-regulated inhibitor) which is activated by iron deficiency, heavy metals, osmotic or oxidative stress and heat shock; PKR (protein kinase activated by double-stranded RNA) which is activated by double stranded RNA from viral infections or interferon-induced apoptosis; GCN2 (general control non-derepressible-2) which is activated by amino acid starvation; and PERK (PKR-like endoplasmic reticulum kinase) which is activated during the unfolded protein response (UPR).

The link between eIF2α phosphorylation and apoptosis is not straightforward, and eIF2α phosphorylation can either be a cause or consequence of the cell's commitment to apoptosis. For instance, in MCF-7 breast cancer cells treated with TNFα or TRAIL (TNFα-related apoptosis-inducing ligand), eIF2α phosphorylation by PKR is dependent on caspase 8 [12, 13]. In contrast, in mouse embryonic fibroblasts treated with TNFα or deprived of serum, eIF2α phosphorylation is necessary to induce caspase 3 activation [14]. Interestingly, forced expression of a phosphomimetic S51D mutant of eIF2α is sufficient to activate caspase 3 and induce apoptosis in the absence of any other triggers, whereas expression of a non-phosphorylatable S51A mutant protects cells from TNFα or serum depriva-

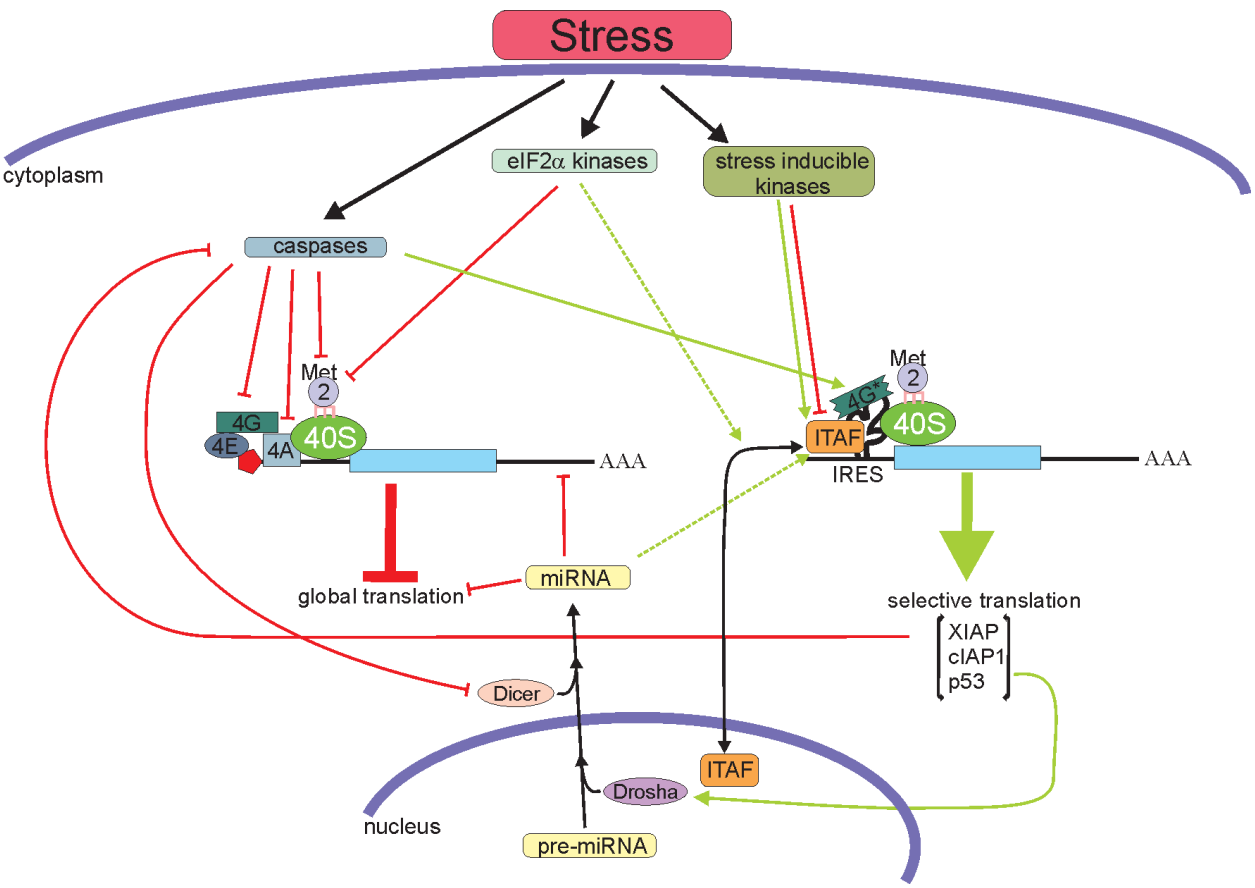

Fig. 2. A model of the interconnectedness of translation and apoptosis. Only factors pertinent to this review are shown. The left side of the model shows regulation of cap-dependent translation; the right side depicts IRES-mediated translation. Green lines indicate positive, while red lines negative interactions. Dotted lines depicts indirect effect

tion. It has also been reported that eIF2α itself can be cleaved in apoptotic cells, mainly by caspase 3, but also by caspases 6, 8 and 10 [15, 16]. The functional relevance of this cleavage product is not fully understood but it has been shown that its GTP exchange rate is higher, independent of eIF2B, and that it may contribute to translation inhibition [15].

In contrast to inhibiting global translation, eIF2α phosphorylation can up- or downregulate selective translation. For example, under hypertonic stress, eIF2α phosphorylation was shown to induce cytoplasmic accumulation of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), a known ITAF, which in turn inhibited IRES-mediated translation of the anti-apoptotic proteins XIAP and Bcl-x_L, thus sensitizing the cells to apoptosis [17]. Interestingly, in the context of the adaptive UPR, eIF2α phosphorylation can be a signal that promotes cell survival rather than apoptosis. In this context, eIF2α phosphorylation by PERK leads to the selective translation of transcription factors, such as ATF4 that controls the expression of pro-survival and anti-apoptotic proteins such as cIAP1 [3, 18].

eIF4E

The cap-binding protein eIF4E is another initiation factor whose availability is regulated by phosphorylation during apoptosis. eIF4E is phosphorylated at Ser209 by the MAPK integrating kinases Mnk1 and Mnk2 in response to different stimuli such as treatment of cells with growth factors, anisomycin or UV, that activate the ERK and p38 MAPK pathways [19]. eIF4E phosphorylation reduces its affinity for the 5' cap structure, thus stimulating translation initiation. Conversely, eIF4E dephosphorylation by protein phosphatase A leads to inhibition of global translation. However, eIF4E binding to the 5'cap is regulated mainly through the phosphorylation status of eIF4E binding proteins (4E-BPs). 4E-BP1, 2, and 3 are proteins that share structural similarities to the fragment of eIF4G responsible for binding to eIF4E. 4E-BPs are phosphorylated in normal growth conditions by the mTOR signaling pathway (reviewed in [20]). However, during apoptosis induced by DNA damage, TRAIL, the protein kinase inhibitor staurosporine, or the mTOR inhibitor rapamycin, there is a decrease in 4E-BPs phosphorylation [12, 16, 21, 22]. Hypo-phosphorylated 4E-BPs have a higher affinity for eIF4E and as a consequence, they competitively prevent eIF4G from binding to eIF4E, thus reducing the availability of the eIF4F complex and resulting in inhibition of translation (reviewed in [1]). Similar to eIF2α phosphorylation, loss of 4E-BPs phosphorylation occurs during the early phase of the apoptosis cascade [12, 16], leading to global translation inhibition and commitment to cell death. For example, ectopic expression of a non-phosphorylatable mutant of 4E-BP1 sensitizes multiple myeloma cells to dexamethasone-induced apoptosis [23]. Furthermore, apoptotic triggers such as staurosporine, etoposide or activation of p53 can lead to caspase-mediated cleavage of 4E-BP1 [21, 22], producing a cleaved form that binds strongly to eIF4E and inhibits cap-dependent translation [24].

eIF4G

The availability and function of the initiation factors eIF4GI and eIF4GII is regulated during apoptosis primarily through cleavage by caspases. Upon treatment of cells with apoptotic triggers such as TNFα, TRAIL, cisplatin or etoposide, eIF4GI and eIF4GII are cleaved by caspase-3 at two different sites. This gives rise to three cleavage products named Fragments of Apoptotic cleavage of eIF4G (N-FAG, M-FAG and C-FAG [9, 16, 25]). The middle fragment, M-FAG, retains its ability to interact with eIF4A, eIF4E and eIF3, and supports cap-dependent translation during the early phase of apoptosis. However, M-FAG is degraded with prolonged exposure to stress resulting in the inhibition of cap-dependent translation and attenuation of the anti-apoptotic response. Interestingly, cleavage of the eIF4G-related protein factor p97/DAP5/NAT1 by caspases releases a p86 isoform that stimulates the IRES-mediated translation of apoptosis regulating factors such as XIAP, cIAP1, c-myc, APAF1 and p97/DAP5 itself [18, 26–28]. Hence, cleavage of eIF4G and p97/DAP5 proteins regulates the fate of the cell by tipping the balance between the translation of pro- and anti-apoptotic factors.

eIF3

eIF3 is a critical factor that bridges the binding between the 43S ribosome and eIF4F-bound mRNA. It has been reported that eIF3j (p35) is cleaved during apoptosis in BJAB cells treated with anti-Fas or cycloheximide [16]. eIF3j cleavage occurs in a caspase-3 dependent manner and results in reduced affinity of the eIF3 complex for the 40S ribosomal subunit and subsequent inhibition of global translation [29]. Similarly, the p47 subunit of eIF3, eIF3f, is phosphorylated by CDK11 during staurosporine-induced apoptosis of the human melanoma cell line A376. eIF3f phosphorylation results in its enhanced association with the core subunits of eIF3 and sequestration in insoluble complexes, leading to an inhibition of protein synthesis and induction of apoptosis [30].

eIF4B

The initiation co-factor eIF4B which stimulates eIF4A helicase activity and ribosome binding to the mRNA, is also modified during apoptosis. eIF4B is cleaved in its C-terminal region by caspase 3 both *in vitro* and in BJAB cells treated with anti-Fas or cycloheximide [16]. However, eIF4B can also be cleaved in a caspase-3 independent manner in apoptotic MCF-7 cells that lack caspase 3 [12]. The N-terminal fragment of eIF4B is still able to interact with eIF4F and eIF3 [16]; however, it lacks the region that mediates its interaction with PABP [31]. The effects of eIF4B truncation on translation and apoptosis are not well characterized. However, a substantial amount of work has been done on elucidating eIF4B's role in cell survival and proliferation (reviewed in [32]). For instance, eIF4B depletion from HeLa cells using RNA interference was shown to selectively reduce the

translation of genes involved in cell proliferation (such as cdc25C, c-myc, and ornithine decarboxylase) and survival (such as Bcl-2 and XIAP). Moreover, eIF4B depletion caused a decrease in HeLa cells proliferation rates, enhanced apoptosis and sensitized these cells to camptothecin-induced cell death [33].

In general, modifications of translation initiation factors, whether they are a cause or a consequence of the initiation of the apoptotic cascade, are aimed at inhibiting global protein synthesis. This general inhibition of translation contributes to the shutdown of all cellular processes, and is believed to conserve cellular energy and prevent the synthesis of protein factors that could stall the apoptotic process. However, in cases of adaptive stress, translation can be reprogrammed such that the translation of specific mRNA transcripts continues and influences the fate of the cell (Fig. 2).

SELECTIVE TRANSLATION *VIA* IRES

Despite the cessation of global protein synthesis during the early phase of apoptosis, selective translation of specific mRNAs can continue *via* the IRES mechanism, as described above. Several key regulators of cell death were shown to be translated *via* IRES elements; here we focus on the regulation of translation of cIAP1, XIAP and p53.

Cellular inhibitor of apoptosis 1 (cIAP1)

The inhibitor of apoptosis (IAP) family of proteins is comprised of eight members in mammals that regulate many key cellular processes including signaling, cell division, and apoptosis, and are the subject of another review in this special issue [34]. cIAP1 is a key regulator of nuclear factor-κB (NF-κB) dependent signaling and capase-8 mediated cell death in mammalian cells. The abundance of cIAP1 in the cell is regulated at multiple levels: at the transcriptional level by the transcription factor NF-κB [35], at the protein stability level by autoubiquitination [36], and at the mRNA stability level by an AU-rich element (ARE) in its 3'UTR [37]. Importantly, cIAP1 expression in response to apoptotic triggers is mainly regulated at the level of protein translation. The cIAP1 mRNA has a long (1.2 kb) and highly structured 5'UTR that contains 23 AUG codons and two upstream open reading frames (uORF) which contribute to inhibition of its basal translation. In fact, the upstream ORF was shown to severely inhibit translation of the downstream ORF, thus explaining the low levels of cIAP1 observed under normal growth conditions [38]. Several studies have now established that cIAP1 expression is selectively upregulated in response to apoptotic stress [18, 39–41]. Indeed, cIAP1 protein expression is upregulated *via* an IRES-dependent mechanism in response to tunicamycin- or thapsigargin-induced endoplasmic reticulum (ER) stress, in conditions where global protein synthesis is inhibited [18, 42]. The relevance of cIAP1 IRES-mediated translation to ER stress induced apoptosis was further shown by the fact that cIAP1 overexpression attenuated tunicamycin-induced death in HeLa cells, whereas cIAP1 depletion by RNA interference enhanced sensitivity to tunicamycin [18]. The importance of an IRES that drives cIAP1 expression to inhibit apoptosis was also demonstrated in the context of etoposide or sodium arsenite treatment [39] and viral infection [41].

Interestingly, activation of the cIAP1 IRES in the context of ER stress is dependent on caspase activation and is accompanied by cleavage of eIF4GI and its homolog p97/DAP5/NAT1 during the early phase of the UPR [18]. As mentioned above, the p97/DAP5/NAT1 cleavage product, p86/DAP5 functions as an ITAF that stimulates the activity of several IRES elements including cIAP1 [18, 26–28]. Indeed, ectopic overexpression of a p86/DAP5 fragment, but not the full length p97/DAP5 protein, in both HEK293T and rabbit reticulocyte lysates was able to specifically drive cIAP1 IRES activity and increase cIAP1 endogenous protein levels [18]. It was later shown that both p97/DAP5/NAT1 and p86/DAP5 bind to the cIAP1 IRES, possibly through association with other accessory proteins [18, 42].

The structure of the cIAP1 IRES and the proteins that specifically interact with this IRES were characterized recently. One of these proteins, NF45, enhances cIAP1 IRES-dependent translation and mediates cIAP1 induction in response to thapsigargin-induced ER stress and UPR [40]. NF45 is an NFAT-related transcription factor that was first identified to regulate interleukin-2 transcription, together with its binding partner NF90 [43]. NF45 and NF90 are involved in several cellular processes such as transcription [44], viral replication [45] and microRNA processing [46] and our study confirmed its role in IRES-mediated translation [40]. More recently, NF45 has been implicated in mitotic control in HeLa cells since depletion of NF45/NF90 complexes by RNA interference in these cells leads to the generation of large multinucleated cells, a result of impaired cytokinesis and cell growth due to defects in DNA break repair [47]. In line with this new role for NF45, we have recently discovered that NF45 preferentially regulates a cohort of AU-rich IRES-containing mRNAs including cIAP1 and XIAP, which are responsible for the multinucleated phenotype of NF45-deficient cells (MDF and MH unpublished observations). Loss of NF45 results in reduced IRES-mediated translation of XIAP and cIAP1 mRNAs. Interestingly, the resulting decrease in XIAP expression causes an increase in Survivin protein levels, likely due to Survivin protein stabilization [48]. Survivin, another member of the IAP family, plays an important role in microtubule spindle checkpoint regulation and its aberrant expression leads to cytokinesis defects [49], thus explaining the multinucleated phenotype of NF45-deficient cells. Similarly, through its control of cIAP1 translation NF45 regulates cyclin E expression. Nuclear cIAP1 was shown to transcriptionally regulate cyclin E [50], and we found that either NF45 or cIAP1 depletion caused a decrease in cyclin E expression that is rescued by NF45 re-expression. Coordinated changes in cyclin E and Survivin expres-

sion in NF45-depleted cells would lead to a block in cell cycle, mitotic catastrophe and defects in cytokinesis thus explaining the senescence-like phenotype of these cells. These observations uncover a novel role for NF45 in controlling ploidy and highlight the importance of IRES-mediated translation in the regulation of mitosis, cell growth and apoptosis.

X-linked inhibitor of apoptosis protein (XIAP)

XIAP, a prototype member of the IAP family is a direct inhibitor of caspases 3, 7, and 9. Given XIAP's key role in inhibiting caspases, it is not surprising that misregulation of its expression is associated with tumourigenesis and cancer. Importantly, elevated levels of XIAP, as is observed in many cancers, have been linked to enhanced chemo- or radiation resistance, whereas reduction of XIAP through chemical inhibitors can restore chemosensitivity [51].

Studies into the regulation of XIAP expression led to the discovery of an IRES element located in its 5' UTR region which mediates XIAP protein translation under conditions of cellular stress such as γ-irradiation or nutrient deprivation, thus providing the cell with protection against apoptosis [53]. Interestingly, XIAP protein is encoded by two mRNA splice variants that differ only in their 5'UTR regions [53]. The more abundant, shorter transcript produces the majority of XIAP protein under normal growth conditions by cap-dependent translation. However, during cellular stress, the longer transcript that contains the IRES element supports efficient translation even though global cap-dependent translation is attenuated [54]. The secondary structure of the XIAP IRES and its associated ITAFs has been determined [55]. Some of these, such as La autoantigen [55], hnRNP C1/C2 [56], and HuR [57] have been shown to enhance XIAP IRES translation, whereas others, such as hnRNP A1 [58], PTB [54] and PDCD4 [59] repress XIAP IRES translation.

Interestingly, cytoplasmic localization of XIAP ITAFs appears to play a key role in the regulation of XIAP translation in response to stress. For example, osmotic shock causes an accumulation of hnRNP A1 in the cytoplasm by activating the mitogen-activated protein kinase kinase 3/6-p38 signaling pathway resulting in phosphorylation of hnRNP A1, thus preventing its import into the nucleus [60]. Once in the cytoplasm, hnRNP A1 binds with the XIAP IRES and inhibits protein expression [58]. Another example of an ITAF being regulated at the level of localization was shown by Gu *et al.* [61] in acute lymphoblastic leukemia (ALL) cells treated with ionizing radiation (IR). They observed that IR treatment resulted in the misregulation of the oncogene MDM2. MDM2 overexpression is observed in many cancers and correlates with poor patient outcome because it binds to and inhibits the activity of the tumour suppressor p53 [61]. It is known that the phosphorylation status of MDM2 dictates its localization such that survival signals promote nuclear localization and cell proliferation whereas cellular stress results in dephosphorylation of MDM2 and subsequent retention in the cytoplasm. However, the cytoplasmic function of MDM2 was not well understood. Upon treatment with IR, dephosphorylated MDM2 is retained in the cytoplasm and is no longer associated with its main target, p53 [61]. Instead, cytoplasmic MDM2 is able to directly and specifically bind to the XIAP IRES. It is interesting to note that many cancers express elevated levels of a mutated form of MDM2 that does not contain the N-terminal p53 binding domain [62] and it is this remaining C-terminal portion of MDM2 that is responsible for interacting with the XIAP IRES and upregulating its IRES-mediated translation. Importantly, the MDM2-mediated increase in XIAP expression leads to enhanced resistance to IR-induced apoptosis.

Similar to DNA damage or osmotic shock, cell proliferative stimulation also results in stimulation of IRES translation. For example, treatment of small cell lung cancer (SCLC) cells with the fibroblast growth factor (FGF) 2 protects them from etoposide induced cell death by upregulating the anti-apoptotic proteins XIAP and Bcl-x_L. It was shown that a complex forms between S6 kinase 2 (S6K2), BRaf, and PKCε leading to activation of S6K2 in response to FGF 2 [63]. We have identified the target of activated S6K2 as programmed cell death 4 (PDCD4; [59]). PDCD4 is a known tumour suppressor and its loss has been correlated with more aggressive and invasive tumours [64]. The FGF2-activated S6K2 phosphorylates PDCD4, leading to its proteasomal degradation. Furthermore, we identified XIAP and Bcl-x_L as two novel translational targets of PDCD4. We showed that the N-terminal portion of PDCD4 was responsible for directly binding to XIAP and Bcl-x_L IRES RNAs both *in vitro* and *in vivo* and the loss of PDCD4 correlated with an increase in XIAP and Bcl-x_L protein expression. This response to FGF-2 is a critical factor in tumour formation and resistance to apoptosis because mutations in cancer cells typically lead to an acquired ability of the cells to produce growth factors and stimulate proliferation through autocrine signalling (reviewed in [65]).

Tumour suppressor p53

p53 is a tumour suppressor that plays a major role in the regulation of cell cycle progression and apoptosis in response to cellular stress, mainly DNA damage and genomic instability [66]. p53 also plays a central role in the process of oncogenesis as its gene is mutated in more than 50% of all human cancers [67] and as such, p53 remains one of the most highly studied genes. It is now well established that p53 protein levels and activity increase in response to DNA damage and that regulation of this process occurs mainly at the level of protein stability by the ubiquitin ligase MDM2 [68]. However in recent years, there has been accumulating evidence that translational control is important in the induction of p53 expression in response to cellular stress (reviewed in [69]). This evidence includes, but is not limited to: (I) the fact that cycloheximide — a protein elongation inhibitor-prevents the increase in p53 protein levels normally observed after IR-induced [70] or etoposide-induced [71] DNA damage, (II) the fact that there is an increase

in p53 mRNA in polyribosomes upon IR exposure [72] or etoposide treatment [71], and (III) the fact that *de novo* protein synthesis rates of the p53 mRNA increase in response to DNA damage caused by IR [72, 73], UVC [74], etoposide [71], doxorubicin or in response to tunicamycin-induced ER stress [75].

In the past ten years, there has been more focus on understanding the mechanisms underlying p53 translation induction in response to cell stress. Yang *et al.* [71] were the first to report that the p53 mRNA can be translated in a cap-independent manner in MCF-7 breast cancer cells and subsequently identified an IRES within the p53 5'UTR that is induced more than 2-fold during etoposide-induced DNA damage. Moreover, a second study showed that the p53 5'UTR was able to direct *in vitro* translation of the p53 mRNA in the absence of a cap structure [76], further confirming the existence of a p53 IRES.

Interestingly, Ray *et al.* [76] proposed a model in which two different IRES structures control the translation of two different p53 isoforms, namely the full-length p53 protein (FL-p53) and the ΔN-p53 (p40/47) isoform. The ΔN-p53 protein is translated from an alternative initiation codon situated within the coding sequence, 40 nucleotides downstream of the FL-p53 translation start site [77, 78]. It has been suggested that ΔN-p53 acts as a dominant-negative form that antagonizes p53-mediated transcription and growth regulation [77]. However it appears that ΔN-p53 functions are much more complex since the protein does not contain an MDM2 binding site, is able to oligomerize with FL-p53 to induce different transcription patterns [78], and induces apoptosis when expressed in p53-null cells [77]. They further showed that expression of the two p53 isoforms is regulated in a cell-cycle dependent manner, *via* an IRES mechanism of translation [76]. In fact, the IRES driving FL-p53 protein expression is more active during the G_2/M transition when p53 activity is required the most, whereas the IRES driving ΔN-p53 expression is active during the G_1/S transition [76]. These findings are more consistent with ΔN-p53 being an antagonist of p53 activity where at the G_1/S phase it would drive the expression of genes necessary for transition through the cell cycle. Differential regulation of FL-p53 and ΔN-p53 *via* translational control may also have an effect on cell sensitivity to apoptosis. For instance, doxorubicin-induced DNA damage and tunicamycin-induced ER stress give rise to different patterns of p53 isoform expression where H1299 lung carcinoma cells overexpressing the ΔN-p53 are less sensitive to doxorubicin treatment and more sensitive to tunicamycin [75]. Thus, translational control *via* the IRES is an important mechanism by which p53 can integrate and respond to the different apoptotic or proliferative cues the cell is exposed to. Another layer of complexity is brought about by the different ITAFs that can bind to the p53 IRES and modulate its activity in response to stress. The ribosomal protein L26 [73], PTB [79], and hnRNP C1/C2 [80] were all shown to enhance p53 expression, whereas nucleolin was shown to repress it [73]. The La autoantigen, hnRNP U and p53 itself may also be potential p53 IRES trans-acting factors [73]. PTB binds specifically to the p53 IRES structure and a reduction in PTB protein levels by RNA interference leads to a decrease in IRES activity and blunting of p53 isoforms induction in the presence of doxorubicin [79]]. Furthermore, treatment of A549 human lung carcinoma cells with doxorubicin causes PTB translocation from the nucleus to the cytoplasm, corresponding with an increase in p53 expression. Interestingly PTB cytoplasmic levels are high at the G_2/M phase and low at the G_1/M transition [81], suggesting that PTB might be the factor contributing to increased p53 translation during the G_2/M checkpoint [79]. These results further support the notion that cell stressors can alter the expression level, cellular localization or status of different ITAFs to modulate the output of p53 protein available to respond to the stress and decide the fate of the cell.

Most of the work done on p53 in the context of carcinogenesis was aimed at characterizing the effects of p53 coding region mutations on the transcriptional activities of the protein. However, it has become apparent that mutations can also occur within the p53 5'UTR and may have relevance to the pathology of cancer [82]. Indeed, a cancer-derived triple silent mutation at positions 185, 188 and 191 that was previously shown to alter MDM2 binding to the p53 mRNA [82], as well as a single silent mutation at position 200 of the p53 5'UTR [82] were found to alter p53 IRES activity, alter the profile of ITAFs binding to the IRES and blunt the IRES induction in response to doxorubicin [83]. Thus, it is possible that mutations within the p53 IRES may lead to carcinogenesis by decreasing p53 induction and protective activity in response to DNA damage. The recent characterization of the p53 IRES structure [83] may help in identifying more cancer-derived mutations that are relevant to p53 function and that could be used as predictors of response to treatments.

Another aspect that may be relevant to the pathology of cancer was the recent finding that p53 IRES-dependent translation is impaired during oncogene-induced senescence (OIS) in DKC1^m cells [84]. The DKC1 gene encodes the dyskerin protein which is responsible for modifying uridines in ribosomal RNA into pseudouridines, and mutations in DKC1 have been linked to the development of X-linked dyskeratosis congenita (X-DC). X-DC patients have increased susceptibility to cancer, as reflected by the fact that more than 50% of DKC1^m mice develop tumours of different origin [85]. Interestingly, Yoon *et al.* [86] showed that DKC1-mutated cells are impaired in IRES-mediated translation, providing one of the first *in vivo* links between IRES-mediated translation and the onset of oncogenesis. In addition, during OIS, in which p53 translation is normally induced to counteract the oncogenic insult [87], p53 IRES-mediated translation is impaired both in DKC1^m mice cells and in X-DC patient derived cells. This results in a significant decrease in p53 protein induction and of its target genes p21 and MDM2 in response to etoposide treatment or γ-irradiation which correlates with a reduction in the number of apoptotic cells as compared to wild-type [84].

These results were corroborated by an independent group that showed that DKC1 knock-down in both MCF-7 breast cancer cells and in primary breast cancer cells caused a decrease in p53 IRES-mediated translation, which led to a decrease in p53 transcriptional activity and apoptosis upon doxorubicin treatment [88]. Together, these findings show that defects in p53 IRES-mediated translation are relevant not only to the OIS process but also to carcinogenesis, particularly in the context of the X-DC pathology.

MICRORNA MEDIATED REGULATION

MicroRNAs (miRNAs) are small, non-coding RNA sequences of approximately 21 nucleotides in length that regulate gene expression post-transcriptionally by binding to target mRNAs to silence their expression. miRNAs play a significant role in regulating processes as diverse as development, metabolism, cell proliferation, and apoptosis [89]. In humans, over 500 miRNAs have been identified so far and each miRNA has multiple targets, therefore it is thought that about 10,000 mRNAs could be regulated by miRNAs.

miRNAs are transcribed from the genome by RNA polymerase II or III as long, double-stranded hairpin transcripts containing a 5'cap and 3' poly-A tail, termed primary miRNA (pri-miRNA [90]). Pri-miRNAs are further processed into a smaller double stranded structure in the nucleus by the RNase III-like enzyme Drosha and DGCR8 to produce the precursor miRNAs (pre-miRNAs) [91] that are subsequently are exported into the cytoplasm by exportin-5 where they are further processed by the RNase III enzyme Dicer, yielding an approximately 22 nucleotide long, double-stranded product [92]. Only one of the miRNA strands is then incorporated into the RNA-induced silencing complex (RISC) containing the argonaute (AGO) protein, while the other strand is degraded [90, 93]. Upon recognition of their target mRNAs *via* near-perfect complementarity, miRNAs can direct the degradation of the target mRNA by the 5'-to-3' mRNA decay pathway which involves deadenylation by the CAF1-CCR4-NOT deadenylase complex, followed by decapping by DCP2, and ultimately degradation by the exonuclease XRN1 [94, 95]. As well, miRNAs that bind with near perfect complementarity can direct endonucleolytic cleavage of their target mRNAs through the catalytically active Argonaute protein present in the RISC complex [96]. On the other hand, miRNAs can also bind their target mRNAs *via* imperfect complementarity resulting in a loss of protein product but no change in mRNA levels suggesting that an inhibition of translation occurs rather than degradation of mRNA. Early studies showed that these inhibited mRNAs were found associated with polysomes indicating that repression occurred at a post-initiation stage, likely during translation elongation [97, 98]. However, in recent years, it has been suggested that miRNAs inhibit translation initiation by interfering with the eIF4F and the poly-A binding complexes. Further evidence for miRNAs affecting the initiation step of translation was provided by Humphreys *et al.* [99] who showed that a construct containing the IRES element of cricket paralysis virus (CrPV) lacking a cap and poly-A structure was unaffected by miRNAs, suggesting that the initiation step was the target of miRNA regulation, since regulation of elongation or termination should still occur in the presence of the CrPV IRES. These data strengthen the link between miRNA function and translational control which is important not only during times of cellular stress, but also during the cell's decision to undergo apoptosis. As mentioned above, many mRNAs that are involved in the cellular stress response contain IRES elements, which not only allow the mRNA to be translated during attenuation of cap-dependent translation, but may also aid in protecting the mRNA from miRNA induced silencing. Furthermore, cytoplasmic processing bodies (P-bodies), which are involved in processes such as translation inhibition and mRNA degradation, have been suggested to be involved in retaining repressed mRNAs in the RISC complex thus preventing translation [100–102]. Interestingly, certain stressors can cause release of some repressed mRNAs from the P-bodies where they are able to re-enter into polysomes for efficient translation [103]. For example, in Huh7 hepatoma cells, the cationic amino acid transporter (CAT-1) mRNA is found in the P-bodies. However, upon exposure to amino acid starvation, the RNA binding protein HuR is relocalized from the nucleus to the cytoplasm where it binds the CAT-1 mRNA and releases it from the P-bodies. This allows translation of the mRNA to respond to the cellular stress. It is likely that this mechanism of release under stress occurs for other mRNAs in response to specific stressors and the combination of these mechanisms allows the cell to quickly respond to its changing environment by translating pro- or anti-apoptotic proteins that are crucial for deciding the fate of the cell.

Interestingly, miRNAs have been identified as both tumour suppressors and oncogenes involved in tumour development, and mis-regulation of miRNA expression has been linked to cellular transformation. It has been suggested that as many as 50% of miRNAs are located in unstable regions of chromosomes that are prone to being amplified or deleted in many cancers [104]. Furthermore, proteins that are frequently mis-regulated or mutated in cancers can affect the levels of miRNAs. For example, upon DNA damage, p53 interacts with the Drosha complex to enhance the processing of select pri-miRNAs involved in apoptosis and cell proliferation [105]. However, inactive p53 mutants that are commonly found in many cancers (for example p53 mutated at C135Y, R175H and R273H) prevent the interaction of p53 with the Drosha complex, therefore attenuating the processing of these miRNAs. Many miRNAs have been implicated in regulating expression of apoptotic proteins, thus altered levels of these miRNAs can have negative effects on the cells ability to respond to apoptotic cues, resulting in a lack of cell death and enhanced proliferation, ultimately leading to tumour growth and survival. (For a detailed review on miRNAs see [106, 107]).

miRNA 21 is the most consistently up-regulated miRNA across many cancer types. Chan *et al.* [108] discovered that reducing miR-21 levels in glioblastoma cells increased apoptosis, which correlated with

a decrease in tumour growth. Of the many targets of miR21 [109], PDCD4 is an important target that is frequently down-regulated in a variety of cancers [110, 111]. The tumour suppressive function of PDCD4 stems from its ability to bind to and inhibit eIF4A, thus blocking cap-dependent translation and attenuating cell growth [112–114]. Upon loss of PDCD4, the cell loses this ability to regulate protein translation, leading to enhanced cell proliferation and increased tumour formation. However, we and others have shown recently that PDCD4 plays a more specific role in translation by regulating translation of a specific set of targets (as described above; [59, 115]. For example, PDCD4 can bind to and negatively regulate the expression of p53 under normal growth conditions. However, upon induction of DNA damage by ultraviolet irradiation (UV), PDCD4 is degraded, thus allowing for an up-regulation in p53 levels [116]. Similarly, PDCD4-dependent repression of XIAP and Bcl-x_L in response to FGF 2 has been described above. This suggest that an increase in miR-21 leading to a loss in PDCD4 may not only result in an increase in overall translation through de-repression of eIF4A, but also to a specific increase in expression of anti-apoptotic proteins, thus leading to enhanced resistance to apoptosis-inducing chemotherapeutics [59].

The Bcl-2 family of proteins has also been identified as being regulated directly by miRNAs. For example, the miR-15-16 cluster of miRNAs can induce apoptosis by inhibiting Bcl-2, an anti-apoptotic factor involved in maintaining mitochondrial membrane homeostasis. As is common for many miRNAs, this cluster is down regulated in many cancers. For example, the miR-15–16 cluster is deleted in B-cell chronic lymphocytic leukaemia (CLL; [117]), pituitary adenoma [118], and prostate carcinoma [119]. This down-regulation of miRNAs contributes to the increased expression of Bcl-2 that is often observed in many cancers, and promotes chemoresistance by inhibiting the release of mitochondrial cytochrome c required for activation of caspase 9.

Interestingly, apoptotic cues can also directly regulate the proteins involved in the miRNA process. For example, Matskevich *et al.* [120] demonstrated that the RNase III enzyme Dicer is cleaved by caspases in response to apoptotic cues, in particular inhibition of protein kinase C (PKC) as well as during HIV infection, resulting in an inhibition of the RNA interference pathway [120]. Furthermore, Nakagawa *et al.* [121] demonstrated that the *C. elegans* Dicer gene, DCR-1, is cleaved specifically by a caspase, CED-3. They identified a novel role for the remaining C-terminal fragment of Dicer that can no longer process double stranded RNA species, but instead gains a deoxyribonuclease activity that can nick DNA leading to DNA degradation and enhanced apoptosis [121].

As mentioned above, many miRNAs play a large role in regulating genes involved in apoptosis or cell proliferation leading to development and progression of cancer. Recently, miRNA profiles have been generated that can be utilized as a tool for the identification and classification of tumours with hopes that this information can help with disease prognosis and predictions of outcomes [122].

CONCLUSIONS

Regulation of translation can be both the cause and the consequence of apoptosis. We have chosen examples to illustrate how this process is highly dynamic and is crucial for the cell's ability to respond to environmental cues (Fig. 2). We have highlighted the critical points of control prior to and during the onset of apoptosis with the hope of convincing the reader that the ability to translate specific proteins in response to stress is essential to decide the fate of the cell. Both the IRES and miRNA-mediated control of translation initiation are emerging as key mechanisms that regulate selective translation. IRES mediated translation allows for a selective translation of a subset of mRNAs in times of attenuation of global cap-dependent translation by bypassing the requirement for canonical initiation factors that are subject to inhibitory modifications during apoptosis. In contrast, miRNA-mediated control of translation may, in addition to regulating the expression of specific target mRNAs, protect IRES containing mRNAs from degradation. These examples demonstrate how misregulation of translation initiation plays a crucial part in tumorigenesis and chemoresistance through enhanced resistance to apoptosis.

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