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Live or let die: posttranscriptional gene regulation in cell stress and cell death

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Summary: Studies of the regulation of gene expression historically focused on transcription. However, during stress and apoptosis, profound gene expression changes occur more rapidly and globally than is possible by regulating transcription. Posttranscriptional changes in mRNA processing and translation in response to diverse stresses shut down most protein translation to conserve energy and lead to rapid remodeling of the proteome to promote repair. Pre-mRNA splicing and mRNA stability are fundamentally altered under some stress conditions. Stress pathways coordinate a cytoprotective repair response, while simultaneously initiating signaling that can ultimately trigger cell death. How the cell mediates the decision between repair and apoptosis is largely not understood. In some stresses, microRNAs may tip the balance. Here, we review what is known about posttranscriptional gene regulation during stress, focusing on what is still unknown and how new technologies might be used to understand what changes are most physiologically important in different forms of stress and death.

Keywords: *stress, apoptosis, pre-mRNA splicing, translation, miRNA*

Introduction

The cell responds to stress rapidly by remodeling protein expression within minutes. Transcriptional changes are too slow to accomplish this task. Most mRNAs have half-lives on the scale of hours [on average 6.9 h in human cells, more than 50-fold longer than in *E. coli* (1, 2)]. Hence, posttranscriptional events play a pivotal role in regulating the initial rapid response to cell stress. A swift, almost global, shutoff of translation occurs in response to diverse cellular insults. This 'integrated stress response' is generally an adaptive program that helps a cell endure stress and renormalize when the stress abates. Concurrent with this rapid response, stresses trigger many slower-acting alterations in gene expression, including changes in transcription, many of which provide protection against potential future insults. However, persistent stresses favor changes in expression that will ultimately tip a cell toward apoptosis. The expression of proapoptotic effectors can be regulated at multiple checkpoints, with each checkpoint requiring continued stress.

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One example of this is upregulation of the proapoptotic CHOP transcription factor during endoplasmic reticulum (ER) stress, as discussed later. How cells respond to stress ultimately depends on the type, severity, and duration of the stress. Although most research on the stress response has focused on inhibition of translation, strong and in some cases very rapid alterations in pre-mRNA splicing and mRNA stability occur in response to some stresses. These events need to be better characterized. Changes in posttranscriptional regulation occur both before and after cells that fail to repair become committed to apoptosis. The best-studied apoptotic change in gene expression is a global translational arrest, but the mechanistic underpinnings of this phenomenon remain poorly understood. Even less is known about changes in mRNA stability and splicing in apoptosis, although recent work from our laboratory suggests that dramatic changes in pre-mRNA splicing occur during programmed cell death that promote the cell's demise.

The basics of cell stress and apoptosis

Eukaryotes have evolved a comprehensive portfolio of mechanisms to cope with cell stress. In this review, we focus on the response to a few stresses in which posttranscriptional regulation has been best studied. The heat shock (HS) response is one of the best characterized. The master regulator of many aspects of the HS response is the transcription factor HSF1, which does not respond to HS *per se* but senses misfolded proteins in the cytoplasm that accumulate during HS and other stresses (3). When activated, HSF1 translocates to the nucleus and transactivates a repair program, most notably inducing the heat shock proteins (HSPs), molecular chaperones that promote proper protein folding. Genotoxic stress (4, 5), which is important in the etiology and treatment of cancer, activates expression of the tumor suppressor TP53, a key transcription factor that induces the expression of genes to promote cell cycle arrest and apoptosis. Unlike other stresses, genotoxic stress does not necessarily directly induce translational arrest, but can trigger profound TP53-dependent and -independent alterations in splicing. TP53 also enhances the transcription of microRNA primary transcripts that play a potentially important role in the genotoxic stress response. ER stress rapidly triggers a translation block through the unfolded protein response (UPR). Excessive protein load in the ER, malfunctioning ER protein-folding chaperones, or genetic protein-folding disorders can all induce the UPR. Three major ER sensors of unfolded proteins are ATF6, PERK, and IRE1 (6). The transcription factor ATF6 migrates from the ER to the Golgi during stress, where it is released by proteolytic cleavage and then traffics to the nucleus to transactivate genes that remediate ER stress. PERK and IRE1 are both kinases, which become activated by homo-oligomerization and autophosphorylation in response to ER stress to mediate profound alterations in translation and mRNA stability. Collectively, these proteins both reduce the protein load in the ER by reducing protein synthesis and activate a slower adaptive response that requires *de novo* transcription and translation to improve ER protein folding (7, 8). If ER stress is irremediable, a back-up program prepares the cell for apoptosis.

Apoptosis is a tightly controlled cell death program used to eliminate unwanted cells during development, infection, or malignant transformation and to eliminate cells experiencing irreparable stress-related damage. Apoptotic cells are rapidly recognized and cleared by macrophages and other scavenger cells without causing inflammation or harm to bystander cells. The best-studied cell death program (classical apoptosis) is mediated by the caspases, but alternate caspase-independent programs of cell death are also triggered by immune killer cells. In all these pathways, dying cells undergo membrane blebbing, mitochondrial damage, DNA degradation, chromatin condensation, and nuclear fragmentation. Executing programmed cell death requires that the cell membrane remain intact maintaining enough ATP to execute the program; apoptosis takes several hours to complete. The major effectors of classical apoptosis are the BCL-2 family proteins and the caspases, which form intertwined cell death pathways (9). The mitochondria are the site of action of the BCL-2 family and a key control point in cell death. The BCL-2 family is divided into three classes: multidomain anti-apoptotic proteins (BCL-2, BCL-X_L, MCL-1), the proapoptotic BH3-only proteins (such as BID and BAD), and the proapoptotic multidomain proteins (BAX and BAK) (10, 11). BAX and BAK form pores in the mitochondrial outer membrane to cause mitochondrial outer membrane permeabilization (MOMP) and the release of apoptogenic factors, including cytochrome c and SMAC/DIABLO, from the intermembrane space to the cytosol. The BH3-only proteins promote BAX and BAK multimerization, while the anti-apoptotic members inhibit it (11). The caspases are cysteine proteases that orchestrate cell death by cleaving a large number of substrates. The initiator caspases are activated by dimerization and autoproteolysis in response to death receptor ligation (caspases 8 and 10), MOMP (caspase 9), or stress signaling (caspase 2). Mitochondrial release of cytochrome c triggers formation of the apoptosome – a scaffold that binds and activates caspase 9. Once activated, caspase 9

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and the other initiator caspases cleave and activate the downstream effector caspase zymogens (caspase 3, 6, and 7), which then cleave hundreds of cellular substrates in an accelerating cascade to complete the cell death program (9, 12). In the extrinsic pathway of cell death, death receptor signaling induces activation of caspase 8 or 10. In addition to activating the effector caspases by direct cleavage, the initiator caspases cleave the BH3-only protein BID to truncated BID (tBID), which activates BAX and BAK. Cell stresses can lead to apoptosis, generally through the intrinsic pathway of apoptosis by enhancing the activity or expression of BH3-only proteins (10). For example, in genotoxic stress, TP53 transactivates the BH3-only proteins NOXA and PUMA (4, 10). Some stresses activate caspase 2 by an unknown mechanism to cleave BID and initiate the mitochondrial pathway (13).

The granzymes (Gzms) are killer lymphocyte serine proteases, stored with other cytotoxic molecules (notably perforin, the pore-forming protein that delivers Gzms into cells targeted for immune elimination) in cytotoxic granules (14). The Gzms (5 distinct enzymes in humans, 10 in mice) activate diverse and redundant pathways of programmed cell death by cleaving many cellular proteins, to guarantee that it will be difficult for infected or transformed cells to evade immune attack. GzmB acts like an initiator caspase by directly cleaving BID and caspase 3. GzmA initiates a distinct pathway of caspase-independent apoptosis with mostly non-overlapping substrates that damages mitochondria without causing MOMP and causes single-stranded, rather than double-stranded, DNA damage (15, 16). The death mechanisms activated by the other Gzms are distinct from these two but not well characterized. The Gzms translocate into the target cell nucleus by an unknown mechanism (17, 18), where they have recently been shown to disrupt pre-mRNA splicing and nuclear export profoundly.

A very robust bistable switch controls the balance between life and apoptosis. A recent single-cell study of apoptotic cells indicates that initiator caspase activity can build slowly before triggering suicide through irreversible MOMP and effector caspase activation (19). Stress pathways simultaneously promote repair and activate molecules that can eventually trigger an irreversible commitment to apoptosis. The distinction between the preapoptotic and commitment phases is not trivial, and conflating the two can be misleading. For example, although it is clear that translation is strongly inhibited after the commitment to apoptosis, preapoptotic signaling events can inhibit translation even without cell death (20). In this review, we seek to draw a clear distinction between events that occur in response to preapoptotic stresses and those restricted to the commitment phase of cell death.

Effects of stress and death on splicing

Pre-mRNA splicing (hereafter referred to simply as splicing) occurs cotranscriptionally to excise introns and join exon ends to generate a mature mRNA (*Fig.* 1). Splicing is controlled by hundreds of constitutive and regulated splicing

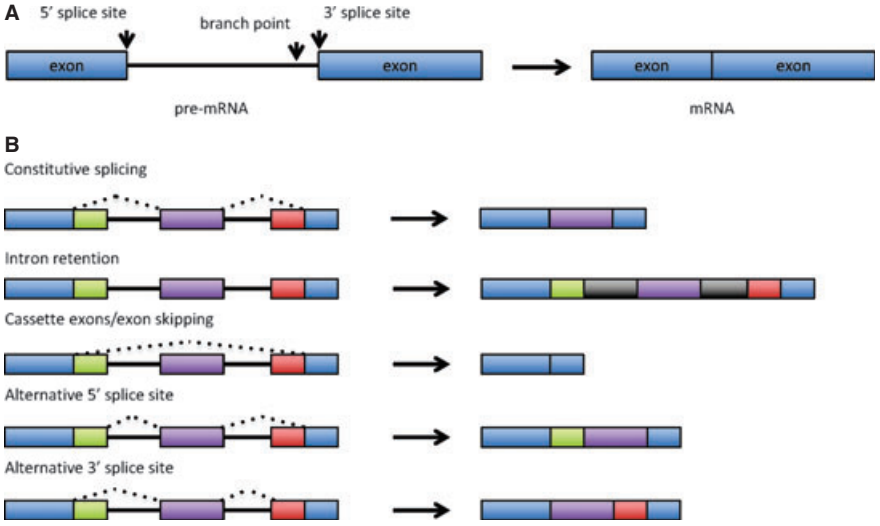

***Fig.* 1. pre-mRNA splicing and alternative splicing.** (A) The *cis*-regulatory elements recognized by the spliceosome are the 5′ and 3′ splice sites and the branch point. Other elements in the mRNA are recognized by splicing regulators to ensure the fidelity of splicing. (B) Four major types of alternative splicing. First and last exons can also vary. Alternative transcription start sites and cleavage and polyadenylation sites also contribute to isoform diversity. Global splicing inhibition is manifested as intron retention, as occurs in apoptosis and HS. Other forms of alternative splicing are more common in genotoxic stress. Note that the 5′ and 3′ splice sites refer to the ends of the intron that is spliced out, not the retained exon.

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factors (21). The basic splicing reaction is catalyzed by the spliceosome, a complex and well-studied RNA-protein machine (21, 22). The splicing reaction involves the recognition of three *cis*-regulatory elements on pre-mRNA – the 5′ splice site, the branch point, and the 3′ splice site (22) (*Fig. 1A*). Through an ordered assembly process, the spliceosome brings together the 5′ and 3′ splice sites and excises the intervening intron. However, the information contained in these three *cis*-regulatory elements is not sufficient to define exon boundaries; most transcripts contain many cryptic splice sites that are rarely or never used (23). The body of the transcript bears other *cis*-regulatory elements bound by splicing accessory factors that provide additional specificity to ensure the fidelity of splicing. In addition, chromatin signatures and nucleosome positioning affect exon recognition (24). The information conferred by these different factors is integrated by the spliceosome to generate mature mRNA. Two classes of abundant splicing regulators are the hnRNPs (heterogeneous nuclear ribonucleoproteins) and SR proteins, which are historically viewed as splicing repressors and enhancers, respectively (25, 26). In agreement with this view, *in vitro* assays on model templates have demonstrated that hnRNP A1 antagonizes exon inclusion promoted by the SR protein SRSF1 (SF2/ASF) (27, 28). These results are supported by recent genome-wide surveys showing that hnRNP C1/C2 binding is depleted around splice sites (29). However, other studies suggest that hnRNPs can either repress or activate splicing in a sequence-dependent manner (30, 31).

The complexity of the splicing code allows for variations in splicing, such that the same genomic locus can generate many different transcripts. Alternative splicing generates considerable transcriptome diversity. In fact, >95% of multiexon genes generate more than one splice isoform (25). Alternative splicing variants include exon skipping, intron retention, and the use of alternative 5′ and 3′ splice sites (*Fig. 1B*). Transcriptome-wide sequencing and microarray studies have focused on the large variation in alternative splicing found across tissues, and the corresponding variations in expression of splicing regulators, such as the hnRNP and SR proteins (25, 32, 33). In contrast with tissue-specific splicing, stress causes transient splicing alterations through rapid, often reversible, changes in *trans*-acting factors. Therefore, cell stress presents an opportunity to understand what factors regulate alternative and constitutive splicing in a physiological setting.

The best-characterized stress-induced alterations in splicing occur in heat shock. In HS, but not other stresses, there is potent, yet transient, global inhibition of splicing (26), leading to intron retention (*Fig. 1B*). Splicing of model templates treated with a nuclear extract from heat-shocked cells is completely inhibited (34). The SR protein SRSF10 (SRp38) is the master splicing inhibitor in HS (35). SRSF10 is normally abundantly phosphorylated, but, when hypophosphorylated during HS, strongly inhibits splicing *in vitro* (35). The HS-mediated block of splicing is independent of the HS master transcriptional regulator HSF1 (36). The SRSF10-mediated block on splicing is transient because SRSF10 is completely rephosphorylated within 1 h of recovery from HS (35). Although splicing inhibition by HS is well characterized in nuclear extracts, much less is known about what happens inside intact cells. As transcription is also strongly inhibited during HS (37), the relative importance of blocking transcription versus splicing is unknown. Furthermore, as most splicing occurs cotranscriptionally (38), *in vitro* studies using pretranscribed model splicing templates could lead to erroneous conclusions (35). One recent study utilized splicing-sensitive microarrays to analyze the transcriptome of HS-treated cells, and found an increase in the usage of downstream 'latent' 5′ splice sites (39) (*Fig. 1B*). However, this analysis filtered out cases of intron retention, which should be ubiquitous in HS. Many HS-induced isoforms contain premature termination codons, which mark transcripts for nonsense-mediated decay in the cytoplasm (40).

Not much is known about alterations in splicing during cell death. Our laboratory recently found that pre-mRNA nuclear export and splicing are profoundly disrupted in cell death mediated by GzmA and GzmB and likely during caspase-mediated apoptosis more generally. Because the Gzm serine proteases accumulate in the nucleus of target cells, we wished to identify their nuclear substrates. The proteomes of intact nuclei that were treated or not with GzmA were analyzed for proteins degraded by GzmA. Of 44 nuclear substrates, 33 were RNA-binding proteins, including 14 hnRNPs (41). Many hnRNPs were also degraded following caspase activation. As hnRNPs assemble on newly synthesized transcripts to orchestrate posttranscriptional processing, this prompted us to look at the fate of newly synthesized RNA in cells undergoing Gzm-mediated apoptosis. Newly synthesized RNA globally was retained in the nucleus and pre-mRNA splicing was severely compromised as assessed by measuring intron retention. Notably, GzmA cleaved hnRNP A1, causing it to mislocalize to the cytoplasm. A non-cleavable mutant of hnRNP A1 restored splicing and partially rescued cells from death, suggesting that the inhibition of pre-mRNA processing is an important component of the cell death program (*Fig. 2A*). Of note,

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alterations in mRNA splicing and nuclear export were absent in cells undergoing oxidative stress, suggesting that global inhibition of splicing is not a general feature of non-apoptotic stress. The splicing defect was likely the reason for nuclear retention of newly synthesized mRNAs. Intronless transcripts of immediate early genes (such as Jun) transcriptionally activated in response to stress were rapidly exported and translated into protein, whereas stress-activated transcripts that contain introns (such as Fos) were not translated into new protein. On the basis of these findings, we hypothesize that disrupted splicing during apoptosis blunts an adaptive stress response and tips the cell toward death.

The great increase in intron retention observed during programmed cell death, which we interpreted as global inhibition of splicing, may seem surprising given that hnRNP proteins are generally considered splicing silencers (25, 27). However, a view of hnRNPs as only splicing silencers is an oversimplification. Most human introns are long, with a mean length of approximately 3.2 kb (42), and hnRNP A1 promotes the excision of long introns ($\geq$ 1 kb). When hnRNP A1-binding activity is inhibited *in vitro*, long introns are almost completely retained (31). In a recent genome-wide study of hnRNP function and targets, intron retention significantly increased when any of six hnRNPs were knocked down (30). Four of these hnRNPs are GzmA targets and several are validated caspase substrates as well (41). GzmA also potentially cleaves other splicing factors, which became undetectable after GzmA treatment. These included U5S1, a component of the spliceosome, and SRSF1, which aids the spliceosome in recognizing the 5′ splice site, and is required for the splicing of at least some transcripts *in vitro* (43). GzmA cleavage of some SR proteins could collaborate with hnRNP cleavage to enhance intron retention. Future studies should characterize what happens to the spliceosome and how splicing changes during different forms of stress and apoptosis. It is not clear whether the Gzms simply inhibit splicing (as evidenced by intron retention) or also activate alternative splicing (*Fig.* 1B).

Alternative splicing in stress and death

In contrast with the broad inhibition of splicing caused by HS and GzmA, other stresses appear to bring about more targeted alterations in splicing. Apoptotic genes in both the mitochondrial and caspase cell death pathways have pro- and anti-apoptotic splice isoforms, whose relative expression is modulated during stress. Genotoxic stress alters splicing by several distinct mechanisms. These include altering the rate of transcription, sequestration of splicing factors, and transactivation of certain splicing factors. Splice site selection is modulated by the transcription rate of RNA polymerase II (PolII), further supporting the idea that transcription and splicing are intimately coupled (44–46). The 'kinetic coupling' model posits that PolII pausing downstream of alternative exons with weak splice sites promotes their inclusion (47) (*Fig.* 2B). The putative mechanism is simple – when PolII is slowed, downstream constitutive splice sites are less available, providing more time for exons with weak splice sites to be included. Stresses that reduce the transcription rate of specific genes should disproportionately alter their splicing. Low-dose ultraviolet (UV) irradiation induces modest phosphorylation of PolII in the heptad repeats of its C-terminal domain (CTD), which reduces the rate of transcription and alters splicing of model transcripts and splicing reporter minigenes (48). Expression of PolII phosphomimetic mutants, which transcribe more slowly, recapitulates some of the UV-induced changes in splicing (48). A recent transcriptomic analysis supports the kinetic splicing model. In Jurkat cells treated with drugs to reduce the PolII elongation rate, downregulated transcripts have more alternative splicing, favoring inclusion of exons with weaker splice sites (49). However, PolII interacts during transcription with many pre-mRNA processing factors (50) in a manner that may also depend on CTD phosphorylation (45). Thus PolII phosphorylation may modulate splicing both by altering the rate of transcription and through changes in its interactions with splicing factors. However, altered cassette exon splicing in response to various genotoxic stresses is usually modest. The functional significance of altered splicing in response to altered PolII elongation rates remains unclear (47–49).

Stress also modulates the expression, localization, and activity of splicing factors. Osmotic shock and high doses of UV drive the phosphorylation and cytoplasmic relocalization of hnRNP A1 in a p38 MAPK-dependent manner (51). Other hnRNPs and SR proteins remain in the nucleus. Osmotic stress or constitutive p38 MAPK activation also alters splicing of a reporter minigene. However, it remains unclear whether altered splicing during these stresses is due to changes in hnRNP A1 localization.

Alternative splicing during cell stress can play a pivotal role in tuning the cellular response to stress. During genotoxic stress, TP53 is upregulated and transcriptionally activates many targets, including MDM2. MDM2 ubiquitinates TP53 and targets it for destruction, thus completing a negative feedback loop that limits TP53 activity. However, as long as the stress persists, despite the increase in MDM2 transcription, exon skipping during the processing of the

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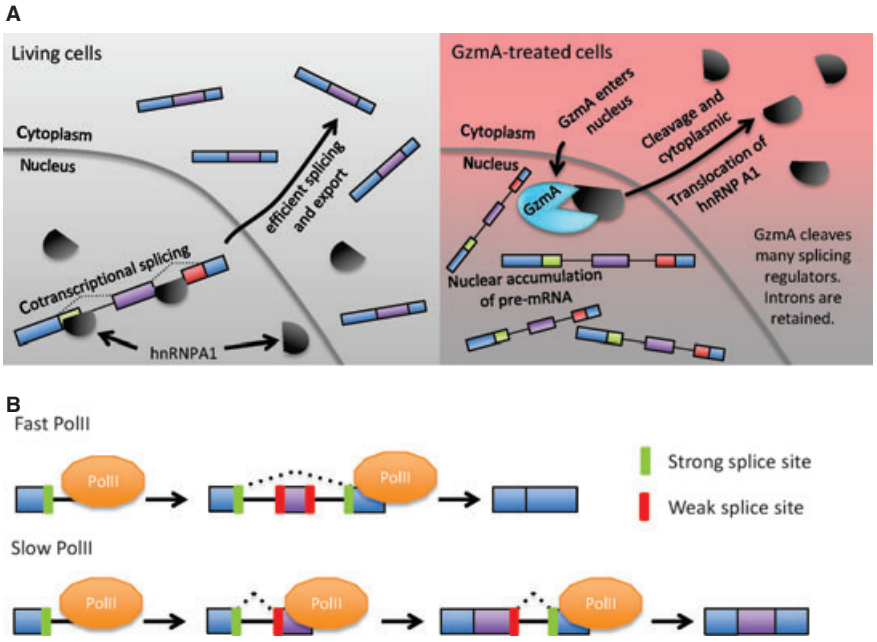

Fig. 2. Some mechanisms that regulate splicing in cell death and stress. (A) In living cells, the hnRNPs help to define intron boundaries. During killer cell attack, GzmA enters the nucleus, cleaving hnRNP A1, which relocalizes to the cytoplasm. A potent block on splicing leads to nuclear retention of nascent RNA and intron retention. Expression of a GzmA-uncleavable form of hnRNP A1 partially rescues both splicing and cell death. (B) The kinetic model of splicing posits that the rate of PolII transcription dictates exon choice. When PolII transcription is fast, strong splice sites are rapidly made available and those sites are preferred. Because splicing occurs cotranscriptionally, when transcription is slow, the spliceosome has time to utilize weak splice sites, permitting the inclusion of exons surrounded by weak sites. During some forms of stress PolII is phosphorylated in its C-terminal domain, which reduces the rate of transcription.

MDM2 pre-mRNA increases and consequently MDM2 mature mRNA and protein levels do not increase. At the same time, other mRNAs are normally processed (52). *MDM2* splicing is restored when the genotoxic stress is removed, producing MDM2 protein, which shuts down the stress response by degrading TP53. *MDM2* exon inclusion is promoted during transcription by an interaction between YB-1 and EWS proteins; this interaction declines during genotoxic damage. YB-1 and EWS also promote proper exon inclusion of other genes that undergo exon skipping in genotoxic stress (52). How stress signaling diminishes the EWS–YB-1 interaction remains unknown. As YB-1 promotes the recognition of weak 3′ splice signals and the inclusion of the associated exons (53), reduced YB-1 activity could explain how certain *MDM2* exons (those with weak splice signals) are skipped during genotoxic stress.

The transcriptional regulation of splicing factors can also affect cell fate in response to stress. E2F1, which is induced by genotoxic stress, transcriptionally activates SRSF2 (SC35), which promotes the expression of the proapoptotic splice variants of *CASP9*, *CASP8*, and *BCL2L1* (BCL-xL/-xS) (54). Knockdown of SRSF2 inhibits the upregulation of all proapoptotic splice variants and consequently inhibits apoptosis. The E2F-driven change in splice isoform ratios and the proapoptotic increase in the ratio of BCL-xS/BCL-xL appears to be much greater than that due to PolII phosphorylation (48) or TP53 signaling (55).

Unanswered questions concerning splicing in stress and cell death

There is no unified understanding of what happens to splicing during cellular stress. Although splicing is strongly inhibited by SRSF10 hypophosphorylation *in vitro*, some transcripts must still be correctly spliced to respond adequately to HS. While some heat shock proteins are encoded by intronless transcripts (*HSP70*), others are not (*HSP27*, *HSP90*). How are these still processed in the context of a potent block on splicing? Most studies in intact cells have assayed a mixture of stable prestress mRNAs and new mRNA produced after the stress (39, 49). This may miss many events because mature mRNAs are relatively stable and many stress-induced alterations to splicing are transient, such as the SRSF10-driven block to splicing in HS. Most studies of splicing and stress have relied on a handful of model transcripts or transfected splicing reporters to draw general conclusions about alternative splicing in stress and cell death

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(41, 48, 52, 54, 55). However, splicing is sensitive to sequence context and is regulated by a complex milieu of *cis*- and *trans*-acting factors (23). New experimental techniques to metabolically label and capture nascent mRNAs (1, 56, 57), when combined with splicing-sensitive microarrays (30) or RNA-seq (32), can now be leveraged to assess the transcriptome-wide impact of HS on splicing and capture transient changes in pre-mRNA processing. These methodologies should be very powerful tools for understanding the global change in splicing that occurs in response to various stresses and apoptosis.

The alterations in alternative splicing observed during stress and cell death provide opportunities to improve our understanding of how splicing is regulated and to understand how changes in splicing contribute to a cell's survival or death. The same stress can manipulate splicing by multiple mechanisms. For example, genotoxic stresses alter the phosphorylation and localization of hnRNP A1 (51), slow PolII elongation (48), disrupt the interaction of YB-1 and EWS with the spliceosome (52), and transcriptionally upregulate splicing factors (54). The best studies have systematically addressed each of these possible mechanisms to assess their relative contributions to observed splicing defects (48, 52). As evidenced by vast differences in splicing driven by HS and genotoxic stress, the type, duration, and severity of the stress will determine the nature and extent of the splicing changes. Crucially, in almost every case, it remains unclear whether altered splicing in stress has functional consequences. Many apoptosis-associated genes undergo altered splicing in response to stress, generating transcript isoforms that produce proteins with significant functional differences (58). However, most stresses induce only modest changes in the ratio of the different splice isoforms (48, 55, 59). In limited cases, manipulating the *trans*-acting factors that control altered splicing after stress substantially alters cell survival (54). Very few studies have shown that a single alternative splicing event in the cell stress response is significant. Antisense oligonucleotides can be used to interfere with individual splice sites to modulate the expression of specific splice isoforms (60). This tool might be useful to manipulate specific splice isoforms during cell stress to assess their importance. Finally, altered splicing in stress cannot be viewed in a vacuum – virtually all stresses induce profound alterations to translation, and some factors (such as hnRNP A1 and SRSF1) regulate both splicing and translation initiation.

Regulation of translation in cell stress

Translational control has been extensively studied in stress and apoptosis (61, 62). Here, we focus on the interplay between stress and cell death, in particular how altered translation during stress protects cells from or predisposes them toward apoptosis. Most stresses cause a rapid and nearly global shutoff of protein synthesis through one of two mechanisms that interfere with translation initiation – phosphorylation of eIF2α and inhibition of the eIF4F complex (*Table* 1). Modifications of these factors are common in almost all stresses, and their effects can be assessed by global snapshots of translation, captured by ^{35}S labeling of newly synthesized proteins and separation of polyribosomes on density gradients. Inhibiting translation serves to conserve cell resources, as translation consumes >50% of cellular energy (61), and to remodel the proteome by selective translation of individual transcripts (63, 64). Recent work suggests that the balance between repair and cell death is regulated by translation.

***Table* 1. Effects of stress on translation**

Stress	eIF2α phosphorylation	Modifications to eIF4F complex	Stress granules	Notes
Apoptosis	Yes PKR is cleaved and activated	Hypophosphorylation of 4E-BPs Caspase cleavage of eIF4G	Unknown	PKR activation occurs after translation shutoff. Mechanism of 4E-BP phosphorylation unknown
Heat shock	Yes PKR and HRI, depending on cell type	HSP27 blocks eIF4G interaction with eIF4E	Yes	Unclear which mechanism(s) are most important for inhibiting translation
Osmotic shock	Yes Responsible kinase is unclear (probably HRI in erythroid cells)	Hypophosphorylation of 4E-BPs	Yes	Mild stress only alters 4E-BPs and eIF4F complex. eIF2a phosphorylation promotes apoptosis in more severe stress
ER stress	Yes PERK is activated	4E-BP1 expression induced by ATF4, which enhances translation inhibition	Yes	Some transcripts are translated to balance repair and stress, notably *ATF4* and *CHOP*

eIF2α phosphorylation occurs during all of these stresses and other stresses including nutrient starvation, UV irradiation, and oxidative stress. Cap-dependent translation is inhibited by disrupting the eIF4F complex, but this is achieved by diverse mechanisms.

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Translation initiation requires a ternary complex composed of the translation initiation factor eIF2, GTP, and the methionine initiator tRNA (Met-tRNA$_i^{Met}$). During translation initiation, GTP is hydrolyzed to GDP, which must be recycled by eIF2B to form a new initiation-competent eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complex. Stresses can activate one of four kinases that phosphorylate the eIF2 regulatory subunit (eIF2α) on serine 51. The kinases and their respective stress activators are PKR (viral infection), PERK (ER stress), GCN2 (nutrient starvation), and HRI (oxidative stress) (64). S51 phosphorylation of eIF2α leads to stable, inhibitory association of the eIF2-GDP complex with eIF2B, blocking recycling of the ternary complex. This results in a fast, potent, and reversible block of translation initiation. eIF2α phosphorylation is the main feature of the integrated stress response, a common protective program activated by most cellular stresses (65) (*Table* 1). A fraction of the proteome escapes translation inhibition through a unique mechanism, while some proteins are even upregulated in response to eIF2α phosphorylation (64). Transcripts that have one or more upstream open reading frames (uORFs) are able to escape eIF2α-dependent translation arrest. When the amount of eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complexes is limited by eIF2α phosphorylation, ribosomes are more likely to scan through the uORFs and initiate translation at a downstream ORF to generate functional protein (61, 63, 64). In contrast, when eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complexes are abundant, the ribosomes initiate first at uORFs and are subsequently less able to initiate at downstream ORFs encoding the relevant protein.

Inhibiting cap-dependent translation in stress

Another way to inhibit translation is to limit formation of the eIF4F complex, which is composed of the cap-binding protein eIF4E, the RNA helicase eIF4A, and the adapter protein eIF4G (66). eIF4F is required for initiating translation of cap-dependent transcripts, which are most mRNAs. The mechanisms of eIF4F inhibition have been reviewed elsewhere (61, 66, 67). Although eIF2α is a common target of almost every stress, only some stresses interfere with eIF4F, and they do so through a diversity of mechanisms (*Table* 1). For example, the heat shock protein HSP27 binds directly to eIF4G to inhibit eIF4F complex formation during HS, which profoundly limits translation to produce very few proteins (68). The best-studied regulators of eIF4F are the eIF4E-binding proteins (4E-BPs), which inhibit eIF4E binding to eIF4G. 4E-BP binding to eIF4E is regulated by phosphorylation: hypophosphorylated 4E-BP binds eIF4E with high affinity, but phosphorylated 4E-BP does not bind (67). The 4E-BPs are phosphorylated by mTORC1, a kinase complex that integrates signaling from multiple mitogenic pathways (67). In the absence of eIF4F, selected mRNAs can be translated through structured elements in their 5′UTRs termed internal ribosomal entry sites (IRES). Ribosome entry at IRESs requires IRES *trans*-acting factors (ITAFs). Numerous apoptosis-associated transcripts (with both pro- and anti-apoptotic functions) contain IRESs that allow them to be translated during cell stress.

Recently, a new mechanism for inhibiting cap-dependent translation has been described (69). tRNA fragments (tiRNAs) are generated during certain stresses by tRNA cleavage at a single site in the anticodon loop by the RNase A family member angiogenin, which is secreted by stressed cells and acts in an autocrine or paracrine fashion. The 5′ fragment tiRNA inhibits cap-dependent translation but not IRES-mediated translation, possibly by binding directly to eIF4G and eIF4E (69, 70).

Regulated translation and the commitment to apoptosis

Whether cells adapt to stress or commit to cell death depends on a delicate balance between the activation of different pathways during stress, which in turn depends on the intensity and duration of the stress. At levels of osmotic stress that induce apoptosis, translation is globally inhibited both through eIF2α phosphorylation and hypophosphorylation of 4E-BP1. Translation inhibition in response to osmotic stress is not rescued in MEFs expressing eIF2α lacking a phosphorylation site at serine 51 (S51 A/A), indicating that eIF2α phosphorylation is not required. Inhibition of the eIF4F complex is probably more important for blocking translation in this setting. In spite of this, S51 A/A MEFs are significantly protected from osmotic stress-induced apoptosis (71). Cytoplasmic relocalization of hnRNP A1 does not occur in S51 A/A MEFs during osmotic stress (51, 72). Cytosolic hnRNP A1 can act as an ITAF or an inhibitor of cap-dependent translation, depending on the transcript. For example, cytosolic hnRNP A1 activates cap-independent translation of the proapoptotic factor APAF-1, but inhibits expression of anti-apoptotic BCL-xL (71, 73). Overexpression of cytosolic hnRNP A1 in S51 A/A MEFs restores the same level of apoptosis during osmotic stress as observed in wildtype MEFs. This study and others suggest that some of the same factors that regulate altered splicing in response to

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stress or cell death can also modulate translation inhibition (41, 51, 71).

Translation of individual mRNAs in the setting of global inhibition is important for balancing cell fate between repair and apoptosis. During hypoxic stress and hepatotoxic ER stress, phosphorylation of eIF2α by PERK protects against apoptosis (74, 75). In contrast, MEFs treated with ER stressors (such as tunicamycin and thapsigargin) undergo apoptosis that relies on eIF2α phosphorylation-dependent expression of the CHOP transcription factor, which upregulates expression of proapoptotic genes, such as *BIM* and *DR5* (76–79). CHOP commits cells to apoptosis during the stress response, as *Chop*$^{-/-}$ MEFs are resistant to death in response to ER stress (78). During ER stress, *CHOP* mRNA is transcriptionally upregulated by the stress transcription factors ATF6 and ATF4, but protein expression requires eIF2α phosphorylation. A single uORF in the *CHOP* 5′ UTR is used during ER stress (79). Prolonged stress leads to apoptosis (6, 7, 80). The proapoptotic upregulation of CHOP requires the coordinated action and sustained signaling of several ER stress pathways. During stress, translation of ATF4 is upregulated through uORFs in its 5′ UTR (81), and then ATF4 protein transactivates *CHOP* mRNA expression (82). In turn, continued eIF2α phosphorylation is required for *CHOP* mRNA translation, and then the protein transactivates target gene expression to exert its proapoptotic function. CHOP protein is therefore downstream in the ER stress response, and the events that lead to its expression require persistent ER stress at multiple points (6) (*Fig.* 3). This built-in delay could allow a cell time to execute a protective response before committing to apoptosis. A similar mechanism induces CHOP during oxidative stress or amino acid starvation (81), suggesting that it is a common means to push cells toward apoptosis in response to sustained insults.

Unanswered questions in cell stress

Although much is known about well-characterized stress response master regulators, such as ATF4, that are upregulated by eIF2α phosphorylation, for most stresses, the full complement of transcripts that escape translation inhibition or are induced by stress remains unknown. New methods of

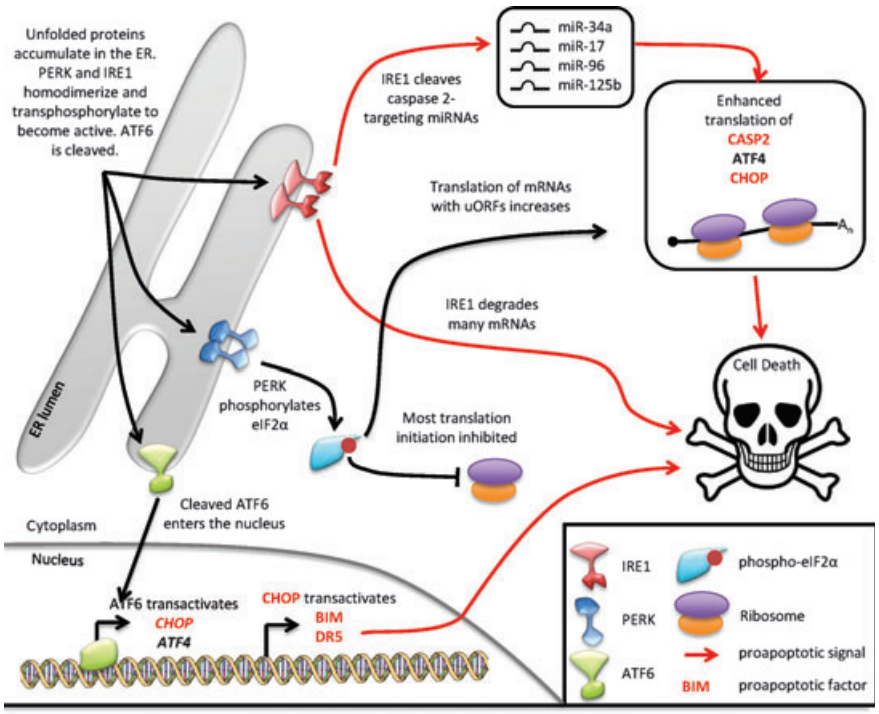

***Fig.* 3. Proapoptotic posttranscriptional events in the ER stress response.** In response to ER stress, the three major ER-localized effectors (ATF6, IRE1, and PERK) all contribute to signaling that can ultimately lead to repair or apoptosis. The ATF6 transcription factor translocates to the Golgi apparatus and is cleaved. The released DNA binding domain enters the nucleus to transactivate expression of repair genes (such as *ATF4*) and proapoptotic genes (such as *CHOP*). PERK and IRE1 kinases become activated. PERK phosphorylates eIF2α, which blocks translation initiation. However, key transcripts, such as *ATF4* and *CHOP*, involved in the stress response escape this translation block through uORFs. The CHOP transcription factor activates expression of the BH3-only protein BIM, the death receptor DR5, and other proapoptotic targets. Latent RNase activity of IRE1 also becomes activated. IRE1 contributes to cell death by cleaving and inactivating multiple miRNAs that normally suppress translation of caspase 2. Caspase 2 translation then increases. IRE1 also cleaves many ER-resident mRNAs to reduce ER stress.

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high-resolution comprehensive ribosome profiling (83) could be used to identify differentially translated proteins in stress. Although the translational responses to diverse stresses share many features, differences between types of stress and how changes in translation help a cell adapt to each type of insult could be better understood.

Inhibited translation in cell death

Translation is drastically inhibited early in cell death (84–86), and a number of mechanisms have been posited, mostly involving alterations to the same initiation factors that regulate the stress response. Although genotoxic stress occurs late during apoptosis, the generation of ROS is an early event that is likely to activate many of the same responses as non-lethal oxidative stress. During apoptosis, the eIF4F complex is disrupted by phosphorylation of 4E-BPs (87) and caspase cleavage of eIF4G (84), whereas eIF2α phosphorylation can be induced by caspase cleavage of the eIF2α kinase PKR, which activates it (85, 88). It remains still unclear which apoptotic alteration(s) are responsible for inhibited translation in cell death. They may vary with the apoptotic insult (62). For example, translation inhibition is caspase dependent in cells treated with death receptor ligands, but independent of the caspases in the same cells treated with the genotoxic agent etoposide (62, 89). Here, we discuss alterations that occur before and after 'commitment' to cell death, which we define as effector caspase activation.

Translation inhibition before the commitment to apoptosis

Stress signaling that occurs before the commitment to apoptosis could play a pivotal role in blocking translation. Jurkat T cells deficient in caspase 8 are almost completely resistant to death receptor-mediated apoptosis, whereas caspase 8-sufficient Jurkat cells activate the effector caspases and die under the same treatment conditions. eIF2α phosphorylation modestly increases shortly after adding anti-Fas, regardless of caspase 8 status (20). However, the impact of non-apoptotic eIF2α phosphorylation is modest because global translation inhibition only occurs in caspase 8-sufficient (dying) Jurkat cells. In MCF-7 cells treated for 4 h with the death receptor ligand TRAIL, global translation is reduced by approximately 50%, at a time when there is very little PARP1 cleavage and little phosphatidylserine externalization on the cell membrane (markers of apoptosis). However, at this early time 4E-BP1 hypophosphorylation and reduced binding of eIF4G to eIF4E inhibits the eIF4F complex to block cap-dependent translation (87). These alterations to 4E-BP1 are independent of caspase 3 but sensitive to the pan-caspase inhibitor zVAD-FMK, suggesting a role for the initiator caspase 8 (89). It remains unclear how 4E-BP1 becomes hypophosphorylated or whether this event is essential for translation inhibition. MCF-7 cells treated with etoposide demonstrate reduced translation even when caspase activity is inhibited. Wildtype MEFs treated with etoposide have increased eIF2α phosphorylation and association of 4E-BP1 with eIF4E concurrent with translation inhibition. However, MEFs deficient in the eIF2α kinase PKR have a comparable block to translation in response to etoposide without any increase in eIF2α phosphorylation or 4E-BP1–eIF4E interaction (89). These results suggest that other unknown events besides eIF2α phosphorylation and disruption of eIF4E binding to eIF4G may be important for inhibiting translation in cell death triggered by DNA damage.

Alterations to translation after commitment to cell death

Once activated, the effector caspases directly cleave many translation factors. Notably, caspase 3 cleaves eIF4G (84, 90, 91). It remains unclear, however, whether eIF4G cleavage products still support translation (62) or how much eIF4G cleavage contributes to translation inhibition, which is often already well underway by the time eIF4G is cleaved. In some studies, eIF4G cleavage correlates well with translation inhibition (84, 91), whereas in others, translation inhibition precedes eIF4G cleavage (85, 86). The relative impact of eIF4G cleavage on translation may depend on the cell type and death-inducing stimulus. eIF4G is not cleaved in caspase-3 null MCF-7 cells treated with TRAIL, but translation is still effectively inhibited (89). PKR is also cleaved by the caspases during death receptor-mediated apoptosis in Jurkat cells, but PKR cleavage and eIF2α phosphorylation are late events that occur well after translation inhibition and are probably not major contributors to translation inhibition in cell death (85).

Although large alterations in translation factors have been cataloged during initiation and execution of apoptosis, it remains still unclear which modifications are responsible for inhibiting translation. Alterations to translation factors have only been studied in bulk cell populations undergoing asynchronous cell death. Even genetically identical sister cells die asynchronously in response to apoptotic stimuli (92), making it difficult to dissect which alterations occur in the

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preapoptotic versus commitment phases. For example, it has been argued that many IRES-containing mRNAs are upregulated in apoptotic cells (87), but markers of the commitment phase of apoptosis remain low at the time IRES-dependent translation was analyzed, raising the possibility that these translation alterations are actually preapoptotic. Synchronous and rapid means of inducing cell death or single-cell studies will be necessary for understanding which processes are truly responsible for blocking translation during apoptosis. New genetic models with key apoptosis effectors ablated may help dissect the contribution of different pathways. At a more fundamental level, although widely assumed, it has not been proven that translation inhibition is necessary for cell death.

MicroRNAs at the nexus of mRNA translation and stability in stress

MicroRNAs (miRNAs), approximately 22-nt RNAs that recognize target mRNAs by partial base-pairing, interfere with mRNA translation and stability when they are bound to the RNA-induced silencing complex (RISC) (93). miRNAs can affect the overall protein expression of many genes, sometimes only by a subtle amount. The regulated targets also vary with the particular cellular context. Recent studies suggest that miRNAs first inhibit translation initiation, then activate mRNA deadenylation and decay (94). Because miRNAs require only limited complementary sequence over a short sequence to recognize targets, target identification remains a major challenge (95). Knockouts of many miRNAs in mice exhibit no overt phenotypes under normal conditions, but phenotypes can emerge after stress. miRNAs can act as important mediators of the balance between repair and apoptosis (96). The miR-15 family is upregulated in the heart following ischemia/reperfusion (IR) injury and promotes apoptosis in response to this stress. In cardiomyocytes subjected to hypoxia and reoxygenation, antagonizing miR-15 increases expression of the antiapoptotic miR-15 target BCL-2 and reduces apoptosis (97, 98). miR-15 suppression *in vivo* significantly reduces the size of the infarct following IR injury. In contrast, miR-214, which is also upregulated in response to IR, protects against apoptosis. The hearts of miR-214 knockout mice resemble their wildtype counterparts under normal conditions, but these mice are much more susceptible to IR, with decreased survival and increased cardiac apoptosis (99).

miRNAs also play a role in the TP53-dependent response to genotoxic stress. miR-34a and other miRNAs are upregulated by diverse genotoxic stresses (100–104). The promoters of these miRNAs contain strong TP53 consensus sites transactivated by TP53. Ectopic expression of miR-34a induces potent cell cycle arrest and/or apoptosis *in vitro*. In one study, knockdown of miR-34a also significantly reduced apoptosis in response to etoposide (104). These and other early studies suggested a central role for the miR-34 family (and miR-34a especially) in promoting cell cycle arrest and cell death following genotoxic stress in a TP53-dependent manner. Multiple negative regulators of TP53 have also been identified as direct targets of miR-34a, suggesting that miR-34a and TP53 might form a positive feedback loop, with each promoting the expression or activity of the other (105) (*Fig.* 4). However, a recent study using miR-34 family knockout mice and mouse embryonic fibroblasts (MEFs) has called into question the importance of miR-34 in the response to genotoxic stress. Wildtype and miR-34 family knockouts tested under a variety of genotoxic stress conditions showed no difference in cell survival *in vitro* or *in vivo* (106). Indeed, the only differences observed were increased proliferation and transformation propensity of miR-34 knockout MEFs compared with wildtype MEFs. This finding agrees with work from our laboratory. We developed a novel biochemical approach to identify miRNA targets without bias by isolating mRNAs bound to a biotinylated miRNA mimic transfected into cultured cells. Although miR-34a pulldown significantly enriched both for genes in the TP53 network and genes involved in cell cycle progression, confirming earlier studies, the most significant enrichment was in genes whose products participate in all aspects of growth factor signaling (105). Cancer cell lines overexpressing miR-34a were less responsive to stimulation by serum growth factors, whereas miR-34a-deficient MEFs were less likely to die after serum starvation. As in the knockout study, we also found that knockdown of the miR-34 family did not affect cell survival after genotoxic stress (authors' unpublished results). In line with its role in regulating growth factor signaling, miR-34a transcription is also regulated by an alternate promoter that is activated by growth factor signaling (107). These new results suggest that the primary role of the miR-34 family may be to suppress growth-stimulating signaling and downstream cell cycle genes to temper the potentially oncogenic response to growth factors (*Fig.* 4). These studies also suggest that the integrated effect of modulating an individual miRNA during stress can be less straightforward than predicted by overexpression studies.

Although the above examples demonstrate the potential of miRNAs to play a central role in mediating cell fate in

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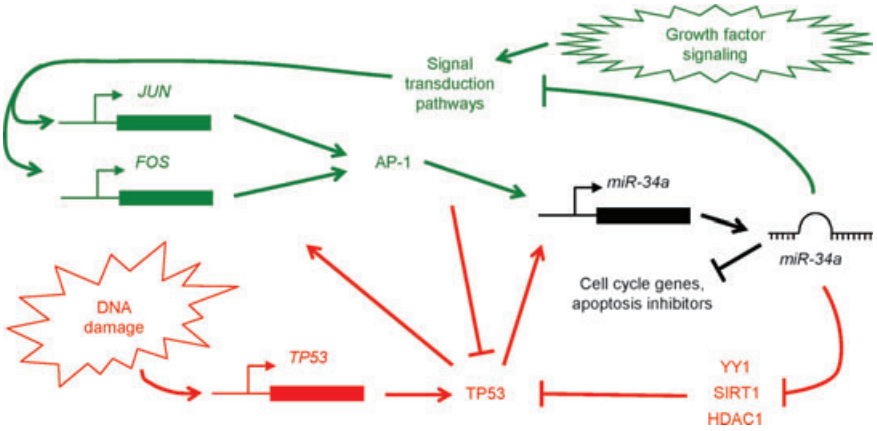

Fig. 4. miR-34a regulates growth factor signaling and the DNA damage response. The miR-34 family participates in the TP53-dependent response to DNA damage (indicated in red). The miR-34a promoter is strongly upregulated by TP53. miR-34a in turn downregulates many TP53 inhibitors, cell cycle genes, and apoptosis inhibitors. This positive feedback loop suppresses cell proliferation. miR-34a is also induced by growth factor signaling (indicated in green) and dampens responsiveness to mitogenic signals by suppressing many growth factor signaling pathways. Results from knockout mice suggest that regulating growth factor signaling is the major biological function of miR-34, as knockout mice have no detectable difference in their response to genotoxic stress.

response to stress, these regulatory events may be fairly slow because they require *de novo* transcription and processing of the miRNA before they can downregulate their targets. Intriguing new studies implicate a fast-acting role for miRNAs in IRE1-dependent cell death in response to ER stress (Fig. 3). IRE1 is an ER kinase composed of an N-terminal lumenal domain, and cytosolic kinase and RNase domains (6). During ER stress, IRE1 aggregation drives its *trans*-phosphorylation, unmasking its RNase activity. IRE1 cleaves a subset of miRNAs (miR-17, miR-34a, miR-96, and miR-125b) that blocks their target-suppressing functions. Normally, miRNAs are extremely stable (108), so the rapid decline in these miRNAs (by approximately 50% after 4 h of ER stress) is quite striking. Caspase 2 (CASP2) is a common target of these miRNAs. Caspase 2 upregulation is an important initiator of the mitochondrial pathway of cell death in response to ER stress (109). Knockdown of these miRNAs increases caspase 2 protein, while their overexpression prevents the accumulation of caspase 2 following ER stress. Although most miRNAs reduce the stability, and thus steady state levels, of their mRNA targets (110), *CASP2* mRNA levels are unchanged by ER stress, while its translation rate, as measured by polyribosomal occupancy, significantly increases. Unlike IRE1-driven mRNA decay, miRNA targeting is dependent on the IRE1 RNase domain but not its kinase domain. However, although kinase-inactive IRE1 induces CASP2 protein, it does not induce apoptosis. Thus, IRE1-dependent mRNA decay (discussed below) appears to be crucial for tripping the apoptotic switch in response to irreparable ER stress (111, 112).

miRNA alteration of mRNA stability in ER stress also can have an inflammatory effect. TXNIP (thioredoxin interacting protein) is a mediator of oxidative stress that may promote insulin resistance in type 2 diabetes induced by a high-fat diet (113). TXNIP protein is upregulated in response to diverse ER stresses in a PERK and IRE1-dependent manner. Signaling downstream of PERK enhances *TXNIP* transcription (114). Moreover, IRE1 cleavage of miR-17 also enhances the stability of its target *TXNIP*. The physiological impact of increased TXNIP following ER stress *in vitro* and *in vivo* is profound. Pancreatic islets activate the inflammasome to cleave the precursor of the proinflammatory cytokine IL-1β in response to ER stress, which is linked to the development of diabetes in this model (115). Inflammasome activation may be secondary to increased ROS generation when TXNIP increases, as pharmacological inhibition of IRE1 RNase activity or knockdown of *TXNIP* fully or partially inhibits, respectively, the increase in IL-1β in response to ER stress (114, 116). In response to pharmacological inducers of ER stress, fewer Txnip-null MEFs die than their wildtype counterparts, while Txnip deletion partially reduces development of diabetes in a model driven by ER stress (116). Together, these results suggest that miRNAs can play a central role in regulating inflammation and apoptosis under stress conditions.

Two consistent themes emerge in the study of miRNAs and stress. First, target identification is central to understanding a miRNA's true biological function, and second, that functions inferred from cell culture experiments must be validated *in vivo*. Many miRNA studies have relied on ectopic expression of miRNA mimics, which can suggest

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functions that are not physiologically relevant. This is highlighted best by recent studies of miR-34a. miRNA levels may change rapidly in response to stress to alter protein levels that are critical to cell survival under stress. One mechanism for this has been described (miRNA cleavage by activated IRE1), but other unknown mechanisms may be at play.

Changes in mRNA stability during cell stress

Little is known about how cell stress affects mRNA stability. Most mRNAs are protected from decay by a 5′ $^{7\text{-meG}}$ cap and a 3′ poly(A) tail (117), and most basal mRNA turnover is mediated by deadenylation followed by decapping and exonucleolytic decay. However, mRNA decay can be initiated by a variety of other mechanisms, including endonucleolytic cleavage (118). Although many of the *trans*-acting factors that regulate mRNA turnover are well characterized (nucleases, RNA-binding proteins), little is known about what signals initiate basal mRNA decay and how individual mRNAs become tagged for decay. Recent work suggests an intimate coupling between mRNA translation and stability. However, although almost all stresses globally shut off translation, there is no evidence for a concomitant global decline in mRNA stability, suggesting that somehow, stability and translation are decoupled following cell stress.

Recent work suggests that altered mRNA stability could play a key role in the commitment to cell death following prolonged ER stress. IRE1 cleaves the *XBP1* mRNA to activate an unusual cytosolic splicing reaction, excising a 26-nt intron from *XBP1* to generate *XBP1-s* (7). This processed transcript encodes a functional XBP1 transcription factor that transactivates many genes that function to reduce the unfolded protein load in the ER and alleviate ER stress. Until recently, this was the only known stress-dependent function of IRE1 (7). However, it is now clear that IRE1 also directly degrades many mRNAs during stress (111, 119, 120). Gene expression profiling of *Drosophila* S2 cells depleted of *IRE1* or *XBP1* by RNAi demonstrated that a subset of transcripts is downregulated in ER stress in an IRE1-dependent, XBP1-independent fashion. Most of these degraded transcripts are normally found at the ER, leading to the hypothesis that IRE1 cleaves these mRNAs to reduce ER-localized translation. mRNA degradation would collaborate with the PERK/eIF2α-dependent inhibition of translation to reduce the protein load in the ER (119). Follow-up studies have confirmed that the IRE1 RNase directly targets some ER-localized mRNAs for decay (111, 120). This mRNA-targeted activity depends on both the kinase and the RNase domains of IRE1, whereas *XBP1* splicing does not require IRE1 kinase activity. As only cells expressing IRE1 protein with functional kinase and RNase domains undergo apoptosis in response to prolonged ER stress, the mRNA decay activities of IRE1 may be essential for committing to apoptosis (111). In support of this hypothesis, expressing an IRE1-binding peptide that enhances *XBP1* splicing activity, but reduces IRE1-dependent mRNA decay, protects cells from ER stress-induced apoptosis (121).

Stress granules: mysterious mRNA-protein reservoirs

No review of mRNA stability in stress would be complete without a discussion of stress granules (SGs) (122–125). SGs, microscopically defined cytoplasmic structures that aggregate in response to a diverse array of stresses, contain the 40S ribosomal subunit, several translation initiation factors and numerous RNA-binding proteins bound to large amounts of mRNA. G3BP1, TIA1, and TIAR proteins are important for SG formation, which is triggered by phosphorylation of eIF2α, a common feature of many stresses (125). Despite intensive research on SGs, it is still not completely clear what role they play in mRNA stability. Some transcripts translated during stress are excluded from SGs, while housekeeping genes are contained in them (122). This finding has led to the hypothesis that SGs 'store' mRNAs in a translationally inactive state during conditions of cell stress. When stress is relieved, SGs disappear and the stored mRNAs are available for translation. However, SGs localize close to P bodies, sites of miRNA-dependent and independent mRNA decay. Moreover, it is not possible to separate SGs from P bodies, and they contain some of the same proteins. SGs might protect mRNAs during transient stress but could hand them over to P bodies for degradation when stress persists. Moreover, the fate of individual mRNAs within SGs need not be uniform. Whether a SG-resident mRNA is stabilized may depend on the *cis*-regulatory sequences in the mRNA and the *trans*-acting factors that bind it. There is circumstantial evidence that localization to SGs can stabilize mRNAs. For example, IGF2BP1 (also known as zipcode binding protein) localizes to SGs in conditions of cell stress, and individual IGF2BP1 target mRNAs are stabilized by this protein during stress (126). Future work will be required to determine if mRNAs are indeed globally or partially stabilized by SGs under stress conditions.

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mRNA stability during apoptosis

In contrast with the very well-characterized changes that occur to proteins, lipids, and DNA during cell death (9), the fate of mRNAs in apoptotic cells has not been extensively studied. In some cells, the 28S rRNA is cleaved by an unknown nuclease to generate distinct fragments (127). Because this occurs late in cell death and is not universally seen, it is unlikely to be important for cell death. Some intriguing studies that have assayed individual mRNA transcripts in apoptotic cells by Northern blotting suggest that mRNA decay may be an early feature of apoptosis (86, 128).

Postmortem: answering old questions with new techniques

Recently developed methods could be deployed to follow the effects of stress and apoptosis on the cellular transcriptome to better define the fate of newly transcribed RNAs. Recent methods to label and capture new transcripts could be paired with next-generation sequencing to track posttranscriptional changes in pre-mRNA processing and half-life (1, 56, 57). These experimental approaches could be coupled with genetic manipulation by knockout, knockdown, or overexpression of mutant or wildtype candidate factors to define the key pathways involved in controlling protein expression in stressed cells. Although global inhibition of translation in response to stress and cell death has been known for decades, other posttranscriptional events need further characterization. In particular, we lack an understanding of how the type, severity, and duration of stress impact mRNA splicing, translation, and stability. Although stress responses share some common features, the differences may prove important in determining how cells adapt to diverse insults. Ultimately, we need to understand how posttranscriptional changes during the stress response influence cell repair versus apoptosis.

Some of the basic mechanisms that regulate gene expression in the stress response remain unknown. Under normal conditions, mRNA translation and stability are intimately coupled, but generally mRNAs remain stable in stressed cells despite blocked translation. It remains to be seen whether the formation of SGs can explain this uncoupling. Similarly, there are major open questions in the study of apoptosis. The true cause(s) of translation inhibition during programmed cell death remain elusive. Is it secondary to the stresses that accompany apoptosis, or do activated caspases play a major role? Is blocking new protein expression necessary for carrying the apoptotic program to completion? More careful studies that separate preapoptotic cells from cells committed to apoptosis will be crucial to answer these questions.

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