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Post-transcriptional control of gene expression through subcellular relocalization of mRNA binding proteins

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Eukaryotic cells have developed multiple mechanisms to respond to different physiological cues, such as cellular stress, which allow the cells to adapt themselves to their new environment. The regulation of post-transcriptional gene expression is an important component of the cellular stress response and is mediated by mRNA binding proteins (mRBPs). Recently, several studies have shown that regulated subcellular localization of mRBPs upon diverse stimuli (such as cellular stress) provides an additional level of regulation for gene expression.

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1. Introduction

Many mRNA binding proteins (mRBPs) can shuttle between the nucleus and the cytoplasm, which allows them to have either a nuclear or cytoplasmic distribution within the cell [1,2]. In several instances deregulated subcellular localization of mRBPs has been shown to be associated with pathology. For example, cytoplasmic relocalization of Hu antigen R (HuR, ELAVL1), an RNA binding protein of the embryonic lethal, abnormal vision, Drosophila-like ELAV family of RNA binding proteins that can stabilize and/or regulate the translation of target mRNAs [3], is associated with a poor histologic differentiation, large tumor size, and poor survival in breast cancer [4]. In addition, HuR cytoplasmic relocalization upon platelet-derived growth factor (PDGF) activation contributes to the regulation of vascular smooth muscle cell growth and homeostasis in pathologies associated with vascular smooth muscle proliferation [5]. Heterogeneous nuclear ribonucleoproteins (hnRNPs) have been shown to play central roles in diverse cellular processes including DNA repair, telomere biogenesis, cell signaling, splicing, mRNA stability and translation [6]. hnRNP A2, a member of the hnRNP family, has been reported as a tumor marker and predictor of neoplastic transformation [7]; hnRNP A2 is normally localized in the cytoplasm of glioblastoma multiform cells whereas cerebral glioblastomas contain low cytoplasmic levels of hnRNP A2 [8]. Thus, the role of the mRBPs in these pathologies is associated with their location within the cell. Because

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mRBPs play a critical role in the regulation of gene expression, dysregulated subcellular localization is expected to modify the expression of key genes linked to these pathologies. In this review we will discuss the role of subcellular localization in regulating the diverse activities of mRNPs, and how this regulation affects cell survival.

2. mRBP relocalization

mRNAs are associated with a plethora of mRNA binding proteins that are involved in several aspects of mRNA metabolism [6,9,10]. Among mRBPs, proteins belonging to the hnRNP family [6] and to the splicing factor serine/arginine-rich (SR) protein family [10,11] were originally described as nuclear factors involved in mRNA splicing. Later it was observed that most of these proteins are able to shuttle between the nucleus and the cytoplasm [2,12].

Interestingly, the shuttling of mRBPs between the nucleus and the cytoplasm is not a constitutive process, but rather is subject to modulation by extracellular stimuli or during different processes such as hypoxia or apoptosis (Table 1). The pathways that regulate the subcellular distribution of

Table 1 – Stimuli/processes leading to a relocalization of mRBPs

Stimuli	mRBP	References
Osmotic shock	hnRNP A1	[20,21,34]
	Hrp1/Nab4	[29]
	HnRNP A18	[35]
Oxidative stress (arsenite)	hnRNP A1	[21]
	RBM4	[19]
Oxidative stress (H_2O_2)	HuR	[28,48]
	hnRNP A18	[35]
Heat shock	hnRNP A1	[21]
	HnRNP A18	[35]
UVC	hnRNP A1	[20,33]
	HuR	[48]
	hnRNP A18	[49]
	HSlu7	[24]
Hypoxia	hnRNP A2	[8,50]
Hyperglycemia	hnRNP A2	[8]
ER stress (Thapsigargin or DTT)	Tra2-β1	[18]
	Sam68	[18]
	hnRNP A18	[49]
Ischemia	Tra2-β1	[18]
Serum	hnRNP K	[16,51]
Viral infection	hnRNP A1	[31,33]
	La autoantigen	[31,52]
	Sam 68	[31]
	Nucleolin	[31]
	hnRNPC	[31]
	hnRNP K	[31]
Chemotoxic drugs	PTB, PCBP1	[47]
Apoptosis	HnRNP C1/C2	[23,53]
	TIAR	[53]
Neurite growth	PTB	[13]
Polyamines	HuR	[25,32,54]
HGF on hepatocytes	HuR	[26]
ATP	HuR	[17,55]
Granulocytic and monocytic differentiation (PMA)	HuR	[56]
Vascular smooth muscle cells proliferation	HuR	[5]
Chronic ethanol exposure (LPS stimulation)	HuR	[57]
Actinomycin D, alkylating agent (MMS), anti-inflamatory agents (atcyclopentenone PGA_2)	HuR	[48]
Myogenesis	HuR	[30]
Apigenin and UVB	TIAR, HuR	[58]
17β-Estradiol	AUF1	[59]
Glucose stimulation	PTB	[14,60]

Table 2 – Signal transduction pathways leading to cytoplasmic relocalization of mRBPs

Pathway	mRBP	References
cAMP/PKA	PTB	[13,14]
MKK-p38-MnK1	hnRNP A1	[20,21]
PKCζ	hnRNP A1	[61]
MKK-p38	RBM4	[19]
ROCK	HnRNP C1/C2	[23]
MAPK/ERK	hnRNP K	[16]
JNK-kinase	HSlu7	[24]
AMPK	HuR	[25–27]
PKC	HuR	[17]
P38-MK2	HuR	[28]

mRBPs have only been described for a few of these proteins (Table 2). In most cases the phosphorylation of the mRBP is correlated with its subcellular redistribution. For example, hnRNP I/PTB is exported from the nucleus of cAMP-activated PC12 cells to the growing neurite terminals and the cytoplasm following the phosphorylation of its serine 16 by PKA [13]. This pathway is also involved in the cytoplasmic accumulation of hnRNP I/PTB in glucose-stimulated β-cells, since it has been shown that elevation of cAMP levels causes the PKA-dependent phosphorylation and nucleocytoplasmic translocation of hnRNP I/PTB [14]. Serine 16 of hnRNP I/PTB is part of the nuclear localization signal (NLS) of the protein, and its phosphorylation in both heterokaryon assay and *Xenopus oocytes* leads to the accumulation of hnRNP I/PTB in the cytosol [15]. Therefore, the phosphorylation status of hnRNP I/PTB serine 16 determines its subcellular localization.

Additional examples of mRBPs whose subcellular distribution is correlated with phosphorylation include the hnRNP K, HuR, tra2-β1, and the RBM4 proteins. Indeed, serum stimulation results in hnRNP K phosphorylation at serines 284 and 353 by mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK) and cytoplasmic accumulation of hnRNPK [16]. The ATP-induced recruitment of HuR to the cytoplasm is under the control of PKCα, which interacts with HuR and phosphorylates its serines 158 and 221 [17]. The change in the subcellular localization of tra2-β1 is concomitant with its hyperphosphorylation [18]. The $MKK_{3/6}$-p38 kinase pathway is involved in the cytoplasmic relocalization of RBM4, which is phosphorylated on its serine 309 following oxidative stress [19]. Moreover, hnRNP A1 is phosphorylated during osmotic, oxidative, and heat shock by Mnk1, which is itself activated by the $MKK_{3/6}$-p38 kinase [20,21]. In this case, phosphorylation of hnRNP A1 leads to its cytoplasmic accumulation following these stress stimuli [22]. Many other signaling pathways are also involved in controlling the relocalization of mRBPs. For example, the apoptotic Rho-associated kinase (ROCK)-dependent pathway in PMA-induced cells and the JNK-kinase pathway regulate the subcellular localization of hnRNP C1/C2 [23] and hSlu7 [24], respectively. Also, HuR cytoplasmic relocalization is induced by the AMP-activated kinase (AMPK) or by the p38-MK2 pathway [25–28].

At least two hypotheses can be proposed to explain how mRBPs accumulate in the cytoplasm: (i) an inhibition of nuclear import or (ii) an activation of nuclear export. In most cases, the former seems to be the case. For example, serine-specific phosphorylation of hnRNP A1 within a C-terminal peptide, which is adjacent to the M9 motif that mediates bidirectional transport of hnRNP A1, weakens the interaction of hnRNP A1 with the Trn1 transportin [22], thus blocking nuclear import of hnRNP A1. Inhibition of Hrp1 nuclear import during osmotic shock is due to a decreased interaction between the Kap104 importer protein and Hrp1. At the same time, Hrp1 can still exit the nucleus by using XpoI/Crm1-dependent export pathway [29]. Disruption of the nuclear import of HuR is due to the dissociation of the HuR-transportin2 (TRN2) complex at the late stages of myogenesis [30]. The increase in cytoplasmic levels of HuR in polyamine-deficient intestinal epithelial cells is a result of inactivation of AMPK-driven importin α1 pathway [25]. Finally, during poliovirus infection two components of the nuclear pore complex (NPC), Nup153 and p62, are proteolyzed, which results in the blockage of the nuclear import of hnRNPA1, hnRNP K, hnRNP C, nucleolin, La and Sam68 [31]. The only study that suggests that the accumulation of an mRBP in the cytoplasm is due to an increase in export from the nucleus concerns hnRNPC1/C2 cytoplasmic relocalization in TNFα or PMA-induced apoptotic cells. The authors defined a novel and transferable NES which exports hnRNPC1/C2 to the cytoplasm, but also heterologous reporter proteins containing this sequence in response to ROCK-mediated signaling [23].

Altogether, there is now compelling evidence that external stimuli regulate mRBPs subcellular localization and the signal transduction pathways that mediate these changes are just beginning to emerge. The consequences of the translocation of mRBPs between the nucleus and the cytoplasm have also been studied and the results demonstrate that the subcellular localization of mRBPs provides a powerful mechanism for the regulation of gene expression.

3. Effects of mRBP relocalization on post-transcriptional control of gene expression

Because of the compartmentalization of gene expression in eukaryotes, it is obvious that the subcellular localization of mRBPs involved in nuclear pre-mRNA splicing and/or cytoplasmic steps of gene expression such as mRNA localization, stability and translation will have consequences on these post-transcriptional events (Table 3).

The accumulation of mRBPs in the cytoplasm indeed affects splicing events in the nucleus. It has been shown that the stress-induced cytoplasmic relocalization of hnRNP A1 modifies the alternative splicing pattern of an adenovirus E1A pre-mRNA splicing reporter [20], and that the ischemia-dependent cytoplasmic redistribution of Tra2-β1 stimulates inclusion of an alternative exon of the ICH-1 pre-mRNA [18]. In these cases, the export of the splicing factors to the cytoplasm changes the abundance of these factors in the nucleus, thereby affecting splicing events.

Once they have accumulated in the cytoplasm, the mRBPs can affect cytoplasmic steps of gene expression. Upon PKA activation, hnRNP I/PTB is exported from the nucleus and accumulates at growing neurite terminals

Table 3 – Post-transcriptional steps regulated by subcellular relocalization of mRBPs

Step	RBP	Target gene	Condition	References
Splicing	hnRNP A1	Splicing reporters	Osmotic shock	[20]
	Tra2-β1	ICH-1	ER stress	[18]
	HSlu7	Minigene, D-aspartate-oxidase	UVC	[24]
mRNA stability	hnRNP A2	Glut1	Hypoxia, hyperglycemia	[8]
	hnRNP A18	Thioredoxin	UV	[49]
	HuR	MyoD, myogenin	Myogenesis	[30]
		Nucleophosmin, p53	Polyamine	[32]
		ATF-2	Polyamine	[54]
		SLC11A1	Granulocytic and monocytic differentiation (PMA)	[56]
		SAT, CDK2, SLC7A7, OSBLP2	Vascular smooth muscle cells proliferation	[5]
		COX2, MMP9	ATP	[17,55]
		Reporter (uPA, uPAR)	Oxydative stress	[28]
		TNF-α	Chronic ethanol exposure	[57]
		P21	UVC; actinomycin D, oxidative stress	[48]
		COX-2	Apigenin and UVB	[58]
		ABIN2, Ier2/pip92	17β-Estradiol	[59]
	PTB	ICA512, PC1/3, PC2, insulin	Glucose stimulation	[14,60]
mRNA localization	PTB	β-actin		[13]
Translation	hnRNP A1	Xiap	Osmotic shock	[34]
		Apaf-1	UV	[33]
		HRV-2	Viral infection	[33]
	La autoantigen	Poliovirus	Viral infection	[52]
	PTB	PC2	Glucose stimulation	[60]
	TIAR	COX-2	Apigenin and UVB	[58]
	RBM4	Transl. reporters, Bcl-2, c-myc	Arsenite	[19]
	hnRNP A18	Transl. reporters	Oxydiative stress, osmotic shock and heat shock	[35]
		Thioredoxine, replication protein A (RPA2)	UV	[49]
	hnRNP A2	Glut1	Hypoxia, hyperglycemia	[8]
		Transl. reporters	Glucose stimulation	[14,60]
	PTB, PCBP1	Bag-1	Chemotoxic drug	[47]
	hnRNP K	Transl. reporters	Serum stimulation	[16]

where it associates with the β-actin mRNA and is critical for the localization of this mRNA at neurite terminals [13]. Decreasing the levels of cellular polyamines in intestinal epithelial cells leads to cytoplasmic accumulation of HuR. HuR then binds to and stabilizes several mRNAs including those encoding nucleophosmin, p53 and other growth-inhibiting factors [32].

Several recent reports have also highlighted a key role for cytoplasmic redistribution of mRBPs in the regulation of translation. hnRNP A1 can activate rhinovirus IRES-mediated translation upon rhinovirus infection. This activation is directly controlled by the cytoplasmic accumulation of hnRNP A1 [33]. UVC or osmotic shock-dependent cytoplasmic relocalization of hnRNP A1 is also correlated to its ability to bind to the Apaf-1 or X-linked inhibitor of apoptosis (XIAP) IRES and to inhibit translation of both mRNAs [33,34]. It appears that these phenomena are not unique to hnRNP A1, since different environmental cues such as oxidative stress, osmotic and heat shock regulate the cytoplasmic accumulation of hnRNP A18 and its ability to control translation of reporter constructs [35]. Interestingly, the stress-induced cytoplasmic accumulation of hnRNP A1 and hnRNP A18 occurs in discrete phase-dense particles, the cytoplasmic stress granules (SGs) [21,35]. These particles occur in eukaryotic cells that are exposed to environmental stress, and they contain abortive translational mRNP complexes that constitute a site of mRNA triage from which mRNAs are directed to the decay machinery or are directed to return to active translation. It is hypothesized that the accumulation of the translation repressors hnRNP A1 and hnRNP A18 in the SGs during stress is a way to control the translatability of their target mRNAs and reflects their role in the stress response.

An unsolved issue is to establish whether the mRBP binds to its target mRNAs in the nucleus or in the cytoplasm. First, the mRBP may be preassembled with its target mRNA in the nucleus and subsequently transported together with the mRNA into the cytoplasm to control the cytoplasmic step of gene expression (Fig. 1A). This hypothesis is supported by experiments showing that several mRNAs (c-Myc, Smad5) cannot support IRES-mediated translation following RNA transfection [36,37]. This suggests that the nuclear binding of the mRBPs to the mRNA may be required for IRES-mediated translation. Furthermore, the nuclear import of hnRNP D

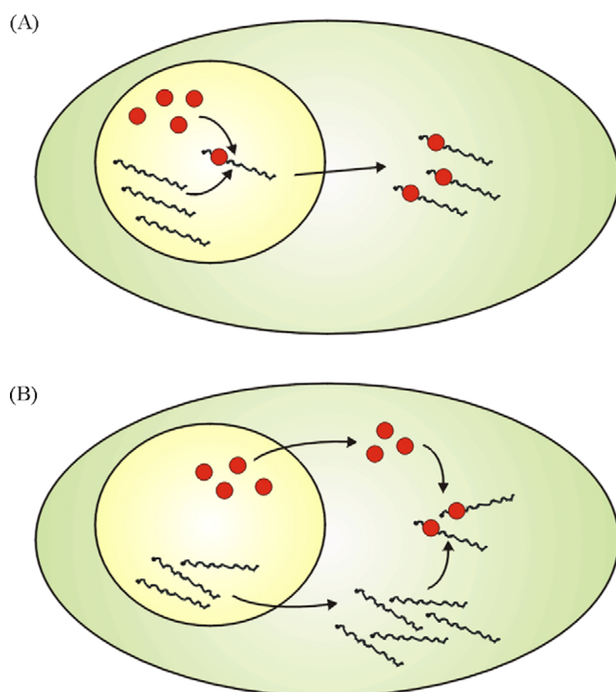

Fig. 1 – Two possible mechanisms for the interaction of target mRNAs with mRBPs. (A) mRNA and mRBPs are preassembled in the nucleus and are transported together into the cytoplasm. (B) mRBPs are sequestered in the nucleus and need to be relocalized into the cytoplasm in order to interact with their target mRNAs (mRBPs are shown as red circles, mRNAs are shown as black lines).

seems to be a prerequisite for this mRBP to exert its cytoplasmic function since a cytoplasmically restricted mutant of hnRNP D is unable to block cytoplasmic mRNA turnover [38]. Conversely, introducing heterologous shuttling domains to this hnRNP D mutant restores its ability to enter the nucleus and its cytoplasmic function. The second hypothesis is that the mRBP, which is primarily located in the nucleus, needs to be relocalized to the cytoplasm in order to encounter its target and regulate its expression (Fig. 1B). In agreement with this hypothesis it has been recently shown that the cytoplasmic relocalization of hnRNP A1 upon rhinovirus infection is necessary for hnRNP A1 to encounter the mRNA of this virus, which has an entirely cytoplasmic replicative cycle, and promote rhinovirus IRES-mediated translation [33]. This hypothesis is also supported by several studies showing that mRBPs can be exported to the cytoplasm independent of mRNA export. Indeed, the lack of binding to nascent mRNA in the nucleus allows a more rapid nucleoplasmic movement and nucleocytoplasmic shuttling of PTB [39]. Furthermore, export of hnRNP A1 from the nucleus is independent of mRNA synthesis since inhibition of transcription still allows continued shuttling of hnRNP A1 and accelerates its export [40]. This second hypothesis of an mRNA-independent control of mRBP localization also suggests an interesting way to regulate the availability of mRBPs in the cytoplasmic compartment and would provide another layer of regulation to the cytoplasmic steps of gene expression.

4. mRBP relocalization, translational control and cell death

The cellular response to physiological or pathophysiological insult is a rapid repression of global protein synthesis. Simultaneously, however, this repression of global translation is accompanied by selective translation of specific mRNAs whose protein products are required to deal with the consequences of the insult [41]. The translation of many proteins involved in the cellular response to stress is mediated by IRES (internal ribosome entry site) present within the 5′ non-coding region of their mRNAs. IRES-mediated translation initiation is one of the alternative means of initiating protein synthesis that allows for the production of new protein when global, mostly cap-dependent translation initiation is attenuated. In particular, IRES-mediated translation promotes the expression of several genes that either allow cell survival (XIAP, Bag-1, Bcl-2, Bcl-Xl, c-myc) or conversely lead to cell death (Apaf-1, c-myc) during stress. The IRES is thought to be a specialized RNA segment that is capable of recruiting the ribosome to the mRNA in a cap-independent manner. While it is not clear if it is the primary sequence or the secondary/tertiary structure of the cellular IRES RNA (or both) that is the determinant of IRES activity [42], numerous studies have shown that IRES-dependent translation requires several *trans*-acting protein factors (collectively known as IRES *trans*-acting factors, or ITAFs) to aid the recruitment of the ribosome and initiation of polypeptide synthesis [43]. Moreover, many ITAFs are mRBPs that have other diverse functions in mRNA metabolism. The demand for ITAFs varies among IRES-containing mRNAs and exactly how ITAFs enable IRES-dependent translation, and how the activities of these proteins themselves are regulated remains to be elucidated. Several recent findings suggest that one mechanism that regulates the activity of ITAFs in IRES-dependent translation initiation is the control of the subcellular distribution of these proteins.

As mentioned above in Section 2 the cytoplasmic accumulation of hnRNP A1, an mRBP that affects pre-mRNA splicing when in the nucleus, was recently shown to affect the IRES-mediated translation of both Apaf-1 and XIAP [33,34], two proteins with critical roles in the apoptotic program [41]. Apaf-1 is a component of the apoptosome and is required for execution of the extrinsic apoptosis pathway [44]; XIAP is an inhibitor of apoptosis protein that can arrest apoptosis by binding to and inhibiting the activity of caspases [45]. Interestingly, the effect that cytoplasmic accumulation of hnRNP A1 exerts on the translation of these apoptosis regulators, and thus cell survival, is different in each case. In the case of Apaf-1, the cytoplasmic accumulation of hnRNP A1 following UVC irradiation limits translation of Apaf-1 and may therefore decrease the level of apoptosis in irradiated cells, which in turn may be necessary to allow cells to survive and repair damaged DNA. Osmotic shock, a severe cellular stress, reduces XIAP IRES-dependent translation through the cytoplasmic accumulation of hnRNP A1 [34]; in this case, a

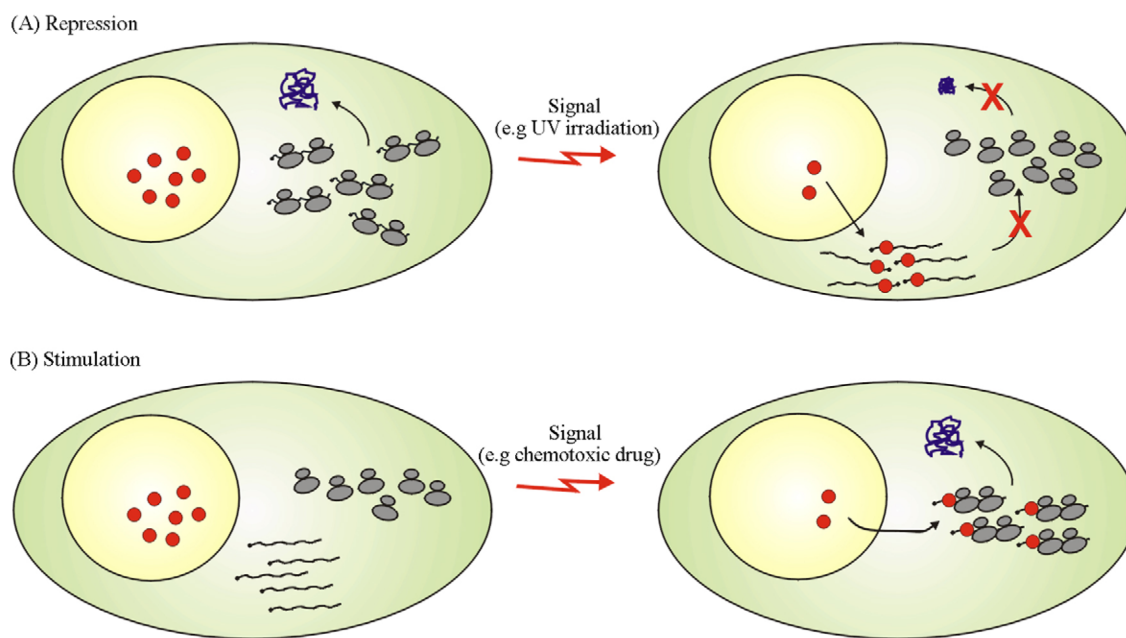

Fig. 2 – Two proposed models for the modulation of translation of specific mRNAs by subcellular localization of mRBPs. (A) Repression: mRBPs are normally localized in the nucleus while their target mRNA readily engage ribosomes in the cytoplasm resulting in the efficient translation. Following the appropriate signal (such as treatment of cells with UV irradiation) the mRBPs accumulate in the cytoplasm where they bind to their target mRNAs, sequestering them in inactive mRNP particles resulting in repression of translation. This mode of regulation is seen for example with hnRNPA1 and Apaf-1 mRNA (see text for details). (B) Stimulation: alternatively, in the absence of mRBPs that are normally localized in the nucleus the target mRNA are unable to efficiently engage ribosomes resulting in poor or no translation. Following the appropriate signal (such as chemotoxic drug treatment) the mRBPs relocalize to the cytoplasm where they associate with their target IRES mRNAs and enable efficient translation, as for example in the case of RBM4 and c-myc mRNA (see text for details) (mRBPs are shown as red circles, ribosomes are depicted in gray, mRNAs are shown as black lines, newly synthesized protein is shown in purple).

reduction in the translation of an antiapoptotic protein (and thus its abundance in the cell) allows cells to commit to cell death. These observations suggest that there may be fine control over the strength of IRES-dependent translation that determines cell fate during cellular stress, and this fine control may be regulated by the subcellular distribution of mRBPs.

RBM4 is another example of an mRBP that is involved in pre-mRNA splicing in the nucleus, but affects translation when in the cytoplasm [19]. The cytoplasmic accumulation of RBM4 is stress-responsive; upon activation of the MKK3/6-p38 pathway by extracellular stress, RBM4 relocalizes to the cytoplasm where it enhances the IRES-dependent translation of c-myc, a transcription factor, and Bcl-2, an antiapoptotic protein. Interestingly, the mechanism employed by RBM4 to modulate IRES-dependent translation specifically requires sequestration of RBM4 from the cytoplasmic translation apparatus to regulate its effects. Lin et al. [19] found that cytoplasmic RBM4 interacts with the RNA helicase eIF4A, a component of the eIF4F complex that mediates cap-dependent translation [41]. Their data suggest that RBM4 interacts with eIF4A and recruits this protein to the IRES of Bcl-2 and c-myc (and perhaps others), where it enhances the IRES-dependent translation of these messages [19]. Furthermore, Lin et al. found the cytoplasmic RBM4 could also decrease cap-dependent translation of messages containing a CU-rich sequence, to which RBM4 is known to bind [46]. Therefore, not only does the cytoplasmic accumulation of RBM4 allow for enhanced IRES-dependent translation during cellular stress, which in turn determines the fate of the cell, it may also be partially responsible for the decrease in global cap-dependent translation that occurs during stress conditions.

The examples of hnRNP A1 and RBM4 also illustrate that relocalization of mRBPs from the nucleus to the cytoplasm can have an opposite effect on the regulation of translation (Fig. 2). hnRNP A1 acts as a repressor of Apaf-1 and XIAP translation and thus its accumulation in the cytoplasm results in reduced expression of Apaf-1 or XIAP. In contrast, since RBM4 functions as an enhancer of c-myc or Bcl-2 IRES translation, its relocalization into the cytoplasm causes an increase in c-myc or Bcl-2 expression.

The subcellular relocalization of mRBPs that act as ITAFs may also play a role in the chemoresistance of cancer cells. A recent report from Dobbyn et al. presents evidence that the treatment of cancer cells with the chemotoxic drug vincristine causes the cytoplasmic accumulation of both PTB and PCBP1 [47]. These mRBPs are ITAFs for the BAG-1S IRES, which

controls translation of the antiapoptotic protein BAG-1S. The cytoplasmic accumulation of both PTB and PCBP1 during vincristine treatment was shown to correlate with the maintenance of BAG-1S IRES-dependent translation, despite the fact that global, cap-dependent protein synthesis was inhibited. These data suggest that some chemotoxic drugs can regulate the subcellular distribution of ITAFs, which in turn mediates the IRES-dependent translation of proteins that affect progression of the apoptotic program. Moreover, the maintained levels of an antiapoptotic protein (such as BAG-1S) that result from subcellular relocalization of mRBPs during treatment with a chemotherapeutic may contribute to the chemoresistance, and therefore survival, of some cancer cells.

5. Conclusions

It has been known for many years that mRBPs shuttle between the nucleus and the cytoplasm. However, several recent reports have highlighted important roles for mRBP subcellular localization in the many diverse facets of mRNA metabolism. Moreover, subcellular relocalization of mRNPs is a key regulatory mechanism for the survival response of cells in the face of pathophysiological stress. It will be interesting to discover the identities of the mRNA targets that are affected by the subcellular distribution of mRBPs, as well as identify and dissect the full spectrum of signaling pathways that regulate mRBP subcellular localization in response to cellular stress.

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