Multifunctional regulatory proteins that control gene expression in both the nucleus and the cytoplasm

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Summary

The multistep pathway of eukaryotic gene expression involves a series of highly regulated events in the nucleus and cytoplasm. In the nucleus, genes are transcribed into pre-messenger RNAs which undergo a series of nuclear processing steps. Mature mRNAs are then transported to the cytoplasm, where they are translated into protein and degraded at a rate dictated by transcript- and cell-type-specific cues. Until recently, these individual nuclear and cytoplasmic events were thought to be primarily regulated by different RNA- and DNA-binding proteins that are localized either only in the nucleus or only the cytoplasm. Here, we describe multifunctional proteins that control *both* nuclear and cytoplasmic steps of gene expression. One such class of multifunctional proteins (e.g., Bicoid and Y-box proteins) regulates both transcription and translation whereas another class (e.g., Sex-lethal) regulates both nuclear RNA processing and translation. Other events controlled by multifunctional proteins include assembly of spliceosome components, spliceosome recycling, RNA editing, cytoplasmic mRNA localization, and cytoplasmic RNA stability. The existence of multifunctional proteins may explain the paradoxical involvement of the nucleus in an RNA surveillance pathway (nonsense-mediated decay) that detects cytoplasmic signals (premature termination codons). We speculate that shuttling multifunctional proteins serve to efficiently link RNA metabolism in the cytoplasmic and nuclear compartments. ***BioEssays*** **23:775–787, 2001.**

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Abbreviations: AHC, adrenal hypoplasia congenita; ARE, AU-rich element; Bcd, Bicoid; Cad, Caudal; C/EBPβ, CCAAT/enhancer-binding protein β; cTNT, troponin T; CUG-BP, CUG-binding protein; EDEN-BP, embryo deadenylation element-binding protein; ELAV, embryonic lethal abnormal vision; Fru, Fruitless; hnRNP, heterogeneous nuclear ribonucleoprotein; Msl2, Male-specific lethal2; NMD, nonsense-mediated decay; Osk, Oskar; Pol, polymerase; PTC, premature termination codon; SMN, survival of motor neurons; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; SR, serine-arginine; Sxl, Sex-lethal; TB-RBP, testis-brain RNA-binding protein; Tra, Transformer; TRA-1, Transformer-1; UTR, untranslated region

Introduction

The individual events that constitute gene expression in eukaryotic cells have historically been considered to be highly compartmentalized. Gene transcription and RNA processing events occur in the nucleus, whereas translation and RNA decay occur in the cytoplasm. Likewise, the regulatory molecules that modulate these events have, until recently, been thought to function in either the nucleus or the cytoplasm but not both. For example, DNA-binding proteins that regulate gene transcription in the nucleus were thought to have little to do with regulation of RNA events in the cytoplasm. Conversely, RNA-binding proteins that regulate translation and RNA stability in the cytoplasm were not thought to regulate transcription or RNA processing events in the nucleus.

It has now become clear that some regulatory proteins are not restricted to a single function in a single compartment but, instead, govern gene expression events in both the nucleus and the cytoplasm. Some members of this new class of regulatory proteins have been shown to shuttle between the nucleus and the cytoplasm. This is a striking observation, as it contradicts the previous view that shuttling proteins merely transport RNA across the nuclear envelope.

As we will discuss, these regulatory proteins that act in both the nucleus and the cytoplasm are important, as genetic mutations rendering them inactive can have severe biological consequences. Multifunctional proteins play critical roles in a wide variety of biological events, including early embryonic development, sex determination, cell-cycle regulation, viral gene regulation, surveillance of aberrant transcripts, and the function of the neurological, muscular, immunological, and reproductive systems. Although this topic has been covered in past reviews,[1–3] here we will focus mostly on recently published studies. We will place an emphasis on those RNA-binding proteins whose roles in both the nuclear and

cytoplasmic compartments are well characterized. Although there are many interesting proteins that regulate multiple activities in only the nucleus or the cytoplasm (e.g., both transcription and RNA splicing or both translation and cytoplasmic RNA-stability), these will not be dealt with here. We will begin by discussing RNA-binding proteins that regulate both transcription in the nucleus and translation in the cytoplasm, and then describe those that have a role in both RNA processing in the nucleus and translation in the cytoplasm. Next, we will briefly overview multifunctional proteins with other novel activities. Finally, we will discuss an RNA surveillance pathway termed nonsense-mediated RNA decay (NMD) that illustrates how events in the nucleus and cytoplasm may be interconnected. Table 1 lists RNA-binding proteins with well-defined activities in the nucleus and the cytoplasm, and Table 2 lists RNA-binding proteins that are good candidates to possess such multifunctional activities.

Multifunctional regulators of transcription and translation

Y-box proteins

One of the first reported examples of multifunctional regulatory proteins acting both in the nucleus and the cytoplasm are the Y-box proteins. These were originally defined as DNA-binding proteins containing cold-shock domains that bind to the DNA consensus sequence ATTGG (Y-box) and thereby regulate the transcription of a wide variety of genes.[57] Then, in the 1990s, it was discovered that many Y-box proteins are also RNA-binding proteins required for translational silencing of some maternal mRNAs (Fig. 1A). This was first demonstrated for FRGY2, which binds to maternal RNAs in the nuclei of *Xenopus laevis* oocytes and then travels with these RNAs into the cytoplasm, where it blocks their translation.[1,16] The fact that FRGY2 inhibits translation provides an explanation for why mRNAs transcribed from DNA injected into the nuclei of *X. laevis* oocytes are translated 50 times less efficiently than mRNA injected directly into the cytoplasm. Thus, this is a striking example of the nuclear history of an mRNA influencing its behavior in the cytoplasm.

The ability of Y-box proteins to regulate both transcription and translation is a conserved feature of these proteins. A variety of Y-box proteins in frogs, chickens, mice, and humans have been shown to possess one or both of these activities.[57] Because most Y-box proteins are highly abundant proteins that bind to RNA in a relatively non-specific manner, it has been proposed that they are the RNA-binding equivalent of DNA-binding histones.[57] It remains for future research to determine the precise degree of selectivity of Y-box proteins in mediating translational inhibition and whether this activity is altered by post-translational modifications in response to physiological cues.

Bicoid (Bcd)

Bcd is another multifunctional protein that regulates both transcription and translation. This maternally expressed homeodomain transcription factor is confined to the anterior end of the *Drosophila melanogaster* embryo, where it regulates the transcription of genes that dictate anterior embryo development. It was a surprise when it was found that Bcd not only binds DNA but also interacts with the 3′ untranslated region (UTR) of mRNA encoding Caudal (Cad), another homeodomain transcription factor.[5,6] Because Bcd resides in the anterior region of the embryo, it blocks Cad translation only in that specific region, thereby restricting Cad protein to the posterior end of the embryo,

TABLE 1. RNA-binding proteins with nuclear and cytoplasmic functions

Protein	Nuclear function	Cytoplasmic function	Reference
Apobec-1 (mammals)	RNA editing	mRNA stability	4
Bcd (*D.melanogaster*)	Transcription	Translational silencer	5–8
CUG-BP / Bruno / EDEN-BP (mammals, *D. melanogaster, X. laevis*)	mRNA splicing	Translational regulation	9–15
FRGY2 *(X. laevis)* and other Y-box proteins (mammals, chicken)	Transcription	Translational silencer	1,16
hnRNP A2/B1 (mammals)	mRNA splicing, trafficking	mRNA localizatioin, translational enhancer	17
hnRNP D (mammals)	Transcription, class switching	mRNA stability	17
hnRNP K (mammals)	Transcription	Translational silencer	17
Sxl (*D. melanogaster*)	mRNA splicing	Translational silencer	18–21
TFIIIA (*X. laevis*)	Transcription	Sequesters RNA in the cytoplasm	1
Tra (*D. melanogaster*)	mRNA splicing	Translational silencer	22

While we have attempted to list all known RNA-binding proteins with well-defined function in both the nucleus and the cytoplasm, we apologize for any we have not included. Journal constraints have also precluded us from listing all references supporting their functions.

TABLE 2. RNA-binding proteins that may possess both nuclear and cytoplasmic functions

Protein	Proposed nuclear function	Proposed cytoplasmic function	Reference
Aly/REF *(S. cerevisiae; mammals)*	mRNA processing, export	Marker protein for NMD	23,24
DAX-1 (mammals)	Transcription	Translation (polysome-associated)	25
eIF-4E (mammals)	nuclear translation, shuttles	Translation initiation	26,27
FMRP (mammals)	mRNA export, processing, shuttles	Translation silencer *in vitro*	28,29
ELAV-like proteins (mammals)	mRNA export, shuttles	mRNA stability	30,31
hnRNP A1 (mammals)	mRNA splicing, export	Translation, mRNA stability	17,32,33
hnRNP E1/2 (mammals)	mRNA export, shuttles	Translational silencer, mRNA stability	17
hnRNP I/PTB (mammals)	Transcription, splicing, polyadenylation,	Internal translation, mRNA localization	2,32
hnRNP L (mammals)	mRNA export	mRNA stability, internal translation	32,34
La (mammals)	RNA stabilization	Translational enhancer, RNA stabilization	35–42
p53 (mammals)	Transcription	Translational silencer *in vitro*	43
RNPS1 (mammals)	mRNA splicing activator	Marker protein for NMD	23,24
SLBP (mammals)	3′-end processing, shuttles	Translation (polysome-associated)	44,45
SMN (mammals)	Splicesome recycling	snRNP assembly	46–48
Sqd A, S (*D. melanogaster*)	mRNA export, shuttles	RNA localization	33
Shuttling SR proteins (mammals)	mRNA splicing	Shuttles	49
TRA-1 (*C. elegans*)	Transcription	Shuttles	50
Translin (mammals)	DNA recombination, DNA repair	Translational silencer, intercellular mRNA transport	51–55
Y14 (mammals)	mRNA processing, export, shuttles	marker protein for NMD	23,24,56

This table provides a partial list of RNA-binding proteins that are candidates to regulate events in both the nucleus and the cytoplasm. The term ''shuttles'' refers to the ability to traverse back-and-forth between the nucleus and the cytoplasm, suggesting a possible function in mRNA export.

where it regulates zygote-segmentation genes. This unique regulatory relationship establishes a new hierarchical paradigm in which one homeodomain protein regulates the expression of the next one by a post-transcriptional rather than by a transcriptional mechanism.

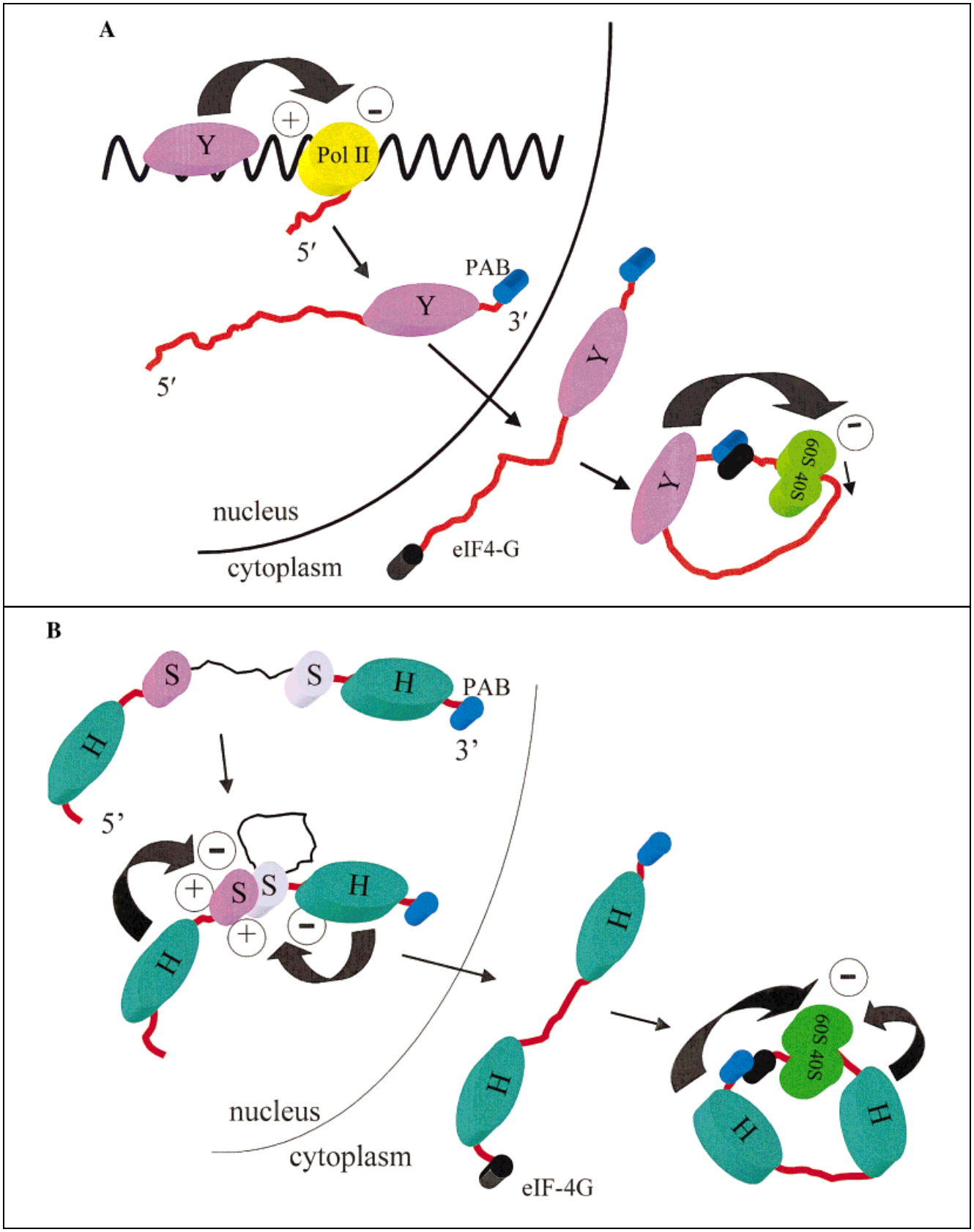
A recent study suggested that Bcd uses different strategies to regulate transcription and translation. A critical amino acid in the Bcd homeodomain (arginine 54) is required for Bcd's translational inhibitory function but not its ability to activate transcription.[7] Thus, distinct subregions in the Bcd homeodomain may specify DNA and RNA binding. The Bcd PEST domain also differentially participates in Bcd's two functions. Amino-acid substitutions in the C-terminal region of the PEST domain abolish Bcd's translation repression activity but still allow it to activate transcription.[8] PEST domains are well-established signals that trigger ubiquitination and subsequent proteolysis, but it is not yet known if this is the case for Bcd.

It remains to be determined how Bcd gets to the two different compartments in which it acts. One possibility is that some Bcd molecules are imported to the nucleus to regulate transcription, whereas the rest remain behind in the cytoplasm to regulate translation. Another possibility is that Bcd travels back and forth between these two compartments. Because Bcd does not have a known nuclear-export signal, it may not traverse the nuclear pore to reach the cytoplasm; instead, it may slip into the cytoplasm when the nucleus breaks down during mitosis. Cells in the early *D. melanogaster* embryo divide very rapidly (about every 30 minutes), and therefore a significant amount of Bcd could reach the cytoplasm in this way.

DAX-1

Recently, a mammalian transcription factor was identified that may also regulate translation. This protein, DAX-1, is a member of the nuclear hormone receptor superfamily and is encoded by an X-linked gene that, when mutated, causes adrenal hypoplasia congenita (AHC), a disease associated with gonadotropin deficiency and small reproductive organs. DAX-1 regulates the function of steroidogenic tissues by virtue of its transcriptional repressor activity in the nucleus. In addition, DAX-1 is in the cytoplasm, where it forms complexes with polyadenylated RNA and cofractionates with polysomes, suggesting that it may also regulate translation.[25] DAX-1 binds directly to RNA using two domains that permit cooperative binding activity and specificity. Importantly, DAX-1 binds to RNA using domains different from those that it uses to bind to DNA.[25]

AHC patients express mutant versions of DAX-1 with impaired RNA-binding ability, suggesting the possibility that RNA binding is critical for DAX-1's biological function.[25] It will therefore be interesting to determine whether mutant DAX-1 proteins from AHC patients are also deficient in translational regulatory activity. Intriguingly, DAX-1 is a nucleocytoplasmic shuttling protein, raising the possibility that DAX-1 regulates gene expression events in a sequential manner: first it could bind to DNA to regulate transcription, and then it could bind to


A
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PAB
3’
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cytoplasm
eIF-4G

the transcribed mRNA and regulate its cytoplasmic export and translation.

Other transcription–translation regulators?

It is possible that only a few transcription factors possess the additional ability to regulate translation. Conversely, this may be a common attribute that has not yet been rigorously assessed. Certainly, there are many candidates that *may* regulate both transcription and translation. For example, the retinoic acid receptor-α and -γ transcription factors share with DAX-1 the ability to bind to RNA and thus they could regulate cytoplasmic RNA events.[25] p53, a key transcriptional factor involved in genome surveillance and apoptosis was recently shown to be capable of also regulating the translation of the cell-cycle regulator Cdk4 in vitro.[43] Many transcription factors have been shown to be sequestered in the cytoplasm until they are needed in the nucleus to activate gene transcription (NF-κB is an example). It will be interesting to determine if the cytoplasm is merely a resting spot for these transcription factors or whether instead it is a target site for translational regulation by some of these factors.

Multifunctional regulators of RNA processing and translation

Translation is controlled not only by nuclear transcription factors but also by regulatory proteins that control RNA processing, including splicing factors. Splicing factors bind to *cis* elements in precursor mRNA to dictate splice-site efficiency and use. The ability of splicing factors to regulate alternative splicing permits a single precursor RNA to encode many proteins with different functions. Because splicing factors regulate an exclusively nuclear event (RNA splicing), it was a surprise when some of these factors were also found to regulate cytoplasmic events such as translation (Fig. 1B).

Sex-lethal (Sxl)

The first and best-characterized splicing factor shown to regulate translation is Sxl, an RNA-binding protein at the beginning of a pathway that controls sex determination and X-chromosome dosage compensation in *D. melanogaster*. Sxl promotes female sex determination; its absence leads to the default male phenotype. Many years ago, it was shown that Sxl controls sex determination by regulating both its own splicing and that of other players in the sex-determination pathway. More recently, Sxl was shown to also have the ability to repress translation. In particular, it represses both the translation of itself and Male-specific lethal 2 (Msl2), one of a set of Msl proteins that promote X-chromosome dosage compensation.[18–21] Sxl inhibits Msl2 translation by binding to the 5′ and 3′ UTRs of Msl2 mRNA. This inhibition of Msl2 is essential in females, as Msl2 prevents overexpression of X-linked genes. To ensure this, the female-specific Sxl inhibits not only Msl2 translation but also Msl2 RNA splicing by binding to an intron within Msl2 precursor mRNA.[18]

Transformer (Tra)

Recently, another RNA-splicing regulator that governs fruit fly sexual differentiation pathway was also shown to have translation regulatory activity. This protein, Tra, regulates the splicing of several downstream targets, including fruitless (Fru). Fru functions in the male central nervous system to control normal male courtship behavior, as shown by the finding that Fru mutant males court males and females indiscriminately. In addition, Fru is required to form the muscle of Lawrence, an abdominal muscle that forms in response to male neuron innervation. Because Fru is only known to be essential for activities in the male, it was anticipated that its expression would be restricted to males. Indeed Fru protein is male specific, but surprisingly Fru transcripts accumulate in the central nervous system of both sexes.[25] This male-specific expression pattern appears to be dictated by translational repression mediated by Tra, which binds to a Tra-binding site in an alternatively spliced form of Fru mRNA that is present only in females. That Tra acts by blocking translation is further supported by transfection experiments in cultured cells.[22] Thus, Tra is an example of a splicing factor that appears to have the additional ability to regulate sex-specific translation.

CUG-binding protein (CUG-BP)

A third example of a multifunctional protein regulating both RNA splicing and translation is CUG-BP. This is an RNA-binding protein, related to the embryonic lethal abnormal vision (ELAV) family, that binds to the triplet CUG tandemly repeated in the 3′ UTR of the *DMPK* gene. The number of CUG repeats in the *DMPK* gene is increased in myotonic dystrophy, the most common form of muscular dystrophy in adult humans. At least some of the pathology observed appears to be due to the deleterious effects of the expanded CUG repeats

Figure 1. Multifunctional regulators that act in both the nucleus and the cytoplasm. **A:** Regulatory proteins (labeled "Y") that regulate transcription in the nucleus and repress translation in the cytoplasm. Such regulatory molecules can bind both DNA and RNA. **B:** RNA-binding proteins (labeled "H") that regulate RNA splicing in the nucleus and repress translation in the cytoplasm. Both classes of molecules (Y and H) may shuttle with RNA from the nucleus to the cytoplasm. However, some of these molecules may only be capable of moving from the cytoplasm (where they are synthesized) into the nucleus, and not back (not shown). Cytoplasmic mRNA is depicted as circular, based on the known interaction between factors bound to each end. Pol II, RNA polymerase II; 40S and 60S, ribosomal subunits; S, splicesomal components; PAB, poly(A)-binding protein; eIF-4G, an essential translation initiation factor.

on the function of CUG-binding proteins.[9] One of CUG-BP's normal functions is to regulate the alternative splicing of human cardiac troponin T (cTNT) mRNA in muscle cells. It does this by binding to a conserved intronic element 27 nucleotides downstream of an alternatively spliced exon in cTNT precursor mRNA.[10] This CUG-BP-binding element is necessary and sufficient for inclusion of a heterologous exon in muscle cells. Recently, several other proteins very similar to CUG-BP have been identified that exhibit different patterns of tissue expression but, like CUG-BP, bind to the cTNT intronic splicing element and promote alternative exon inclusion.[11]

CUG-BP's role in the cytoplasm was suggested by experiments indicating that it regulates the translation of the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ).[12] CUG-BP binds to specific sites in the 5′ UTR of C/EBPβ mRNA in vitro, thereby switching translation initiation to downstream alternative start AUG codons so that shorter C/EBPβ isoforms are made. The physiological relevance of this in vitro activity of CUG-BP was supported by in vivo studies showing that partial hepatectomy caused CUG-BP to associate with polysomes that translate shorter isoforms of C/EBPβ. Direct evidence for a role in translation comes from studies on *D. melanogaster* and *X. laevis* CUG-BP-like (possibly orthologous) proteins. *D. melanogaster* bruno binds to several discrete sites in the 3′ UTR of Oskar (Osk) mRNA, where it represses Osk translation during the migration of Osk transcripts to the posterior end of the fly embryo.[13] This translational repression is lifted when Osk mRNA reaches its final destination, thus ensuring localized Osk protein expression only in the posterior portion of the fly embryo. Embryo deadenylation element-binding protein (EDEN-BP), the *X. laevis* protein related to CUG-BP, also binds to the 3′ UTR of its target transcripts, where it inhibits translation as a secondary consequence of triggering cytoplasmic deadenylation.[14,15]

La

Another RNA-binding protein that appears to be a multifunctional regulator is the human autoantigen La. Unlike the other three examples of regulatory proteins that we have discussed, La does not directly regulate RNA processing, rather it stabilizes newly synthesized small RNAs and thereby appears to indirectly promote their processing.[35,36] In the nucleus, La associates primarily with nascent transcripts synthesized by RNA polymerase (Pol) III, such as pre-tRNAs and some spliceosomal U small nuclear RNAs (snRNAs), by binding the polyuridylates found at the 3′ end of all Pol III-derived RNAs. In *Saccharomyces cerevisiae*, La also binds to precursors of small RNAs synthesized by Pol II, including some U snRNAs and the U3 small nucleolar RNA, all of which contain uridylates at the 3′ end. Binding by La protects many of these nascent RNAs from exonucleases in the nucleus, thus allowing proper maturation, ribonucleoprotein assembly, or both.

In the cytoplasm, La appears to function as a positive regulator of translation. In particular, La stimulates the translation of poliovirus and other viruses containing internal ribosome entry sites.[37,38] Consistent with this role, La relocates to the cytoplasm upon poliovirus infection.[37] La stimulates the translation of the human immunodefiency virus-1 retrovirus by another mechanism - by alleviating the repression mediated by its 5′ leader sequence.[39] Although these effects of La on viral translation are intriguing, they are somewhat controversial because very large amounts of La are required to achieve them.[40] La has also been implicated in the selection of the appropriate start AUG codon for translation and has been shown to stabilize cytoplasmic histone transcripts in vitro.[41,42] Although the precise mechanism by which La stimulates translation is unknown, an intriguing possibility is that it stabilizes higher-order RNA structures that are required for optimal initiation.[39]

Stem-loop binding protein (SLBP)

Another protein that may regulate both RNA processing and translation is SLBP, which promotes nuclear 3′ processing of histone poly(A) − tail-lacking transcripts by binding to their unique 3′ stem loops.[44] SLBP has been implicated in regulating translation by virtue of its ability to shuttle out of the nucleus and into the cytoplasm, where 90% of it cofractionates with polysomes.[45] Given its association with polysomes, SLBP is a good candidate to regulate translation, but this has not yet been definitely demonstrated.

Candidate marker proteins for RNA surveillance

An intriguing phenomenon in which multifunctional proteins play a role is nonsense-mediated decay (NMD). In NMD, aberrant transcripts containing premature termination codons (PTCs) are selectively degraded, thereby preventing the expression of potentially deleterious truncated proteins.[58–62] How the NMD surveillance pathway distinguishes between PTCs and normal termination codons has been puzzling. It turns out that most mammalian mRNAs undergo NMD only if the nonsense codon is followed by at least one intron.[62–64] Thus, NMD in mammalian cells is governed by a two-signal rule: a stop codon (signal 1) and a downstream intron (signal 2) are required to trigger mRNA decay. Because the vast majority of normal stop codons are in the terminal exon (and thus are not followed by an intron), they do not trigger NMD. In contrast, PTCs are typically in middle exons followed by one or more introns and therefore they trigger the NMD response.

Although teleologically satisfying, the two-signal rule leads to a paradox: because introns are spliced out in the nucleus, how can they act as a second signal for a process that is

triggered by stop codons, which are only known to be read by the translation machinery in the cytoplasm? A model that explains this apparent paradox is the marker model, which posits that signal 2 is not an intron itself but rather a mark left at exon–exon junctions after RNA splicing (Fig. 2) .[62–64] The introduction of the mark absolutely requires RNA splicing, as NMD is abolished when the intron following a nonsense codon is rendered defective by splice-site mutations.[63] Following splicing, the mark remains bound to exported mRNAs, allowing both signals 1 and 2 to be read in the cytoplasm, the only place where the translational machinery is known to function.

What is the mark? It has been widely speculated that it is one or more proteins left over after RNA splicing, such as a component of the spliceosome or splicing regulators such as shuttling SR proteins.[23,24,49,60,62–64] Given that such a marker protein would remain bound to fully processed mRNAs, it could also have further functions beyond NMD, such as an involvement in mRNA export and the subsequent fate of the mRNA in the cytoplasm (translation, localization, and decay). Recently, several proteins have been identified that remain bound to mRNAs after RNA splicing and thus may serve as marker proteins. At least two of these proteins, the RNA export factor Aly/REF and the Y14 shuttling protein, are deposited on transcripts in the nucleus and then appear to shuttle with these transcripts to the cytoplasm.[24,56] Others are the general splicing activator RNPS1, the nuclear matrix-localized serine-arginine-containing (SR) protein SRm160, and the oncoprotein DEK.[23,24] Interestingly, many of these proteins appear to be part of a multiprotein "E–E complex"

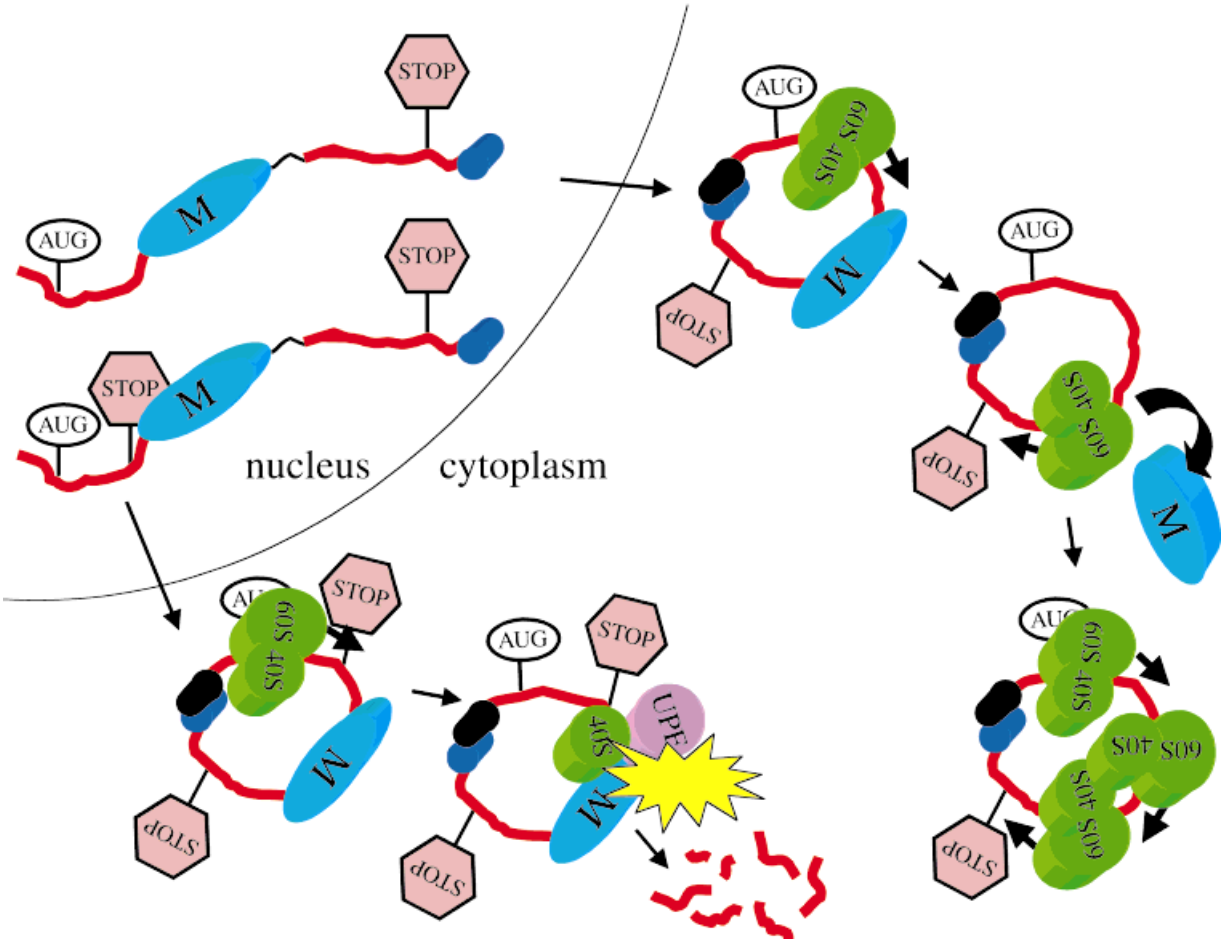

Figure 2. A solution to the RNA surveillance paradox: a shuttling intron-marker protein. Transcripts containing PTCs are recognized and rapidly degraded by the NMD RNA surveillance mechanism. Paradoxically, the stop codon must be followed by an intron to trigger NMD. Shown is a model that explains how introns (which are spliced out in the nucleus) might be essential for a mechanism mediated by the cytoplasmic translational machinery. The model posits that a marker protein (M), which remains bound to transcripts after RNA splicing, demarcates the location of introns. This marker shuttles with mature mRNA to the cytoplasm, where it serves as the second signal for NMD. The nature of the shuttling marker protein is not known (see text for candidates).

deposited at a conserved position 20–24 nucleotides upstream of exon–exon junctions.[23,24]

A set of proteins that may interact transiently with the E–E complex are the human UPF1, UPF2, UPF3a, and UPF3b proteins. When any one of these UPF proteins is tethered (via the RNA-binding protein MS2) downstream of a termination codon, they are sufficient to trigger NMD.[24] Because these four UPF proteins interact with each other, any one of them is capable of recruiting the other two, thus explaining why they can all trigger NMD. UPF3a and UPF3b are particularly interesting members of this foursome, as it is a shuttling proteins that accumulate primarily in the nucleus at steady state, suggesting that they may function in both the nuclear and cytoplasmic compartments.[24] Loss-of-function experiments have demonstrated the importance of UPF proteins for NMD. Mutation of the genes encoding any one of these proteins in *S. cerevisiae* completely abolishes NMD and a dominant-negative mutant of human UPF1 partially inhibits NMD in mammalian cells.[59–62] It remains for future studies to determine which (if any) of the UPF and E–E complex proteins actually function as signal 2 for NMD and whether this signaling event occurs in the nucleus, the cytoplasm, or both.

Multifunctional proteins with other novel activities

Heterogeneous nuclear ribonucleoproteins (hnRNPs)

hnRNPs have long been known to bind to nuclear precursor mRNAs and regulate their splicing. Some hnRNPs have also been shown to shuttle from the nucleus to the cytoplasm, leading to the notion that hnRNPs might be involved in transporting mRNAs to the cytoplasm. Until recently, however, it was not appreciated whether hnRNPs performed any additional functions once they reached the cytoplasm. Now it is clear that several cytoplasmic events can be regulated by hnRNPs; not only translation, but also cytoplasmic RNA localization and cytoplasmic RNA stability.[17,32–34] For instance, hnRNP-A2 stimulates the translation and directs the cytoplasmic localization of mRNAs that contain hnRNP-A2-binding sites in their 3′ UTRs. In contrast, hnRNP-D (AUF1) and hnRNP-K elicit an inhibitory effect on gene expression, as they destabilize cytoplasmic mRNAs and inhibit translation, respectively.

Apolipoprotein B mRNA-editing enzyme (Apobec-1)

Apobec-1 is a multifunctional protein possessing RNA-editing activity in the nucleus and RNA-stabilizing activity in the cytoplasm. In the nucleus, it is a cytidine deaminase that converts C residues to U residues. This activity requires that Apobec-1 bind AU-rich sequences in its target mRNA. Because AU-rich sequences are well-known RNA-destabilizing *cis* elements, this binding specificity raised the possibility that Apobec-1 also had a role in cytoplasmic mRNA turnover. Indeed, this possibility was recently supported by a study showing that overexpression of Apobec-1 stabilizes *c-myc* mRNA, which contains two AU-rich elements in its 3′ UTR.[4] In contrast, a mutant form of Apobec-1 lacking RNA-binding activity failed to stabilize *c-myc* mRNA. It will be interesting to determine whether Apobec-1 shuttles back and forth between the nucleus and the cytoplasm to mediate its two functions, and whether its activities and localization are regulated by post-translational modifications.

Survival of motor neurons (SMN)

Novel nuclear and cytoplasmic regulatory activities have been ascribed to SMN, which is encoded by two genes deleted or mutated in 98% of patients with spinal muscular atrophy, one of the most common fatal autosomal recessive human diseases. In most cell types, SMN is concentrated in two nuclear structures called coiled (Cajal) bodies.[46] The function of coiled bodies has been an enigma ever since their discovery in 1903 by Cajal. One suggestion has been that coiled bodies are spliceosome assembly factories, as coiled bodies have high concentrations of small nuclear ribonucleoprotein (snRNPs), the building blocks of spliceosomes. Until fairly recently, however, there was little evidence for this possibility, nor was it known whether SMN participates in this process. It was therefore an important development when Dreyfuss and colleagues found that a dominant-negative form of SMN causes widespread reorganization of snRNPs in the nucleus of intact cells and inhibits mRNA splicing in vitro.[47] This provided strong evidence that SMN plays a crucial role in maintaining the splicing machinery in the nucleus, probably for the regeneration or recycling of snRNPs within coiled bodies.

Although SMN is predominantly nuclear in most cell types, it also accumulates diffusely in the cytoplasm, opening up the possibility that SMN also possesses a cytoplasmic function. Indeed, two lines of evidence indicate that SMN is involved in snRNP assembly, which occurs in the cytoplasm of mammalian cells. First, dominant-negative SMN inhibits the assembly of snRNPs in the cytoplasm of somatic cells.[47] Second, antibodies against SIP1, a protein that forms a complex with SMN, inhibit snRNP assembly in *X. laevis* oocytes, a cell type in which SMN is mainly cytoplasmic.[48] Thus, it appears that SMN is involved in both the assembly of snRNPs in the cytoplasm and their recycling or use in the nucleus. It remains for future studies to determine precisely how SMN participates in the assembly and recycling of these building blocks of RNA splicing, whether it is absolutely required for these events, and why motor neurons are particularly susceptible to SMN deficiency.

Translin/Testis-Brain RNA-binding protein (TB-RBP)

Translin is a DNA- and RNA-binding shuttling protein originally identified on the basis of its interaction with consensus

sequences at the breakpoint junctions of human chromosomal translocations in lymphoid tumors.[52] Although ubiquitously expressed in the cytoplasm of most cell types, translin selectively localizes to the nuclei of human lymphoid cell lines, suggesting that it may play a role in immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements.[52] Translin's mouse orthologue, TB-RBP, which is 99% identical with human translin, also binds specifically to DNA.[53] TB-RBP accumulates in the nuclei of male germ cells initiating meiosis, providing further support for an involvement of TB-RBP in DNA recombination and repair.[55] TB-RBP shuttles to the cytoplasm of male germ cells after metaphase I of meiosis, suggesting that it may also function in mRNA transport between individual spermatids, which are connected by intercellular bridges.[54,55] In addition, it appears to play a role in translation control once it reaches the cytoplasm, as testicular extracts enriched for TB-RBP repress the translation of mRNAs containing TB-RBP-binding sites in their 3′ UTRs.[51] It will be intriguing to see whether TB-RBP is responsible for the repression of the stored mRNAs that accumulate in differentiated male germ cells in vivo.

Translation factors functioning in the nucleus?

Although ribosomal subunits are assembled in the nucleus, they are widely believed to only function in translation after they are exported to the cytoplasm. This central dogma of cell biology has recently shown some signs of erosion, as several lines of evidence have suggested the possibility that translation not only occurs in the cytoplasm but also in the nucleus.

First, several studies have suggested that the nucleus is the site of action of the NMD RNA surveillance pathway, which, as discussed earlier, degrades transcripts harboring nonsense codons (PTCs). Evidence for this notion is that PTCs decrease mRNA levels in mammalian nuclei purified by a variety of methods, including techniques that yield non-aggregated nuclei with no detectable cytoplasmic remnants.[65,66] Although it has not been ruled out NMD occurs in a cytoplasmic compartment that co-fractionates with nuclei, the available data are at least consistent with the notion of a nuclear translation-like scanning mechanism.[58,67]

A second line of evidence for nuclear translation comes from studies demonstrating that nonsense codons increase the levels of nuclear precursor mRNAs. Precursor (unspliced) transcripts from Ig-κ, Ig-μ, TCR-β , and minute virus of mice genes have all been shown to be upregulated in response to PTCs.[68–70] In the case of Ig-μ and TCR-β, it has been shown by fluoresence in situ hybridization analysis that precursor mRNAs from these genes are present at elevated levels at or near the site of transcription, making this the first clear demonstration that nonsense codons affect events in the nucleus proper.[70] A critical issue for future studies is to determine how nonsense codons increase precursor mRNA levels (by inhibiting RNA splicing or by increasing precursor mRNA stability?) and whether this occurs by a direct mechanism involving nuclear translation or is instead an indirect feedback response from the cytoplasmic translation machinery.

Third, numerous human genetic studies have demonstrated that nonsense and frameshift mutations are associated with increased levels of alternatively spliced (usually exon-skipped) transcripts that have removed the offending PTC.[71,72] This is intriguing, as it brings up the possibility that nonsense codons can influence RNA splicing. It should be noted, however, that in many cases, it appears that nonsense and frameshift mutations do not act by disrupting reading frame but instead act by disrupting splicing enhancers.[71,72] Only in two cases has sufficient evidence been obtained to suggest that bona fide in-frame termination codons increase alternatively spliced mRNA levels. One case is that of the fibrillin gene, which if mutated to have a PTC at a particular position in exon 51, expresses dramatically increased levels of an alternatively spliced transcript that lacks exon 51.[73,74] The other case is that of a splicing enhancer-debilitated version of MVM that responds to a nonsense mutation in the NS2-specific exon by increasing the level of an alternatively spliced transcript that has skipped this exon.[69] In both cases, a frameshift in the upstream exon abolished the induction of the exon-skipped transcript, suggesting that this response is frame dependent. Thus, these two studies are consistent with the notion of a nuclear translation-like process influencing splice-site selection. However, because of the conceptual difficulties of imagining how a stop codon could regulate an upstream alternative splicing event, it is important to consider other post-transcriptional mechanisms (involving, for example, RNA stability) that could explain how nonsense codons increase the levels of alternatively spliced transcripts.

Fourth, many components necessary for translation have been found in the nucleus. In particular, eIF2α, eIF3, eIF4γ, eIF-4E, eIF-4G, EF-1, and charged tRNAs have been shown to accumulate in mammalian nuclei.[26,75–78,85] The translation initiation factors eIF-4E and eIF-4G are essential for the very early steps of translation, as they assemble with the 40S ribosomal subunit on the 5′ mRNA cap. eIF-4E binds directly to the cap, and eIF-4G is the central scaffolding protein that interacts with eIF-4E and many other factors important for translation. Interestingly, rather than being randomly distributed in the nucleus, both eIF-4E and eIF-4G are present in a speckled pattern in the nucleus.[26,75] eIF-4E colocalizes with snRNPs in these speckles, suggesting that it may have some involvement with RNA splicing.[26] Rather than passively entering the nucleus, eIF-4E piggy-backs on 4E-T, a recently identified nucleocytoplasmic shuttling protein.[27]

Lastly, pulse-labeling localization and fractionation studies have provided evidence that some translation occurs in the nucleus. The original studies suggesting this were performed in the 1960s and 1970s, when the localization of protein

synthesis was first being determined.[79] Although these studies showed a small proportion of amino-acid incorporation in the nuclear fraction, this was usually attributed to contamination with cytoplasmic ribosomes.

Fresh evidence for nuclear translation has come from recent experiments with the slime mold *Dictyostelium discoideum*, in which polyribosome-like entities were identified in nuclei, based on their sedimentation in sucrose gradients, their ability to be pulse-labeled with [^{3}H]uracil, and their sensitivity to the protein synthesis inhibitor puromycin.[80] These putative nuclear polyribosomes cosedimented with both nascent mRNAs and nascent polypeptides. Based on this set of correlative data, it was concluded that transcription and translation are probably coupled in *D. discoideum*. Although intriguing, clearly further work is required to demonstrate conclusively that the large sedimenting structures ($\sim$200S) in isolated *D. discoideum* nuclei are in fact translating polysomes, and that they derive from the nucleoplasm rather than from cytoplasmic contamination.

Recent further support for the notion of coupled transcription-translation in the nucleus comes from work in permeabilized mammalian cell lines (85). Permeabilized cells briefly incubated with any of three different tags ([3H]lysine, biotin-lysisne-tRNA, and BODIPY-lysyl-tRNALys) were found to incorporate amino acids in a speckled pattern in the nucleus. Amino-acid incorporation also occurred in isolated nuclei, suggesting that proteins were not simply being translated in the cytoplasm and then being transported to the nucleus. Further evidence against this artifact was that nuclear labeling occurred in the presence of the nuclear import blocker thapsigargin. Furthermore, nascent polypeptides were not localized at the nuclear periphery (nearest the cytoplasm), nor were they randomly distributed, but instead they colocalized with nascent transcripts, mRNA processing components (SR proteins), and translation factors (L7,eIF-4E) in the nucleus. Nuclear labeling was inhibited by the translation elogation inhibitor cyclobeximide but not by aurintricarboxylic acid, which at low concentrations only inhibits translation initiation. This latter result made it unlikely that nuclear labeling was merely due to translation initiation by cytoplasmic-displaced ribosomes that entered nuclei during cell permeabilization. This apparent nuclear translation was coupled with transcription, as the addition of nucleotides increased amino-acid incorporation, whereas the transcriptional inhibitors α-amanitin and 3dATP blocked it. Collectively, the data make a good case that coupled transcription-translation can occur in the nucleus of permeabilized cells. A future challenge is to determine whether nuclear translation occurs in intact cells.

The possibility of coupled transcription and translation in eukaryotes is intriguing from an evolutionary perspective, in that it is consistent with a scenario in which an ancient bacterium engulfed by another became the present-day eukaryotic nucleus.[81] According to this scenario, the *engulfed* bacterium retained its ability to undergo coupled transcription–translation (for the purpose of scanning messages for mistakes), but as it evolved into a modern nucleus, it handed over the responsibility for most translation to the *engulfing* bacterium's ribosomes in the cytoplasm. The symbiotic origin of the eukaryotic nucleus would not only explain nuclear translation but also provides an explanation for why ribosomal proteins regulate so many nuclear events, including DNA repair, DNA replication, transcription, and RNA processing.[82]

Even though the possibility that mRNAs are proofread in the nucleus by a mechanism involving nuclear translation is an intriguing idea, it should be stressed that this notion is still quite uncertain. One stumbling block is that there is no electron microscope evidence for active ribosomes or polysomes in the nucleus.[79] Thus, another possibility is that translation factors such as eIF-4E, eIF-4G, EF-1, and charged tRNAs reside in the nucleus because they perform a non-translation-related function. Future studies will hopefully resolve whether translation factors in the nucleus are participating in non-translational activities or are instead directing a nuclear translation-like proofreading mechanism.

The evolution of multifunctional proteins

How have RNA-binding regulatory proteins evolved to control different events in the nucleus and the cytoplasm? One obvious characteristic that such multifunctional proteins must possess is the ability to travel to both the nucleus and the cytoplasm. For example, in order for a primordial cytoplasmic regulatory protein to gain a nuclear function, it must evolve a nuclear import signal. Likewise, selection for a nuclear export signal would allow a primordial nuclear regulator to also have the potential to control events in the cytoplasm.

After having gained access to a new compartment, how does an RNA-binding protein acquire a new function? We speculate that this would only require that its binding site be available in a new context. For example, an RNA-binding protein that initially had the ability to regulate RNA splicing by binding to the sequence CACAGAR near a splice site in transcript X could acquire the ability to also regulate translation if this same target sequence was acquired in the 3′ UTR of transcript Y. Thus, we propose that a major driving force for the generation of multifunctional regulatory molecules is selection for new binding-sites.

We further speculate that most multifunctional RNA-binding proteins have evolved to inhibit (rather than stimulate) post-transcriptional events. This follows from the idea that inhibition of most functions only requires that a given regulatory protein binds to RNA; it does not necessitate that this protein possesses a different domain to mediate each function. In agreement with this notion, most of the multifunctional RNA-binding proteins that have so far been identified have inhibitory activity, particularly those involved in translational control (Tables 1 and 2). In contrast,

stimulatory regulatory factors will not only require an RNA-binding domain but may also need multiple activation domains to attract all of the specific factors required for each of the post-transcriptional steps controlled by the regulatory factor.

In conclusion, we believe that the evolution of multi-functional RNA-binding regulatory proteins is probably a common event. It only requires that an RNA-binding protein acquire nuclear import and/or export signals and that its binding sites arise in appropriate contexts on target mRNAs for different types of regulation. Given these rather simple requirements, we speculate that many RNA-binding proteins that spend time in both the nucleus and the cytoplasm will turn out to be regulatory proteins controlling events in both of these compartments. For example, the HuR and HuA ELAV-class RNA-binding proteins, which are known to regulate cytoplasmic RNA stability, may ultimately be found to also regulate nuclear events by virtue of their ability to shuttle between the cytoplasm and the nucleus.[30,31] Conversely, the *C. elegans* TRA-1 protein, which functions in the nucleus as a DNA-binding transcription factor, may also be found to regulate cytoplasmic events, based on its recent assignment as an RNA-binding protein that shuttles to the cytoplasm (note that *C. elegans* TRA-1 is not related to *D. melanogaster* Tra) .[50] Another candidate multifunctional protein is FMRP (encoded by the fragile-X syndrome gene *FMR1*), which associates with a subset of RNAs in the nucleus but also shuttles to the cytoplasm where it associates with polysomes and appears to inhibit translation.[28,29]

Concluding remarks

Why have multifunctional proteins evolved? One advantage conferred by this arrangement is that fewer proteins are then required to regulate the large number of varied events that transpire in eukaryotic cells. A second advantage is that multifunctional proteins can achieve more efficient regulation than can nuclear and cytoplasmic-restricted regulatory molecules. Because multifunctional proteins remain bound to their target RNAs during both transcription and translation, the number of binding and dissociation steps is reduced, thus streamlining the process. That some multifunctional proteins may also shuttle their target mRNAs across the nuclear membrane provides an additional level of efficiency. A third advantage is that it permits feedback regulation between the nuclear and cytoplasmic compartments. Several ribosomal proteins, including human S14 and yeast L30 (formerly, L32), have been shown to feedback inhibit their own synthesis in the nucleus.[82,83] Nuclear feedback control is an efficient and responsive means of generating an optimal amount of cytoplasmic protein in a given circumstance. Lastly, multi-functional shuttling proteins may prevent chaos. By directing RNA traffic in both the nucleus and the cytoplasm, these RNA-binding proteins may be critical for coordinating the discrete events that occur in different compartments of eukaryotic cells. Some facets of gene expression in eukaryotic cells may have evolved from this interconnected web. For instance, to be expressed at normal steady-state levels in the cytoplasm, most genes from higher eukaryotic cells must possess at least one intron. This has been puzzling, as intron splicing requires energy and thus one might have expected decreased mRNA levels as a result of introns, rather than the reverse. We propose that this obligate requirement for RNA splicing results from multifunctional proteins acting on both splicing and later steps of gene expression. An obvious set of candidates for this role are some of the proteins in the E–E complex, which are deposited on exons near RNA splice sites and then may go on to promote cytoplasmic mRNA transport and subsequent cytoplasmic events.[23,24]

The intriguing interconnectedness of the nucleus and the cytoplasm is further illustrated by the following two examples. First, some genes must have introns for efficient translation of their spliced mRNA products in the cytoplasm.[84] Second, as mentioned earlier, introns are necessary to engage the NMD RNA surveillance pathway. These examples of intron dependence could be explained by the activity of multifunctional RNA-binding proteins that regulate intron-dependent events in the nucleus and then go on to the cytoplasm to regulate subsequent events. Future studies will be required to test this theory and to identify the specific RNA-binding shuttling proteins involved and how they communicate with each other.

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