

MESSENGER-RNA-BINDING PROTEINS AND THE MESSAGES THEY CARRY

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From sites of transcription in the nucleus to the outreaches of the cytoplasm, messenger RNAs are associated with RNA-binding proteins. These proteins influence pre-mRNA processing as well as the transport, localization, translation and stability of mRNAs. Recent discoveries have shown that one group of these proteins marks exon–exon junctions and has a role in mRNA export. These proteins communicate crucial information to the translation machinery for the surveillance of nonsense mutations and for mRNA localization and translation.

PRE-mRNA

The primary transcript of the genomic DNA, which contains exons, introns and other sequences.

SPLICING

The removal of introns from the pre-mRNA.

TERMINATION CODONS

The stop signals for translation: UAA, UAG and UGA.

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DOI: 10.1038/nrm760

To function properly, eukaryotic messenger RNAs must contain, in addition to a string of codons, information that specifies their nuclear export, subcellular localization, translation and stability. An important theme to emerge over the past few years is that much of this information is provided by specific RNA-binding proteins. These proteins — collectively referred to as heterogeneous nuclear ribonucleoproteins (hnRNP proteins) or mRNA–protein complex proteins (mRNP proteins) — are PRE-mRNA/mRNA-binding proteins that associate with these transcripts and profoundly influence their function and fate.

The ribonucleoprotein complexes (RNPs) are the functional forms in which pre-mRNAs and mRNAs exist in cells. The protein components of the RNPs participate in pre-mRNA processing and are important determinants of mRNA export, localization, translation and stability. The association of hnRNP proteins with pre-mRNAs initiates co-transcriptionally, as they are still nascent transcripts. Many of them remain bound to the resulting mRNAs all the way to ribosomes, and shuttle back and forth between the nucleus and the cytoplasm. The RNP is highly dynamic — some proteins dissociate whereas others bind only later, as a consequence of specific processes such as SPLICING. At every step of the pathway, the mRNA–protein complex (mRNP) is further metamorphosed until a distinct mRNP emerges in the cytoplasm to engage the translation machinery.

Recent discoveries showed that this mature mRNP contains proteins that it acquired strictly in the wake of the splicing reaction. These proteins, which are arranged in the form of a complex called the exon–exon junction complex (EJC), mark the position of exon–exon junctions. EJC proteins have a role in the nuclear export of mRNAs that are produced by splicing, and several of the mRNP's components persist in the same position after export of the mRNP to the cytoplasm. This indicates that the mRNP proteins — some acquired by virtue of the RNA sequence and some by the processes that it has experienced — mould a unique mRNP that is an information-rich packet. This communicates information to the cytoplasm about the overall structure of the gene from which the mRNA was formed, and possibly other signals too. The specific structure of the mRNP complex is probably crucial for the fate of the mRNA in the cytoplasm — including surveillance for potentially deleterious premature TERMINATION CODONS (nonsense mutations) — and for its localization and translation.

HnRNP and mRNP proteins

The primary protein-coding transcripts that are produced by RNA POLYMERASE II are termed pre-mRNAs (or, using the historical term that describes their size heterogeneity and cellular location, heterogeneous nuclear RNAs; hnRNAs). The pre-mRNAs are associated with

RNA POLYMERASE II

The enzyme that transcribes mRNA and most of the small nuclear RNAs (snRNAs) of eukaryotes, in conjunction with various transcription factors.

Table 1 | **Characteristics of representative hnRNP and mRNP proteins**

Proteins	Domain structure	kDa*	Possible functions	Shuttling	References
A1	2XRBD, RGG	34	mRNA splicing mRNA export Telomere biogenesis	+	8,24–26,29,35 43,44 20,22
A2/B1	2XRBD, RGG	36/38	mRNA splicing mRNA localization	+	1,2 45,49
C1/C2	1XRBD	41/43	mRNA splicing mRNA stability	–	1,2 56
D(AUF1)	2XRBD, RGG	44–48	Telomere biogenesis mRNA stability Recombination	+	19,21 55,59,60 23
E1/E2 (α CP1,2 or PCBP1,2) [‡]	3XKH	38,39	mRNA stability Translational control	+	54,57 50,51,53
F	3XRBD	53	mRNA splicing	N/K [§]	27
H/H'(DSEF-1)	3XRBD	56	mRNA splicing Polyadenylation	N/K [§]	31,33 40
I (PTB)	4XRBD	59	mRNA splicing mRNA localization Polyadenylation	+	28,30 47 38
K	3XKH, RGG	62	Transcription Translational regulation	+	16–18 10,50,51,53
L	4XRBD	68	mRNA export mRNA stability	N/K [§]	7 42
Q	3XRBD, RGG	55–70	mRNA splicing	N/K [§]	34
U	RGG	120	Nuclear retention	–	1,2
PABPI	4XRBD	72	mRNA translation and stability	+	64–67
HuR	3XRBD	36	mRNA stability mRNA export	+	109 44
Yra1	1XRBD	27	mRNA export	–	123,124
Npl3/Nop3	2XRBD, RGG	60	mRNA export Pre-rRNA processing	+	41 36
Hrp1/Nab4	2XRBD, RGG	73	Polyadenylation	+	37
Squid/hrp40	2XRBD, RGG	40	mRNA localization	N/K [§]	46
ASF/SF2 (SRp30a)	2XRBD, RS domain	30	mRNA splicing	+	63
SC35 (SRp30b)	1XRBD, RS domain	30	mRNA splicing	–	63
SRp20	1XRBD, RS domain	20	mRNA splicing mRNA export	+	63 116
9G8	1XRBD, RS domain	35	mRNA splicing mRNA export	+	63 116
magoh	N/H	17	EJC/mRNA localization	(+) [¶]	79,83,127,128
Y14	1XRBD	24	EJC/NMD mRNA localization	+	78–80 90,91
Aly/REF	1XRBD	32	EJC/mRNA export	+	79,82,88
RNPS1	1XRBD, RS domain	50	mRNA splicing EJC/NMD	+	81 79,89
DEK	N/H	60	EJC/mRNA splicing	–	79,87
Upf3	1XRBD	66	EJC/NMD	+	85,86,89
SRm160	RS domain	160	EJC/mRNA splicing	–	77,79

*Molecular mass (kDa) is estimated from SDS-polyacrylamide gel electrophoresis.

[‡]Although α CP1/PCBP1 and α CP2/PCBP2 have been identified as hnRNP E1/E2 proteins, it has not been verified that these proteins are identical to hnRNP E proteins in the human hnRNP complexes^{1,2}.

[§]N/K, not known.

^{||}N/H, no significant homology to other known motifs.

[¶]This protein is strongly proposed to shuttle^{79,83}, but it has not been proved.

proteins and with SMALL NUCLEAR RNPS (snRNPs). The collective term for the proteins that bind hnRNAs, and which are not stable components of other classes of RNP complex such as snRNPs, is hnRNP proteins^{1–3}. This operational definition, like that of other RNPs, including snRNPs and ribosomes, does not make a distinction between the different proteins based on their structure, abundance, tissue specificity, specific functions, steady-state subnuclear localization or the time they join the complexes. So, for example, splicing ‘factors’ (such as U2AF and ASF/SF2) and ‘traditional’ hnRNP proteins (such as hnRNP A1 or hnRNP H; TABLE 1) are components of hnRNP complexes if they bind directly to and co-purify with the same hnRNAs. In this vein, it is important to keep in mind that hnRNP complexes are unusually large and diverse RNA–protein complexes. They typically contain numerous proteins and are highly dynamic. Some hnRNP proteins are extremely abundant (~100 million copies per nucleus); others are present only in small amounts^{4–6}.

The chromatin-associated hnRNA–hnRNP–snRNP complexes are not soluble, so are not readily amenable to conventional biochemical analyses¹. hnRNP complexes can be purified from the post-chromatin nucleoplasm fraction, and immunopurification using monoclonal antibodies against hnRNP proteins has been particularly useful.

The most detailed picture of the protein composition of hnRNP complexes is for human cells. At least 20 major proteins have been identified, and are designated from A1 (34 kDa) to U (120 kDa)¹ (TABLE 1). For many of these, there are several isoforms, and numerous less abundant proteins are also present. hnRNP complexes isolated with antibodies against different hnRNP proteins show a similar pattern of protein composition, albeit with different relative stoichiometry, which indicates that these proteins are part of the same supramolecular complexes that are common to many, if not all, transcripts. But this composition is an average, and the protein composition of individual complexes cannot be determined from it.

Co-purification of the proteins depends on the presence of RNAs, as RNase digestion results in dissociation of the proteins. Many of the hnRNP proteins can be efficiently crosslinked with ultraviolet light to POLY(A)-containing hnRNA *in vivo*, and their steady-state localization, as determined by immunofluorescence microscopy, is nuclear (excluding nucleoli). Some hnRNP proteins are among the most abundant proteins in the nucleus of vertebrate cells. For example, the hnRNP proteins A1 and C1 are about ten times more abundant than the U1 snRNP. Only small amounts of snRNPs co-immunopurify with hnRNPs from the nucleoplasm, and vice versa. However, there is clear cytological evidence that hnRNP proteins and snRNPs co-localize on (almost all) nascent transcripts⁷, and it could be that the interaction between hnRNP proteins and snRNPs is transient, and has already ended by the time the transcripts are released from chromatin.

Several of the abundant hnRNP proteins can form distinct assemblies *in vitro* or in nuclear spreads

(sometimes referred to as core particles and 30–40S beads in the early literature). However, there is no evidence for a defined unit of composition or a repeating structural unit of organization of hnRNP proteins on pre-mRNAs *in vivo*. Rather, a unique combinatorial arrangement of hnRNP proteins is thought to form on each mRNA. The factors that determine the specific constellation of hnRNP proteins that assembles on each mRNA probably depend on the mRNA sequence and on the repertoire of hnRNP proteins (including their relative abundance and the specific post-translational modifications that they receive) in the nucleus during transcription^{8–10}.

Most, if not all, hnRNP proteins contain one or more of a small number of RNA-binding motifs. The most common of these are the RNP motifs (RBD, also called RNA-recognition motif; RRM), KH domains and RGG (Arg–Gly–Gly) boxes¹¹. The overall structure of the hnRNP protein is modular; as well as the RNA-binding domain(s), each contains one or more domains that mediate protein–protein interactions and determine the localization of the protein (TABLE 1). For example, hnRNP A1 contains two RBDs at the amino terminus, and an auxiliary domain at the carboxyl terminus that contains an RGG box and a glycine-rich nuclear import/export domain called M9 (REFS 12–14).

hnRNP proteins have RNA-binding specificity. However, an hnRNP protein might not be bound exclusively to the highest-affinity sequence if it is present in molar excess over its highest-affinity binding sites (as is the case for many hnRNP proteins). In this case, it may be bound ‘nonspecifically’ (that is, at lower affinity) to other RNA sequences. The binding of hnRNP proteins to their cognate RNAs strongly influences the structure and interactions of the pre-mRNAs; and, therefore, their fate and processing to mRNAs. hnRNP proteins participate in various nuclear events, such as transcriptional regulation^{15–18}, telomere-length maintenance^{19–22}, immunoglobulin gene recombination²³, splicing^{8,24–35}, pre-ribosomal-RNA processing³⁶ and 3′-end processing^{37–40}. hnRNP proteins are also important in nucleo-cytoplasmic transport of mRNA^{41–44}, and in mRNA localization^{45–49}, translation^{10,50–53} and stability^{54–60}.

Although many of the abundant hnRNP proteins are localized at steady state in the nucleus, they in fact shuttle continuously between the nucleus and the cytoplasm. This finding brought about a fundamental change in our view of how these proteins act. It indicated that they could not be regarded merely as ‘packaging’ proteins, but that they could also have a role in mRNA export, function in the cytoplasm, and transduce signals between the nucleus and the cytoplasm⁶¹. Similarly, splicing factors such as the SR (SERINE–ARGININE) PROTEINS have also been found to shuttle with exported mRNAs^{62,63}.

So, the distinction (based largely on historical nomenclature) between hnRNP proteins, SR splicing factors and mRNP proteins is no longer meaningful. The characteristics of many hnRNP proteins — including their structural motifs, nuclear localization, ability to shuttle between the nucleus and the cytoplasm, direct

SMALL NUCLEAR RNPS
Low-molecular-weight RNAs, associated with proteins. They mediate the splicing of primary RNA transcripts.

POLY(A)
Newly synthesized mRNAs are poly-adenylated to generate a 3′ poly(A) tail. In eukaryotes, the number of residues added can vary from 50–250.

SR PROTEINS
A family of splicing factors, so-named because they are rich in serine (S) and arginine (R) residues. These proteins select splice sites and form part of the spliceosome complex.

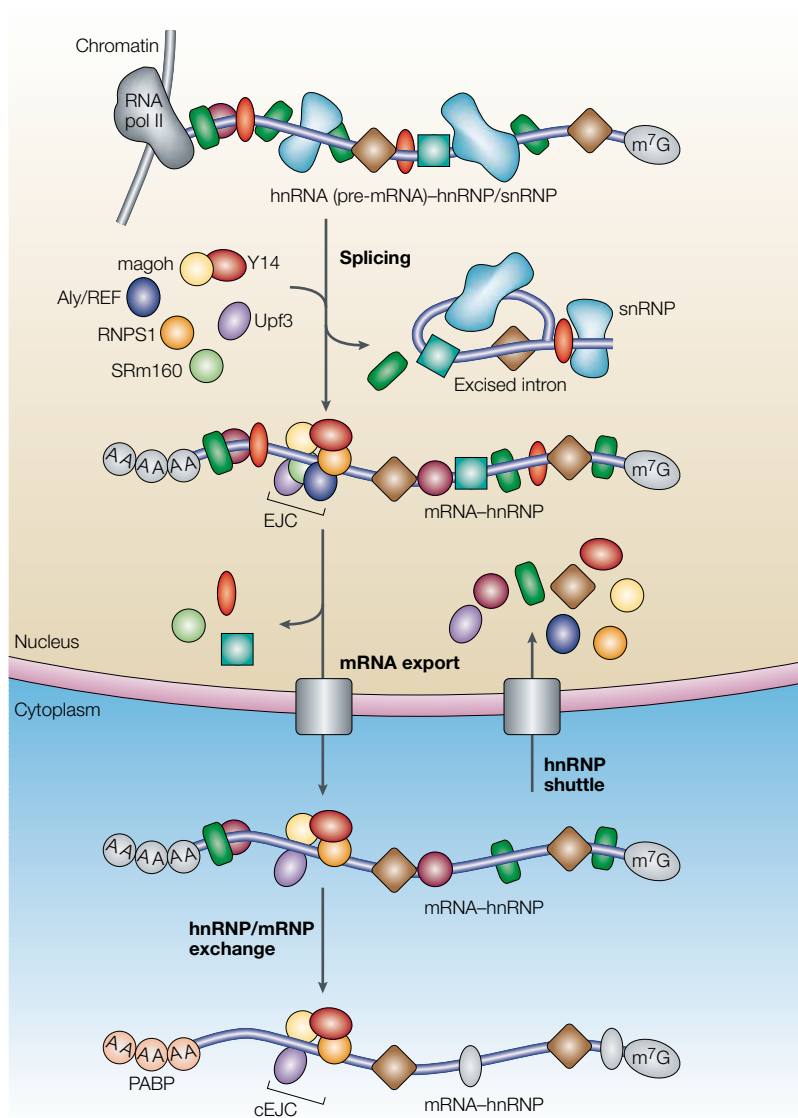


Figure 1 | HnRNP proteins and mRNP proteins along the pathway of mRNA biogenesis. Overview of the interaction and dynamics of the myriad of RNA-binding proteins with pre-mRNA and mRNA. EJC, exon–exon junction complex; NMD, nonsense-mediated mRNA decay; RNA pol II, RNA polymerase II; hnRNP, heterogeneous nuclear ribonucleoprotein; snRNP, small nuclear ribonucleoprotein; mRNP, mRNA–protein complex; PABP, poly(A)-binding protein; m⁷G, 5' 7-methylguanosine cap.

binding to pre-mRNA and mRNA, capacity for sequence-specific RNA binding and their role in pre-mRNA processing — make the distinction between the different classes of RNP proteins difficult, and not entirely valid. For example, glycine-containing hnRNP A1 and SR-containing ASF/SF2 both affect splicing and are both abundant shuttling proteins that bind pre-mRNAs and mRNAs. Yet hnRNP A1 is often referred to as a ‘core’ hnRNP protein, whereas ASF/SF2 is often referred to as a splicing factor. This mindset is potentially misleading, as the unifying thread tends to be missed; namely, that what the various RNP proteins have in common is more instructive than their differences in understanding how they operate in concert and

how they contribute their functions in a connected pathway.

In the cytoplasm, mRNAs are also associated with proteins. Some of these are shuttling hnRNP proteins, which are exported with the mRNAs to the cytoplasm, whereas others are predominantly cytoplasmic mRNP proteins. Overall, in comparison to nuclear poly(A)-containing RNAs, cytoplasmic mRNPs are not as protein-rich. Only a few mRNP proteins are consistently associated with most cytoplasmic mRNAs. Of these, the poly(A)-binding protein I (PABPI) is the most abundant and most extensively studied^{64–67}. This 72-kDa protein shares many characteristic features with hnRNP proteins, including the presence of RBDs, a modular structure, and shuttling activity between nucleus and cytoplasm. Furthermore, the observation that many hnRNP proteins, particularly the shuttling ones, also have important functions in the cytoplasm and are found on translating mRNAs in POLYSOMES⁶⁸ makes the distinction between hnRNP and mRNP proteins rather superficial. This is further complicated by the fact that several proteins that were previously thought of as classic cytoplasmic mRNP proteins have been found to shuttle into the nucleus.

Coupling between the various steps of the pathway of mRNA formation is evident at several stages. The polymerase itself, particularly the carboxy-terminal domain (CTD) of RNA polymerase II (pol II), has a central role in coupling transcription with mRNA processing (capping, splicing and polyadenylation)⁶⁹ by recruiting hnRNP proteins and processing factors to the nascent pre-mRNAs. In a process that is coupled with transcriptional termination, the 3' end of mRNA is cleaved and polyadenylated⁷⁰. The poly(A)-binding protein II (PABPII) then binds to the poly(A) tail in the nucleus. In the cytoplasm, a different poly(A)-binding protein (PABPI) is found on the poly(A) tail, although the exact place and timing of exchange is not known. A similar exchange occurs on the cap of the mRNA, where the cap-binding protein complex (CBC) is replaced by eIF4E, presumably during or immediately after export to the cytoplasm.

In conclusion, hnRNP proteins are an inextricable part of the pathway of gene expression from the transcription complex to mRNA in the cytoplasm. They are bound to, and accompany, the mRNA, and participate in every known aspect of the biogenesis, transport and function of mRNA in eukaryotic cells. Because they associate with pre-mRNAs as they transcribe, and can remain with them until their demise in the cytoplasm, hnRNP proteins can link the various steps into a connected pathway. This can be most vividly seen by microscopy on the amphibian oocyte LAMPBRUSH CHROMOSOMES, and has been recognized for decades⁷¹. Another extensively studied system is the BALBIANI RING transcript in the larval salivary glands of *Chironomus tentans*^{68,72–74}.

A further layer of complexity in the pathway from transcription to translation became apparent with the discovery that splicing adds and removes proteins from the pre-mRNA; namely, that there are extra entry and

POLYSOMES
Also known as polyribosomes. Two or more ribosomes that are attached to different points on the same strand of mRNA.

eIF4E
This is part of the translation-initiation complex, in which eIF4E is the cap-binding component.

exit points for more proteins along this route. Pre-mRNA splicing alters the composition of proteins that are bound to the mRNA, such that general pre-mRNP complexes (hnRNPs) are somewhat different from mRNP complexes. The hnRNP proteins that are bound on intronic sequences are removed after splicing, leaving behind only the hnRNP proteins that are bound to exonic sequences. Some splicing factors that are bound on exonic sequences also remain on the mRNA⁶³. Recently, a new group of proteins was found to be recruited to the mRNAs by splicing, and bound to spliced mRNAs in a sequence-independent manner.

The exon–exon junction complex

Splicing alters the protein composition of mRNPs, as some proteins bind mRNAs in a splicing-dependent manner. *In vitro* splicing experiments showed that mRNP complexes that are produced by splicing have a different mobility on a non-denaturing gel compared with that of mRNP complexes that contain intronless mRNAs with identical sequences⁷⁵. Although no specific proteins were identified in this study, the findings indicated that spliced mRNAs could acquire and/or lose factors in a splicing-dependent, sequence-independent manner. Ultraviolet crosslinking after *in vitro* splicing showed that several proteins become associated with spliced mRNAs⁷⁶ — one of which was identified as **SRm160** (REF. 77). Characterization of a new RNA-binding protein, **Y14**, showed that it binds preferentially to mRNAs that are produced by splicing, both *in vitro* and *in vivo*⁷⁸. Further studies identified further protein components that bind mRNAs produced by splicing, and showed that they are organized into a complex near exon–exon junctions (~20 nucleotides upstream of the junctions) *in vitro* and *in vivo*^{79,80} (FIG. 1).

The known components of the exon–exon junction complex (EJC) include at least six proteins — **SRm160**, **RNPS1**, **Aly/REF**, **Y14**, **magoh** and **Upf3** (FIG. 1; TABLE 1). **SRm160** was originally identified as a nuclear-matrix protein that contains Ser–Arg repeats⁷⁷. **SRm160** enhances splicing of specific pre-mRNAs through interactions with snRNPs and other SR proteins. **RNPS1** contains an RBD and an SR domain, and is a splicing co-activator⁸¹. **Y14** and **Aly/REF** each contain a single RBD^{78,82}. Recently, two more EJC components have been identified (FIG. 1): **magoh**, the vertebrate homologue of the *Drosophila melanogaster* **mago nashi** protein^{83,84}, and **Upf3**, the mammalian homologue of the *Saccharomyces cerevisiae* **Upf3** protein^{85,86}. **DEK**, a protein that co-purifies with SR proteins, has also been proposed to be a component of the EJC⁸⁷. However, the specificity of its association with spliced mRNA is less convincing than that of the other components⁸⁷.

Aly/REF, **RNPS1**, **Upf3**, **Y14** and **magoh** are predominantly nuclear. However, they shuttle between the nucleus and cytoplasm, which indicates that they could have a role in the export of mRNA and/or function in cytoplasmic processes. Indeed, **Aly/REF** was shown to stimulate mRNA export^{88,89}. **RNPS1**, **Upf3** and (to a lesser extent) **Y14** have been shown to have a role in NONSENSE-MEDIATED mRNA DECAY (NMD)⁹⁰. In addition, **Y14**

and **magoh** have been implicated in the cytoplasmic localization of mRNA in *Drosophila*^{91,92}. The functions of **SRm160** and **DEK** in post-splicing events are not known.

The EJC was estimated to be 335 kDa *in vitro*, and the shortest RNA fragment to which it is bound is approximately 8 nucleotides (nt) in length⁷⁹. It is intriguing that such a large, multi-protein complex can bind to a relatively short segment of RNA in a sequence-independent manner. Most of the components dissociate rapidly during or immediately after export, whereas **Y14** and **magoh** persist in the same position on mRNAs in the cytoplasm^{80,83,84}. It will be interesting to determine how the EJC components organize to form this stable yet dynamic complex.

The identification of the individual components of the EJC and knowledge of their functions indicated that this complex probably carries out several processes. In particular, mRNA export and NMD have emerged as likely biological roles of the EJC, and further functions such as cytoplasmic localization of mRNA are also possible.

EJC proteins and the export of spliced mRNAs

The export of mRNAs occurs by one or more pathways, which might be different from those that are used to export other RNAs and proteins. Most nucleo-cytoplasmic transport of proteins, TRANSFER RNA and U snRNAs is mediated by a single family of nuclear-transport receptors^{93–95}. Members of this family share a key co-factor, the small GTPase **Ran**. However, an important factor for mRNA export, termed **TAP** in vertebrates, or **Mex67** in *S. cerevisiae*, does not belong to the nuclear-transport receptor family. This evolutionarily conserved protein^{96–99} interacts with both mRNPs and the NUCLEAR PORE COMPLEX (NPC), and shuttles between the nucleus and cytoplasm^{100–102}. Its function in mRNA export was shown by using temperature-sensitive mutants in yeast⁹⁶, and by knocking down its expression in *Caenorhabditis elegans* by RNA INTERFERENCE¹⁰³. In addition, when tethered to unspliced mRNAs, **TAP**, together with its co-factor **p15/NXT1**, facilitated mRNA export^{104–107}. At least three more genes encode proteins that are similar to **TAP** in humans, which indicates a multigene family of mRNA export factors^{108,109}.

Other effectors that probably have a role in mRNA export include the shuttling hnRNPs, especially hnRNP **A1** and related proteins^{13,43,68}, **HuR**^{44,110}, **CRM1/exportin 1** (REF. 44), **RAE1/mRNP41/GLE2** (REFS 111–114), **Dbp5/Rat8** (REFS 115,116) and shuttling SR proteins^{63,117}. These components might have a role at different steps of a single export pathway, or several alternative mRNA-export pathways might exist¹¹⁸. Indeed, using cell-permeable inhibitory peptides, **Gallouzi** and **Steitz**⁴⁴ recently showed that there are several export pathways for specific mRNAs that involve hnRNP **A1**, **HuR** or **CRM1**. However, inhibitory peptides (**M9**, **HNS** and **NES**) did not cause accumulation of poly(A) RNAs, which indicates that most of the mRNA is exported by another — probably **TAP**-mediated — pathway.

It has been proposed that assembly of the SPLICEOSOME causes retention of pre-mRNAs in the nucleus^{119–121}.

LAMPBRUSH CHROMOSOMES

These are giant chromosomes that are found in oocytes — generally in amphibians. These chromosomes contain large loops that are active in RNA synthesis.

BALBIANI RING

A ‘puff’ or bloated segment of a lampbrush chromosome that shows especially intense activity (in this case, RNA transcription).

NONSENSE-MEDIATED mRNA DECAY (NMD)

A pathway that ensures that mRNAs bearing premature stop codons are eliminated as templates for translation.

TRANSFER RNA

A small RNA molecule that is responsible for the transfer of specific amino acids to the growing end of a polypeptide chain during translation.

U snRNA

A uridine-rich small nuclear RNA.

NUCLEAR PORE COMPLEX

A protein complex that is involved in the import of proteins to the nucleus.

RNA INTERFERENCE

The process by which an introduced double-stranded RNA specifically silences the expression of genes through degradation of their cognate mRNAs.

SPLICEOSOME

A protein–U snRNA complex that is required for folding of the pre-mRNA into the correct conformation for the removal of introns.

However, release from retention factors does not seem to be sufficient for mRNA export. For some mRNAs at least, splicing enhances the efficiency of export compared with that of unspliced mRNAs that are transcribed from intronless complementary DNAs. This implies a link between splicing and export. Removal of a single intron from a two-intron-containing mRNA is sufficient for its efficient export, which indicates that mRNAs acquire a positively acting export factor as a result of splicing⁷⁸.

The candidate factors that are likely to mediate such a link between splicing and export are Aly/REF in vertebrates^{86,88} and Yra1 in yeast^{82,122,123}. Aly/REF can interact directly with TAP⁸², which indicates that Aly/REF might recruit TAP to mRNA. A function for Aly/REF in the export of mRNAs was shown by microinjection of recombinant Aly/REF protein or anti-Aly/REF antibodies into *Xenopus laevis* oocytes^{88,89}, and by genetic screening for mutants that were defective in mRNA export in yeast¹²³. These experiments showed that Aly/REF facilitates the export not only of spliced mRNAs, but also of unspliced mRNAs, although spliced mRNAs are affected to a greater extent.

A member of the DEAD-BOX FAMILY of splicing factors, UAP56, was recently shown to interact with Aly/REF. This evolutionarily conserved interaction could explain how Aly/REF can be recruited to mRNAs^{124,125}. UAP56 homologues in *S. cerevisiae* (Sub2) and *Drosophila* (HEL) are required for the export of poly(A) RNA^{125–127}. Other EJC proteins — including Y14, magoh and Upf3 — also bind TAP, so it is likely that Aly/REF is not the only factor that can recruit TAP to mRNAs^{83,90}. So, by including a high local concentration of several TAP-binding proteins, the EJC could recruit several TAP molecules and enhance the export of spliced mRNAs.

Cytoplasmic localization of mRNAs

Studies on mago nashi and Y14 in *Drosophila* indicated another role for the EJC. The *Mago nashi* gene was originally identified as a gene that is required for axis formation during oogenesis, and for the localization of *OSKAR* mRNA to the posterior pole of oocytes^{128,129}. Two later studies^{130,131} showed that the human homologue of mago nashi, magoh, interacts with Y14. This interaction is highly conserved; magoh and Y14 co-localize in nuclear speckles and, more importantly, both are components of the EJC that accompany mRNAs to the cytoplasm^{83,84}. Mutations in *Drosophila* Y14 (also referred to as Tsunagi) show a similar phenotype to mutations in mago nashi; that is, a defect in cytoplasmic localization of *oskar* mRNA^{91,92}. It is not clear, however, whether this effect on mRNA localization is a separate function of Y14 and magoh, or whether it is secondary to the roles of these proteins in the EJC, including mRNA export and mRNA stability. Considering the apparently sequence-independent binding of Y14 and magoh to spliced mRNAs, these general components of the EJC probably have a more global role than localizing only a few specific mRNAs.

EJC proteins in NMD

Splicing is also linked to another post-splicing process, NMD (also called mRNA surveillance), whereby aberrant mRNAs that contain premature translation termination codons (PTCs) are rapidly degraded^{132,133}. But how do cells distinguish premature from bona fide stop codons? In mammalian cells, intronless mRNAs are not subject to NMD¹³⁴. For spliced mRNAs, the position of stop codons relative to the last exon–exon junction is crucial^{135–138}. Normal stop codons are almost always found in the last exon in mammalian genes¹³⁹. Termination codons that are located more than 50–55 nt upstream of the last exon–exon junction are subjected to rapid decay, whereas mRNAs with PTCs downstream of this boundary are usually stable.

The recognition of stop codons as either normal or premature also requires the translational machinery, as PTC-containing mRNAs are degraded only if they are translated^{136,140–142}. Furthermore, mutation of the initiation codon that defines the reading frame can rescue PTC-containing mRNAs from NMD¹⁴³. These observations raised the question of how cells integrate splicing and translation when these processes occur in separate compartments — the nucleus and cytoplasm, respectively. The exact cellular location of NMD remains controversial, as both nuclear^{136,144–146} and cytoplasmic^{137,147} NMD have been documented.

To explain nuclear NMD, a ‘nuclear-scanning model’ was proposed¹⁴⁵, in which there is a nuclear ribosome-like apparatus (‘nuclear scanner’). In accordance with this model, some components of translation machinery have been found in the nucleus^{148,149}, and nuclear translation has been reported (see, for example, REF. 150). However, the functional significance of these observations for NMD remains unclear, and the evidence for cytoplasmic NMD seems more persuasive. For example, NMD was specifically abolished when translation was inhibited using the interaction of the iron-regulatory RNA element with iron regulatory protein, which is restricted to the cytoplasm¹³⁷.

In an attempt to integrate the available data into a common model, it was proposed that the first round of translation occurs concomitantly with mRNA export. Indeed, the first round of translation might be distinguishable from the following cycles of translation in terms of the factors involved¹⁵¹. This idea is compatible with a ‘marker model’, according to which a marker is established by the spliceosome on the exon–exon junction in the nucleus. While the mRNA passes through the NPC, translating ribosomes displace the marker from the mRNA during the first round of translation. So, if a nonsense mutation occurs more than 50–55 nt upstream of the last exon–exon junction, the ribosomes will dissociate from the mRNA before they reach and remove the remaining marker. This remaining marker then recruits the NMD factors to form a surveillance complex, probably with translation-termination factors¹⁵², and triggers degradation of this mRNA (FIG. 2).

The factors responsible for NMD were originally identified in *S. cerevisiae* and named Upf1, Upf2/Nmd2,

DEAD-BOX FAMILY
A family of ATP-dependent helicases that can stabilize mRNA and facilitate translation.

OSKAR
A gene that is involved in determining cell fate in *Drosophila*. The *oskar* mRNA localizes to the posterior end of an oocyte.

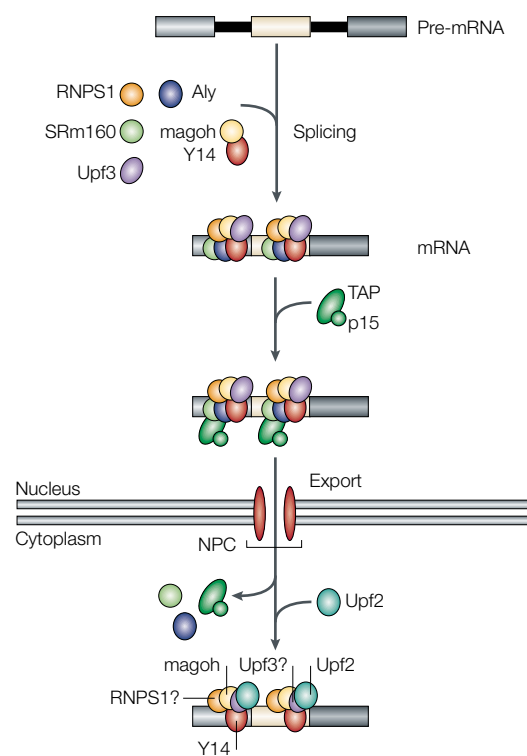


Figure 2 | Model for the functional coupling of pre-mRNA splicing and nonsense-mediated decay. The exon–exon junction complex (EJC) assembles on messenger RNA as a result of splicing. TAP/p15 binds to components of the EJC and mediates the interaction of the mRNP with the nuclear-pore complex (NPC). During export, and immediately afterwards, the composition of the complex is changed as several of its components dissociate, leaving behind the remnants — the cytoplasmic EJC (cEJC), which includes Y14 and magoh, and possibly Upf3 and RNPS1 — at the same position as the complex was assembled in the nucleus. Upf2 probably binds to Upf3 after export.

Upf3 and Hrp1^{153–155}. Human orthologues of the Upf proteins (Upf1, Upf2, Upf3a and Upf3b) have also been found^{90,156–158}. When tethered to the 3′ UNTRANSLATED REGION of β -globin mRNA, human Upf proteins can induce NMD. Upf1/RENT1 (a cytoplasmic RNA helicase) interacts with Upf2, which in turn binds to Upf3. Although Upf proteins can exist as a complex, Upf2 is concentrated in the perinuclear area whereas Upf3 is mainly in the nucleoplasm, and shuttles between the nucleus and the cytoplasm.

Recent studies showed that Upf3 is part of the splicing-dependent EJC^{85,86}, and that it interacts with RNPS1 and Y14 (REF. 90). Tethering RNPS1 (and to a lesser extent, Y14) to the 3′ untranslated region also triggered degradation of the mRNA⁹⁰. So, the splicing-dependent, position-specific binding of Upf3 to mRNAs through interactions with RNPS1 and/or Y14 probably provides the long-sought link between splicing and the NMD pathway. Whereas antibodies to Upf3 immunoprecipitate spliced mRNA from the nucleus but not from the cytoplasm, the converse is true for Upf2 (REF. 86). This indicates that, on export, Upf3 recruits Upf2 to the EJC,

and Upf3 then either becomes shielded from the antibodies by Upf2 or perhaps dissociates from the EJC, leaving Upf2 behind. The way we envisage this process is depicted in FIG. 1. As part of the EJC, splicing produces an mRNA that bears Y14–magoh–Upf3 complexes as ‘death tags’, in effect exporting mRNAs that are ‘guilty’ until proven ‘innocent’. The cell lets the first round of translation be the judge — if not all of the Y14–magoh–(Upf3)–Upf2 complexes (which are now the cytoplasmic EJCs; cEJCs) are removed during that first round of translation (namely, by the time the leading ribosome reaches a termination codon), then the mRNA will be executed by the NMD machinery.

Several questions remain. Is there an EJC on every exon–exon junction? Are all EJCs identical in composition? How is degradation triggered by the remaining EJC proteins that are not removed? What other factors are involved in the process? And, finally, is the NMD pathway related to nonsense-mediated alteration of splicing, which apparently occurs^{159–162} within the nucleus?

A unified pathway: the message is the mRNP

Biochemical experiments and high-resolution immunoelectron microscopy identified many of the proteins that associate with pre-mRNAs and mRNAs in the nucleus and cytoplasm. They show a highly dynamic series of rearrangements that involve the binding and dissociation of numerous proteins along the entire pathway of mRNA biogenesis (FIG. 1).

Most of the proteins — the hnRNP proteins — associate with the pre-mRNAs co-transcriptionally, whereas others associate later as a consequence of the processing reactions that form the mRNAs. Splicing, in particular, reshapes the mRNP. Many hnRNP proteins (and snRNPs) are removed with the excision of introns, and the spliceosome clears away proteins from the vicinity of exon–exon junctions, leaving the EJC in its wake. Yet many of the hnRNP proteins remain on the mRNAs after splicing and, together with the EJC proteins, accompany the mRNAs to the cytoplasm.

In vitro splicing experiments showed that many of the proteins (for example, hnRNP proteins) that bind pre-mRNAs are no longer found on mRNAs after splicing, and led to speculation that mRNAs are entirely depleted of them¹⁶³. However, these conclusions are based on results obtained *in vitro* with very small mRNAs, and do not consider other data, which show that many of these proteins are still found on exported mRNPs and cytoplasmic mRNAs, as vividly visualized on Balbiani ring transcripts⁷².

The hnRNP and mRNP proteins provide a means to integrate the steps in the pathway, with each transformation in their composition or specific arrangement having the capacity to influence the downstream events. At every step, the RNPs are the functional form, and the specific constellation of proteins on a given mRNA carries an impression of its childhood experiences and profoundly influences its fate. What results is an mRNP that is much richer in information than the sequence of

3′ UNTRANSLATED REGION
Non-coding region that lies 3′ to the protein-coding part of an mRNA. This often contains sequences that are involved in RNA regulation.

GERM PLASM

A special cytoplasmic region in (dividing) eggs that contains primary germ-cell-determining factors.

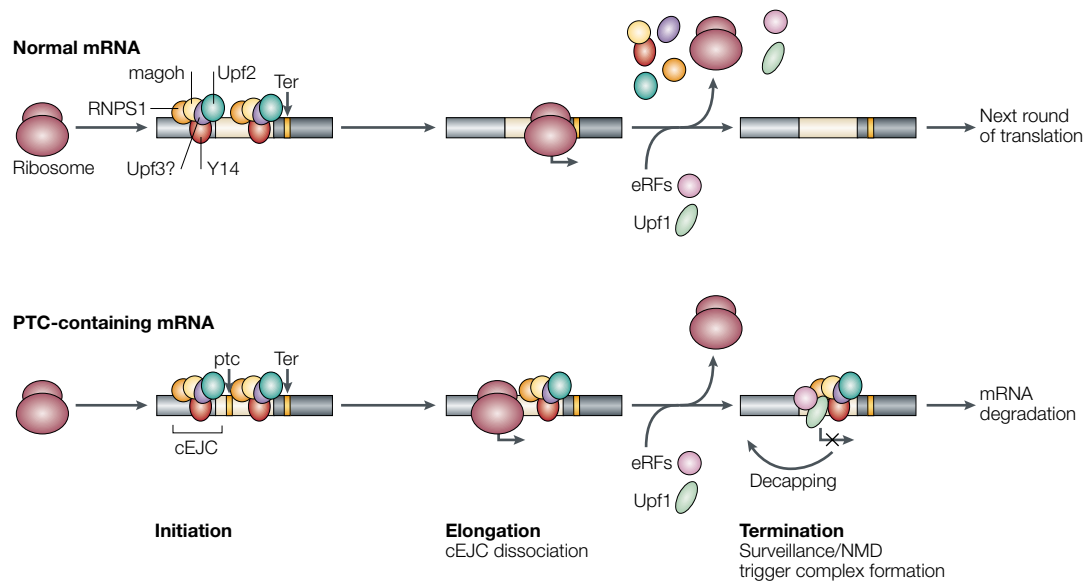


Figure 3 | **Model for the role of the exon-exon junction on nonsense-mediated mRNA decay.** In normal mRNAs, termination codons (Ter) are usually located downstream of the boundary; that is, ~55 nucleotides upstream of the last exon-exon junction. So, translating ribosomes remove all the Y14-Upf2-Upf3 complexes (cEJCs) by the time of termination. During termination, Upf1 and the release factors (eRFs) are recruited and form the surveillance complex, which scans for the remaining Y14-Upf complex. Unless the surveillance complex encounters a Y14-Upf complex, translation proceeds to the next round. In the case of mRNA that contains a premature termination codon (PTC) more than 55 nt upstream of the last exon-exon junction, translation termination occurs before all the Y14-Upf complexes are removed. The remaining Y14-Upf complex interacts with the surveillance complex and triggers decapping of the mRNA. EJC, exon-exon junction complex; NMD, nonsense-mediated mRNA decay.

the mRNA itself. In this vein, the EJC is of particular interest. Its proteins can efficiently interact with the mRNA-export factor TAP, thereby enhancing the export of mRNAs produced by splicing. The mRNP is further modified by the transport process and by exposure of the mRNPs to the environment of the cytoplasm. Because several proteins, including Y14, magoh and Upf2, are bound at exon-exon junctions after export to the cytoplasm, they provide a molecular memory that documents the overall structure of the pre-mRNA, and hence the gene, from which the mRNA was produced.

It is interesting to consider an entirely new function for introns — particularly their position in a gene — in setting up a distinct mRNP. This information is likely to be detected by the translation machinery in the cytoplasm, and seems to be the key for the surveillance of premature termination codons. This ‘no-nonsense’

approach is presumably designed not to take any chances with mRNAs that might produce potentially harmful, carboxy-terminally truncated proteins. It is conceivable that specific microenvironments in the cytoplasm, in which the mRNA can encounter localized factors (for example, near the plasma membrane or intracellular organelles, at specific poles in the GERM PLASM or near synapses of neurons), further alters the mRNP by modifying or dissociating its components, or binding more proteins. Alternative splicing might thereby influence not only the primary sequence of proteins, but also the time, efficiency and place of protein expression. In cells, the upstream events of gene expression influence, and provide a link to, the downstream events. So, not only the nucleotide sequence of mRNAs, but also the RNA-binding proteins, carry important messages for the proper expression and regulation of genes.

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Acknowledgements

We are grateful to members of the Dreyfuss laboratory, especially J. Dostie, A. Gubitz, Z. Mourelatos and L. Wan, for critical reading of and comments on this manuscript. V.N.K. is supported by a BK21 Research Fellowship from the Ministry of Education and Human Resources Development of Korea. Work in G.D.'s laboratory is supported by grants from the National Institutes of Health and the Human Frontier Science Program Organization. G.D. is an Investigator of the Howard Hughes Medical Institute.

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