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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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 (αCP1,2 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 αCP1/PCBP1 and αCP2/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 analyses[1]. 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 transcripts[7], 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) boxes[11]. 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 recombination[23], splicing[8,24–35], pre-ribosomal-RNA processing[36] 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 cytoplasm[61]. 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.

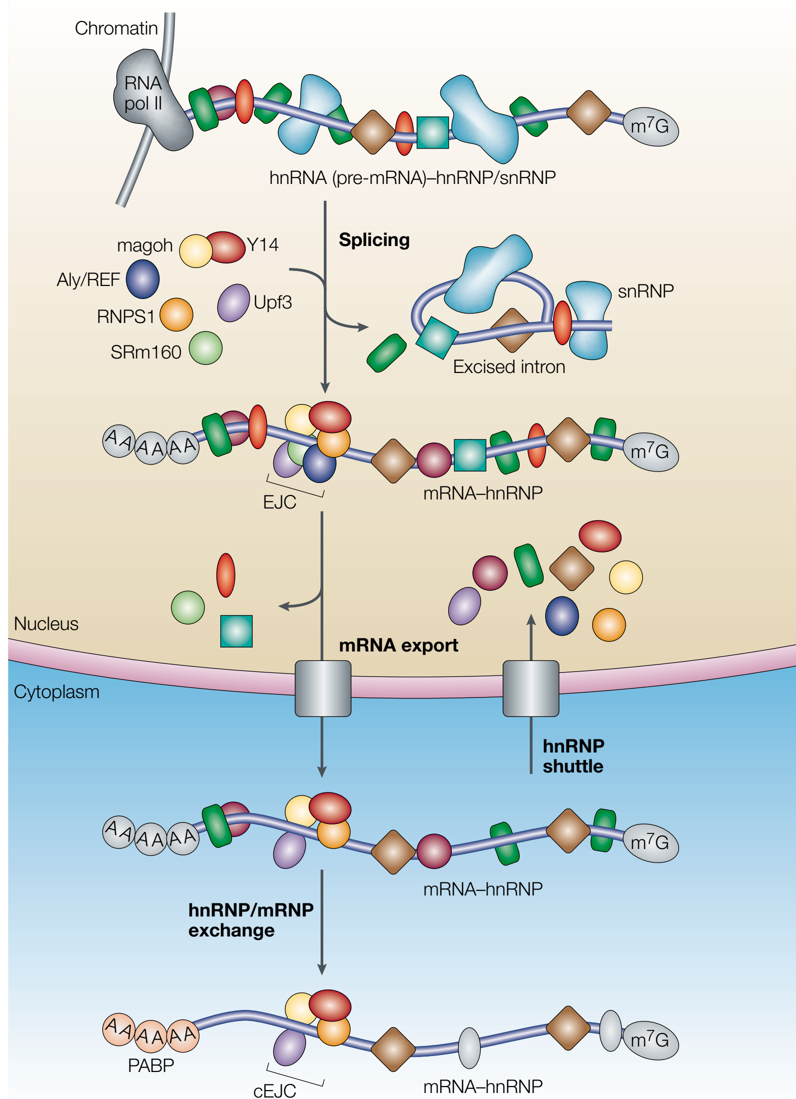

Figure 1 | **HnRNP proteins and mRNP proteins along the pathway of mRNA biogenesis.** Overview of the interaction and dynamics of the myriad of RNA-binding proteins with pre-mRNA and mRNA. EJC, exon–exon junction complex; NMD, nonsense-mediated mRNA decay; RNA pol II, RNA polymerase II; hnRNP, heterogeneous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

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.

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 POLYSOMES[68] 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 polyadenylated[70]. 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 decades[71]. 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

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 mRNA[63]. 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 sequences[75]. 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 mRNAs[76] — 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 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 repeats[77]. 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-activator[81]. 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 EJC[87]. However, the specificity of its association with spliced mRNA is less convincing than that of the other components[87].

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 length[79]. 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 yeast[96], and by knocking down its expression in *Caenorhabditis elegans* by RNA INTERFERENCE[103]. 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 exist[118]. 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 splicing[78].

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 TAP[82], 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 yeast[123]. 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 orginally 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.

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.

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 NMD[134]. 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 genes[139]. 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 NMD[143]. 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 proposed[145], 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 cytoplasm[137].

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 involved[151]. 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 factors[152], and triggers degradation of this mRNA (FIG. 2).

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

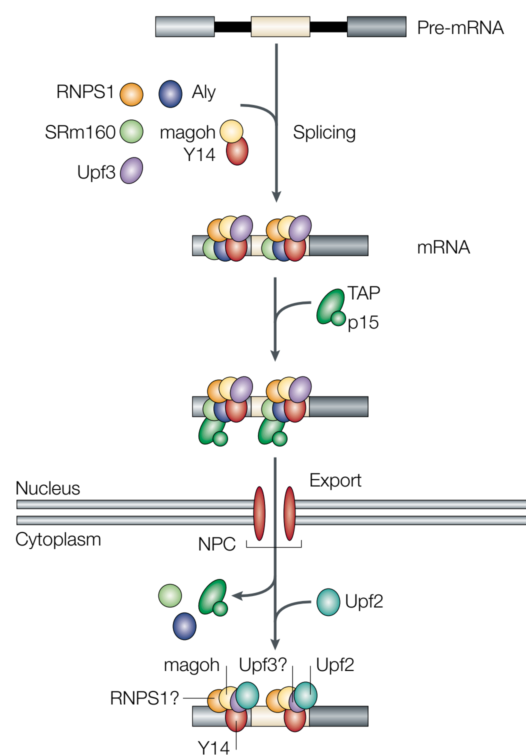

Figure 2 | **Model for the functional coupling of pre-mRNA splicing and nonsense-mediated decay.** The exon–exon junction complex (EJC) assembles on messenger RNA as a result of splicing. TAP/p15 binds to components of the EJC and mediates the interaction of the mRNP with the nuclear-pore complex (NPC). 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 mRNA[90]. 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.

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.

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 them[163]. 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 transcripts[72].

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

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

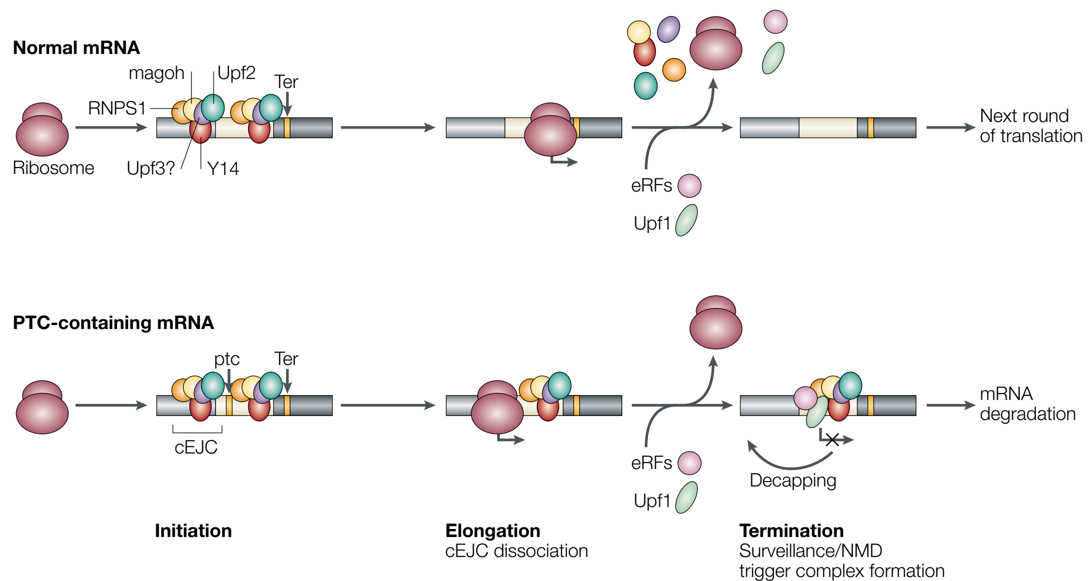

Figure 3 | **Model for the role of the exon–exon junction on nonsense-mediated mRNA decay.** In normal mRNAs, termination codons (Ter) are usually located downstream of the boundary; that is, ~55 nucleotides upstream of the last exon–exon junction. So, translating ribosomes remove all the Y14–Upf2–Upf3 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 machinary 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.

1. Dreyfuss, G., Matunis, M. J., Pinol-Roma, S. & Burd, C. G. hnRNP proteins and the biogenesis of mRNA. *Annu. Rev. Biochem.* **62**, 289–321 (1993).
2. Krecic, A. M. & Swanson, M. S. hnRNP complexes: composition, structure, and function. *Curr. Opin. Cell Biol.* **11**, 363–371 (1999).
3. Shyu, A. B. & Wilkinson, M. F. The double lives of shuttling mRNA binding proteins. *Cell* **102**, 135–138 (2000).
4. Kamma, H., Portman, D. S. & Dreyfuss, G. Cell type-specific expression of hnRNP proteins. *Exp. Cell Res.* **221**, 187–196 (1995).
5. Hanamura, A., Caceres, J. F., Mayeda, A., Franza, B. R. Jr & Krainer, A. R. Regulated tissue-specific expression of antagonistic pre-mRNA splicing factors. *RNA* **4**, 430–444 (1998).
6. Markovtsov, V. *et al.* Cooperative assembly of an hnRNP complex induced by a tissue-specific homolog of polypyrimidine tract binding protein. *Mol. Cell. Biol.* **20**, 7463–7479 (2000).
7. Pinol-Roma, S., Swanson, M. S., Gall, J. G. & Dreyfuss, G. A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. *J. Cell Biol.* **109**, 2575–2587 (1989).
8. Van der Houven van Oordt, W. *et al.* The MKK(3/6)–p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation. *J. Cell Biol.* **149**, 307–316 (2000).
This study shows that the MKK(3/6)–p38 pathway can mediate the cytoplasmic accumulation of hnRNP A1, and indicates that signal transduction mechanisms can regulate pre-mRNA splicing *in vivo*.
9. Mandal, M. *et al.* Growth factors regulate heterogeneous nuclear ribonucleoprotein K expression and function. *J. Biol. Chem.* **276**, 9699–9704 (2001).
10. Habelhah, H. *et al.* ERK phosphorylation drives cytoplasmic accumulation of hnRNP-K and inhibition of mRNA translation. *Nature Cell Biol.* **3**, 325–330 (2001).
This study shows that hnRNP K is phosphorylated by mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK) both *in vitro* and *in vivo*. This phosphorylation leads to the cytoplasmic localization of hnRNP K, which is required for its ability to silence mRNA translation. These findings indicate a role for hnRNP proteins in signal-transduction pathways.
11. Burd, C. G. & Dreyfuss, G. Conserved structures and diversity of functions of RNA-binding proteins. *Science* **265**, 615–621 (1994).
12. Siomi, H. & Dreyfuss, G. A nuclear localization domain in the hnRNP A1 protein. *J. Cell Biol.* **129**, 551–560 (1995).
13. Michael, W. M., Choi, M. & Dreyfuss, G. A nuclear export signal in hnRNP A1: a signal-mediated, temperature-dependent nuclear protein export pathway. *Cell* **83**, 415–422 (1995).

14. Weighardt, F., Biamonti, G. & Riva, S. Nucleo-cytoplasmic distribution of human hnRNP proteins: a search for the targeting domains in hnRNP A1. *J. Cell Sci.* **108**, 545–555 (1995).
15. Tomonaga, T. & Levens, D. Heterogeneous nuclear ribonucleoprotein K is a DNA-binding transactivator. *J. Biol. Chem.* **270**, 4875–4881 (1995).
16. Michelotti, E. F., Michelotti, G. A., Aronsohn, A. I. & Levens, D. Heterogeneous nuclear ribonucleoprotein K is a transcription factor. *Mol. Cell. Biol.* **16**, 2350–2360 (1996).
17. Du, Q., Melnikova, I. N. & Gardner, P. D. Differential effects of heterogeneous nuclear ribonucleoprotein K on Sp1- and Sp3-mediated transcriptional activation of a neuronal nicotinic acetylcholine receptor promoter. *J. Biol. Chem.* **273**, 19877–19883 (1998).
18. Miau, L. H., Chang, C. J., Shen, B. J., Tsai, W. H. & Lee, S. C. Identification of heterogeneous nuclear ribonucleoprotein K (hnRNP K) as a repressor of C/EBPβ-mediated gene activation. *J. Biol. Chem.* **273**, 10784–10791 (1998).
19. Ishikawa, F., Matunis, M. J., Dreyfuss, G. & Cech, T. R. Nuclear proteins that bind the pre-mRNA 3′ splice site sequence r(UUAG/G) and the human telomeric DNA sequence d(TTAGGG)n. *Mol. Cell. Biol.* **13**, 4301–4310 (1993).
20. La Branche, H. *et al.* Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and telomerase. *Nature Genet.* **19**, 199–202 (1998).
21. Eversole, A. & Maizels, N. *In vitro* properties of the conserved mammalian protein hnRNP D suggest a role in telomere maintenance. *Mol. Cell. Biol.* **20**, 5425–5432 (2000).
22. Fiset, S. & Chabot, B. hnRNP A1 may interact simultaneously with telomeric DNA and the human telomerase RNA *in vitro*. *Nucleic Acids Res.* **29**, 2268–2275 (2001).
23. Dempsey, L. A., Sun, H., Hanakahi, L. A. & Maizels, N. G4 DNA binding by LR1 and its subunits, nucleolin and hnRNP D, A role for G–G pairing in immunoglobulin switch recombination. *J. Biol. Chem.* **274**, 1066–1071 (1999).
24. Mayeda, A. & Krainer, A. R. Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. *Cell* **68**, 365–375 (1992).
This paper reports that the relative concentrations of hnRNP A1 and the essential splicing factor SF2 modulate 5′ splice site selection *in vitro*.
25. Caceres, J. F., Stamm, S., Helfman, D. M. & Krainer, A. R. Regulation of alternative splicing *in vivo* by overexpression of antagonistic splicing factors. *Science* **265**, 1706–1709 (1994).
26. Yang, X. *et al.* The A1 and A1B proteins of heterogeneous nuclear ribonucleoparticles modulate 5′ splice site selection *in vivo*. *Proc. Natl Acad. Sci. USA* **91**, 6924–6928 (1994).
27. Min, H., Chan, R. C. & Black, D. L. The generally expressed hnRNP F is involved in a neural-specific pre-mRNA splicing event. *Genes Dev.* **9**, 2659–2671 (1995).
28. Chan, R. C. & Black, D. L. The polypyrimidine tract binding protein binds upstream of neural cell-specific c-*src* exon N1 to repress the splicing of the intron downstream. *Mol. Cell. Biol.* **17**, 4667–4676 (1997).
29. Chabot, B., Blanchette, M., Lapierre, I. & La Branche, H. An intron element modulating 5′ splice site selection in the hnRNP A1 pre-mRNA interacts with hnRNP A1. *Mol. Cell. Biol.* **17**, 1776–1786 (1997).
30. Ashiya, M. & Grabowski, P. J. A neuron-specific splicing switch mediated by an array of pre-mRNA repressor sites: evidence of a regulatory role for the polypyrimidine tract binding protein and a brain-specific PTB counterpart. *RNA* **3**, 996–1015 (1997).
31. Chou, M. Y., Rooke, N., Turck, C. W. & Black, D. L. hnRNP H is a component of a splicing enhancer complex that activates a c-*src* alternative exon in neuronal cells. *Mol. Cell. Biol.* **19**, 69–77 (1999).
32. Del Gatto-Konczak, F., Olive, M., Gesnel, M. C. & Breathnach, R. hnRNP A1 recruited to an exon *in vivo* can function as an exon splicing silencer. *Mol. Cell. Biol.* **19**, 251–260 (1999).
33. Chen, C. D., Kobayashi, R. & Helfman, D. M. Binding of hnRNP H to an exonic splicing silencer is involved in the regulation of alternative splicing of the rat β-tropomyosin gene. *Genes Dev.* **13**, 593–606 (1999).
34. Mourelatos, Z., Abel, L., Yong, J., Kataoka, N. & Dreyfuss, G. SMN interacts with a novel family of hnRNP and spliceosomal proteins. *EMBO J.* **20**, 5443–5452 (2001).
35. Tange, T. O., Damgaard, C. K., Guth, S., Valcarcel, J. & Kjems, J. The hnRNP A1 protein regulates HIV-1 *tat* splicing via a novel intron silencer element. *EMBO J.* **20**, 5748–5758 (2001).
36. Russell, I. D. & Tollervey, D. NOP3 is an essential yeast protein which is required for pre-rRNA processing. *J. Cell Biol.* **119**, 737–747 (1992).
37. Kessler, M. M. *et al.* Hrp1, a sequence-specific RNA-binding protein that shuttles between the nucleus and the cytoplasm, is required for mRNA 3′-end formation in yeast. *Genes Dev.* **11**, 2545–2556 (1997).
38. Moreira, A. *et al.* The upstream sequence element of the C2 complement poly(A) signal activates mRNA 3′ end formation by two distinct mechanisms. *Genes Dev.* **12**, 2522–2534 (1998).
39. Minvielle-Sebastia, L. *et al.* Control of cleavage site selection during mRNA 3′ end formation by a yeast hnRNP. *EMBO J.* **17**, 7454–7468 (1998).
40. Bagga, P. S., Arhin, G. K. & Wilusz, J. DSEF-1 is a member of the hnRNP H family of RNA-binding proteins and stimulates pre-mRNA cleavage and polyadenylation *in vitro*. *Nucleic Acids Res.* **26**, 5343–5350 (1998).
41. Lee, M. S., Henry, M. & Silver, P. A. A protein that shuttles between the nucleus and the cytoplasm is an important mediator of RNA export. *Genes Dev.* **10**, 1233–1246 (1996).
42. Liu, X. & Mertz, J. E. HnRNP L binds a *cis*-acting RNA sequence element that enables intron-dependent gene expression. *Genes Dev.* **9**, 1766–1780 (1995).
43. Izaurralde, E. *et al.* A role for the M9 transport signal of hnRNP A1 in mRNA nuclear export. *J. Cell Biol.* **137**, 27–35 (1997).
44. Gallouzi, I. E. & Steitz, J. A. Delineation of mRNA export pathways by the use of cell-permeable peptides. *Science* **294**, 1895–1901 (2001).
This paper provides evidence that there are many export pathways for specific mRNAs. Cell-permeable peptides fused to nuclear-export signals inhibit interactions between particular receptor and adaptor pairs, and block the corresponding export pathway. This allowed the authors to attribute the export of *c-myc* mRNA to two independent pathways; one mediated by CRM1, and another by transportin 2.
45. Hoek, K. S., Kidd, G. J., Carson, J. H. & Smith, R. hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. *Biochemistry* **37**, 7021–7029 (1998).
46. Matunis, E. L., Kelley, R. & Dreyfuss, G. Essential role for a heterogeneous nuclear ribonucleoprotein (hnRNP) in oogenesis: hrp40 is absent from the germ line in the dorsoventral mutant squid. *Proc. Natl Acad. Sci. USA* **91**, 2781–2784 (1994).
47. Cote, C. A. *et al.* A *Xenopus* protein related to hnRNP I has a role in cytoplasmic RNA localization. *Mol. Cell* **4**, 431–437 (1999).
Localization of *Vg1* RNA within the *Xenopus* oocyte is mediated by recognition of a localization element within its 3′ untranslated region. VgRBP60, which is homologous to a human hnRNP I (PTB), is shown to be a specific binder of this sequence and to co-localize with *Vg1* RNA.
48. Mouland, A. J. *et al.* RNA trafficking signals in human immunodeficiency virus type 1. *Mol. Cell. Biol.* **21**, 2133–2143 (2001).
49. Carson, J. H., Cui, H. & Barbarese, E. The balance of power in RNA trafficking. *Curr. Opin. Neurobiol.* **11**, 558–563 (2001).
50. Ostareck, D. H. *et al.* mRNA silencing in erythroid differentiation: hnRNP K and hnRNP E1 regulate 15-lipoxygenase translation from the 3′ end. *Cell* **89**, 597–606 (1997).
51. Collier, B., Goobar-Larsson, L., Sokolowski, M. & Schwartz, S. Translational inhibition *in vitro* of human papillomavirus type 16 *L2* mRNA mediated through interaction with heterogenous ribonucleoprotein K and poly(rC)-binding proteins 1 and 2. *J. Biol. Chem.* **273**, 22648–22656 (1998).
52. Silvera, D., Gamarnik, A. V. & Andino, R. The N-terminal K homology domain of the poly(rC)-binding protein is a major determinant for binding to the poliovirus 5′-untranslated region and acts as an inhibitor of viral translation. *J. Biol. Chem.* **274**, 38163–38170 (1999).
53. Ostareck, D. H., Ostareck-Lederer, A., Shatsky, I. N. & Hentze, M. W. Lipoxygenase mRNA silencing in erythroid differentiation: the 3′ UTR regulatory complex controls 60S ribosomal subunit joining. *Cell* **104**, 281–290 (2001).
***LOX* mRNA translation is controlled by the differentiation control element in the 3′ untranslated region and by hnRNPs K and E1. This paper shows that the crucial step is joining of the 60S subunit to mRNA.**
54. Kiledjian, M., Wang, X. & Liebhaber, S. A. Identification of two KH domain proteins in the α-globin mRNP stability complex. *EMBO J.* **14**, 4357–4364 (1995).
55. Kiledjian, M., DeMaria, C. T., Brewer, G. & Novick, K. Identification of AUF1 (heterogeneous nuclear ribonucleoprotein D) as a component of the α-globin mRNA stability complex. *Mol. Cell. Biol.* **17**, 4870–4876 (1997).
56. Rajagopalan, L. E., Westmark, C. J., Jarzembowski, J. A. & Malter, J. S. hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA. *Nucleic Acids Res.* **26**, 3418–3423 (1998).
57. Chkheidze, A. N. *et al.* Assembly of the α-globin mRNA stability complex reflects binary interaction between the pyrimidine-rich 3′ untranslated region determinant and poly(C) binding protein αCP. *Mol. Cell. Biol.* **19**, 4572–4581 (1999).
58. Shih, S. C. & Claffey, K. P. Regulation of human vascular endothelial growth factor mRNA stability in hypoxia by heterogeneous nuclear ribonucleoprotein L. *J. Biol. Chem.* **274**, 1359–1365 (1999).
59. Loflin, P., Chen, C. Y. & Shyu, A. B. Unraveling a cytoplasmic role for hnRNP D in the *in vivo* mRNA destabilization directed by the AU-rich element. *Genes Dev.* **13**, 1884–1897 (1999).
60. Xu, N., Chen, C. Y. & Shyu, A. B. Versatile role for hnRNP D isoforms in the differential regulation of cytoplasmic mRNA turnover. *Mol. Cell. Biol.* **21**, 6960–6971 (2001).
61. Pinol-Roma, S. & Dreyfuss, G. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. *Nature* **355**, 730–732 (1992).
This paper shows that several of the abundant hnRNP proteins, such as A1, shuttle rapidly and continuously between the nucleus and the cytoplasm. It further shows that these shuttling proteins are also bound to mRNAs in the cytoplasm, and proposes functions for shuttling proteins in mRNA export, mRNA function in the cytoplasm, and nucleo-cytoplasmic signal transduction.
62. Alzhanova-Ericsson, A. T. *et al.* A protein of the SR family of splicing factors binds extensively to exonic Balbiani ring pre-mRNA and accompanies the RNA from the gene to the nuclear pore. *Genes Dev.* **10**, 2881–2893 (1996).
63. Caceres, J. F., Screaton, G. R. & Krainer, A. R. A specific subset of SR proteins shuttles continuously between the nucleus and the cytoplasm. *Genes Dev.* **12**, 55–66 (1998).
This paper shows that some, but not all, of the SR proteins shuttle between the nucleus and the cytoplasm.
64. Burd, C. G., Matunis, E. L. & Dreyfuss, G. The multiple RNA-binding domains of the mRNA poly(A)-binding protein have different RNA-binding activities. *Mol. Cell. Biol.* **11**, 3419–3424 (1991).
65. Imataka, H., Gradi, A. & Sonenberg, N. A newly identified N-terminal amino acid sequence of human eIF4G binds poly(A)-binding protein and functions in poly(A)-dependent translation. *EMBO J.* **17**, 7480–7489 (1998).
66. Tarun, S. Z. Jr & Sachs, A. B. A common function for mRNA 5′ and 3′ ends in translation initiation in yeast. *Genes Dev.* **9**, 2997–3007 (1995).
67. Piron, M., Vende, P., Cohen, J. & Poncet, D. Rotavirus RNA-binding protein NSP3 interacts with eIF4GI and evicts the poly(A) binding protein from eIF4F. *EMBO J.* **17**, 5811–5821 (1998).
68. Visa, N. *et al.* A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. *Cell* **84**, 253–264 (1996).
This study describes how electron microscopy with Balbiani ring pre-mRNA for *C. tentans* was used to describe hrp36, which is similar to the mammalian hnRNP A1. When hrp36 is added to Balbiani ring RNA during transcription, it stays on the nucleoplasmic Balbiani ring particles, and remains associated with Balbiani ring RNA after export.
69. Hirose, Y. & Manley, J. L. RNA polymerase II and the integration of nuclear events. *Genes Dev.* **14**, 1415–1429 (2000).
70. Shatkin, A. J. & Manley, J. L. The ends of the affair: capping and polyadenylation. *Nature Struct. Biol.* **7**, 838–842 (2000).
71. Wu, Z. A., Murphy, C., Callan, H. G. & Gall, J. G. Small nuclear ribonucleoproteins and heterogeneous nuclear ribonucleoproteins in the amphibian germinal vesicle: loops, spheres, and snurposomes. *J. Cell Biol.* **113**, 465–483 (1991).
72. Daneholt, B. Assembly and transport of a premessenger RNP particle. *Proc. Natl Acad. Sci. USA* **98**, 7012–7017 (2001).
73. Visa, N., Izaurralde, E., Ferreira, J., Daneholt, B. & Mattaj, I. W. A nuclear cap-binding complex binds Balbiani ring pre-mRNA cotranscriptionally and accompanies the ribonucleoprotein particle during nuclear export. *J. Cell Biol.* **133**, 5–14 (1996).
74. Sun, X. *et al.* The hrp23 protein in the balbiani ring pre-mRNP particles is released just before or at the binding of the particles to the nuclear pore complex. *J. Cell Biol.* **142**, 1181–1193 (1998).
75. Luo, M. J. & Reed, R. Splicing is required for rapid and efficient mRNA export in metazoans. *Proc. Natl Acad. Sci. USA* **96**, 14937–14942 (1999).

The authors show that spliced mRNAs are exported at a higher rate than intronless mRNAs, and that the spliced mRNP complex has a different protein composition from that of the intronless mRNP complex. This indicates the presence of an export-promoting factor(s) that is recruited to the mRNA by the process of splicing.

76. Le Hir, H., Moore, M. J. & Maquat, L. E. Pre-mRNA splicing alters mRNP composition: evidence for stable association of proteins at exon–exon junctions. *Genes Dev.* **14**, 1098–1108 (2000).
The authors use an ultraviolet-induced crosslinking technique for mRNAs that contain a single photoreactive group and ^{32}P at exon–exon junctions. After *in vitro* splicing and crosslinking, only proteins that are bound to the junction can be labelled. The results show that splicing recruits a new set of proteins near the exon–exon junction.
77. Blencowe, B. J., Issner, R., Nickerson, J. A. & Sharp, P. A. A coactivator of pre-mRNA splicing. *Genes Dev.* **12**, 996–1009 (1998).
78. Kataoka, N. *et al.* Pre-mRNA splicing imprints mRNA in the nucleus with a novel RNA-binding protein that persists in the cytoplasm. *Mol. Cell* **6**, 673–682 (2000).
This study shows that a new RNA-binding protein, Y14, binds preferentially to mRNAs that are produced by splicing both *in vitro* and *in vivo*. These results also show that pre-mRNA splicing results in the binding of specific proteins to the mRNAs. Binding of these proteins persists on the same mRNAs after export to the cytoplasm.
79. Le Hir, H., Izaurralde, E., Maquat, L. E. & Moore, M. J. The spliceosome deposits multiple proteins 20–24 nucleotides upstream of mRNA exon–exon junctions. *EMBO J.* **19**, 6860–6869 (2000).
This study identifies the binding site of mRNA-specific proteins (SRm160, DEK, Y14, Aly and RNPS1) on spliced mRNAs near exon–exon junctions, and further characterizes the multi-protein complex.
80. Kim, V. N. *et al.* The Y14 protein communicates to the cytoplasm the position of exon–exon junctions. *EMBO J.* **20**, 2062–2068 (2001).
81. Mayeda, A. *et al.* Purification and characterization of human RNPS1: a general activator of pre-mRNA splicing. *EMBO J.* **18**, 4560–4570 (1999).
82. Stutz, F. *et al.* REF, an evolutionarily conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. *RNA* **6**, 638–650 (2000).
The authors show that Mex67 directly interacts with Yra1, and that this interaction is evolutionarily conserved, as Yra1 can also interact with TAP, the mammalian homologue of Mex67. Using database searches with Yra1, it turned out that Yra1 belongs to an evolutionarily conserved family of hnRNP-like proteins across several species. These proteins were named REF-bps, as they bind to both RNA and export factors.
83. Kataoka, N., Diem, M. D., Kim, V. N., Yong, J. & Dreyfuss, G. Magoh, a human homolog of *Drosophila* mago nashi protein, is a component of the splicing-dependent exon–exon junction complex. *EMBO J.* **20**, 6424–6433 (2001).
This paper shows that a human homologue of *Drosophila* mago nashi protein is a new component of the exon–exon junction complex, and that it binds specifically to Y14. It further shows that magoh and Y14 bind the mRNA-export factor TAP.
84. Le Hir, H., Gatfield, D., Braun, I. C., Forler, D. & Izaurralde, E. The protein Mago provides a link between splicing and mRNA localization. *EMBO Rep.* **2**, 1119–1124 (2001).
85. Kim, V. N., Kataoka, N. & Dreyfuss, G. Role of the nonsense-mediated decay factor hUpf3 in the splicing-dependent exon–exon junction complex. *Science* **293**, 1832–1836 (2001).
This study shows that Upf3 is a bona fide component of the exon–exon junction complex, and provides a probable molecular link between splicing and an effector of nonsense-mediated mRNA decay.
86. Le Hir, H., Gatfield, D., Izaurralde, E. & Moore, M. J. The exon–exon junction complex provides a binding platform for factors involved in mRNA export and nonsense-mediated mRNA decay. *EMBO J.* **20**, 4987–4997 (2001).
This shows that both mRNA export factors (TAP and p15) and nonsense-mediated mRNA decay factors (Upf2 and 3) are enriched near exon–exon junctions, which indicates that the exon–exon junction complex might function in both pathways.
87. McGarvey, T. *et al.* The acute myeloid leukemia-associated protein, DEK, forms a splicing-dependent interaction with exon-product complexes. *J. Cell Biol.* **150**, 309–320 (2000).
88. Zhou, Z. *et al.* The protein Aly links pre-messenger-RNA splicing to nuclear export in metazoans. *Nature* **407**, 401–405 (2000).
This study reports direct evidence for a function for Aly/REF in mRNA export based on oocyte-injection studies. Now it seems, however, that Aly/REF might not be the only component of the exon–exon junction complex that has this activity, and also that Aly/REF might also facilitate the export of intronless mRNAs.
89. Rodrigues, J. P. *et al.* REF proteins mediate the export of spliced and unspliced mRNAs from the nucleus. *Proc. Natl Acad. Sci. USA* **98**, 1030–1035 (2001).
90. Lykke-Andersen, J., Shu, M. D. & Steitz, J. A. Communication of the position of exon–exon junctions to the mRNA surveillance machinery by the protein RNPS1. *Science* **293**, 1836–1839 (2001).
RNPS1 and, to a lesser extent, Y14 are found to have a role in nonsense-mediated mRNA decay, presumably through their interactions with Upf3.
91. Mohr, S. E., Dillon, S. T. & Boswell, R. E. The RNA-binding protein Tsunagi interacts with Mago Nashi to establish polarity and localize *oskar* mRNA during *Drosophila* oogenesis. *Genes Dev.* **15**, 2886–2899 (2001).
92. Hachet, O. & Ephrussi, A. *Drosophila* Y14 shuttles to the posterior of the oocyte and is required for *oskar* mRNA transport. *Curr. Biol.* **11**, 1666–1674 (2001).
93. Gorlich, D. & Kutay, U. Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* **15**, 607–660 (1999).
94. Mattaj, I. W. & Englmeier, L. Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.* **67**, 265–306 (1998).
95. Nakielny, S. & Dreyfuss, G. Transport of proteins and RNAs in and out of the nucleus. *Cell* **99**, 677–690 (1999).
96. Segref, A. *et al.* Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)+ RNA and nuclear pores. *EMBO J.* **16**, 3256–3271 (1997).
This paper identifies an essential cellular factor for nuclear mRNA export in yeast, called Mex67, through its genetic interaction with nucleoporin Nup85. In the thermosensitive *mex67* mutant, accumulation of poly(A) RNA in intranuclear foci is detected shortly after a shift to the restrictive temperature. These results strongly indicate that Mex67 is likely to participate directly in the export of mRNA from the nucleus to the cytoplasm.
97. Gruter, P. *et al.* TAP, the human homolog of Mex67p, mediates CTE-dependent RNA export from the nucleus. *Mol. Cell* **1**, 649–659 (1998).
The constitutive transport element (CTE) of the type D retroviruses has been shown to promote nuclear export of unspliced viral RNAs. This paper identifies TAP as the cellular factor that specifically binds to wild-type CTE and mediates CTE-dependent export. As the excess amount of CTE blocks mRNA export and TAP can overcome this effect, TAP was thought to have a function in mRNA export, like its yeast homologue Mex67.
98. Katahira, J. *et al.* The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. *EMBO J.* **18**, 2593–2609 (1999).
99. Yoon, J. H., Love, D. C., Guhathakurta, A., Hanover, J. A. & Dhar, R. Mex67p of *Schizosaccharomyces pombe* interacts with Rae1p in mediating mRNA export. *Mol. Cell. Biol.* **20**, 8767–8782 (2000).
100. Bear, J. *et al.* Identification of novel import and export signals of human TAP, the protein that binds to the constitutive transport element of the type D retrovirus mRNAs. *Mol. Cell. Biol.* **19**, 6306–6317 (1999).
101. Braun, I. C., Rohrbach, E., Schmitt, C. & Izaurralde, E. TAP binds to the constitutive transport element (CTE) through a novel RNA-binding motif that is sufficient to promote CTE-dependent RNA export from the nucleus. *EMBO J.* **18**, 1953–1965 (1999).
102. Kang, Y. & Cullen, B. R. The human Tap protein is a nuclear mRNA export factor that contains novel RNA-binding and nucleocytoplasmic transport sequences. *Genes Dev.* **13**, 1126–1139 (1999).
103. Tan, W., Zolotukhin, A. S., Bear, J., Patenaude, D. J. & Felber, B. K. The mRNA export in *Caenorhabditis elegans* is mediated by Ce-NXF-1, an ortholog of human TAP/NXF and *Saccharomyces cerevisiae* Mex67p. *RNA* **6**, 1762–1772 (2000).
104. Braun, I. C., Herold, A., Rode, M., Conti, E. & Izaurralde, E. Overexpression of TAP/p15 heterodimers bypasses nuclear retention and stimulates nuclear mRNA export. *J. Biol. Chem.* **276**, 20536–20543 (2001).
105. Guzik, B. W. *et al.* NXT1 (p15) is a crucial cellular cofactor in TAP-dependent export of intron-containing RNA in mammalian cells. *Mol. Cell. Biol.* **21**, 2545–2554 (2001).
106. Levesque, L. *et al.* RNA export mediated by tap involves NXT1-dependent interactions with the nuclear pore complex. *J. Biol. Chem.* **276**, 44953–44962 (2001).
107. Wiegand, H. L. *et al.* Formation of Tap/NXT1 heterodimers activates Tap-dependent nuclear mRNA export by enhancing recruitment to nuclear pore complexes. *Mol. Cell. Biol.* **22**, 245–256 (2002).
108. Herold, A. *et al.* TAP (NXF1) belongs to a multigene family of putative RNA export factors with a conserved modular architecture. *Mol. Cell. Biol.* **20**, 8996–9008 (2000).
109. Jun, L. *et al.* NXF5, a novel member of the nuclear RNA export factor family, is lost in a male patient with a syndromic form of mental retardation. *Curr. Biol.* **11**, 1381–1391 (2001).
110. Fan, X. C. & Steitz, J. A. HNS, a nuclear-cytoplasmic shuttling sequence in HuR. *Proc. Natl Acad. Sci. USA* **95**, 15293–15298 (1998).
111. Brown, J. A. *et al.* A mutation in the *Schizosaccharomyces pombe rae1* gene causes defects in poly(A)+ RNA export and in the cytoskeleton. *J. Biol. Chem.* **270**, 7411–7419 (1995).
112. Murphy, R., Watkins, J. L. & Wente, S. R. GLE2, a *Saccharomyces cerevisiae* homologue of the *Schizosaccharomyces pombe* export factor RAE1, is required for nuclear pore complex structure and function. *Mol. Biol. Cell* **7**, 1921–1937 (1996).
113. Bharathi, A. *et al.* The human *RAE1* gene is a functional homologue of *Schizosaccharomyces pombe rae1* gene involved in nuclear export of Poly(A)+ RNA. *Gene* **198**, 251–258 (1997).
114. Pritchard, C. E., Fornerod, M., Kasper, L. H. & van Deursen, J. M. RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains. *J. Cell Biol.* **145**, 237–254 (1999).
115. Snay-Hodge, C. A., Colot, H. V., Goldstein, A. L. & Cole, C. N. Dbp5p/Rat8p is a yeast nuclear pore-associated DEAD-box protein essential for RNA export. *EMBO J.* **17**, 2663–2676 (1998).
116. Tseng, S. S. *et al.* Dbp5p, a cytosolic RNA helicase, is required for poly(A)+ RNA export. *EMBO J.* **17**, 2651–2662 (1998).
117. Huang, Y. & Steitz, J. A. Splicing factors SRp20 and 9G8 promote the nucleocytoplasmic export of mRNA. *Mol. Cell* **7**, 899–905 (2001).
118. Yang, J., Bogerd, H. P., Wang, P. J., Page, D. C. & Cullen, B. R. Two closely related human nuclear export factors utilize entirely distinct export pathways. *Mol. Cell* **8**, 397–406 (2001).
119. Chang, D. D. & Sharp, P. A. Regulation by HIV Rev depends upon recognition of splice sites. *Cell* **59**, 789–795 (1989).
120. Legrain, P. & Rosbash, M. Some *cis*- and *trans*-acting mutants for splicing target pre-mRNA to the cytoplasm. *Cell* **57**, 573–583 (1989).
121. Custodio, N. *et al.* Inefficient processing impairs release of RNA from the site of transcription. *EMBO J.* **18**, 2855–2866 (1999).
122. Portman, D. S., O'Connor, J. P. & Dreyfuss, G. *YRA1*, an essential *Saccharomyces cerevisiae* gene, encodes a novel nuclear protein with RNA annealing activity. *RNA* **3**, 527–537 (1997).
123. Strasser, K. & Hurt, E. Yra1p, a conserved nuclear RNA-binding protein, interacts directly with Mex67p and is required for mRNA export. *EMBO J.* **19**, 410–420 (2000).
This paper shows that an RNA-binding protein Yra1 binds directly to Mex67. Mutants of *YRA1* are impaired in nuclear-poly(A)-RNA export at the restrictive temperature. These results indicate that Yra1 is the mRNA-export factor that bridges the export factor Mex67/Mtr2 to mRNA transport cargoes.
124. Luo, M. L. *et al.* Pre-mRNA splicing and mRNA export linked by direct interactions between UAP56 and Aly. *Nature* **413**, 644–647 (2001).
This paper shows that the conserved DEAD-box helicase UAP56, which is a splicing factor, interacts directly and specifically with Aly/REF. These data indicate that UAP56 can couple pre-mRNA splicing and mRNA export by recruiting Aly/REF to the spliced mRNP.
125. Strasser, K. & Hurt, E. Splicing factor Sub2p is required for nuclear mRNA export through its interaction with Yra1p. *Nature* **413**, 648–652 (2001).
This paper shows a genetic interaction in yeast between mRNA-export factor Yra1 and Sub2, a DEAD-box helicase involved in splicing. Sub2 and Mex67/Mtr2 bind to the same domains of Yra1, and compete with each other for binding. These results indicate that the splicing factor Sub2 (the yeast homologue of the mammalian UAP56) is also

important in mRNA export, probably by recruiting Yra1 to the mRNA.
126. Jensen, T. H., Boulay, J., Rosbash, M. & Libri, D. The DECD box putative ATPase Sub2p is an early mRNA export factor. *Curr. Biol.* **11**, 1711–1715 (2001).
127. Gatfield, D. *et al.* The DExH/D box protein HEL/UAP56 is essential for mRNA nuclear export in *Drosophila*. *Curr. Biol.* **11**, 1716–1721 (2001).
128. Micklem, D. R. *et al.* The *mago nashi* gene is required for the polarisation of the oocyte and the formation of perpendicular axes in *Drosophila*. *Curr. Biol.* **7**, 468–478 (1997).
129. Newmark, P. A., Mohr, S. E., Gong, L. & Boswell, R. E. mago nashi mediates the posterior follicle cell-to-oocyte signal to organize axis formation in *Drosophila*. *Development* **124**, 3197–3207 (1997).
130. Zhao, X. F., Nowak, N. J., Shows, T. B. & Aplan, P. D. MAGOH interacts with a novel RNA-binding protein. *Genomics* **63**, 145–148 (2000).
131. Mingot, J. M., Kostka, S., Kraft, R., Hartmann, E. & Gorlich, D. Importin 13: a novel mediator of nuclear import and export. *EMBO J.* **20**, 3685–3694 (2001).
132. Hentze, M. W. & Kulozik, A. E. A perfect message: RNA surveillance and nonsense-mediated decay. *Cell* **96**, 307–310 (1999).
133. Wilusz, C. J., Wang, W. & Peltz, S. W. Curbing the nonsense: the activation and regulation of mRNA surveillance. *Genes Dev.* **15**, 2781–2785 (2001).
134. Neu-Yilik, G. *et al.* Splicing and 3′ end formation in the definition of nonsense-mediated decay-competent human β-globin mRNPs. *EMBO J.* **20**, 532–540 (2001).
By using human β-globin mRNA as a model system, this paper shows that the formation of nonsense-mediated mRNA decay-competent mRNP particles in higher eukaryotes depends on splicing, but does not require the presence of a poly(A) tail.
135. Cheng, J., Belgrader, P., Zhou, X. & Maquat, L. E. Introns are *cis* effectors of the nonsense-codon-mediated reduction in nuclear mRNA abundance. *Mol. Cell. Biol.* **14**, 6317–6325 (1994).
136. Carter, M. S., Li, S. & Wilkinson, M. F. A splicing-dependent regulatory mechanism that detects translation signals. *EMBO J.* **15**, 5965–5975 (1996).
137. Thermann, R. *et al.* Binary specification of nonsense codons by splicing and cytoplasmic translation. *EMBO J.* **17**, 3484–3494 (1998).
138. Sun, X., Moriarty, P. M. & Maquat, L. E. Nonsense-mediated decay of glutathione peroxidase 1 mRNA in the cytoplasm depends on intron position. *EMBO J.* **19**, 4734–4744 (2000).
This paper shows that a premature termination codon elicits nonsense-mediated mRNA decay when it is located more than 50–55 nt upstream of the last exon–exon junction.
139. Nagy, E. & Maquat, L. E. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Biochem. Sci.* **23**, 198–199 (1998).
140. Qian, L., Vu, M. N., Carter, M. S., Doskow, J. & Wilkinson, M. F. T cell receptor-β mRNA splicing during thymic maturation *in vivo* and in an inducible T cell clone *in vitro*. *J. Immunol.* **151**, 6801–6814 (1993).
141. Belgrader, P., Cheng, J. & Maquat, L. E. Evidence to implicate translation by ribosomes in the mechanism by which nonsense codons reduce the nuclear level of human triosephosphate isomerase mRNA. *Proc. Natl Acad. Sci. USA* **90**, 482–486 (1993).
142. Li, S., Leonard, D. & Wilkinson, M. F. T cell receptor (*TCR*) mini-gene mRNA expression regulated by nonsense codons: a nuclear-associated translation-like mechanism. *J. Exp. Med.* **185**, 985–992 (1997).
143. Naeger, L. K., Schoborg, R. V., Zhao, Q., Tullis, G. E. & Pintel, D. J. Nonsense mutations inhibit splicing of *MVM* RNA in *cis* when they interrupt the reading frame of either exon of the final spliced product. *Genes Dev.* **6**, 1107–1119 (1992).
144. Dietz, H. C. & Kendzior, R. J. Jr. Maintenance of an open reading frame as an additional level of scrutiny during splice site selection. *Nature Genet.* **8**, 183–188 (1994).
145. Li, S. & Wilkinson, M. F. Nonsense surveillance in lymphocytes? *Immunity* **8**, 135–141 (1998).
146. Gersappe, A. & Pintel, D. J. A premature termination codon interferes with the nuclear function of an exon splicing enhancer in an open reading frame-dependent manner. *Mol. Cell. Biol.* **19**, 1640–1650 (1999).
147. Moriarty, P. M., Reddy, C. C. & Maquat, L. E. Selenium deficiency reduces the abundance of mRNA for Se-dependent glutathione peroxidase 1 by a UGA-dependent mechanism likely to be nonsense codon-mediated decay of cytoplasmic mRNA. *Mol. Cell. Biol.* **18**, 2932–2939 (1998).
148. Lund, E. & Dahlberg, J. E. Proofreading and aminoacylation of tRNAs before export from the nucleus. *Science* **282**, 2082–2085 (1998).
149. Dostie, J., Lejbkowicz, F. & Sonenberg, N. Nuclear eukaryotic initiation factor 4E (eIF4E) colocalizes with splicing factors in speckles. *J. Cell Biol.* **148**, 239–247 (2000).
150. Iborra, F. J., Jackson, D. A. & Cook, P. R. Coupled transcription and translation within nuclei of mammalian cells. *Science* **293**, 1139–1142 (2001).
151. Ishigaki, Y., Li, X., Serin, G. & Maquat, L. E. Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. *Cell* **106**, 607–617 (2001).
152. Czaplinski, K. *et al.* The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs. *Genes Dev.* **12**, 1665–1677 (1998).
153. Leeds, P., Peltz, S. W., Jacobson, A. & Culbertson, M. R. The product of the yeast *UPF1* gene is required for rapid turnover of mRNAs containing a premature translational termination codon. *Genes Dev.* **5**, 2303–2314 (1991).
154. Cui, Y., Hagan, K. W., Zhang, S. & Peltz, S. W. Identification and characterization of genes that are required for the accelerated degradation of mRNAs containing a premature translational termination codon. *Genes Dev.* **9**, 423–436 (1995).
155. Gonzalez, C. I., Ruiz-Echevarria, M. J., Vasudevan, S., Henry, M. F. & Peltz, S. W. The yeast hnRNP-like protein Hrp1/Nab4 marks a transcript for nonsense-mediated mRNA decay. *Mol. Cell* **5**, 489–499 (2000).
156. Perlick, H. A., Medghalchi, S. M., Spencer, F. A., Kendzior, R. J. Jr & Dietz, H. C. Mammalian orthologues of a yeast regulator of nonsense transcript stability. *Proc. Natl Acad. Sci. USA* **93**, 10928–10932 (1996).
157. Mendell, J. T., Medghalchi, S. M., Lake, R. G., Noensie, E. N. & Dietz, H. C. Novel Upf2p orthologues suggest a functional link between translation initiation and nonsense surveillance complexes. *Mol. Cell. Biol.* **20**, 8944–8957 (2000).
158. Serin, G., Gersappe, A., Black, J. D., Aronoff, R. & Maquat, L. E. Identification and characterization of human orthologues to *Saccharomyces cerevisiae* Upf2 protein and Upf3 protein (*Caenorhabditis elegans* SMG-4). *Mol. Cell. Biol.* **21**, 209–223 (2001).
159. Dietz, H. C. *et al.* The skipping of constitutive exons *in vivo* induced by nonsense mutations. *Science* **259**, 680–683 (1993).
160. Lozano, F., Maertzdorf, B., Pannell, R. & Milstein, C. Low cytoplasmic mRNA levels of immunoglobulin κ light chain genes containing nonsense codons correlate with inefficient splicing. *EMBO J.* **13**, 4617–4622 (1994).
161. Aoufouchi, S., Yelamos, J. & Milstein, C. Nonsense mutations inhibit RNA splicing in a cell-free system: recognition of mutant codon is independent of protein synthesis. *Cell* **85**, 415–422 (1996).
162. Muhlemann, O. *et al.* Precursor RNAs harboring nonsense codons accumulate near the site of transcription. *Mol. Cell* **8**, 33–43 (2001).
163. Reed, R. & Magni, K. A new view of mRNA export: separating the wheat from the chaff. *Nature Cell Biol.* **3**, E201–E204 (2001).

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