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A Conserved mRNA Export Machinery Coupled to pre-mRNA Splicing

Review

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Summary

Recent advances have led to a new understanding of how mRNAs are exported from the nucleus to the cytoplasm. This process requires a heterodimeric mRNA export receptor that is part of an elaborate machinery conserved from yeast to humans. Export of mRNAs is coupled to upstream steps in gene expression, such as pre-mRNA splicing, and to downstream events, including nonsense-mediated decay.

The nuclear and cytoplasmic compartmentalization of different steps in gene expression is a fundamental property of eukaryotic cells. As a consequence, a high volume of RNA and protein traffic flows between these compartments. Nucleocytoplasmic transport occurs through numerous nuclear pore complexes that span the nuclear envelope. These complexes, which consist of proteins known as nucleoporins, contain a central channel through which the cargo is transported. In its closed position, the channel is ~9 nm in diameter, but can expand to a diameter of 26 nm during active translocation of cargo. Other features of the nuclear pore complex include the nuclear basket, nuclear and cytoplasmic rings, short cytoplasmic fibrils, and a central spoke complex (Vasu and Forbes, 2001). Transport through the channel requires binding of the cargo to soluble transport receptors. These receptors mediate transport by interacting with the phenylalanine-glycine (FG) repeats of a class of nucleoporins that line the channel of the nuclear pore complex (Rout et al., 2000; Stewart et al., 2001).

A general paradigm for nuclear transport was established primarily through studies of protein import and export (Mattaj and Englmeier, 1998; Pemberton et al., 1998; Görlich and Kutay, 1999). These studies revealed that transport of most cargoes requires members of the conserved family of transport receptors called "karyopherins" (also known as importins/exportins). These receptors interact with protein cargoes via specific import and export signals, designated nuclear localization signals (NLSs), and nuclear export signals (NESs). A hallmark of karyopherins is their requirement for the small GTPase Ran, which regulates cargo/receptor interactions. For import, the cargo and importin are translocated into the nucleus. The cargo is then released from the receptor upon binding of RanGTP. For export, the cargo and exportin bind cooperatively to RanGTP. After this ternary complex is translocated to the cytoplasm, the cargo is released concomitant with hydrolysis of the GTP on Ran. Thus, import and export are essentially the reverse of each other, with the directionality maintained by the presence of RanGTP in the nucleus and RanGDP in the cytoplasm.

The best characterized exportin, exportin-1, is required for export of proteins. This receptor binds directly to "leucine-rich" NESs in the protein cargoes and mediates their export (Mattaj and Englmeier, 1998). Karyopherin family members are also required for transport of several classes of RNAs, including tRNA, rRNA, and snRNAs (Nakielny and Dreyfuss, 1999). tRNA is exported by a mechanism involving direct binding to its receptor, exportin-t (Görlich and Kutay, 1999). In contrast, rRNA, which requires exportin-1, is exported in pre-ribosomal particles containing ribosomal proteins, several rRNA species, and non-ribosomal proteins (Warner, 2001). Finally, snRNAs are transiently transported to the cytoplasm for assembly into snRNP particles and require exportin-1 for export (Ohno et al., 2000).

Unlike the transport processes discussed above, a karyopherin family member that functions in general mRNA export has not been identified. Until recently, the primary candidates for mRNA export factors were the highly abundant hnRNP proteins, some of which shuttle between the nucleus and cytoplasm. The shuttling hnRNP proteins were proposed to bind to mRNAs and mediate their export. However, new studies in yeast and metazoans have shifted the focus of attention to several highly conserved proteins that are exclusively required for mRNA export. Surprisingly, karyopherins and Ran are not among them.

In addition to providing a new understanding of the general mechanisms for mRNA export, recent studies in metazoans led to the unexpected finding that components of this conserved mRNA export machinery physically and functionally couple splicing to mRNA export. The discovery of this link in turn has led to a new understanding of the mechanism for coupling splicing to nonsense-mediated decay (NMD), an mRNA surveillance mechanism used to degrade mRNAs containing premature stop codons. In contrast to metazoans, most *S. cerevisiae* genes lack introns, and it is not yet known how the conserved mRNA export machinery is recruited to these mRNAs. Studies over the past several years indicate that there is extensive coupling between the different steps in gene expression. Indeed, there is already evidence that transcription is involved in recruiting export factors to pre-mRNAs, and this may be the case for mRNAs derived from both intron-containing and intron-lacking pre-mRNAs. Other steps in pre-mRNA processing, such as polyadenylation, may also play a role in recruiting the export machinery to both types of mRNAs.

Here, we review recent work on the key players in mRNA export and then discuss how these factors couple export to splicing and NMD. In addition, we briefly dis-

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cuss coupling of mRNA export to other steps in the gene expression pathway.

Key Players

A Conserved mRNA Export Receptor?

The best candidate for an mRNA export receptor is the protein Mex67, whose conserved metazoan counterpart is known as Tap or NXF. Mex67 was first detected in yeast via a synthetic lethal screen with Nup85, a nucleoporin that functions in mRNA export (Segref et al., 1997). Mutants of the Mex67 gene result in rapid accumulation of poly(A)$^+$ RNA in the nucleus, and the Mex67 protein interacts with both poly(A)$^+$ RNA and nuclear pore complexes. Mex67 forms a heterodimer with a small protein designated Mtr2 (Kadowaki et al., 1994), and this Mex67-Mtr2 heterodimer is essential for mRNA export (Santos-Rosa et al., 1998). The metazoan ortholog of Mtr2 is known as p15 or NXT. For simplicity, we will refer to the metazoan heterodimer as Tap-p15.

Evidence that Tap is a key player in mRNA export in metazoans was first provided by studies of the constitutive transport element (CTE), an RNA element required for export of unspliced genomic retroviral RNA (Grüter et al., 1998 and references therein). These studies revealed that Tap binds directly to the CTE and mediates its export (Grüter et al., 1998). Moreover, excess CTE blocks mRNA export, presumably by titrating Tap. Subsequent work showed that the Tap-p15 heterodimer directly stimulates the export of cellular mRNAs, confirming its role in mRNA export (Katahira et al., 1999; Braun et al., 2001; Guzik et al., 2001). Both Mex67-Mtr2 and Tap-p15 also shuttle between the nucleus and cytoplasm (Santos-Rosa et al., 1998; Bear et al., 1999; Kang and Cullen, 1999; Katahira et al., 1999; Schmitt and Gerace, 2001). This observation, together with the finding that the conserved heterodimer interacts directly with the nuclear pore complex and (directly or indirectly) with the mRNA export cargo, strongly suggests that this factor is a general mRNA export receptor. In contrast to karyopherins, the Mex67/Tap-Mtr2/p15 heterodimer does not require the GTPase Ran (Clouse et al., 2001). Below, we will refer to this conserved complex as the mRNA export heterodimer.

Mex67/Tap is a member of a family of structurally related proteins that are distinct from Ran-dependent karyopherins (Herold et al., 2000). Both classes of receptors have in common binding directly to the phenylalanine residues of FG repeat nucleoporins that line the pore channel (Conti and Izaurralde, 2001). Mex67/Tap consists of three conserved modules: (1) a leucine-rich repeat (LRR) domain, (2) a middle (M) domain with an NTF2-fold (NTF2 is an import receptor for RanGDP), and (3) a carboxyl (C) domain with a UBA (ubiquitin-associated) fold (Figure 1A) (Conti and Izaurralde, 2001). The p15 protein also contains an NTF2-fold (Fribourg et al., 2001).

The C and M domains of Mex67/Tap function in shuttling and contact the FG nucleoporins (Bear et al., 1999; Kang and Cullen, 1999; Katahira et al., 1999; Bachi et al., 2000; Strasser et al., 2000; Tan et al., 2000; Fribourg et al., 2001; Schmitt and Gerace, 2001). The C domain competes with karyopherins for binding to FG nucleoporins, indicating that both types of receptors may use the same binding sites for translocation through the pores (Bear et al., 1999; Bachi et al., 2000; Schmitt and Gerace, 2001). Insights into how Tap-p15 interacts with FG nucleoporins were provided by recent structural work. These studies revealed that the NTF2-folds in Tap and p15 interact with each other (Fribourg et al., 2001). Interestingly, this complex resembles the homodimeric import receptor NTF2 (Bullock et al., 1996). The FG repeats in nucleoporins bind to a hydrophobic pocket on the surface of the NTF2 homodimer (Bayliss et al., 2000). Likewise, FG repeats bind to an analogous hydrophobic cavity present on the M domain of Tap (Fribourg et al., 2001). A hydrophobic patch present on the surface of the C domain of Tap is thought to constitute the second FG nucleoporin binding site found in the mRNA export heterodimer (R.Grant, submitted).

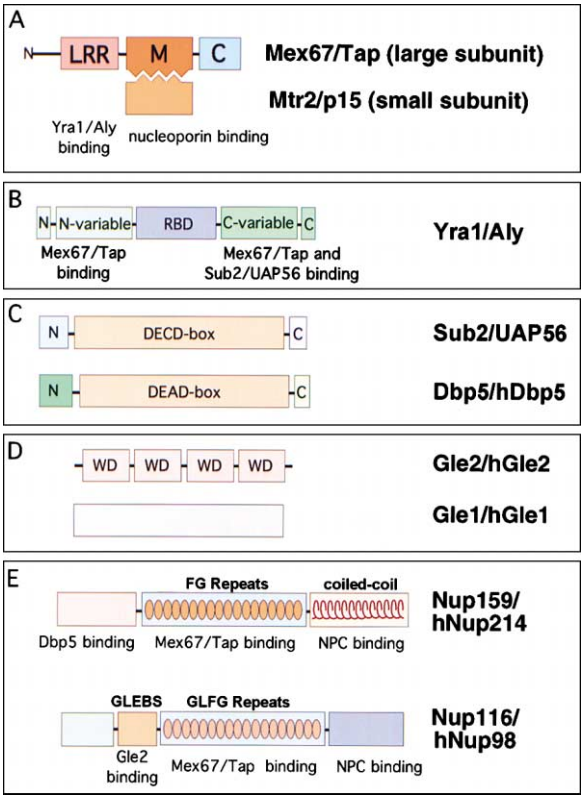

Figure 1. Conserved mRNA Export Factors

(A) The large (Mex67/Tap) and small subunits (Mtr2/p15) of the mRNA export heterodimer are indicated. LRR: leucine rich repeat, M: middle domain, C: C-terminal domain. Amino-terminal extensions of variable length in different family members are indicated by a line. The regions where Yra1/Aly and nucleoporins bind are shown.

(B) Yra1/Aly (REF family). RBD: RNA binding domain, N and C: highly conserved amino- and carboxy-terminal motifs, respectively. N-variable and C-variable: degenerate regions of variable length. The regions where the heterodimer and Sub2/UAP56 bind are indicated.

(C) Dead box RNA helicases. Sub2/UAP56 has a DECD, Dbp5/hDbp5 a DEAD box motif. N and C: N- and C-terminal extensions.

(D) Gle1 and Gle2: Gle2 contains 4 WD-repeats. No motifs are present in the conserved export factor Gle1.

(E) Nucleoporins: Nup159/hNup214 showing FG repeats and Nup116/hNup98 showing GLFG repeats. GLEBS: GLE2 binding sequence. The regions where nucleoporins bind to Dbp5, the heterodimer, and nuclear pore complexes (NPC) are indicated.

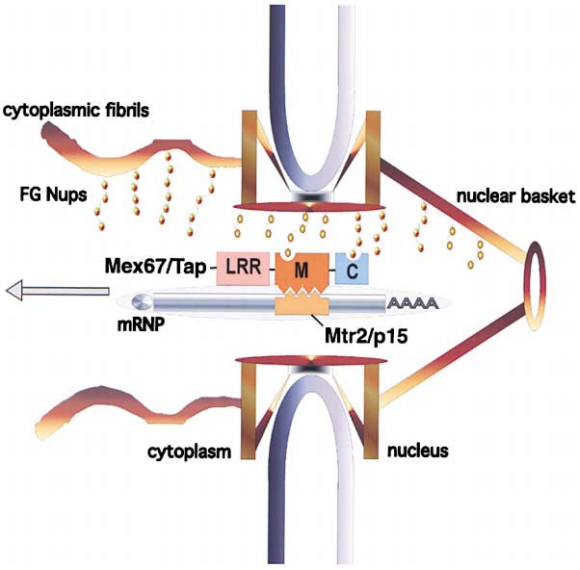

Figure 2. Model for mRNP Transport through the Nuclear Pore Complex

FG Nups: FG repeat-containing nucleoporins. For simplicity, FG Nups were omitted on the lower half of the nuclear pore complex. Mex67/Tap with LRR, M and C domain, and Mtr2/p15 are indicated. mRNP: mRNA (grey cylinder) with 5′ cap (circle) and poly(A) tail. Other RNA-binding proteins bound to the mRNA are indicated by a gray shell surrounding the mRNA. Please note that the drawing is not to scale.

As shown in Figure 2, current models for translocation of cargoes through the nuclear pore channel propose that the consecutive array of FG nucleoporins provides transient docking sites, or "stepping stones," for moving the receptor/cargo complex through the pore (Bayliss et al., 2000; Rout et al., 2000; Allen et al., 2001; Conti and Izaurralde, 2001). In the case of transport of the mRNP, the FG repeats would contact the M and C domains of Mex67/Tap as described above (Figure 2). Certain conserved FG nucleoporins play a role in mRNA export (Vasu and Forbes, 2001 and references therein). Of these, some are located exclusively on the cytoplasmic fibrils whereas others are primarily associated with the nuclear basket. Thus, transport via the mRNA export heterodimer may involve docking to the nucleoporins on the nuclear basket and release from the nucleoporins on cytoplasmic fibrils (Figure 2).

In principle, the mRNA export heterodimer can translocate across the nuclear pores in both directions, and it remains to be determined how directionality of mRNA export is established. Considering that Ran does not appear to play a role in general mRNA export, the Ran-GTP/GDP gradient that is thought to impart directionality on the karyopherins is not likely to be involved. Assuming that nuclear mRNA export follows a receptor-mediated diffusion mechanism, a concentration gradient with a higher amount of mRNA in the nucleus than in the cytoplasm could drive vectorial mRNP translocation. Electron microscopic studies of the giant Balbiani ring mRNAs from *Chironomous tetans* show that mRNAs exit the pore and first enter the cytoplasm with the 5′ cap leading (Daneholt, 1997). These studies also revealed that ribosomes associate with the mRNA immediately after it enters the cytoplasm. Thus, association of the mRNA with the translational machinery would decrease the pool of free cytoplasmic mRNAs and facilitate diffusion. Another possibility is that the mRNP undergoes conformational changes and/or dissociates from mRNA export factors as it exits the pore and enters the cytoplasm, and these changes could be a driving force for vectorial translocation (for discussion, see Snay-Hodge et al., 1998; Tseng et al., 1998).

Although the conserved mRNA export heterodimer appears to be required for transport of bulk poly(A)$^+$ mRNA (Segref et al., 1997; Tan et al., 2000; Gallouzi and Steitz, 2001), it is likely that other mechanisms for mRNA export exist. Indeed, some exceptions have already been reported in metazoans. One case involves a Tap family member (NXF3) that is expressed in human testes and is thought to play a role in male germ cell development (Yang et al., 2001). The NXF3 protein lacks the C-terminal domain that is typically required for transport in Tap family members. Instead, NXF3 contains an NES and is thought to be exported by the karyopherin exportin-1 and RanGTP (Yang et al., 2001). A second case involves mRNAs that rapidly turn over due to the presence of an AU-rich element in their 3′UTR (e.g. proto-oncogene, cytokine, and lymphokine mRNAs). One of these mRNAs, *c-fos*, is thought to be exported via two distinct pathways, both involving karyopherin family members (exportin-1 and transportin-2) (Brennan et al., 2000; Gallouzi and Steitz, 2001). Considering the complexity of metazoan gene expression, further studies are needed to determine how widely this conserved mRNA export the heterodimer is used and the significance of the exceptions.

Yra1/Aly—A Coupling Protein

Another key player in mRNA export is the conserved nuclear protein known as Yra1 in yeast and Aly in metazoans. Yra1 was first discovered as an essential factor with RNA binding and annealing activity (Portman et al., 1997). This protein was subsequently identified as an mRNA export factor in a screen for proteins that interact genetically with Mex67 (Strasser and Hurt, 2000). Additional studies revealed that Yra1 also interacts physically with Mex67 (Strasser and Hurt, 2000; Stutz et al., 2000). The protein Aly was first discovered as a cofactor of two transcription factors (LEF-1 and AML) (Bruhn et al., 1997) and soon after as a protein chaperone designated BEF (Virbasius et al., 1999). Subsequently, Aly (also renamed REF) was shown to interact directly with Tap, suggesting a conserved role for Yra1/Aly in mRNA export (Stutz et al., 2000). Direct functional evidence that Aly is an mRNA export factor in metazoans was provided by the observation that recombinant Aly promotes export of mRNA in *Xenopus* oocytes (Zhou et al., 2000; Rodrigues et al., 2001). In addition, microinjection of antibodies to Aly blocks mRNA export without affecting other transport pathways (Rodrigues et al., 2001). Although Aly is primarily nuclear, it shuttles between the nucleus and cytoplasm, consistent with a nuclear export function (Zhou et al., 2000; Rodrigues et al., 2001).

Additional insight into the function of Aly came from the observation that pre-mRNA splicing in metazoans is directly coupled to mRNA export, and Aly plays a key role in this coupling. Evidence that splicing and export

are coupled was initially provided by the observation that spliced mRNAs assemble into a distinct complex that does not assemble on cDNA transcripts, and this "spliced mRNP" enhances mRNA export (Luo and Reed, 1999). Subsequent characterization of the spliced mRNP revealed that it specifically recruits Aly, providing an explanation for the enhanced mRNA export (Zhou et al., 2000; Le Hir et al., 2001). Consistent with the observation that Aly is recruited to mRNAs during splicing, studies in mammalian cells reveal that Aly colocalizes with pre-mRNA splicing factors in nuclear "speckles" which are storage sites for components of the pre-mRNA processing machinery (Zhou et al., 2000; Rodrigues et al., 2001 and references therein). When present in excess, recombinant Aly not only promotes export of spliced mRNAs but also promotes export of cDNA transcripts and random-sequence RNAs (Zhou et al., 2000; Rodrigues et al., 2001). In contrast, at normal physiological levels, Aly is most efficiently recruited to mRNAs during splicing (Zhou et al., 2000). Thus, splicing-dependent recruitment of Aly may function as a quality control mechanism to reduce export of mutant or otherwise inappropriate RNA export substrates.

Recent studies have also revealed that several components of the spliced mRNP, including Aly and possibly the Tap-p15 heterodimer, form a stable complex located ~20 nucleotides upstream of the exon-exon junction (Le Hir et al., 2000, 2001). This exon junction complex (EJC) was shown to be the component of the spliced mRNP that promotes mRNA export (Le Hir et al., 2001). Remarkably, these studies also revealed that the EJC contains several factors involved in NMD, providing significant new insights into the mechanism of this process (see below) (Kim et al., 2001a, 2001b; Le Hir et al., 2001; Lykke-Andersen et al., 2001).

Yra1 and Aly are members of a conserved protein family (Strasser and Hurt, 2000), designated the REF family (Stutz et al., 2000). Some REF family members have distinct functions while other members appear to be redundant (Rodrigues et al., 2001; Zenklusen et al., 2001). As shown in Figure 1B, REF family members have a central RNA binding domain (RBD) flanked by degenerate RGG-rich regions of variable length. The short N and C termini of REF family members are similar in sequence and are highly conserved. In vitro studies indicate that the termini serve as two separate binding sites for Mex67/Tap (Rodrigues et al., 2001; Strasser and Hurt, 2001; Zenklusen et al., 2001). They also bind to RNA in vitro and mediate multimerization of the REF proteins (Virbasius et al., 1999; Rodrigues et al., 2001; Zenklusen et al., 2001).

Sub2/UAP56—A Spliceosome Component and mRNA Export Factor

Recent studies revealed that Yra1/Aly forms a stoichiometric complex with a conserved protein designated Sub2 in yeast (Strasser and Hurt, 2001) and UAP56 in mammals (Luo et al., 2001), respectively. Sub2/UAP56 is a member of the DEAD box family of ATP-dependent helicases (Figure 1C). This protein was initially identified in mammals as a nuclear helicase of unknown function called p47 or BAT1 (Peelman et al., 1995). More recently, it was renamed UAP56 when it was found as a spliceosomal component required for U2 snRNP binding to pre-mRNA (Fleckner et al., 1997). Its essential yeast ortholog Sub2 is thought to function during multiple steps of spliceosome assembly (Kistler and Guthrie, 2001; Libri et al., 2001; Zhang and Green, 2001). Data from both yeast and metazoans show that Sub2/UAP56 interacts primarily with the C terminus of Yra1/Aly (Luo et al., 2001; Strasser and Hurt, 2001). Moreover, studies in yeast revealed that the Mex67-Mtr2 heterodimer competes with Sub2 for binding to Yra1 (Strasser and Hurt, 2001). These data have led to the model that Mex67-Mtr2 may bind to Yra1 and thereby release Sub2 from Yra1 prior to mRNA export.

Functional evidence that Sub2 and UAP56 play a role in mRNA export in both yeast and metazoans was provided by mutational analysis, RNA interference, and overexpression studies (Jensen et al., 2001a; Strasser and Hurt, 2001; Gatfield et al., 2001; Luo et al., 2001). In metazoans, UAP56 is present in the spliced mRNP and plays a role in recruiting Aly to the mRNA during splicing (Gatfield et al., 2001; Luo et al., 2001). Surprisingly, in both yeast and metazoans, Sub2/UAP56 is required for export of mRNAs derived from intronless genes as well as for export of spliced mRNAs (Gatfield et al., 2001; Jensen et al., 2001a; Strasser and Hurt, 2001). Thus, an important unanswered question is how Sub2/UAP56 and Yra1/Aly are recruited to mRNAs derived from intronless genes.

Other Conserved mRNA Export Factors

Several other proteins involved in mRNA export have also been identified. These include Dbp5 (Figure 1C), Gle1 (Figure 1D), and Gle2 (Figure 1D), all of which have metazoan counterparts that were recently shown to function in mRNA export. The most is known about Dbp5, which was originally identified in a screen for yeast DEAD-box helicase proteins (Tseng et al., 1998 and references therein). A role for Dbp5 in mRNA export was subsequently revealed when it was detected genetically in a collection of yeast mRNA export mutants (Snay-Hodge et al., 1998). Although Dbp5 is a shuttling protein, it is mainly cytoplasmic and interacts with the cytoplasmic fibrils of the nuclear pore complex. The conserved nucleoporin yeast Nup159 and human Nup214 recruit Dbp5 to these fibrils (Hodge et al., 1999; Schmitt et al., 1999; Strahm et al., 1999).

The metazoan ortholog of Dbp5 was identified based on its similarity to the yeast protein (Schmitt et al., 1999). Antibodies to hDbp5 or mutants in the helicase domain of hDbp5 specifically inhibit export of mRNA in *Xenopus* oocyte microinjection assays, indicating that the role of hDbp5 in mRNA export is conserved (Schmitt et al., 1999). The Dbp5/hDbp5 protein has ATP-dependent RNA unwinding activity (Schmitt et al., 1999). This activity depends on an additional factor(s) present in cytoplasmic extracts. Further studies revealed that Dbp5/hDbp5 interacts with Yra1/Aly, but the significance of this interaction is not yet known (Schmitt et al., 1999). The observation that Dbp5/hDbp5 is a conserved DEAD-box RNA helicase that shuttles and is located on the cytoplasmic face of the pore has led to the proposal that this protein may function in remodeling the mRNP during or shortly after translocation through the nuclear pore complex (Snay-Hodge et al., 1998; Tseng et al., 1998; Schmitt et al., 1999). More recent studies using RNA interference indicate that this protein is not essential for mRNA export in *Drosophila* (Gatfield et al., 2001).

Thus, further work is needed to clarify the role of this protein in metazoans.

Yeast Dbp5 also interacts genetically with Gle1, a conserved nucleoporin-like protein also located on the cytoplasmic face of the nuclear pore complex (Murphy and Wente, 1996; Watkins et al., 1998; Strahm et al., 1999). Gle1 is thought to function at a terminal step of mRNA export by interacting with a complex containing Nup159 (Hurwitz et al., 1998; Strahm et al., 1999). Gle1 also interacts genetically with phospholipase C and two inositol polyphosphate kinases that regulate nuclear mRNA export through production of inositol hexakisphosphate (IP6) (York et al., 1999). Recent work shows that IP6 may also play a role in mRNA export in humans (Feng et al., 2001).

The conserved mRNA export factor Gle2 was found in the same synthetic lethal screen that identified Gle1 (Murphy et al., 1996). Gle2 was also detected in *S. pombe* as a protein designated Rae1 that is essential for mRNA export (Brown et al., 1995), and the human ortholog is called mrnp41 (Kraemer and Blobel, 1997). There is also a conserved interaction between Gle2 and Mex67/Tap, but the significance of this interaction remains to be determined (Bachi et al., 2000; Yoon et al., 2000; Zenklusen et al., 2001).

hnRNP Proteins—The Jury Is Still out

Studies in metazoans indicate that the ~20 distinct, highly abundant hnRNP proteins package nascent pre-mRNA as it is transcribed. These proteins became the focus of attention as potential mRNA export factors with the discovery that some of them, such as hnRNP A1 and hnRNP K, shuttle between the nucleus and the cytoplasm, while others such hnRNP C, are exclusively retained in the nucleus (Nakielny and Dreyfuss, 1999). Moreover, nuclear export signals were identified in shuttling hnRNP proteins, with one of the best characterized of these being the M9 sequence in hnRNP A1. The M9 sequence also functions as an NLS to mediate import of hnRNP A1 via the karyopherin transportin-1 (Nakielny and Dreyfuss, 1999). To date, a role in mRNA export for the M9 sequence and its NES has not been directly demonstrated. Nevertheless, some observations are consistent with the possibility that hnRNP proteins play a role in mRNA export. For example, excess hnRNP A1 or M9 peptides inhibit export of DHFR mRNA (Izaurralde et al., 1997; Gallouzi and Steitz, 2001). In addition, hnRNP A1-like proteins bind to the giant Balbiani ring mRNA in *Chironomus tentans* and accompany this mRNA to the cytoplasm (Daneholt, 1997). Finally, distinct nuclear mRNP complexes have been isolated from mammalian cells that contain shuttling hnRNP proteins and Aly but lack non-shuttling hnRNP proteins (Mili et al., 2001).

HnRNP proteins have also been proposed to play a role in packaging the vast intron sequences present in metazoan pre-mRNAs (Reed and Magni, 2001). After splicing, the excised introns are retained in the nucleus and ultimately degraded. The possibility that hnRNP proteins package excised introns is supported by the recent observation that antibodies against hnRNP A1 efficiently immunoprecipitate excised introns, but not spliced mRNA (Kataoka et al., 2000). It is also well established that pre-mRNAs defective in spliceosome assembly in vitro are packaged into hnRNP complexes and subsequently degraded (Reed and Magni, 2001 and references therein). Assuming that the same is true in vivo, hnRNP complexes may function as part of a nuclear RNA surveillance system to retain and degrade both excised introns and defective RNAs.

In summary, the jury is still out regarding the role of hnRNP proteins in mRNA export. The possibilities that remain to be sorted out include whether hnRNP proteins play an active or passive role in mRNA export, are involved in export of only specific mRNAs, and/or whether they package excised introns and mutant pre-mRNAs and retain them in the nucleus. These possibilities are not mutually exclusive.

SR Proteins—Shuttling Splicing Factors Bound to Exon Sequences

SR proteins are a family of serine-arginine-rich splicing factors that are essential for spliceosome assembly in metazoans (Graveley, 2000). Distinct members of the SR protein family bind to degenerate sequence elements within exons and promote spliceosome assembly on the flanking intron sequences (Graveley, 2000). The possibility that SR proteins play a role in mRNA export first came to light with the observation that these proteins shuttle between the nucleus and cytoplasm and require RNA synthesis for shuttling (Cáceres et al., 1998). Moreover, SR proteins, including RNPS1 and SRm160, bind exclusively to exon sequences in pre-mRNA and remain bound to the exons after the mRNA is spliced (Graveley, 2000).

More recent studies have provided direct evidence that SR proteins play a role in mRNA export. Ironically, these studies involved an mRNA derived from a naturally intronless pre-mRNA (Huang and Steitz, 2001). In this case, the mRNA contains specific sequence elements that are required for export and binds to SR proteins. The SR proteins are thought to mediate export either directly or indirectly. Further studies are needed to determine whether SR proteins also function in export of mRNAs derived from pre-mRNAs containing introns.

The closest relative to SR proteins in *S. cerevisiae* is the shuttling protein Npl3, which binds to poly(A)$^+$ mRNA and is involved in mRNA export (Lee et al., 1996). In contrast to SR proteins, Npl3 is not required for splicing. However, Npl3 has been linked to the polyadenylation machinery, suggesting that 3′ end processing and mRNA export are coupled (Kessler et al., 1997 and references therein). In addition, Npl3 has been linked to the nuclear cap-binding complex consistent with a role for packaging the mRNA for export (Shen et al., 2000). Recent studies revealed that Npl3 is phosphorylated by the cytoplasmic SR protein kinase Sky1 (Yun and Fu, 2000; Gilbert et al., 2001). These and other observations led to the proposal that phosphorylation of Npl3 causes release of the mRNA after translocation through the nuclear pore (Gilbert et al., 2001).

Splicing-Coupled and Splicing-Independent mRNA Export

Studies over the past few years have revealed that virtually all of the steps in the gene expression pathway are extensively coupled to one another (Bentley, 1999; Hirose and Manley, 2000; Proudfoot et al., 2002). Consistent with this emerging concept, mRNA export is thought

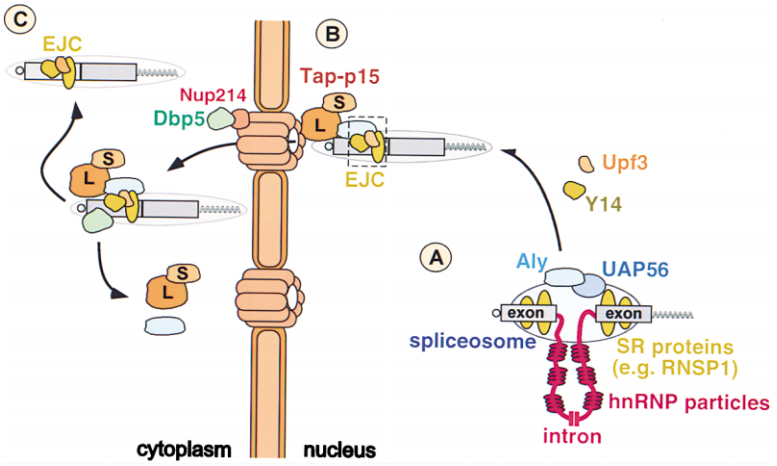

Figure 3. Model for Splicing-Coupled mRNA Export in Metazoans

(A) UAP56 and Aly associate with the spliceosome. Simplified pre-mRNA with a 5′ cap, 2 exons and an intron, and a poly(A) tail. hnRNP proteins package the pre-mRNA, and SR proteins associate with exons.

(B) The Tap-p15 heterodimer targets the mRNP to the nuclear pores. Aly acts as bridging protein between the exon junction complex (EJC) and Tap-p15.

(C) mRNA export factors dissociate from the mRNP after export to the cytoplasm. Factors involved in NMD (e.g. Upf3, Y14, and RNPS1) remain bound to the mRNP.

to be coupled to transcription, pre-mRNA splicing, and other steps in gene expression. At present, the most progress has been made in understanding the coupling between splicing and mRNA export in metazoans. This progress can be attributed in large part to the isolation and characterization of the spliced mRNP complex, which has revealed that some of the key conserved factors required for mRNA export are recruited to the mRNA by the splicing machinery. In contrast, the mechanism by which the conserved machinery is recruited to yeast mRNAs, the vast majority of which are derived from intronless genes, is unknown. A great deal of insight should be gained when the yeast mRNP is isolated and direct analysis of this complex is carried out. Below, we discuss a working model for splicing-coupled export in metazoans, as well as proposals for export of mRNAs derived from intronless genes in both lower and higher eukaryotes.

In metazoans, most pre-mRNAs contain multiple introns and exons. The exons have an average small size of ~140 nucleotides, whereas the introns are enormous by comparison (10^3–10^5 nucleotides). A simplified pre-mRNA containing two exons and one intron is shown in the model for splicing-coupled export (Figure 3). Initially, SR proteins associate with exon sequences and recruit the spliceosome, while the intron is packaged into hnRNP complexes. The splicing factor UAP56 associates with the spliceosome at an early step in assembly. Subsequently, UAP56 plays a role in recruiting Aly to the spliced mRNP (Figure 3A). These two factors, as well as several factors involved in NMD (e.g., Y14, RNPS1, hUpf3, and see below) interact with the spliced mRNA ~20 nucleotides upstream of the exon-exon junction to form a stable exon junction complex (EJC) (Figure 3B). In a subsequent step, UAP56 may be released upon binding of the Tap-p15 heterodimer to Aly (Figure 3B). The spliced mRNP is then translocated through the nuclear pore to the cytoplasm. Prior to translation, the components of the EJC that function in NMD remain stably bound, whereas the mRNA export factors dissociate (Figure 3C).

For export of mRNAs derived from naturally intronless pre-mRNAs, two scenarios can be envisioned. In the first, the early acting components of the export machinery (e.g., Yra1/Aly, Sub2/UAP56, and Npl3/SR proteins) are loaded onto the mRNA by a transcription-coupled mechanism. Evidence for this possibility has come from studies showing that both Npl3 and Yra1 associate with the mRNA during transcription (Lei et al., 2001). In addition, Npl3 is thought to interact directly with the transcription machinery (Lei et al., 2001). Likewise, there is evidence that SR proteins interact with the transcription machinery and may be recruited to exon sequences during transcription (Hirose and Manley, 2000). It is likely that other steps in gene expression, such as polyadenylation, are also coupled to mRNA export and may be critical for export of mRNAs derived from genes lacking introns (Brodsky and Silver, 2000; Hilleren and Parker, 2001; Jensen et al., 2001b).

In the second scenario, the mRNA contains specific sequence elements that recruit either the same conserved machinery that is used for splicing-coupled export or a distinct machinery (Otero and Hope, 1998; Huang and Steitz, 2001 and references therein). Studies of NMD in yeast indicate that downstream sequence elements (DSEs) functionally substitute for the exon/exon junctions that recruit the NMD machinery in metazoans (Ruiz-Echevarria et al., 1998). Thus, by analogy, DSEs are potential candidates for recruiting the export machinery in yeast. Alternatively, distinct sequence elements could be involved in stably recruiting the export and NMD machineries. For example, in a metazoan mRNA derived from a naturally intronless gene, export machinery appears to be recruited to specific sequence elements in mRNA (Huang and Steitz, 2001).

Coupling Splicing, Export, and NMD

Throughout the pathway of gene expression, numerous mechanisms exist to insure the quality control of mRNA and hence the final protein product. Nonsense-mediated decay is a conserved mechanism used to detect and selectively degrade mRNAs that contain premature stop codons. In metazoans, it had been known for several years that the presence of a stop codon greater than 50 nucleotides upstream of an exon-exon junction triggers NMD in the cytoplasm and that this phenomenon was in some way coupled to splicing in the nucleus (Lykke-Andersen et al., 2000 and references therein). Insight into the mechanism for splicing-coupled NMD came from studies of hUpf3, the human ortholog of a

yeast protein essential for NMD (Lykke-Andersen et al., 2000). These studies showed that NMD was triggered in metazoans by artificially tethering hUpf3 downstream from a termination codon (Lykke-Andersen et al., 2000). However, the natural mechanism for recruiting hUpf3 to the mRNA was not known.

A compelling answer to this question came from the discovery that the EJC contains components of the conserved export machinery as well as factors involved in NMD (Kim et al., 2001a; Le Hir et al., 2001; Lykke-Andersen et al., 2001). Of most significance to NMD are a novel protein designated Y14 (Kataoka et al., 2000), a splicing factor RNPS1 (Mayeda et al., 1999), and hUpf3 (Lykke-Andersen et al., 2000). All three of these proteins shuttle between the nucleus and cytoplasm (Kataoka et al., 2000; Lykke-Andersen et al., 2000, 2001). Additional studies showed that Y14 and RNPS1 play direct roles in recruiting hUpf3 to the EJC during splicing (Kim et al., 2001a; Lykke-Andersen et al., 2001). Moreover, Y14, hUpf3, Aly, and Tap-p15 are thought to form a stable protein-protein complex (Kim et al., 2001a). Thus, this complex functions in both mRNA export and NMD to transmit a positional mark of the exon-exon junction from the nucleus to the cytoplasm. Following mRNA export to the cytoplasm, other NMD factors including hUpf1 and hUpf2 are recruited to the mRNA through interaction with hUpf3. This NMD complex triggers mRNA decay if the translating ribosome encounters a premature stop codon. In normal mRNAs, the NMD complex is thought to be stripped from the mRNA by the translating ribosome thereby preventing mRNA degradation (Kim et al., 2001a; Lykke-Andersen et al., 2001 and references therein).

Concluding Remarks

Studies over the past few years have led to the identification of an mRNA export machinery that is conserved from yeast to humans. A fundamental component of this machinery is the mRNA export heterodimer, which has the key features of an export receptor. Thus, we propose designating this heterodimer the m-exporter (*m*RNA-exporter). Recent advances discussed here have also revealed that the m-exporter and other conserved components of the mRNA export machinery are intimately coupled to the pre-mRNA splicing machinery. Considering that most genes in yeast and some genes in metazoans lack introns, a critical unanswered question is how the conserved export machinery is recruited to mRNAs derived from these genes. Finally, numerous studies indicate that there is extensive coupling among the multiple steps in the gene expression pathway. Further work is required to clarify how these steps are integrated with mRNA export.

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