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***Review Article***

# ARRIVAL OF RNAs TO CYTOPLASM IS ESSENTIAL FOR PROTEIN PRODUCTION

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## ABSTRACT
All movement of molecules and macromolecules between the cytoplasm and the nucleus takes place through nuclear pore complexes (NPCs), very large macromolecular complexes that are the only channels connecting these compartments. mRNA export is mediated by multiple, highly conserved protein factors that couple steps of nuclear pre-mRNA biogenesis to mRNA transport. Mature messenger ribonucleo proteins (mRNPs) diffuse from sites of transcription to NPCs, although some active genes are positioned at the nuclear periphery where they interact physically with components of NPCs. As properly processed mRNPs translocate through the pore, certain mRNP proteins are removed, probably through the enzymatic action of the DEAD-box helicase Dbp5, which binds to Nup159 and Gle1, components of the cytoplasmic filaments of the NPC. Gle1 and the phosphoinositide $IP_6$ activate Dbp5's ATPase activity *in vitro* and this could provide critical spatial regulation of Dbp5 activity *in vivo*.

***Keywords:*** *mRNA Export, mRNP Assembly, snRNAs, Export Factors*

## INTRODUCTION
The TREX (transcription/export) complex is a key player in the transport of mRNA from the nucleus to the cytoplasm. This complex is conserved from yeast to human, and a *Drosophila* counterpart was recently characterized. The two main constituents of the TREX complex are the stable multi-subunit THO complex and the mRNA export proteins UAP56 (Sub2 in yeast) and Aly (Yra1 in yeast). As discussed below, the THO complex plays a central role in recruiting these export proteins to the mRNA in both yeast and human. Despite the striking conservation in the structure and function of the TREX complex, studies in yeast provide strong evidence linking the TREX complex to transcription elongation and to co-transcriptional recruitment of the mRNA export machinery. By contrast, studies in mammals provide compelling evidence linking the TREX complex to the splicing machinery and suggesting that that the TREX complex is recruited to mRNA during a late step in splicing.
This review focuses on the evidence for the different TREX complex recruitment mechanisms as well as on the puzzles that remain regarding the export of naturally intronless mRNAs in mammals and spliced mRNAs in yeast. Further, it discusses about exciting new studies of the SR (serine/arginine-rich protein) family of splicing factors in mammals and related proteins in yeast that reveal a role for dephosphorylation of these proteins in mRNA export and the identification of a specific nuclear phosphatase in yeast. For a comprehensive discussion of the coupling between transcription, splicing and mRNA export, readers are referred to excellent reviews of these topics (Dimaano & Ullman, 2004; Erkmann & Kutay, 2004; Jensen *et al.*, 2003; Vinciguerra & Stutz, 2004).

### *mRNP Assembly is Essential for Export*
We have only a partial understanding of how the cell distinguishes an export-competent mRNP from one that should be retained in the nucleus for storage or degraded (Prasanth *et al.*, 2005). Forming an mRNP is a complex process that begins co-transcriptionally and involves dozens of factors that participate in pre-mRNA processing and packaging. Legrain and Rosbash (Legrain & Rosbash, 1989) showed that in yeast defects in the earliest steps of splicing, which prevented the pre-mRNA from associating with the splicing machinery, permitted export of unspliced mRNA, suggesting that association with the splicing machinery sequesters the mRNP physically until splicing has been completed. Several studies demonstrate that mRNA export requires proper 3′ processing, including addition of the poly(A) tail and binding of multiple molecules of the poly(A) binding protein, Pab1 (Brune *et al.*, 2005; Dower *et al.*, 2004; Dunn *et al.*, 2005;

Hammell *et al.,* 2002; Hilleren *et al.,* 2001). Although some mRNAs can be exported if their 3′ ends are generated through the action of a ribozyme (Dower *et al.,* 2004), this is a non-physiological situation.

Two systems have been described in yeast that detect defective mRNPs (for reviews, see (Hieronymus *et al.,* 2004; Milligan *et al.,* 2005; Saguez *et al.,* 2005)). One of these results in retention at sites of transcription in a process that is dependent on the nuclear exosome, a complex of several 3′-5′ exoribonucleases that ultimately degrades mRNPs that are not released for export (Hilleren *et al.,* 2001; Jensen *et al.,* 2001). A second surveillance system operates at NPCs and includes the myosin-like Mlp1 and Mlp2 proteins (Galy *et al.,* 2004; Vinciguerra *et al.,* 2005). Structurally, Mlp proteins resemble the metazoan Tpr proteins to which they may be orthologous, and extend into the nuclear interior from the nuclear basket of the NPC, where they interact with Nup60 and the nuclear envelope protein Pml39 (Palancade *et al.,* 2005). Mlp1 interacts with Nab2, an mRNP protein important for proper pre-mRNA processing (Green *et al.,* 2003). Through this and perhaps other interactions, mRNPs containing introns are retained in the nucleus.

Karyopherins function as transport receptors by recognizing and binding to cargoes that carry nuclear transport signals and interact with NPCs (Harel & Forbes, 2004). Yeast Mex67 (TAP in metazoans) functions as the export receptor for mRNAs and becomes associated with the pre-mRNA through the actions of Sub2 (UAP56) and Yra1 (ALY/REF) (Aguilera, 2005; Reed & Cheng, 2005). mRNA export differs from transport of proteins and spliceosomal pre-RNAs in that there is no direct role for the small GTPase, Ran, in mRNA export.

***Transport of snRNAs***

Very little is known about the biogenesis of snRNAs in yeast (Lygerou *et al.,* 1999). In higher eukaryotes, snRNAs are monomethylated at the 5′ end, which is a signal for nuclear export. Both the CBP80/20 complex, which binds to the 5′ monomethyl cap, and Crm1 (snRNA export is inhibited by Rev NES peptides) appear to be involved in the export process (Fischer *et al.,* 1995; Fischer & Luhrmann, 1990; Hamm & Mattaj, 1990). After arrival in the cytoplasm, the snRNA is hypermethylated and the Sm proteins assemble. This creates a bipartite nuclear import signal. Recently, one of the import factors that binds to the trimethyl cap has been identified and named snurportin (Huber *et al.,* 1998). As importin α, snurportin acts as an adapter by binding to the hypermethylated 5′ cap structure and to importin β. In yeast, no evidence has been obtained that snRNAs leave the nucleus. Thus, yeast snRNPs may assemble inside the nucleus without export and re-import steps (Lygerou *et al.,* 1999).

***Export Factors***

A group of evolutionarily conserved proteins classified as nuclear export factors or NXFs is responsible for exporting the majority of cellular mRNAs and a subset of viral RNAs to the cytoplasm (Izaurralde, 2002). NXFs bear no resemblance to prototypical nuclear transport receptors of the importin–exportin (karyopherin) family (Fried & Kutay, 2003) and lack the characteristic Ran-binding domain found in all karyopherins. Binding of the GTP-bound form of the small GTPase Ran to importins and exportins determines the directionality of cargo transport through the NPC, and, importantly, this interaction with Ran is seminal to substrate binding and hence the efficient export of cargo molecules by exportins. In contrast, NXFs are recruited to nuclear mRNPs independently of Ran by different mechanisms. Similar to the prototypical export receptors, NXFs interact with nucleoporins thus mediating the interaction between the mRNA export cargo and components of the NPC required for translocation.

Most NXF family members share the following functional properties: they associate with nuclear pores, have the ability to shuttle, and require heterodimerization with p15 for efficient interaction with NPC components (Herold *et al.,* 2000; Katahira *et al.,* 1999; Santos-Rosa *et al.,* 1998). However, the reason behind the diversity of the metazoan NXF family has not yet been resolved. Despite the similarities in domain organization between different *Drosophila* NXFs, Dm NXF2 and NXF3 do not perform a redundant function to NXF1 because these two NXFs are insufficient to support growth of *Drosophila* S2 cells in the absence of NXF1. Moreover, depletion of Dm NXF2 and NXF3 by RNAi did not cause an mRNA export phenotype. Therefore, it is currently unclear what the function, if any, of the remaining Dm NXFs in mRNA export is. Using reporter RNAs (Herold *et al.,* 2000) and tethered RNAs (Yang *et al.,*

2001), human NXF2 and NXF3, respectively, have been shown to possess mRNA export activity. However, the role of Hs NXF2 and 3 in the export of endogenous mRNA substrates has not yet been investigated. Both of these genes are highly expressed in the testis (Yang *et al.,* 2001) and may therefore have a cell type-specific function in mRNA export. Little is known about the other human NXFs; however, in an interesting study, Hs NXF5 has been linked to a syndromic form of mental retardation (Jun *et al.,* 2001), suggesting that it may function in the export of a specific subset of mRNAs or has a critical function in the brain.

***mRNA Export is Highly Regulated Event***

An important question is how mRNA export is regulated and how assembly and disassembly of export complexes is controlled. Interestingly, Tom1p, an ubiquitin E3 ligase associated with the SAGA complex (Saleh *et al.,* 1998), is required for mRNA export. Indeed, mutations in Tom1p block the export of transcripts containing the shuttling mRNA binding protein Nab2p (Duncan *et al.,* 2000; Green *et al.,* 2002). Genetic interactions further indicate a connection between Nab2p and the Sus1p–Thp1p–Sac3p complex, suggesting that post-translational modification by ubiquitin may regulate this pathway (Gallardo *et al.,* 2003; Rodriguez-Navarro *et al.,* 2004). Recent evidence by the Hurt and Dargemont labs shows that another ubiquitin E3 ligase, called Rsp5p, is also essential for mRNA export (Neumann *et al.,* 2003; Rodriguez *et al.,* 2003). The identification of specific substrates should indicate whether Rsp5p and Tom1p control distinct pathways and reveal how ubiquitin regulates mRNP biogenesis and export.

A recent report by the Guthrie lab demonstrates that post-transcriptional modification by phosphorylation also contributes to mRNA export regulation (Gilbert & Guthrie, 2004). As mentioned above, this study identified Npl3p, an SR-like protein essential for mRNA export, as a new adaptor for the export receptor Mex67p. Npl3p is recruited to nascent mRNPs in its phosphorylated form but interacts with Mex67p only in its unphosphorylated form. Importantly, the authors show that Glc7p, a phosphatase essential for mRNA export, coordinates dephosphorylation of Npl3p with the release of the mRNP from the 3′-end processing machinery and the recruitment of Mex67p to the mRNP. Such a mechanism may ensure that only correctly 3′-end processed mRNPs become associated with the export receptor. After translocation, the cytoplasmic kinase Sky1p phosphorylates Npl3p, promoting the dissociation of Mex67p from the mRNP (Gilbert *et al.,* 2001). These observations show that a cycle of cytoplasmic phosphorylation and nuclear dephosphorylation of Npl3p, and perhaps other shuttling SR proteins, regulates Mex67p-dependent mRNA export (Izaurralde, 2004).

***Conclusions***

The TREX complex is conserved from yeast to human and functions in mRNA export. Despite this conservation, the complex appears to be recruited to mRNA by the transcription machinery in yeast and the splicing machinery in human. It makes more sense to load the export machinery onto spliced mRNA than onto unspliced pre-mRNA in the case of higher eukaryotes, which have numerous introns. And, conversely, it makes sense in yeast to make use of the transcription machinery for loading the export machinery, as most transcripts are not spliced. The obvious question raised is how to deal with the intron-containing genes in yeast and the intron-lacking genes in higher organisms. Studies over the past year have revealed a conserved role for dephosphorylation of SR proteins in mRNA export, but how they, the TREX complex, or some as yet undetected adaptors interact and function in these processes remains to be determined.

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