

MINIREVIEW

Nucleocytoplasmic Transport: Integrating mRNA Production and Turnover with Export through the Nuclear Pore

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Separation of the nucleus and cytoplasm, maintained by two membrane bilayers that form the nuclear envelope, allows for spatial control over transcription factors and signaling molecules. This compartmentalization further ensures the presence of specialized environments for different stages of gene expression, such as transcription and protein production. Selective exchange between these two compartments is clearly important as well. Whereas many types of active transport between the nucleus and cytoplasm rely on transport receptors in the importin- β superfamily, export of mRNA utilizes distinct soluble machinery (6, 92). Moreover, in general mRNA export does not depend on a specific motif in the cargo, as has been demonstrated in many other cases of receptor-cargo interactions (22). Recent progress in identifying soluble factors important to mRNA trafficking is beginning to reveal the molecular basis for functional coupling between steps in mRNA biogenesis and how such coupling, rather than a consensus motif, brings specificity to mRNA export.

Studies with *Saccharomyces cerevisiae* revealed that the key modulators of cellular mRNA export are unrelated to canonical importin- β -related receptors. Specifically, yeast deficient in a gene called *MEX67* accumulate poly(A)⁺ RNA in the nucleus (73). A second protein, Mtr2p, binds Mex67p, and this interaction is required for the export of poly(A)⁺ RNA in yeast (71, 77). In an independent avenue of investigation, involving metazoan cells and the simian type D retrovirus Mason Pfizer monkey virus, the cellular protein TAP was found to facilitate export of RNA containing the viral constitutive transport element (CTE) (8, 32). TAP, confirmed to be the human orthologue of Mex67p, has been redesignated NXF1 (nuclear export factor 1).

NXF1 interacts with p15/NXT1, the presumed functional homologue of Mtr2p (33, 44). Although Mtr2p and p15 share no sequence similarity, the Mex67p-Mtr2p complex displays similar structural architecture to the NXF1-p15 heterodimer (21). Indeed, the mRNA export defect in yeast cells deficient in both Mex67p and Mtr2p can be rescued by expression of human NXF1 and its cofactor p15 (44). Expression knock-down studies using RNA interference have demonstrated that NXF1 and p15 are required for poly(A)⁺ RNA export (35, 84, 93), further strengthening the case for an evolutionarily conserved

system of mRNA export that is distinct from the importin- β superfamily. Likewise, studies with *Xenopus* oocytes support the conclusion that the small GTPase Ran, a key modulator of importin- β -type receptors, is not key to mRNA export (10). However, the distinction between mRNA export and the importin- β family/Ran network is not absolute, as an importin- β family member has recently been implicated in mRNA export as well (74).

mRNA EXPORT IS COUPLED TO SPLICING

In contrast to CTE-mediated transport, NXF1 does not bind directly to cellular mRNA. Therefore, deciphering how NXF1 is recruited to mRNA is crucial to understanding mRNA export and has been the focus of much current work. Experiments with *Xenopus* oocytes demonstrated that the process of splicing can contribute to the efficiency of mRNA export; the spliced product from adenovirus major late (Adml) mRNA was shown to export more efficiently than an identical mRNA engineered to lack an intron (58). In addition, the spliced Adml mRNA and its engineered counterpart form distinct RNP complexes in splicing extracts, suggesting that recruitment of a unique set of proteins to the spliced mRNA may promote export competency. Indeed, specific proteins, including the factor Aly (renamed REF for RNA export factor), the acute myeloid leukemia-associated protein DEK, and the RNA binding protein Y14, were individually found to preferentially interact with processed mRNA (46, 60, 97). The identification and characterization of an entire protein complex, the exon junction complex (EJC), associated with spliced mRNA soon followed (53). Components originally identified in the EJC (53) include REF, SRm160, RNPS1, DEK, Y14, and later its protein partner Magoh (45).

The notion that EJC deposition leads to recruitment of NXF1 is an attractive model to explain the stimulatory effect of splicing on export. Direct interactions between REF and NXF1 have been observed in both human and yeast systems (69, 81). REF also shuttles between nucleus and cytoplasm and enhances mRNA export when injected into *Xenopus* oocyte nuclei as a recombinant protein (69, 97). Studies with yeast demonstrated a high degree of functional conservation since Yra1p (the yeast orthologue of REF)-deficient cells accumulate poly(A)⁺ RNA; Yra1p also physically interacts with mRNA in vivo (79). The enhanced placement of REF onto mRNA in a splicing-dependent fashion, as well as its associa-

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tion with NXF1, made REF a prime candidate for recruiting NXF1 to mRNA.

A connection between splicing and mRNA export was further solidified with the characterization of a novel role for the putative RNA helicase UAP56 [56-kDa U2AF(65)-associated protein]. UAP56 was originally characterized for its contribution to spliceosome assembly (20). More recently, independent from its function in splicing, a role for Sub2p (yeast orthologue of UAP56) in mRNA export has also been identified (78). Sub2p directly interacts with Yra1p *in vitro* and *in vivo*, a characteristic conserved in metazoan counterparts (59, 78). Interestingly, Sub2p and Mex67p bind to the same domain of Yra1p, suggesting that Sub2p may recruit Yra1p to mRNA and may subsequently be displaced by Mex67p (78). Moreover, recruitment of REF to spliced mRNA is dependent upon its interaction with UAP56 (59) and UAP56 is essential for mRNA export in *Drosophila* (25). From these data, a very simple yet elegant mode of coupling splicing with mRNA export became evident. Namely, REF is recruited to spliced mRNA through direct interactions with UAP56, and consequently, REF (and the EJC in general [94]) recruits the export factor NXF1 to promote exit from the nucleus by mediating docking and presumably movement through the pore.

SPlicing IS SUFFICIENT BUT NOT NECESSARY TO PROMOTE mRNA EXPORT

Despite the observation that splicing promotes export of mRNA, such processing is not the only or even the major route for export factor recruitment. For example, antibodies directed against REF block the export of both spliced and intronless mRNA in *Xenopus* oocytes (69). Yeast cells deficient in Sub2p show arrest of bulk poly(A)⁺ mRNA (42, 78) despite the lack of an intron in most genes. Moreover, the interaction between Yra1p and mRNA occurs in the absence of splicing in yeast (55) and the same is thought to be true for UAP56 in *Chironomus tentans* (48). RNA interference studies with *Drosophila* also showed that depletion of UAP56 causes the export inhibition of both spliced and nonspliced RNAs (25). In fact, additional studies with *Drosophila* demonstrated essential roles in bulk mRNA export for NXF1, p15, and UAP56, while the EJC components Y14, RNPS1, SRm160, and REF appear to be dispensable (24).

Recent functional analysis of mammalian cells also suggested that splicing does not always have a major effect on mRNA export *per se*. When compared to intron-containing counterparts, intronless constructs did not show a specific decrease in cytoplasmic RNA levels but rather were found to result in a general decrease in both nuclear and cytoplasmic RNA (57). This result was corroborated in *Xenopus* oocytes, where the relative RNA distribution of a luciferase reporter construct did not significantly change if an intron was present to recruit EJC components (64). In fact, in these studies, members of the EJC complex appear to be involved in increasing the translational yield or in enhancing mRNA abundance rather than influencing export.

Together, these observations suggest that the coupling between splicing and export factor recruitment that has been documented may represent only one way that export factors load onto mRNA and indeed may make a significant contri-

bution at this step only when the RNA is particularly short. This conclusion is further illustrated by the work of Mattaj and colleagues, who engineered a hybrid U1 snRNA harboring an intron flanked by minimal exonic sequences and demonstrated that splicing endows this hybrid molecule with hallmark features of mRNA export (65). They further observed that hybrid U1 snRNA molecules containing sufficiently long, unstructured stretches of exonic sequences are also exported using mRNA export machinery instead of U1 snRNA-associated export factors (65). The function of this inserted sequence is independent of orientation and has thus defined a stretch of approximately 300 nucleotides of any unstructured RNA to be an mRNA identity element (reviewed in reference 86). A previously observed correlation between export and mRNA length requirements also supports this connection (69). This insight, however, begs the question of exactly which are the critical proteins that recognize and bind unstructured stretches of mRNA. REF is a good candidate, but the observation that REF is dispensable for mRNA export (24, 56) suggests that other proteins work in conjunction or in parallel at this step.

CREATING EXPORT COMPETENT mRNPs: DIFFERENT MEANS TO THE END?

Presumably, export factors can load onto mRNA in both a relatively nonspecific way as well as via splicing-coupled mechanisms. If an mRNA is sufficiently long, generic loading of factors appears to be no more limiting than splicing-dependent mechanisms. The above-mentioned results provide insight into how a diverse array of mRNAs can be recognized by export machinery and point toward the influence of mRNA cargo size and global appearance on export requirements. However, while a baseline for mRNA export requirements may be met by an mRNA identity element or by splicing, additional mechanisms could promote efficiency in connecting to either NXF1 or other transport factors. For instance, RNA elements that recruit export factors have been characterized (39) and, as discussed in more detail below, export factors are also recruited during very early events in mRNA biogenesis (Fig. 1).

Baseline requirements for mRNA export have been illustrated by a recent genome-wide analysis of mRNA export. *Drosophila* cells were depleted of export factors via RNA interference, and RNA levels were then assessed by microarray (36). In the absence of NXF1, p15, or UAP56, a large population of mRNA showed decreased cytoplasmic levels and, at early time points, levels in the cytoplasm had decreased more than in total RNA, suggestive of a block in mRNA export. Moreover, mRNA expression profiles of cells independently depleted of NXF1, p15, or UAP56 are markedly similar, indicating an underlying global pathway in mRNA biogenesis in which these three factors function (24, 36). However, although the roles of these proteins intersect, there may still be diversity in how each protein is recruited to different transcripts.

In fact, a recent study of a different system demonstrated that specific mRNAs are associated with distinct mRNA export factors (37). Hieronymus and Silver utilized a coimmunoprecipitation strategy to pull out either Yra1p or Mex67p from yeast extracts and analyzed mRNAs associated with these proteins by microarray. Surprisingly, these general export factors associated with distinct populations of mRNAs (37). Although

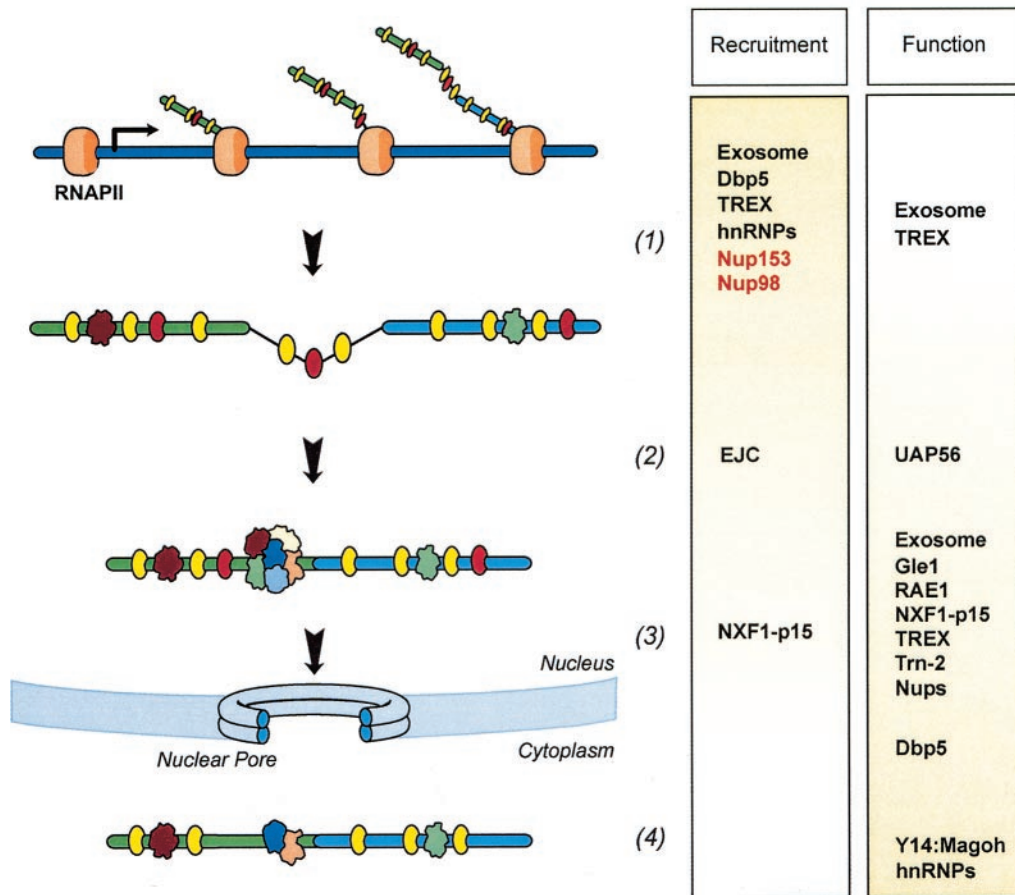


FIG. 1. Interconnections between steps in mRNA biogenesis. A schematic illustration of mRNA biogenesis is depicted, with the proposed times of recruitment and functions for specific proteins indicated in the boxes. (1) Transcription. Much evidence points to cotranscriptional loading of factors involved in RNA processing, export, and quality control to the nascent transcript. The mobile pore proteins, Nup153 and Nup98, are candidates (indicated in red text) for loading onto mRNA early in its biogenesis, although this is yet to be demonstrated. RNAPII, RNA polymerase II. (2) Splicing. The splicing factor UAP56 interacts with REF, which is one of a group of proteins referred to as the EJC that is deposited on mRNA in conjunction with splicing. Loading of certain transport factors, such as REF, can also occur independent of splicing as a part of TREX or if the RNA is sufficiently long. (3) Remodeling and export. NXF1-p15 is recruited to the mRNA via protein-protein interactions, readying export-competent mRNA for mobilization out of the nucleus. At this step, other proteins, such as Gle1, RAE1, Trn-2 (transportin-2), and TREX components, are also thought to function. Certain hnRNPs and EJC components are shed from the mRNP prior to export, and proper mRNP formation appears to be monitored at this stage by the exosome. Specific pore proteins, or Nups, are implicated in moving mRNA cargo through the pore (Fig. 2). Although loaded onto the transcript early in biogenesis, Dbp5 may play a late role in remodeling and/or moving the mRNP complex. (4) Cytoplasmic function. Factors remaining on the mRNA, such as Y14 and Magoh, influence translation and localization of mature mRNPs.

other interpretations are also possible, these results are consistent with the idea that there may be different ways to get a shared set of export factors loaded onto mRNA and, depending on how factors get loaded onto a transcript, the association may be of different duration and/or stability.

COORDINATING mRNA EXPORT WITH TRANSCRIPTION AND TURNOVER

The addition of a 5' cap, splicing, polyadenylation, and cleavage all occur in close connection to transcription. Concurrent with the processing events, mRNAs are also packaged with a number of proteins specific to this class of RNA (Fig. 1). A significant subset of such proteins was originally classified as hnRNPs for their ability to associate with heterogeneous nu-

clear RNA (reviewed in reference 15). Interestingly, hnRNP A1 was later implicated in mRNA export, as a fragment of hnRNP A1 injected into *Xenopus* oocytes inhibited the export of dihydrofolate reductase mRNA (41). However, the extent to which hnRNP proteins function in export has not yet been elucidated. Mutation of the yeast protein Npl3p, which bears characteristics of both hnRNP and SR (serine-arginine)-type proteins, causes an mRNA export defect (52). Npl3p has been found in a complex with RNA polymerase II and is recruited to genes in a transcription-dependent manner (54).

Other proteins, not necessarily classified originally in the hnRNP category but loaded onto mRNA, have been functionally connected to both export and transcription. Yra1p and Sub2p display both genetic and physical interactions with all members of the yeast THO complex, a protein complex iden-

tified originally for a role in transcription elongation (9). REF and UAP56 along with the vertebrate counterparts of THO and a new protein of unknown function, Tex1, make up the TREX (transcription and export) complex (80). In yeast, specific TREX components associate with genes during transcription and, individually, their deletion results in nuclear poly(A)⁺ accumulation. Together, this suggests that TREX proteins may be important in mediating cotranscriptional recruitment of factors important in export. For example, one protein in the TREX complex, Hpr1p, is required for efficient targeting of Yra1p and Sub2p to genes undergoing active transcription (95). Therefore, cotranscriptional recruitment and splicing-dependent recruitment represent two broad mechanisms by which mRNA export factors can associate with RNA cargo. For instance, REF may be efficiently loaded onto specific RNA cargos via cotranscriptional targeting of the TREX complex and/or through splicing-dependent deposition of the EJC.

Another example of the connection between transcription and export is found in the DEAD box helicase Dbp5 (16). Dbp5 is localized at steady state to the cytoplasmic fibrils of the nuclear pore complex (NPC) and has been hypothesized to be involved in a terminal step of mRNA release from the NPC, possibly acting in a remodeling step to unwind mRNPs entering the cytoplasm (see reference 16 and references therein). On the other hand, yeast Dbp5p shuttles (38), and in *C. tentans*, Dbp5 associates with mRNA as early as during its transcription, accompanying the mRNA through the NPC and into the cytoplasm (96). Recent work with *S. cerevisiae* has shown that Dbp5p both genetically interacts with genes involved in early transcription events and physically interacts with multiple subunits of transcription factor TFIID (16). Thus, Dbp5 may load onto mRNA cargo very early in biogenesis, although this does not preclude (and in fact may enhance) a later role for Dbp5 in remodeling the mRNP. Overall, much evidence is arising to support a link between mRNA synthesis and the effective recruitment of export factors to the nascent transcript.

An emerging theme is that coupling of mRNA cargo recognition to other important aspects of its physiology, such as transcription or splicing, is a general strategy to generate specificity in mRNA export, perhaps compensating for the generic nature of identifying features of mRNA export cargo. Likewise, proofreading mechanisms appear to be in place to rid the cell of any mRNAs that have been produced but not properly coupled to the next step. Notably, studies have identified functional interactions between the mRNA export factor Yra1p and Rrp6p and Rrp45p, components of the nuclear exosome, which is a protein complex containing multiple RNA exonucleases (95). Failure to correctly form mRNPs results in the nuclear retention and degradation of aberrant mRNA species, serving as an additional level of control to regulate export and ultimately efficient gene expression (recently reviewed in references 43, 68, and 82). This complicated network interconnecting transcription, export, and RNA stability additionally underscores the importance of having functional read-outs for export to complement the bulk poly(A)⁺ accumulation assay.

MOVING ON TO THE NUCLEAR PORE

NPCs span the nuclear envelope and serve as gateways of communication between the nucleus and cytoplasm. The pore is a huge structure with ~30 individual nuclear pore proteins or nucleoporins (Nups) present several times, creating octagonal symmetry. The pore also has asymmetric features on its nuclear and cytoplasmic faces (for recent reviews, see references 6 and 83). Although much of the process of mRNA export is being deciphered, there is still little known about how mRNPs interface with pore machinery. Some recent studies have focused on the roles of proteins that are closely associated with the pore, such as Gle1 and RAE1/Gle2. Gle1 is essential for mRNA export in both yeast and human cells (13, 62, 63, 91), and hGle1 is a dynamic factor that shuttles between nucleus and cytoplasm (47). The shuttling domain of hGle1 acts as a dominant-negative export inhibitor of both bulk poly(A)⁺ RNA and specific mRNA transcripts (47). Docking of hGle1 at the NPC was recently shown to depend on an interaction with the pore protein Nup155 (67).

Murine RAE1 is essential; however, cells from mice bearing targeted disruption of RAE1 do not have a detectable defect on bulk mRNA export (1). In contrast, RAE1 deletion in yeast results in nuclear accumulation of poly(A)⁺ RNA (61). Although there appear to be redundant factors in vertebrates, hRAE1 interacts with NXF1 and the nucleoporin Nup98, as well as with mRNA itself (51), and has been speculated to be involved in delivering mRNA cargo-receptor complexes to Nup98 (7). Nup98, in turn, has been implicated through antibody inhibition studies in the export of mRNA as well as other classes of RNA (66). Nup98 shares similarity with yeast nuclear pore proteins Nup145, Nup116, and Nup100. Deletion of yNup145 causes the nuclear accumulation of poly(A)⁺ RNA (17).

Vertebrate pore proteins have not been exhaustively screened and individually tested for roles in mRNA export. However, along with Nup98, five other vertebrate pore proteins, Nup153, Nup160, Nup133, Tpr, and CAN/Nup214, have so far been implicated in the export of mRNA (Fig. 2). Mouse embryos deficient in CAN/Nup214 not only show arrest in the G₂ phase of the cell cycle but also demonstrate nuclear accumulation of poly(A)⁺ RNA (88). Nup159/Rat7, the yeast orthologue of CAN/Nup214, is similarly implicated in mRNA export, with a temperature-sensitive mutation causing very rapid onset of accumulation of poly(A)⁺ RNA in the nucleus (26). CAN/Nup214 associates with the mRNA export factor Dbp5, an interaction conserved from yeast to vertebrates (38, 72). In addition, CAN/Nup214 is the only vertebrate nucleoporin with a steady-state localization exclusively on the cytoplasmic side of the pore that has been implicated in mRNA export thus far.

The nucleoporin Nup153 has a complicated localization pattern but is found primarily at the nuclear pore basket (reference 19 and references therein) (Fig. 2; see also below). Overexpression of Nup153 fragments in cultured cells causes nuclear poly(A)⁺ accumulation (5), and perturbation of Nup153 function in *Xenopus* oocytes blocks specific RNA species, including mRNA, from being exported (87). Independent overexpression of either Nup160 or Nup133 fragments in culture also causes the nuclear accumulation of poly(A)⁺ RNA

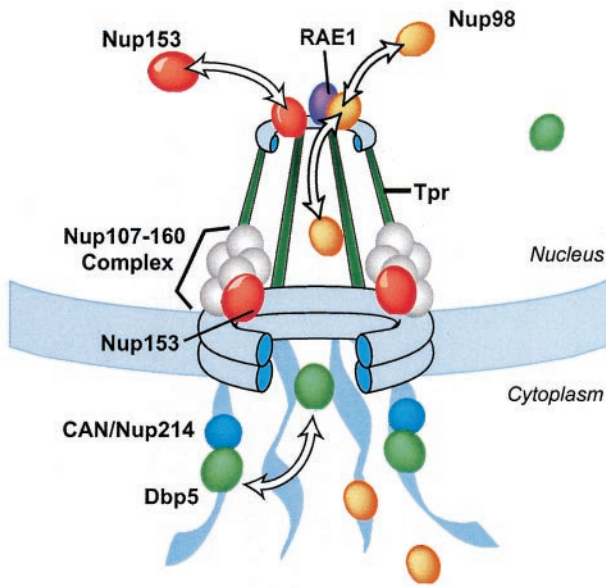


FIG. 2. Distribution and dynamics of pore proteins and associated factors implicated in mRNA export. Nup153 and Nup98 are both dynamically associated with the nuclear pore in a manner dependent on ongoing transcription. However, in the case of Nup153, there is also evidence for a stable population, which is schematically illustrated here proximal to the inner nuclear membrane. The presence of distinct populations of Nup153 is consistent with epitope exposure of this protein: different regions are exposed at the distal and proximal ends of the pore basket. An extended conformation of Nup153 at the pore is an alternative explanation for epitope distribution. Regardless of exactly how Nup153 is arranged at the pore basket, there is evidence that the C-terminal region of this pore protein can extend into the cytoplasm, although Nup153 does not appear to be released from this face of the pore. In contrast, Nup98 exists in equilibrium with a cytoplasmic pool and is known to interact with components of both sides of the pore. RAE1/Gle2 is a partner protein of Nup98. Both Nup153 and Nup98 associate with the Nup107-160 complex, a stable component of the pore. Tpr is also a component of the nuclear pore basket and relies on interaction with Nup153 for correct localization. CAN/Nup214 is localized to fibrils extending from the cytoplasmic ring of the pore and is a docking site for the dynamic DEAD box helicase, Dbp5.

(89). Nup160 is found in a complex (termed the Nup107-160 complex) with Nup133, Nup107, and Nup96, which parallels a network of nucleoporins in yeast (83, 89). The Nup107-160 complex associates with both Nup153 and Nup98. However, whereas the Nup107-160 complex is a stable component of the pore (90), Nup98 and Nup153 are both mobile pore proteins (11, 29). Studies using FRAP (fluorescence recovery after photobleaching) have demonstrated that Nup98 is highly dynamic and its mobility is dependent on ongoing transcription by RNA polymerases I and II (29, 31). Photobleaching studies indicated that Nup153 is also highly mobile (11, 30). As seen with Nup98, mobility of Nup153 is arrested in the presence of transcription inhibitors, although transcription-dependent dynamics are mediated by distinct domains within each protein (30). Transcription could be critical because the dynamic association of these nucleoporins with the pore is coupled to movement of RNA cargo through the pore. Given the links between transcription and export, it is also possible that these mobile pore proteins load onto RNAs destined for the pore at the time of transcrip-

tion and require such association for ongoing mobility (Fig. 1). Although much remains to be elucidated, Nup98 and Nup153 are prime candidates for coupling the production of mRNA to its transport into the cytoplasm.

A novel RNA binding domain identified within Nup153 (14) preferentially interacts with unstructured RNA, independent of orientation (3). Such features match the criteria that define an mRNA identity element. The RNA binding domain within Nup153, therefore, may be involved in the interface between this pore protein and mRNA during the export process. Another possibility is that Nup153 aids in recognizing RNAs that have an anomalous stretch of unstructured RNA. In either case, understanding the functional contribution of RNA binding will go hand-in-hand with integrating other information about Nup153 since the RNA binding domain (amino acids 250 to 400) overlaps binding sites for the Nup107-160 complex (amino acids 1 to 339) (89) and Tpr (amino acids 228 to 439) (34). Surprisingly, in light of the rapid dynamics of Nup153, Tpr relies on this interaction with Nup153 for pore localization (34). If this interaction facilitates anchorage rather than delivery of Tpr, it would suggest that there is a population of Nup153 stably associated with the pore. The existence of such a nonmobile fraction of Nup153 is also supported by photobleaching studies (11, 30). Indeed, multiple populations of Nup153, present at different parts of the pore and in the nuclear interior itself, may be part of the explanation for how the N-terminal region of this protein can interact with so many partners. In terms of RNA export, the functional connection between Nup153 and Tpr is not well defined, although Tpr has also been implicated in the export of both protein and RNA in metazoan cells (4, 23, 75).

CONNECTIONS BETWEEN THE PORE AND QUALITY CONTROL

Studies with yeast have determined that Mlp1p and Mlp2p, proteins with similarity to mammalian Tpr, have a role in mRNA export. Overexpression of Mlp1p causes poly(A)⁺ RNA nuclear accumulation (50). In addition, the yeast hnRNP proteins Npl3p and Nab2p also interact with Mlp1p and may facilitate Mlp function. For example, Nab2p, previously shown to be required for poly(A)⁺ export in yeast (28), accumulates in the nucleus of cells overexpressing Mlp1p (27). In an intriguing recent development, Mlp interactions with the exosome component Rrp6p have also been determined (A. Corbett, personal communication), suggesting that Mlps could participate in a proofreading step that ensures export occurs only for properly formed mRNPs. The connection between the exosome and transport is further underscored by the observation that, in yeast, inhibition of RNA export leads to exosome (Rrp6p)-dependent accumulation of a heat shock transcript near its site of synthesis (85). The extent to which pore proteins and the exosome machinery are functionally connected in vertebrates has not yet been addressed. In this regard, however, it is intriguing that, upon heat shock, *Drosophila* Tpr has been seen to accumulate at a particular chromosomal puff region (93D), a heat shock locus encoding an RNA (hsr-[omega]) that is not exported (98). Future studies are needed to address the full significance of this localization as well as its relationship to the exosome.

TRANSPORT THROUGH THE PORE: PUTTING INDIVIDUAL COMPONENTS INTO CONTEXT

In recent years, several models to explain the mechanism of movement through the pore have been proposed (18, 22, 70, 92). In each, the FG repeat regions found in several nucleoporins play a prominent role, both in contributing to an exclusion barrier as well as in serving as binding sites for cargo-receptor complexes. Consistent with this, NXF1 directly interacts with the FG repeat domains of several nucleoporins in vitro (2). A challenge in thinking about the mechanisms involved in mRNA translocation through the pore is that mRNPs represent a large and heterogeneous type of cargo and, further, appear to undergo remodeling in conjunction with transport. The large Balbiani ring mRNP of *C. tentans* has been shown by electron microscopy to first dock and then unwind as it traverses the pore (12, 49). Elegant immunoelectron microscopy studies have gone on to illustrate that certain proteins are shed from the mRNP, while others accompany the RNA through the pore (reviewed in references 12 and 15). Complicating things further, the nuclear pore basket itself has been observed to adopt different conformations when the Balbiani ring mRNA is traversing the pore. While this large RNA may utilize some specialized mechanisms to promote export, other features of its export are predicted to fit a general paradigm, raising the question of how dynamics of the nuclear pore basket fit into mRNA export. The dynamic nature of specific pore basket components themselves (Nup98 and Nup153), as well as the sensitivity of such mobility to the transcriptional status of the cell, suggests that basket remodeling may normally be ongoing in a manner linked to RNA trafficking. New information derived from reconstructing images of native NPCs suggests that the distal ring of the pore basket is not an open hole but rather a dense structure (76), further underscoring the necessity of remodeling events for this point of entry. Although not mutually exclusive with the idea of pore remodeling, an alternative point of entry into the pore, in between the fibers of the pore basket, has also been proposed (40). Much work is still needed to understand how mRNA enters and translocates through the pore. Future approaches that provide high-resolution real-time imaging as well as more precise functional assays are sure to yield a very interesting story about how the complicated network of mRNA biogenesis connects with translocation through the nuclear pore and the downstream fate of the mRNA.

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ADDENDUM IN PROOF

During the review of the manuscript, two papers demonstrating further connections between pore proteins and distinct steps in mRNA biogenesis were published. Mlplp was found to

play a role in retention of unspliced mRNA. Nup60p, a binding partner of Mlplp, was also implicated in this quality control process (V. Galy, O. Gadal, M. Fromont-Racine, A. Romano, A. Jacquier, and U. Nehrbass, *Cell* **116**:63–73, 2004). Forler et al. found that RNAi knock-down of Nup358/RanBP2 in *Drosophila* cells led to nuclear poly(A)⁺ accumulation. They also independently targeted Nup214 and saw a similar effect on mRNA. However, reduction of Nup358 and not Nup214 led to mislocalization of NXF1, suggesting that Nup358 plays a key role in keeping NXF1 concentrated at the cytoplasmic face of the pore (D. Forler, G. Rabut, F. D. Ciccarelli, A. Herold, T. Kocher, R. Niggeweg, P. Bork, J. Ellenberg, and E. Izaurralde, *Mol. Cell. Biol.* **24**:1155–1167, 2004).

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