

Ratcheting mRNA out of the Nucleus

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Export of mature mRNA to the cytoplasm is the culmination of the nuclear portion of eukaryotic gene expression. After transport-competent mature mRNP export complexes are formed in the nucleus, their passage through nuclear pore complexes (NPCs) is facilitated by the Mex67:Mtr2 heterodimer. At the NPC cytoplasmic face, mRNP remodeling prevents its return to the nucleus and so functions as a molecular ratchet imposing directionality on transport. In budding yeast, recent work suggests that the DEAD-box helicase Dbp5 remodels mRNPs at the NPC cytoplasmic face by removing Mex67 and that the Dbp5 ATPase is activated by Gle1 and inositol hexaphosphate (IP₆).

Export of mRNA is more complex than many nuclear trafficking pathways because it is important to ensure that transcription, splicing, and processing have been completed before mRNA is exported (Rodriguez et al., 2004; Moore, 2005; Cole and Scarcelli, 2006a). However, although the mRNA nuclear export machinery has a number of unique features, it retains the same basic sequence of steps (Figure 1) that are common to other nuclear trafficking pathways, such as those involved in nuclear protein import and export and the export of smaller RNAs, in which cargoes are transported through nuclear pore complexes (NPCs) by soluble carrier molecules (reviewed by Pemberton and Paschal [2005]). Nuclear trafficking pathways involve three key steps (Figure 1): generation of a cargo:carrier complex in the donor compartment, translocation of this complex through NPCs, and release of the cargo in the target compartment and recycling of the carrier. Passage through NPCs is facilitated by interactions between the carriers and NPC proteins that contain distinctive sequence repeats (FG-nucleoporins) that have cores rich in phenylalanine and glycine (such as GLFG and FxFG) separated by hydrophilic linkers of varying sequence and length (reviewed by Tran and Wentz [2006]). Most nuclear trafficking pathways use members of the β -karyopherin superfamily as carriers, but mRNA export is unique in employing primarily the Mex67:Mtr2 heterodimer (TAP:p15 or NXF1:NXT1 in metazoans). A crucial feature of all nuclear trafficking pathways is the recognition of the donor and target compartments to ensure appropriate assembly of transport complexes and cargo release. With β -karyopherins, this recognition is mediated by the nucleotide state of the Ran GTPase, but the nature of this recognition step, together with the way in which transport is initiated and cargo released, is less clear for mRNA export in which both steps are associated with extensive mRNP remodeling. Recent work has given new insight by showing that in budding yeast the DEAD-box helicase Dbp5 facilitates removal of Mex67 after transport through the pores (Lund and Guthrie, 2005) and that the Dbp5 ATPase is activated by Gle1 and inositol hexaphosphate (IP₆), which probably occurs when Dbp5 and Gle1

are bound to nucleoporins located at the NPC cytoplasmic face (Alcazar-Roman et al., 2006; Weirich et al., 2006).

Assembly of mRNP Export Complexes

Nuclear export of mRNA is intimately linked to controlled remodeling of mRNP complexes (Cole and Scarcelli, 2006a, 2006b). A myriad of different proteins bind to mRNA as it progresses to form a mature mRNP in the nucleus. Many of these proteins are associated with particular steps of the gene-expression pathway, such as transcription, splicing, or polyadenylation, and are generally removed before nuclear export. However, many proteins still accompany mRNPs to the cytoplasm, although not all are involved directly in nuclear export. For example, the exon junction complex (EJC) has a crucial role in nonsense-mediated decay (Bono et al., 2006), whereas others function in translation or mRNA targeting. Although the precise way in which completion of mRNP maturation is recognized is unclear, binding of Mex67:Mtr2 is necessary for export. Although Mex67 has an mRNA-binding domain, adaptor/accessory proteins such as REF/Aly/Sub2, EJC components, or SR proteins (Moore, 2005; Huang and Steitz, 2005) also promote its binding to mRNPs.

Translocation through Nuclear Pores

Translocation of mRNP export complexes through NPCs is thought to be mediated primarily by weak interactions between the Mex67:Mtr2 heterodimer and FG-nucleoporins (Braun et al., 2002; Rodriguez et al., 2004). Work on other nuclear trafficking pathways has indicated that transport complexes generally move through the NPC transport channel by simple diffusion (Yang et al., 2004). Thus, the function of the carrier is to facilitate equilibration of the transport complex between the nuclear and cytoplasmic faces of the NPC by an essentially passive mechanism. Transport is driven by preventing the return of transport complexes to the donor compartment. For karyopherin-mediated transport, this is achieved by transport complex disassembly in the target compartment. By Le Chetelier's principle, when transport complexes are dissociated, new complexes move to the target compartment to restore the equilibrium. Transport thus becomes

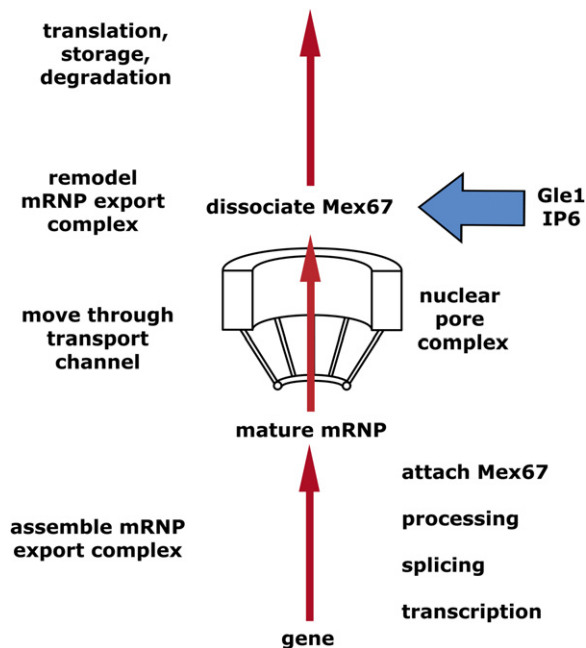


Figure 1. Steps along the Gene-Expression Pathway

A mature mRNP is formed after a number of coordinated nuclear steps, including transcription, splicing, and processing. In addition to a large number of other proteins, the mature mRNP also binds the Mex67:Mtr2 complex, which facilitates its passage through NPCs via weak interactions with FG-nucleoporins. Removal of the Mex67:Mtr2 complex at the cytoplasmic face of the NPC by Dbp5 could act as a Brownian ratchet to prevent return of the mRNP to the nucleus and so generate transport directionality.

a form of rectified Brownian (thermal) motion, where energy is required primarily for the recognition steps involved in transport complex formation and disassembly. Transport complex disassembly thus acts as a molecular Brownian ratchet, imposing directionality of movement through the pores. Although mRNPs are generally larger than the cargoes transported by karyopherins, a Brownian ratchet can still in principle generate transport. The distances traversed through NPCs (~70 nm) are considerably smaller than the steps observed with much larger particles undergoing Brownian motion, and, moreover, movement through NPCs is “lubricated” by the FG-nucleoporins. Even for gigantic mRNPs, such as the Balbiani rings of *Chironomus* that thread through NPCs from their 5' end, thermal motion backward and forward combined with a molecular ratchet could move it through the NPC (Figure 2). An attractive way a ratchet could be generated would be to remove molecules that facilitate passage through NPCs such as Mex67:Mtr2, although other forms of mRNP remodeling could also prevent backsliding. For very large mRNPs it would probably be necessary to have a number of ratchet steps, which need not all employ the same mechanism. Naturally, a thermal ratchet mechanism may also be complemented by some form of pulling, which could be provided by, for example, the translation machinery or by binding cytoplasmic proteins.

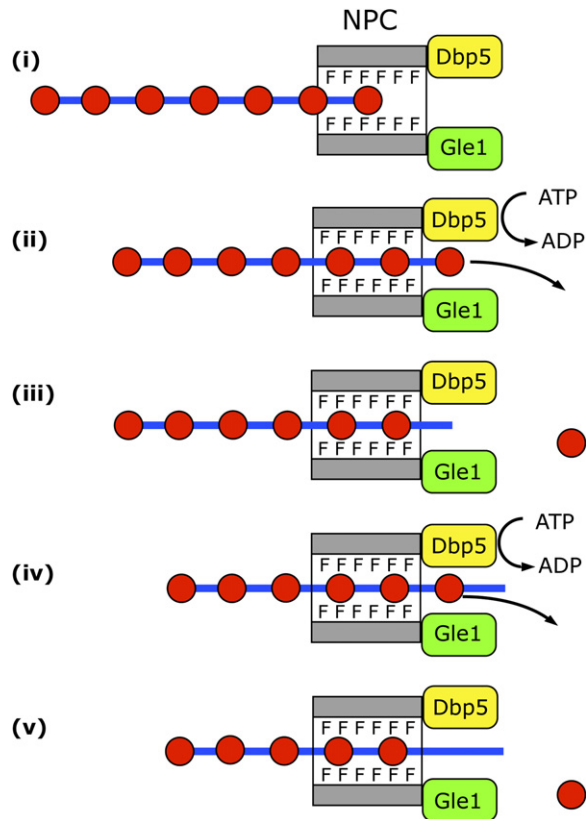


Figure 2. Schematic Illustration of How a Brownian Ratchet Could Transport Large mRNPs through NPCs

(i) A large mature mRNP (shown schematically as a blue line although it will have extensive secondary structure) probably has several Mex67:Mtr2 carrier complexes (red) attached along its length. Interactions between Mex67:Mtr2 and FG-nucleoporins (F) that line the NPC transport channel (gray) facilitate movement of the mRNP backward and forward by thermal motion (Brownian movement). (ii) When one of the Mex67:Mtr2 complexes reaches the cytoplasmic face of the NPC, it is removed from the mRNP by the DEAD-box helicase Dbp5, the ATPase activity of which is stimulated by Gle1 and IP₆. (iii) Removal of Mex67:Mtr2 prevents this segment of the mRNP from moving back into the transport channel and so functions as a molecular ratchet. (iv) Brownian motion soon results in another Mex67:Mtr2 complex reaching the cytoplasmic face, where again it is removed by Gle1:IP₆-stimulated Dbp5, and now (v) a longer segment of the mRNP is prevented from returning. By iteration of this cycle, even an extremely large mRNP can be ratcheted into the cytoplasm using the ATPase activity of Dbp5 to rectify thermal motion. The Mex67:Mtr2 liberated is recycled to the nucleus to participate in another mRNP export cycle. Although this mechanism envisages Mex67 release through the Gle1:IP₆ stimulation of the Dbp5 ATPase, other mRNP-remodeling processes at the NPC cytoplasmic face could generate directionality in an analogous manner.

Removal of Mex67:Mtr2

Although removal of Mex67:Mtr2 from the mRNP could act as a molecular ratchet preventing return to the nucleus by inhibiting translocation through the NPC, the mechanism by which Mex67:Mtr2 is removed has been obscure. One possibility would be that it is removed by the first “pioneer” round of translation. Although several shuttling RNA-binding proteins remain associated with mRNPs during translation, Mex67 appears to be removed before

mRNA associates with the translation machinery (Lund and Guthrie, 2005). Although phosphorylation releases Mex67 bound to SR proteins (Huang and Steitz, 2005), new evidence suggests that, in budding yeast, Dbp5 can remove Mex67 from mRNPs at the cytoplasmic face of NPCs (Lund and Guthrie, 2005). Dbp5 is essential and is one of a host of DEAD-box helicases that perform a variety of functions in the gene-expression pathway (reviewed by Cordin et al. [2006]). These helicases are ATPases and, whereas some remodel mRNPs through their helicase activity, others are thought to function primarily by dissociating proteins. Dbp5 shuttles between nucleus and cytoplasm and is recruited to mRNPs early during transcription (Cole and Scarcelli, 2006a). The N-terminal domain of Nup159, a nucleoporin located only on the NPC cytoplasmic face (Tran and Wenthe, 2006), has a β propeller fold that binds Dbp5 (Weirich et al., 2004), and so its steady-state localization is to the cytoplasmic face of NPCs (Cole and Scarcelli, 2006a, 2006b). However, because Dbp5 is attached to the mRNP during transcription, it has been unclear why it should remove Mex67 only after transit through NPCs and not in the nucleus, where both are attached to the mRNP.

Recent work (Alcazar-Roman et al., 2006; Weirich et al., 2006) has suggested a solution to this paradox by showing that the Dbp5 ATPase is stimulated by Gle1, an essential mRNA export factor also located primarily at the cytoplasmic NPC face, together with IP₆. Gle1 is linked genetically to both Dbp5 and to components of the IP₆ biosynthetic pathway (Alcazar-Roman et al., 2006) and binds to nucleoporin Nup42 that, like Nup159, is located at the cytoplasmic face of NPCs and so is ideally located to provide a marker to indicate that the cytoplasm has been reached (Alcazar-Roman et al., 2006; Weirich et al., 2006). Dbp5 probably requires additional factors for activity under physiological conditions. In this context, the observation that Gle1 stimulates the Dbp5 ATPase in an IP₆- and RNA-dependent manner suggests a mechanism by which mRNP export complexes may be remodeled at the NPC cytoplasmic face (Alcazar-Roman et al., 2006; Weirich et al., 2006). Although Gle1 alone increases both the ATPase efficiency of Dbp5 and its affinity for RNA, IP₆ enhances this effect. IP₆ appears to function directly in the Gle1:Dbp5 interaction and not by promoting Gle1 binding to Nup42. Point mutations in either Dbp5 or Gle1 enable the enzyme complex to bypass the requirement for IP₆, both in vivo and in vitro, consistent with both Gle1 and IP₆ functioning primarily to stimulate the Dbp5 ATPase in nuclear mRNP export. The specific interaction of Dbp5 and Gle1 with Nup159 and Nup42, respectively, suggests that the Dbp5 ATPase is activated locally at the NPC cytoplasmic face, although this has not been demonstrated directly. In addition, Gle1 also binds to mRNPs through Gfd1, which binds to both Gle1 and mRNP-bound Nab2 (Suntaralingam et al., 2004). Furthermore, Ipk1, the enzyme that generates IP₆, is also located at the nuclear periphery.

Although the Dbp5:Gle1:IP₆ interaction is important in mRNA export, how it functions is not yet completely clear.

In principle, Dbp5 could be acting as a processive motor winding RNA through the pore, but this seems unlikely. Few DEAD-box helicases are processive (Cordin et al., 2006), and it would probably require an extravagant amount of ATP to move an RNA chain base-by-base through the helicase. More likely, Dbp5 functions as a ratchet to remodel mRNPs in the cytoplasm, preventing their return to the nucleus. Removing Mex67:Mtr2 would be one way this could be achieved, although this would not preclude Dbp5 having additional remodeling functions. By analogy with the DEAD-box helicase eIF4AIII in the EJC (Bono et al., 2006), Dbp5 probably wraps around the RNA and ATP hydrolysis probably generates a considerable conformational change, although it is not clear how this might be harnessed. One possibility is that it may enable Dbp5 to dissociate Mex67 from mRNPs by competing directly for a binding site either on RNA or on other proteins bound to the mRNP, such as the EJC. Alternatively, the Dbp5 helicase activity could alter mRNA structure locally and so function by changing the conformation of the RNA to which Mex67 is bound. Dbp5 might also function indirectly by displacing other proteins to which Mex67:Mtr2 is bound, although this would perhaps seem less likely as some of these proteins, such as Yra1, are removed in the nucleus before export (Lund and Guthrie, 2005) whereas others, such as the EJC, remain attached until translation.

IP₆ appears to function primarily as a cofactor. Such a small molecule would not be well suited as a marker by which the export complex could recognize the cytoplasm because it would diffuse rapidly throughout the cell and, moreover, it has a number of defined functions in the nucleus. Although primarily bound to Gle1, IP₆ could still function as a Dbp5 ATPase cofactor in a Gle1:Dbp5 complex. Alternatively, it could be important for maintaining Gle1 in a conformation in which it can stimulate Dbp5, and indeed an analogous structural role for IP₆ has been observed in the human RNA-editing enzyme ADAR2 (Macbeth et al., 2005). Another possible function could be related to the large negative charge of IP₆ that might contribute to neutralizing the positive charge of mRNA-binding proteins released during mRNP remodeling.

Although the roles of Gle1, IP₆, and Dbp5 were investigated in budding yeast, an analogous series of factors appear to be important for mRNA export in metazoans. Dbp5 and Gle1 homologs are found in humans, although here nucleoporins hCG1 and Nup155 are involved in tethering hGle1 to the NPC cytoplasmic face (Kendirgi et al., 2005), whereas Dbp5 is tethered by Nup214 (Rodriguez et al., 2004). Similarly, the principal metazoan mRNA export factor, the NXF1:NXT1 (TAP:p15) heterodimer, is homologous to Mex67:Mtr2 (Rodriguez et al., 2004) and so the mRNA export mechanism appears to be strongly conserved.

Future Directions

Although a Brownian ratchet based on removing Mex67 through the Gle1:IP₆-stimulated ATPase activity of Dbp5

is an attractive mechanism for mRNP nuclear export and is consistent with recent observations, evidence is currently lacking or indirect for several steps in this pathway. Both Dbp5-mediated Mex67 release (Lund and Guthrie, 2005) and Gle1:IP₆ stimulation of the Dbp5 ATPase (Alcazar-Roman et al., 2006; Weirich et al., 2006) are important for mRNP export, but, although the binding of Gle1 and Dbp5 to Nup42 and Nup159, respectively, makes it very likely that this stimulation occurs at the NPC cytoplasmic face, this has not been demonstrated directly. It is also important to understand the molecular basis for both attachment and removal of Mex67, the extent to which other mRNP-bound proteins (such as EJC and SR proteins) are involved, and also the numbers of these proteins attached to export-competent mRNPs. Moreover, although Dbp5 dissociates Mex67 from mRNPs, it has not been demonstrated directly that this requires Gle1:IP₆ stimulation of the Dbp5 ATPase. It is also unclear whether Dbp5:Gle1:IP₆-mediated Mex67 release is sufficient for mRNA export or if it is only one of several mRNP-remodeling steps involved. Another unanswered question is how Dbp5 is able to find Mex67 on the mRNP. Are they located near one another or could the activity of Dbp5 be propagated over a considerable distance by, for example, modifying mRNP structure? What role do other proteins, such as Nab2 and Gfd1, play? Because Mex67 appears to bind to several different mRNP components, it may also be that only part is removed by Dbp5 and complete removal requires additional steps. Because of the complexity of the process, deciphering precisely how Gle1, IP₆, and Dbp5 contribute to mRNP export and the extent to which other remodeling processes are involved will be challenging, but holds the prospect of giving major insight into the mechanism of mRNA export.

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