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Mechanisms of nuclear mRNA export: a structural perspective

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Abstract

Export of mRNA from the nucleus to the cytoplasm is a critical process for all eukaryotic gene expression. As mRNA is synthesized, it is packaged with a myriad of RNA-binding proteins to form ribonucleoprotein particles (mRNPs). For each step in the processes of maturation and export, mRNPs must have the correct complement of proteins. Much of the mRNA export pathway revolves around the heterodimeric export receptor yeast Mex67•Mtr2/ human NXF1•NXT1, which is recruited to signal the completion of nuclear mRNP assembly, mediates mRNP targeting/translocation through the nuclear pore complex (NPC), and is displaced at the cytoplasmic side of the NPC to release the mRNP into the cytoplasm. Directionality of the transport is governed by at least two DEAD-box ATPases, yeast Sub2/human UAP56 in the nucleus and yeast Dbp5/human DDX19 at the cytoplasmic side of the NPC, which respectively mediate the association and dissociation of Mex67•Mtr2/NXF1•NXT1 onto the mRNP. Here we review recent progress from structural studies of key constituents in different steps of nuclear mRNA export. These findings have laid the foundation for further studies to obtain a comprehensive mechanistic view of the mRNA export pathway.

Keywords

mRNA export; structural biology; mRNP remodeling; DEAD-box ATPase; export receptor

With the arrival of the nucleus in cellular evolution, intricate mechanisms to transport macromolecules between the nucleus and the cytoplasm became essential. Nucleocytoplasmic transport events occur through the nuclear pore complex (NPC), the cell's largest and most versatile transport channel, which allows transport of proteins and various RNA families such as mRNA, rRNA, tRNA, miRNA, snRNA[1,2]. With respect to mRNAs, transport through the NPC is unidirectional: mRNAs are synthesized in the nucleus, and are exported to the cytoplasm for translation. The central channel of the NPC is filled with thousands of phenylalanine-glycine (FG) peptide repeats[3]. As a general principle for all transport across the NPC, cargo needs to acquire at least one transport receptor to overcome the permeability barrier of the FG milieu[4]. Most nucleocytoplasmic transport events employ the karyopherin family of transport receptors. To establish the transport directionality, the small GTPase Ran, via a concentration gradient of RanGTP across the

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nuclear envelope maintained by the NTF2 protein, drives the assembly/disassembly of the cargo-receptor complex[4,5]. Overall, Ran-dependent transport of protein and several RNA families is well understood (readers are referred to an excellent review by Güttler and Görlich[6]). Bulk mRNA export, however, employs a unique mechanism that does not depend on karyopherins or Ran, and the underlying molecular basis is less well understood. This review discusses ongoing research, mainly from a structural perspective, to provide an overview of the key steps in nuclear mRNA export.

1 The principal export receptor Mex67•Mtr2/NXF1•NXT1 mediates bulk mRNA export

As mRNA is synthesized and processed in the nucleus, it is packaged with RNA-binding proteins (RBPs) to form ribonucleoprotein particles (mRNPs)[7]. mRNPs are formidably diverse. Human cells carry tens of thousands of different mRNAs, and the protein composition of each individual mRNP is unique and highly dynamic throughout its life cycle. Despite the complexity in mRNP protein composition, export of the vast majority of mRNAs utilizes a non-karyopherin export receptor, the heterodimeric Mex67•Mtr2 in budding yeast/NXF1•NXT1 (TAP•p15) in humans[8–11]. NXF1•NXT1 was found to be an mRNA export receptor in light of the observation that NXF1•NXT1 mediates nuclear export of CTE (constitutive transport element) containing RNA, which resides in some retroviral genomes and promotes export of unspliced retroviral RNA[9]. Expression of NXF1•NXT1 in yeast rescues growth of the otherwise lethal *mex67 mtr2* double null strain, revealing a conserved function of the export receptor from yeast to humans[8].

Two key aspects of transport receptor function are cargo recognition and nucleoporin FG-repeat recognition. Both yeast Mex67 and human NXF1 have a modular architecture that includes an RRM (RNA recognition motif), LRR (leucine-rich region), NTF2L (NTF2-like), and UBA (ubiquitin-associated) domains. Mtr2/NXT1, which also exhibits an NTF2-like fold, binds to the NTF2L domain of Mex67/NXF1 (Figure 1A). With respect to cargo recognition, RRM, LRR, and NTF2L domains are all capable of binding RNA[12,13]. Mex67•Mtr2/NXF1•NXT1 binds to RNA without sequence specificity[14,15], which is consistent with the role as a general export receptor. Transcriptome-wide analysis of the RNA-binding landscape of Mex67 suggests a uniform coverage on mRNAs[14,15], but the exact binding mode and copy number of export receptor(s) on an individual mRNP remain unclear. In regard to FG-repeat recognition, Mex67/NXF1 features two FG-repeat binding sites on the NTF2L and UBA domains[16,17] (Figures 1B and 1C). Binding between Mex67/NXF1 and FG-repeats is weak in nature[17], enabling rapid exchange of FG-repeats from the export receptor as the mRNP translocates through the central channel of NPC.

For a long time, the four domains of NXF1 were thought to be arranged like beads on a string, and there was no clear understanding of how the export receptor functions as a whole. However, a recent structure of yeast Mex67•Mtr2 containing the RRM, LRR, and NTF2L domains of Mex67 has established that the LRR domain has a defined orientation relative to the NTF2L domain, while RRM and UBA domains are mobile[12] (Figure 1D). Intriguingly, two copies of human NXF1•NXT1 can also form a higher-order assembly via domain

swapping, where two NXT1 molecules are juxtaposed and the LRR and NTF2L of each NXF1 are connected by a linker that traverses along the surface of both NXT1s[18] (Figure 1E). This configuration generates a 2-fold symmetric platform, featuring a continuous RNA binding surface on one side of the protein complex with the FG-repeat binding sites on the opposite side. Intriguingly, the higher-order NXF1•NXT1 assembly is targeted by the influenza A virus NS1 protein to block nuclear export of host mRNAs[19]. Mutations engineered to disrupt the formation of the higher-order NXF1•NXT1 assembly reduces the nuclear export of CTE-RNA *in vivo*, which also exhibits a 2-fold symmetry, but does not seem to affect bulk poly(A) RNA export. One hypothesis is that the higher-order NXF1•NXT1 assembly may facilitate nuclear export of a subset of structured RNAs, but the precise function and the prevalence of the higher-order form of NXF1•NXT1 in human cells remain unclear.

Although NXF1 can directly bind to RNA, its association and dissociation on the transcripts are regulated by DEAD-box ATPases. These energy-spending processes govern the directionality of the mRNA export process. Overall, nuclear mRNA export can be broken down into three steps: 1) Assembly of an export competent mRNP marked with Mex67•Mtr2/NXF1•NXT1, mediated by the Sub2/UAP56 ATPase in the nucleus; 2) mRNP targeting to and translocation through the NPC, and 3) Disassembly of the mRNP export complex by displacing factors including Mex67•Mtr2/NXF1•NXT1, mediated by the Dbp5/DDX19 ATPase at the cytoplasmic face of the NPC[20–22] (Figure 1F and Table 1). Below we highlight recent work on each of these steps in mRNA export.

2 Assembly of export competent mRNP

2.1 Sub2 mediated nuclear mRNP remodeling

In the nucleus, transcription, basic pre-mRNA processing (capping, splicing, and 3' end processing), and nuclear transport are integral steps in the nuclear phase of gene expression. A wealth of studies indicate that proper mRNA export is first set in motion at the steps of cotranscriptional RBP loading and pre-mRNA processing[23–26]. Association of Mex67/NXF1 onto a nuclear mRNP is driven by the RNA-dependent ATPase Sub2/UAP56[27] with assistance of a multisubunit complex THO[28–30] and an RBP Yra1/ALY[31,32]; Sub2/UAP56, THO, and Yra1/ALY together form the TRanscription-EXport (TREX) complex[33] (Table 1). The Sub2/UAP56 ATPase belongs to the family of DEAD-box RNA helicases, named based on a characteristic motif Asp-Glu-Ala-Asp (DEAD in single-letter code)[34]. DEAD-box proteins participate in all steps of RNA metabolism, in a manner that resembles the activities of protein chaperones. In particular, DEAD-box proteins promote rearrangement of RNA structures or assembly/disassembly of RNA-protein complexes at the expense of ATP hydrolysis. In addition to mRNA export, Sub2/UAP56 has additional roles in splicing and piRNA biogenesis[35–37]. Sub2/UAP56 contains two RecA like domains (NTD and CTD) and a short N-terminal extension. *In vitro*, Sub2/UAP56 like many DEAD-box proteins has ATP-dependent RNA helicase activity in a non-processive manner, and RNA-dependent ATPase activity[34,38,39].

Sub2-mediated nuclear mRNP remodeling occurs in a step wise fashion[27,40] (Figure 2A and 2B). Within the yeast TREX complex, THO exists as a robust structural and functional unit

comprised of the Tho2, Hpr1, Mft1, Thp2, and Tex1 proteins[28,29]. THO travels with transcribing RNA pol II by binding to phosphorylated Pol II CTD and recruits Sub2 to the transcription machinery[23,24]. Recombinant hetero-pentameric THO complex has been shown to stimulate Sub2 ATPase activity[41]. Crystal structure of a 360 kDa THO•Sub2 complex has been determined at 6 Å resolution, revealing the overall architecture of the hetero-pentameric THO complex and how THO activates the Sub2 ATPase[41] (Figure 2C). In particular, the THO complex forms an elongated scaffold, approximately 25 nm in length. It makes contact with both Sub2-NTD and Sub2-CTD, and induces a "half-open" configuration, in which the conserved motifs for ATP/RNA binding are pre-aligned in Sub2. The configuration of Sub2 bound to THO is structurally similar to Dbp5 bound to its activator Gle1[42–44]. Thus, the DEAD-box ATPases mediated nuclear and cytoplasmic mRNP remodeling share a similar activation mechanism. The THO•Sub2 crystal structure likely captures how THO recruits Sub2 to the transcription machinery, with the Sub2 primed in a "half-open" state for subsequent mRNP engagement.

Sub2 has been implicated in loading of Yra1 onto the mRNP, which in turn acts as an adaptor protein to recruit the export receptor Mex67•Mtr2[27]. Sub2 and Yra1 cooperatively bind to RNA in vitro[41]. A crystal structure of Sub2 in complex with the C-terminal region of Yra1 (Yra1-C) and poly (U) RNA reveals that Yra1-C folds into a helix and binds to the Sub2-NTD (Figure 2D)[41]. Sub2, in a "closed" configuration, recognizes the sugar-phosphate backbone of RNA, indicating a sequence non-specific binding. Interestingly, the RNA binding region of Yra1 that precedes Yra1-C would be located close to the bound RNA. The juxtaposition of RNA binding regions of Sub2 and Yra1 could generate an extended RNA binding site, in line with their cooperative RNA binding *in vitro*. In addition, Yra1-C is able to stimulate Sub2 ATPase activity *in vitro*. Together, the Sub2•Yra1-C•RNA complex likely represents a key assembly for recruitment of Yra1 onto the mRNP, which is coupled to the Sub2 engagement with RNA. Sub2 is thought to be later displaced, presumably after ATP hydrolysis, by the export receptor Mex67•Mtr2, as binding of Sub2 and Mex67•Mtr2 to Yra1 is mutually exclusive (Figure 2B)[27,31]. Yra1 does not accompany the mRNP to the cytoplasm; it is ubiquitinated by the E3 ligase Tom1, resulting in Yra1 dissociation form the mRNP, by an yet to define mechanism, prior to nuclear export (Figure 2A)[45].

To date, structural evidence for how the TREX complex facilitates the recruitment of the export receptor is still lacking. Recent studies of human TREX indicate that THO directly interacts with ALY and together they coordinately facilitate RNA binding of the export receptor[46,47]. This implies a more sophisticated mechanism than originally envisioned for this important step in mRNP maturation. When does THO associate with the mRNP and when is THO displaced? Although the THO•Sub2 structure suggests that THO does not favor binding to a "closed" form of Sub2, THO could remain anchored to one of the Sub2 domains when Sub2 is bound to RNA. In fact, evidence suggests that, as discussed in section 4.2, Gle1 can bind to the CTD of Dbp5 alone or to both the NTD and CTD together, indicating a dynamic nature of the Gle1-Dbp5 interaction. In addition, Yra1, as suggested by the human THO-ALY interaction, could potentially serve as another anchor for THO to associate with the mRNP. Furthermore, THO itself has been shown to bind RNA directly in vitro[29]. To date, the dynamics of the interaction between THO and the mRNP remain to be elucidated. Of note, THO makes up the most mass of the TREX complex, containing five

subunits in yeast and six subunits in humans[28–30,48]. Yet surprisingly, no atomic resolution structure has been reported for any THO subunit from yeast to humans, underscoring the need for further structural studies.

Sub2/UAP56-powered nuclear mRNP remodeling is a conserved mechanism from yeast to humans. Perhaps due to the more complex gene expression in humans, multiple adaptors including UIF and CHTOP share similar features with ALY, and are considered dynamic components of the human TREX complex[49,50]. Intriguingly, TREX mediates the export of both intronless and intron-containing genes[30,46,51–53]. One major difference in gene expression between yeast and humans is the prevalence of splicing. While only a small population (~5%) of yeast genes contain introns, the opposite is true in humans[54,55]. The recruitment mechanism of TREX likely reflects this difference, as yeast TREX is recruited by the transcriptional machinery, while human TREX is recruited to mRNA in a splicing dependent manner[23,24,30]. Much remains to be studied to elucidate how the same core machineries are employed in different ways in yeast and humans.

2.2 Integration of pre-mRNA processing and nuclear mRNP remodeling

How does the cell ensure that only properly processed transcripts will be exported into the cytoplasm for translation? There is compelling evidence that TREX is physically and functionally linked to mRNA biogenesis factors involved in every step of pre-mRNA processing. For example, Human TREX is recruited to the 5’ end of the mRNP through the interaction between ALY and the mRNA Cap binding complex (CBC)[25]. CBC is one of the earliest factors deposited on a growing mRNA chain. It associates with various RNAs transcribed by RNA Pol II, including mRNA, snRNA, and miRNA. CBC recruits discrete factors to promote processing and nuclear export for different RNA families[56]. With respect to mRNA, ALY association may be the first step to direct mRNA to the UAP56 mediated export pathway. In addition, the connection of TREX function to splicing has been found in both yeast and humans. Yeast THO associates with the two SR (serine/arginine-rich) proteins Hrb1 and Gbp2, which have been proposed to function as surveillance factors for the selective export of spliced mRNAs[57,58]. In humans, loading of TREX to spliced mRNA occurs by a splicing-coupled mechanism[30]. Furthermore, TREX function is connected to pre-mRNA 3’-end processing. Yra1 interacts with Pcf11, the Pol II CTD binding subunit of the cleavage-polyadenylation factor CF1A[49]. The Yra1-Pcf11 interaction is conserved from yeast to humans, and has been suggested to modulate the assembly of the 3’-end processing machinery[59,60]. Recent work also shows that human THO interacts with the poly(A) RNA binding protein ZC3H14, and their interaction is required for proper control of bulk poly(A) tail length[61]. Together, this multitude of connections between TREX and pre-mRNA processing steps suggests that decision-making during mRNA export is concurrent with pre-mRNA processing. These connections may direct, step by step, the ATPase-powered remodeling reactions to ultimately mark mature transcripts with the export receptor, thereby ensuring the fidelity of gene expression specifically at the stage of mRNP nuclear maturation.

3 mRNP targeting to the NPC

Export competent mRNPs first encounter the nuclear basket of the NPC preceding translocation through the central channel. The nuclear basket is composed of Mlp1, Mlp2, Nup1, Nup2, and Nup60 in yeast[62]. One mechanism that targets export-competent mRNPs to the NPC nuclear basket is transcription-coupled mRNA export, mediated by the yeast TREX-2 complex[63–66] (Figure 3A). TREX-2 is linked to transcription machinery via the SAGA complex, which is a chromatin-modifying transcriptional coactivator, and the Mediator complex, which is an essential regulator of RNA Pol II[67,68]. TREX-2 associates with the nuclear basket and promotes the targeting of actively transcribed genes to the NPC[67,69]. TREX-2 may thereby mediate a fast track from transcription to mRNA export for these transcripts. It remains unclear whether TREX-2 has a global function to tether mRNPs to the NPC, including those that are not transcribed at the NPC peripheral.

TREX-2 is composed of Sac3, Thp1, Sem1, Sus1, and Cdc31 in yeast[63–66]. The entire complex is arranged based on a Sac3 scaffold (Figure 3B and 3C). The Sac3 N-terminal region features FG-repeats that recognize Mex67•Mtr2 associated mRNPs[70]. The Sac3 middle region binds to Thp1 and Sem1, forming an architectural platform that can bind RNA *in vitro*[71,72]. In addition, the Sac3 C-terminal CID (cdc31 interacting domain) region folds into an extended helix where two Sus1 molecules and one Cdc31 molecule bind. This $Sac3^{C}$•Sus1•Cdc31 subcomplex mediates the interaction with the basket nucleoporin Nup1[73,74]. Together, structural characterization of TREX-2 reveals an architecture that is ideally suited to tether an export-competent mRNP to the nuclear basket of the NPC.

The nuclear basket of the NPC has been suggested to implement a quality control step for mRNA export. In particular, deletion of the coiled-coil protein Mlp1 causes leakage of intron-containing mRNAs into the cytoplasm[75]. Mlp1 interacts with the poly(A) RNA binding protein Nab2, which is required for proper poly(A) tail length control and mRNA export[76]. Nab2 contains an N-terminal PWI-like domain and C-terminal tandem zinc finger domains. The N-terminal domain of Nab2 interacts with Mlp1 and a key Phe73 residue is shown to be critical for their interaction[77] (Figure 3A and 3D). The Nab2-Mlp1 interaction could serve as a means of mRNP targeting to the nuclear basket, and also contribute to the Mlp1-mediated quality control.

The molecular basis for mRNP targeting to the NPC and quality control in human cells is poorly understood. Human TREX-2 contains all the orthologous proteins corresponding to yeast including GANP, PCID, DSS1, ENY2, and CENT2/CENT3 (homologues of yeast Sac3, Thp1, Sem1, Sus1, and Cdc31, respectively)[78]. However, in contrast to yeast, most transcription in human cells takes place in the nucleoplasm[79]. Therefore, mRNP must travel from the nuclear interior to the nuclear periphery to find a NPC. Nevertheless, TREX-2 seems to have a conserved role in human cells. The Sac3 homologue GANP partitions between the nuclear interior and the NPC[80]. Of note, NPC association of GANP requires both the CID region and the C-terminal MCM3AP domain (not present in yeast Sac3)[78]. GANP depletion inhibits bulk mRNA export, with retention of mRNPs and NXF1 in punctate foci within the nucleus[80]. This observation is consistent with a model in which GANP contributes to the movement of NXF1-containing mRNPs from the nuclear interior to

the NPC. In regard to the quality control mediated by the NPC nuclear basket, human TPR (Mlp1 homologue) appears to be the main player in retaining aberrant mRNAs like yeast Mlp1[81]. However, it is not known how TPR distinguishes normal and aberrant mRNAs. Overall, the mechanism underlying mRNP concentration and quality control at the NPC nuclear face in humans will require further study.

4 Disassembly of the mRNP export complex

4.1 The mRNA export platform at the NPC cytoplasmic face

The actual translocation of mRNP through the NPC channel is not inherently directional. At the terminal step in nuclear mRNA export, the DEAD-box ATPase Dbp5/DDX19-mediated remodeling releases the export receptor and other RBPs from the exporting mRNP, prohibiting the mRNP from sliding back to the nucleus and thereby ensuring directional movement[20,21]. Dbp5/DDX19 localizes to the cytoplasmic side of the NPC, and is part of the mRNA export platform that also contains Gle1/GLE1, Nup42/NUP42, Nup159/NUP214, Nup116/NUP98, and Gle2/RAE1[82] (Figure 4A and Table 1). Among them, Nup42/NUP42, Nup159/NUP214, and Nup116/NUP98 all contain FG repeat domains, which facilitate docking of the exporting mRNP to the vicinity of Dbp5/DDX19[83,84]. Recent work demonstrates that the mRNA export platform is positioned right over the NPC's central channel, in contrast to the traditional view of the export factors being localized at the distal end of the NPC cytoplasmic filament[85]. This spatial configuration allows efficient cargo capture and remodeling once the exporting mRNP emerges from the central channel of the NPC.

4.2 Dbp5/DDX19 activation by Gle1/GLE1, IP_6, Nup42/NUP42

In the mRNA export platform, Gle1, IP_6 (inositol hexakisphosphate), and Nup42 provide spatial and temporal regulation of Dbp5 by stimulating its ATPase activity[42,43,86,87] (Figure 4B). Dbp5 contains two RecA like domains (NTD and CTD) and a short N-terminal extension (NTE). The NTE seems to be auto-inhibitory, as deletion of the NTE in human DDX19 yields a more active ATPase[88]. In line with this observation, structures of DDX19 reveal that an alpha-helix from the NTE occupies the cleft between NTD and CTD when DDX19 is bound to ADP, whereas this helix is displaced when DDX19 is bound to ATP and RNA[88] (Figure 4C and 4D). Early evidence about the mechanism of Dbp5 activation came from a genetic screen with a *gle1* mutant, which identified Ipk1 as the enzyme that phosphorylates IP_5 to generate IP_6, and revealed that Ipk1 is required for mRNA export[89]. Gle1 and IP_6 were later shown to activate the ATPase activity of Dbp5 *in vitro*[42,43]. Of note, both Dbp5 and Gle1 are also required for protein translation, probably in a manner distinct from their roles in mRNA export[90–92]. Furthermore, Nup42 is recently found to be an integral component, along with Gle1 and IP_6, to Dbp5 activation, and this mechanism is conserved from yeast to humans[86].

Structural studies on the Dbp5•Gle1•IP_6 complex reveal that Dbp5 is activated through a conserved mechanism that is shared with another DEAD-box ATPase eIF4A, which is activated by eIF4G during translation initiation[93]. This notion is reinforced by the subsequent structural studies on THO-mediated Sub2 activation in nuclear mRNP

remodeling[41], and CNOTI-mediated DDX6 stimulation in miRNA-mediated translational repression[94]. In particular, the C-terminal domain of Gle1 resembles the middle domain of eIF4G, and contacts both the NTD and CTD of Dbp5[44] (Figure 4E). A unique feature of the Gle1-Dbp5 interaction is that IP_6 bridges the protein interaction at the interface of Gle1 and Dbp5-CTD. Gle1-mediated Dbp5 activation is conserved from yeast to humans[86,87]. However, IP_6 activation of human DDX19 was only observed using recombinant DDX19 expressed in the baculovirus-insect cell expression system which carries most of the post-translational modification pathways present in mammalian systems, but not observed using DDX19 expressed in E. coli[86]. Future studies are needed to determine potential DDX19 modifications that enable the IP_6 activation of human DDX19.

The mechanism by which Nup42 coordinates Gle1 stimulation of Dbp5 is not entirely clear. Nup42 interacts with Gle1, and they both are required for the export of heat shock mRNAs following stress[86,95–97]. Structure of the human DDX19•GLE1•NUP42 complex reveals that NUP42 does not contact DDX19 directly, and causes no significant conformational change in GLE1 (Figure 4F)[87]. The effect of NUP42 is suggested to be attributed to increasing GLE1 thermostability; the melting temperature of GLE1 increased from 37 to 50 °C in the presence of NUP42[87]. Of note, in the human DDX19•GLE1•NUP42 complex, only DDX19-CTD is engaged in GLE1 binding, whereas Gle1 contacts both Dbp5 NTD and CTD in the yeast Dbp5•Gle1•IP_6 complex. This difference likely reflects the dynamic nature of the Dbp5/DDX19-Gle1/GLE1 interaction. The CTD of Dbp5/DDX19 serves as the primary anchor for Gle1/GLE1, and the NTD provides a secondary binding site that may sample between Gle1/GLE1 binding and non-binding modes. Interestingly, eIF4A also features a primary anchor on CTD and a secondary weaker binding site on NTD for eIF4G[98]. This configuration may be a conserved feature among the interactions between DEAD-box ATPases and their activators.

4.3 Dbp5/DDX19 catalytic cycle

Activation of Dbp5/DDX19 drives the mRNP remodeling and yields a ADP-bound Dbp5/DDX19 which needs to be recycled for the next round of remodeling events. ADP is not efficiently released from full length Dbp5 *in vitro*, and Nup159 has been shown to enhance the release of ADP through direct interaction with Dbp5[99]. Consistently, a *dbp5-R256D/R259D* mutant with reduced ADP binding bypasses the need for Nup159 interaction in yeast. A structure of the NUP214•DDX19 complex reveals that NUP214, the human homologue of Nup159, binds to the NTD of DDX19, and NUP214 and RNA occupy overlapping binding sites on DDX19[100,101] (Figure 4G). Together, these results place the role of Nup159/NUP214 in the post-ATP hydrolysis state(s), facilitating nucleotide exchange from ADP to ATP (Figure 4B) and allowing Dbp5/DDX19 to perform multiple cycles of mRNP remodeling.

While it is clear that Gle1, IP_6, and Nup42 act in the pre-ATP hydrolysis state(s), and Nup159 functions in the post-ATP hydrolysis state(s) (Figure 4B), a consensus of how the catalytic cycle of Dbp5 is orchestrated has not been reached. In particular, the aforementioned dynamic nature of the interaction between Gle1 and Dbp5 introduces more variables to the system. Further studies will be needed to pinpoint the precise molecular

steps during the Dbp5/DDX19 enzymatic cycle. To date, there is still a substantial gap between our biochemical understanding of the protein machinery and what happens in cells. For example, what proteins are displaced *in vivo* during Dbp5/DDX19-mediated remodeling? In addition to the export receptor, poly(A) RNA binding protein Nab2 is thought to be another physiological target of Dbp5. Recombinant Dbp5 is able to displace Nab2 from a Nab2-RNA complex *in vitro*[102]. *In vivo*, Nab2 accompanies mRNPs through the NPC, but is not found associated with mRNA in polysomes[103–105]. A *dbp5* mutant shows an accumulation of Nab2 on poly(A) RNA[102]. Together, these studies are consistent with a model in which Dbp5 mediates release of Nab2 from mRNPs at the cytoplasmic side of the NPC. It remains to be determined how many more proteins are physiological targets for the Dbp5/DDX19-mediated remodeling.

4.4 NUP98 and RAE1

Nup116/NUP98 and Gle2/RAE1 are constituents of the mRNA export platform that have not been shown to directly regulate the activity of Dbp5/DDX19. Nup116/NUP98 contains an FG-repeat domain that can be recognized by nuclear transport receptors[84,106–109]. Nup116/NUP98 binds to Gel1/RAE1 and their interaction is required for Gle2/RAE1 localization at the NPC[110–113] (Figure 4H). Yeast Gle2 was identified along with Gle1 in the same genetic screen for genes that are synthetically lethal with a *nup100* null mutant[114]. Nup100 and Nup116 are highly homologous, with the exception that only Nup116 contains the Gle2 binding region. A further advance in our understanding of RAE1 function came from the observation that RAE1 is targeted by the Matrix (M) protein of the vesicular stomatitis virus (VSV) to block host mRNA export[115]. A structure of the VSV M protein in complex with RAE1 and NUP98 shows the M protein occupying the nucleic acid binding site on RAE1[116], indicating that RAE1 function is RNA-binding dependent. The precise role of RAE1 in mRNA export remains to be determined. Given its spatial proximity to DDX19 and dependence on NUP98 for localization, together with the putative role NUP98 plays in docking mRNPs through interaction with the export receptor, it is plausible that RAE1 and NUP98 also contribute to DDX19-mediated terminal steps of mRNA export.

5 Future directions

To date, studies have elucidated the cast of proteins involved in bulk mRNA export and have laid out the core principles. In particular, the spatiotemporal regulation of Mex67•Mtr2/NXF1•NXT1 receptor association and dissociation on an mRNP by DEAD-box ATPases has been shown to be the key to mRNA export. Although considerable progress has been made to obtain structural snapshots of the DEAD-box ATPases in action, a comprehensive mechanistic understanding is lacking. Deciphering the precise nature of mRNP remodeling in the biological context is still a challenge. This will also require further structural knowledge of the mRNA export machinery and novel functional approaches to capture protein exchanges on mRNPs in cells.

A largely unexplored area in the field is how mRNA export is tuned to accommodate the specific needs of a cell with respect to developmental stages, tissue specificity, extracellular stimuli, etc. Accumulating evidence in recent years has revealed that post-transcriptional

gene regulation plays a critical role in shaping gene expression profiles[117]. Indeed, mRNA export can selectively modulate critical biological processes such as DNA repair, stress response, maintenance of pluripotency, etc[118–122]. Our molecular understanding of the general mRNA export pathway has provided a valuable toolset to investigate the control of gene expression by selective mRNA export.

Not surprisingly, given the essential role of mRNA export in gene expression, the integrity of the pathway is critical for human health. For example, mutations in the gene encoding GLE1 are causally linked to human motor neuron diseases, including lethal congenital contracture syndrome 1 (LCCS-1), lethal arthrogryposis with anterior horn cell disease (LAAHD), and amyotrophic lateral sclerosis (ALS)[123–126]. In addition, aberrant expression of mRNA export factors has been found in many different forms of cancer[127]. A plausible hypothesis is that dysregulation of these factors may alter the export of specific transcripts that are critical for cell proliferation and oncogenesis. Furthermore, many viruses interfere with host mRNA export to block host gene expression and/or facilitate essential viral processes. Key constituents of the mRNA export machinery (NXF1•NXT1, UAP56, REF, RAE1•NUP98, etc) are exploited by a wide range of viruses including herpesvirus, adenovirus, VSV, and influenza virus[128]. Overall, further advances in elucidating the mRNA export pathway will shed light on the pathogenesis of relevant diseases.

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Synopsis:

Nuclear export of mRNA, in the form of ribonucleoprotein particles (mRNPs), is governed by the highly regulated alterations in mRNP protein composition known as mRNP remodeling. Central to mRNP remodeling are two DEAD-box ATPases, Sub2 and Dbp5, which respectively mediate the assembly of export competent mRNPs in the nucleus and the release of mRNPs into the cytoplasm. Here we provide an overview on structural studies of key steps in mRNA export.

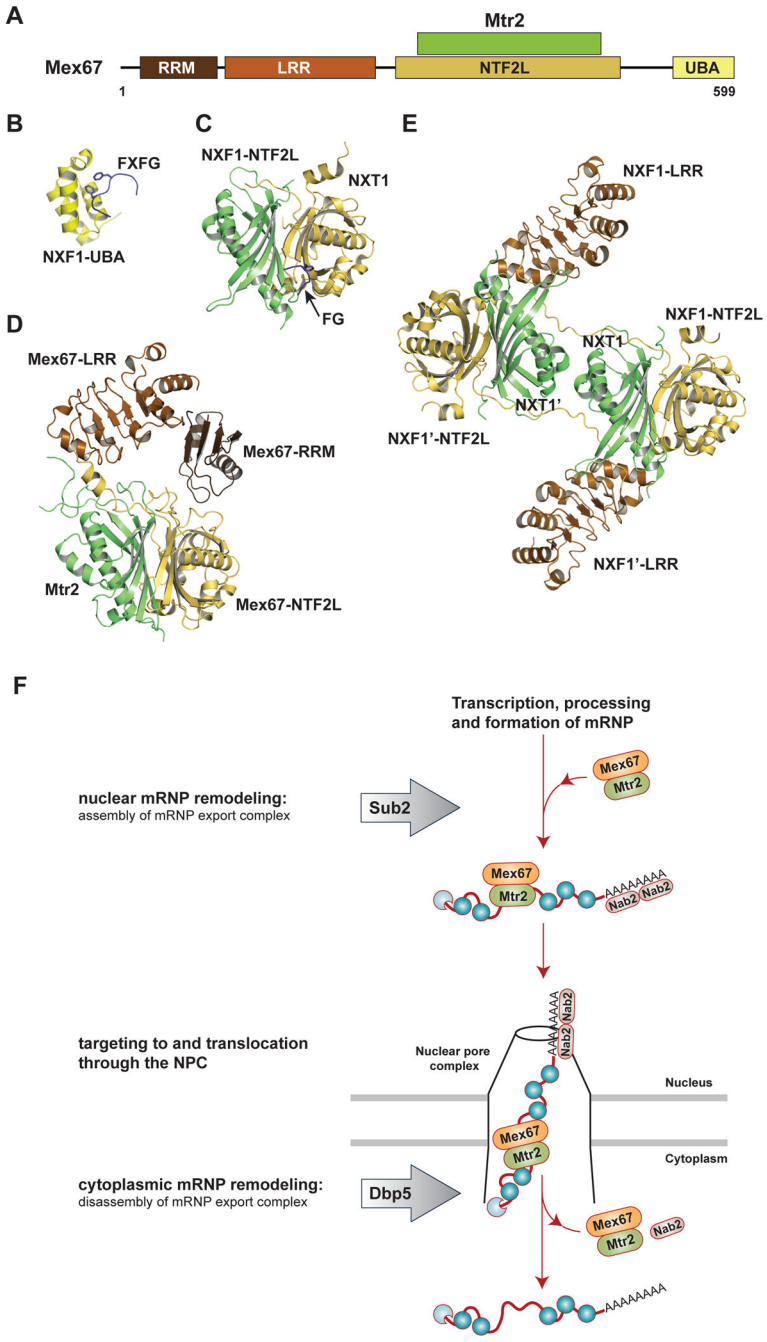

Figure 1.

Nuclear mRNA export is mediated by the principal export receptor yeast Mex67•Mtr2/human NXF1•NXT1. (A) Domain schematic of yeast Mex67•Mtr2. (B) Structure of the NXF1-UBA domain bound to a FXFG peptide (PDB ID 1OAI). (C) Structure of the NXF1-NTF2L domain bound to a FG peptide (PDB ID 1JN5). (D) Structure of yeast Mex67 (RRM, LRR, and NTF2L domains) associated with Mtr2 (PDB ID 4WWU). (E) Structure of NXF1 (LRR and NTF2L domains) associated with NXT1 (PDB ID 4WYK). (F) A working model of Mex67•Mtr2 mediated mRNA export. Export is driven by specific alterations of

the mRNP protein composition. In the nucleus, Sub2 facilitates the assembly of export-competent mRNPs by recruiting the principal mRNA export receptor Mex67•Mtr2. At the cytoplasmic side of the NPC, Dbp5 mediates disassembly of the export complex by displacing proteins including Mex67•Mtr2 and the poly(A) RNA binding protein Nab2 from the mRNP.

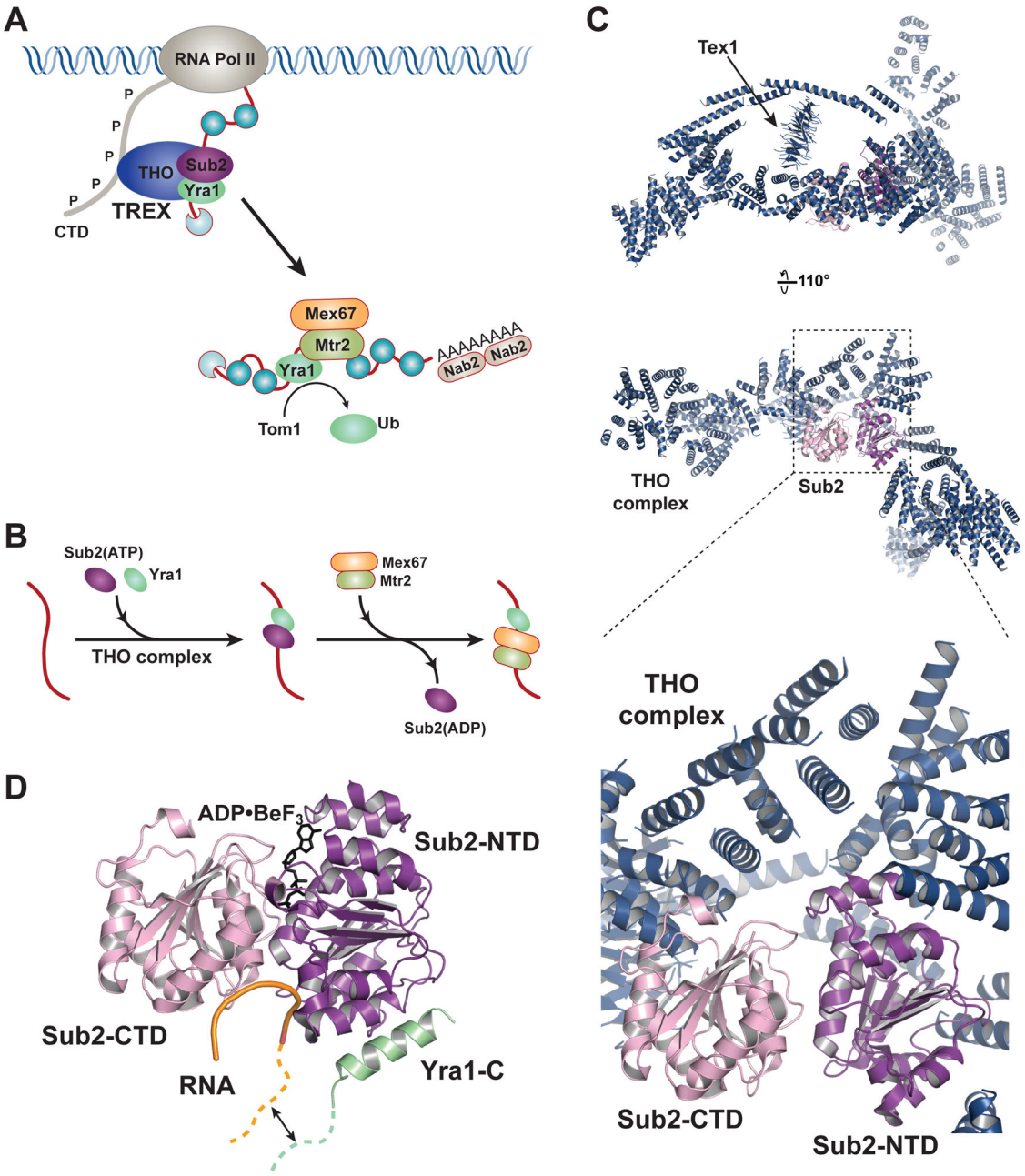

Figure 2.

ATPase mediated nuclear mRNP assembly. (A) Yeast TREX complex THO•Sub2•Yra1 travels with RNA Pol II and facilitates loading of the export receptor onto mRNA. (B) A detailed view of the stepwise remodeling reactions driven by the Sub2 ATPase. THO is omitted from the RNA because the dynamics of THO association with mRNA is not known. (C) A 6.0 Å resolution structure of Sub2 bound to a THO core complex which contains S. cerevisiae $Tho2_{1–1207}$, $Hpr1_{1–603}$, $Mft1_{1–256}$, and $Thp2_{1–26}$, as well as S. bayanus $Tex1_{1–380}$ (PDB ID 5SUQ). Top two panels show the overall architecture of the complex. THO is represented by a polyalanine model and only the Tex1 subunit is assigned. The bottom panel highlights the THO-Sub2 binding interface. (D) Structure of Sub2 in association with a

truncated Yra1 (Yra1-C, a.a. 208–226) and poly (U) RNA in the presence of ADP•BeF_3 (PDB ID 5SUP). The bound RNA is sharply bent, which is characteristic of DEAD-box proteins. The Yra1 region preceding the crystallized fragment is capable of binding RNA (depicted by a green dashed line), and has been proposed to extend the RNA binding site in the Sub2•Yra1 complex.

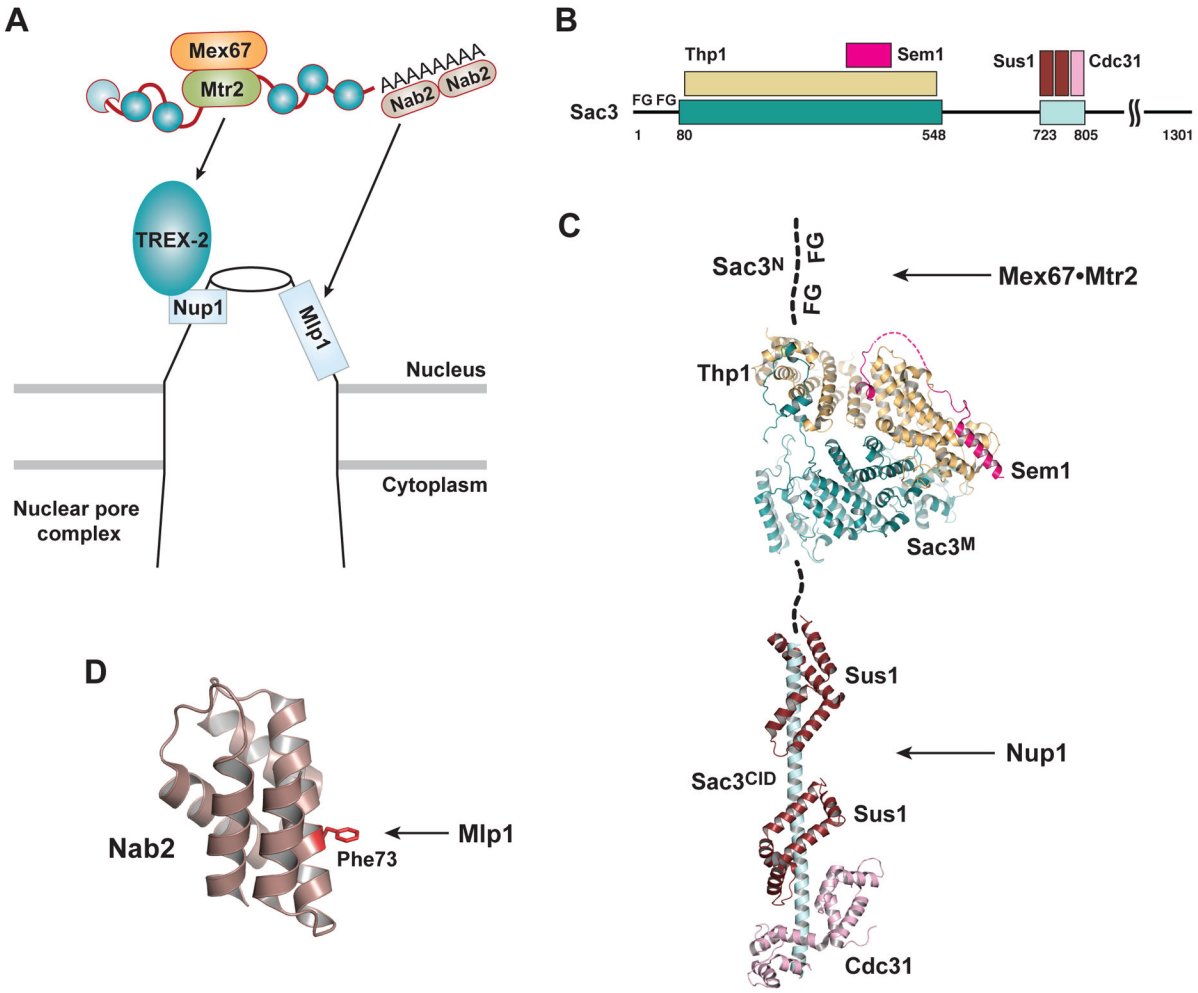

Figure 3.

mRNP targeting to the nuclear basket of the NPC in yeast. (A) Schematic of mRNP targeting mediated by the TREX-2 complex and Mlp1 at the nuclear basket of the NPC. (B) Schematic of the TREX-2 complex. (C) Structural basis for the TREX-2 mediated mRNP targeting. TREX-2 complex is built on a scaffold of the Sac3 subunit. The N-terminal region of Sac3 ($Sac3^{N}$) features FG-repeats that are recognized by the export receptor on mRNP. The middle region of Sac3 ($Sac3^{M}$) binds to the Thp1 and Sem1 subunits of TREX-2 (PDB ID 5UBP). The C-terminal CID region of Sac3 ($Sac3^{CID}$) binds to the Sus1 (two copies) and Cdc31 subunits of TREX-2 (PDB ID 3FWC), together mediating NPC association through interaction with the nuclear basket protein Nup1. (D) Structure of the N-terminal Mlp1-binding domain of Nab2 (PDB ID 2V75). The Phe73 residue is critical for the Nab2-Mlp1 interaction.

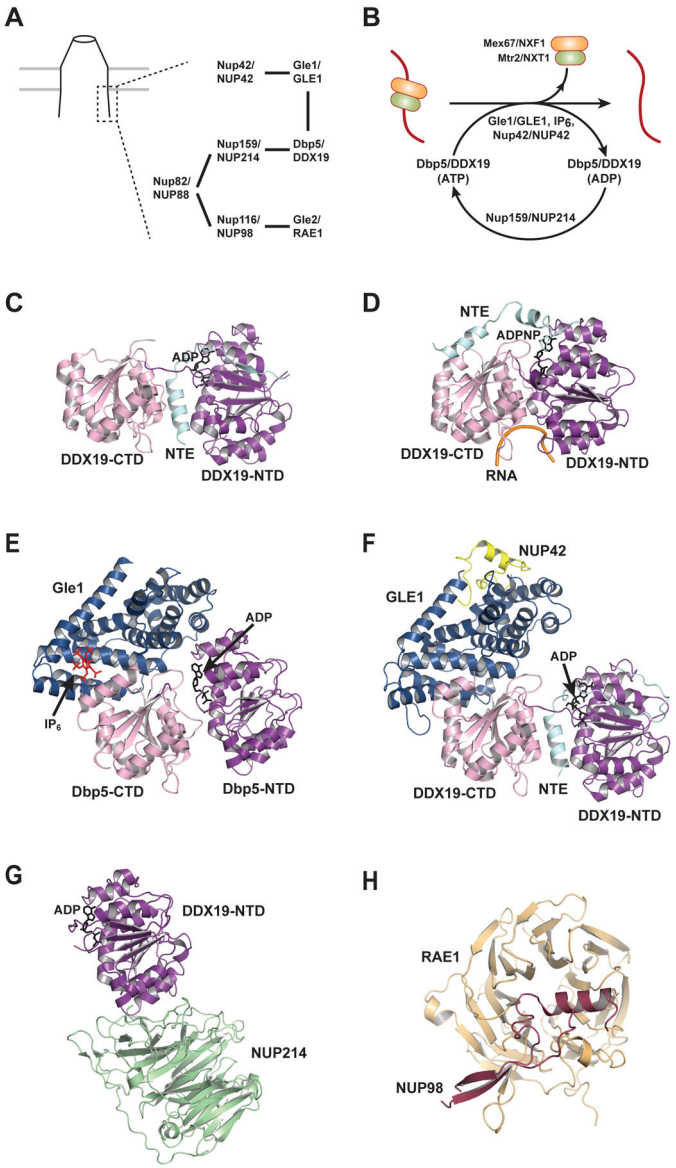

Figure 4.

ATPase mediated mRNP remodeling at the cytoplasmic side of the NPC. (A) Schematic diagram of the interaction network of the mRNA export platform at the cytoplasmic side of the NPC. (B) Schematic of Dbp5/DDX19 mediated dissociation of the export receptor from the mRNP, and regulation of the Dbp5/DDX19 catalytic cycle. (C) Structure of DDX19 bound to ADP (PDB ID 3EWS). (D) Structure of DDX19 bound to an ATP-analogue and poly(U) RNA (PDB ID 3G0H). (E) Structure of the Dbp5•Gle1•IP_6 complex in the presence of ADP (PDB ID 3RRN). (F) Structure of the DDX19•GLE1•NUP42 complex in the presence of ADP (PDB ID 6B4I). (G) Structure of the DDX19-NTD•NUP214 complex in the presence of ADP (PDB ID 3FMO). (H) Structure of RAE1 in complex with a NUP98 fragment (PDB ID 3MMY).

Table 1.

Key constituents of the mRNA export machinery

Yeast	Human	Function
Mex67•Mtr2	NXF1•NXT1 (TAP•p15)	Principal export receptor for bulk mRNA export
In the nucleus		
Sub2	UAP56	DEAD-box ATPase that remodels nuclear mRNP, component of the TREX complex
Yra1	ALY (REF, THOC4)	Adaptor for the export receptor, component of the TREX complex
THO	THO	A multi-subunit complex, component of the TREX complex
Nab2	ZC3H14	Poly(A) RNA binding protein
TREX-2	TREX-2	A multi-subunit complex that targets actively transcribed genes to the NPC
At the NPC cytoplasmic face		
Dbp5	DDX19	DEAD-box ATPase that remodels mRNP at the cytoplasmic side of the NPC
Gle1	GLE1	Activator of Dbp5/DDX19, requires IP_6 for mRNA export
Nup42	NUP42 (hCG1)	FG-Nucleoporin to which Gle1/GLE1 binds
Nup159	NUP214 (CAN)	FG-Nucleoporin to which Dbp5/DDX19 binds
Nup116	NUP98	FG-Nucleoporin, binds to the export receptor
Gle2	RAE1	mRNA export factor, binds to Nup116/NUP98