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RNA nuclear export: from neurological disorders to cancer

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Abstract The presence of a nuclear envelope, also known as nuclear membrane, defines the structural framework of all eukaryotic cells by separating the nucleus, which contains the genetic material, from the cytoplasm where the synthesis of proteins takes place. Translation of proteins in *Eukaryotes* is thus dependent on the active transport of DNA-encoded RNA molecules through pores embedded within the nuclear membrane. Several mechanisms are involved in this process generally referred to as RNA nuclear export or nucleocytoplasmic transport of RNA. The regulated expression of genes requires the nuclear export of protein-coding messenger RNA molecules (mRNAs) as well as non-coding RNAs (ncRNAs) together with proteins and pre-assembled ribosomal subunits. The nuclear export of mRNAs is intrinsically linked to the co-transcriptional processing of nascent transcripts synthesized by the RNA polymerase II. This functional coupling is essential for the survival of cells allowing for timely nuclear export of fully processed transcripts, which could otherwise cause the translation of abnormal proteins such as the polymeric repeat proteins produced in some neurodegenerative diseases. Alterations of the mRNA nuclear export pathways can also lead to genome instability and to various forms of cancer. This chapter will describe the molecular mechanisms driving the nuclear export of RNAs with a particular emphasis on mRNAs. It will also review their known alterations in neurological disorders and cancer, and the recent opportunities they offer for the potential development of novel therapeutic strategies.

1 Eukaryotic expression of genes

The tightly controlled expression of eukaryotic genes into RNA molecules and proteins is essential to cell survival and homeostasis. This is a complex process that integrates several mechanisms: activation, repression, cleavage, processing, directionality, surveillance and regulated degradation. The physiological expression of mammalian genes relies on a very large number of mRNAs (estimated between 100,000 and 1,000,000 molecules per cell), protein factors and ncRNAs

implicated in the composition of ribosomes and the synthesis of proteins, as well as in the regulation of gene expression at the post transcriptional and translational levels.

1.1 Eukaryotic expression of mRNAs and ncRNAs

Three nuclear RNA polymerases are implicated in the expression of genes embedded in chromosomes. In the nucleolus, RNA polymerase I transcribes the mammalian 45S pre-ribosomal RNA precursor that is subsequently processed into mature 28S, 18S and 5.8S ribosomal RNAs (rRNAs). Together with the 5S rRNA transcribed by RNA polymerase III in the nucleoplasm, they constitute the major catalytic and architectural components of the ribosome. RNA polymerase III is also involved in the transcription of other ncRNAs including U6/ U6atac small nuclear RNAs (snRNAs) that form components of the splicing machinery and transfer RNAs (tRNAs) required for recognition of codons and attachment of the corresponding amino-acid moieties to the neo-synthesized polypeptide chain.

The nucleoplasmic RNA polymerase II (RNAPII) transcribes the largest portion of the genome synthesizing the precursors of mRNAs (pre-mRNAs) and multiple classes of ncRNAs. The later are subdivided into small and long ncRNA classes if larger than 200 nucleotides. Small ncRNAs include U1, U2, U4, U4atac, U5, U7, U11 and U12 snRNAs which participate in pre-mRNA splicing by the major (U2-dependent) or minor (U12-dependent) spliceosomes and small nucleolar RNAs (snoRNAs) involved in the processing and modification of rRNAs. RNAPII also transcribes the precursors of micro-RNAs (miRNAs), small-interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs). These are implicated in the post transcriptional or translational silencing of mRNAs and proteins by respectively promoting the degradation of mRNAs or interfering with the translation process. Long ncRNAs (lncRNAs) comprise natural antisense transcripts (NATs) which are transcribed from the antisense DNA strand opposing 50-70% of protein coding genes. They regulate the expression of the sense gene at both transcriptional and translational levels. Other lncRNAs include very long intergenic non-coding RNAs (vlincRNAs). Their transcripts span approximately 10% of the human genome ranging from 50kb to 1Mb in size and are thought to also play a regulatory function. Long intergenic non-coding RNAs (lincRNAs) resemble mRNAs with capped and poly-adenylated transcripts that contain small open reading frames and co-purify with ribosomes. 8,000 to 10,000 lincRNAs have been characterised so far. They have established regulatory functions at epigenetic and transcriptional control levels with roles reported in development, cell differentiation and diseases. The list of ncRNAs presented here is not exhaustive.

It is important to note that the repertoire of ncRNAs is very diverse and expands quickly with the rise of Next Generation RNA sequencing technologies. Discovery of new RNA species keeps on challenging the complex classification of ncRNAs as some previously characterised species may become shared between several groups.

1.2 Eukaryotic expression of mRNAs and proteins: mechanistic overview

Remodeling of the tightly packed heterochromatin into relaxed euchromatin is often a pre-requisite to transcriptional activation. It facilitates the recruitment of transcription factors along with an RNA polymerase onto the promoters of genes to form a closed-state complex named the pre-initiation complex (PIC). The RNA polymerase core enzymes are unable to recognize promoters or initiate transcription from a DNA template. They require general transcription factors for the local melting of the double helix of DNA near the transcription start site downstream of promoters. Recognition of the TATA box element by the TATA box binding protein (TBP) subunit of TFIID in the promoters of RNAPII-transcribed genes initiates the assembly of the pre-initiation complex. The TFIIA and TFIIB factors are recruited next to stabilize the DNA-TFIID complex allowing for recruitment of the hypo-phosphorylated form of the RNA polymerase II (RNAPIIA) in association with the other general transcription factors TFIIE, TFIIF, TFIIH and TFIIS. TFIIB interacts with both the promoter and RNAPII. It triggers bending of the DNA molecule near the transcription start site while the Ssl2/ERCC3 DNA helicase subunit of TFIIH unwinds the double helix to generate a flexible single strand of DNA that can reach the active cleft center for the polymerization of ribonucleotides and promoter escape (Murakami et al. 2015). Stable initiation of transcription by RNAPII requires the polymerization of approximately 25 nucleotides.

Transcription elongation and co-transcriptional processing of pre-mRNAs is orchestrated by the recruitment of several RNA-processing machineries via differential phosphorylation of the heptapeptide (YSPTSPS) repeats composing the carboxyl-terminal domain (CTD) of the RNAPII (Hsin and Manley 2012; Svejstrup 2013). TFIIH-dependent phosphorylation of serine 5 in the heptapeptide repeats of the CTD triggers the start of transcription and phosphorylation of serine 5 remains high for the first few hundred polymerized nucleotides. A 7-methylguanosine CAP (m7G) is added to the 5'-end of nascent transcripts shortly after transcription initiation (<100 nucleotides) by recruitment of the capping enzyme to the highly phosphorylated serine 5 residues in the CTD. The CAP structure protects against RNA degradation and plays additional roles by interacting with the CAP-binding complex, which provides 5’ to 3’ directionality during the mRNA nuclear export

process (Cheng et al. 2006), and by recruiting the eukaryotic initiation factor 4E (eIF4E) for translation initiation (Sonenberg and Hinnebusch 2009). The CDK9 kinase subunit of the elongation factor P-TEFb phosphorylates CTD-serine 2 residues and the elongation factor SPT4/SPT5 to maintain the processivity of transcription elongation for complete synthesis of the nascent pre-mRNAs (hyper-phosphorylated RNAPII under form IIO). Removal of introns by splicing and exon skipping by alternative splicing are linked to phosphorylation of the CTD predominantly on serine 2 but also on serine 5 residues. Pre-mRNA splicing occurs co-transcriptionally in the vicinity of nuclear speckles, small organelles enriched in pre-mRNAs and splicing factors, for approximately 80% of human transcripts (Girard et al. 2012). The final stage of transcription is achieved by endonucleolytic cleavage of the transcript and addition of approximately 200 adenosines at poly-adenylation sites composed of the consensus sequence AAUAAA (Proudfoot 2011). Nuclear export of the bulk mammalian mRNAs is coupled to co-transcriptional splicing (Masuda et al. 2005) and release from nuclear speckles (Girard et al. 2012) via recruitment of the evolutionary conserved transcription-export (TREX) complex and the nucleoporin-interacting export receptor NXF1 (Wickramasinghe and Laskey 2015; Heath et al. 2016).

It remains unclear whether completion of the nuclear mRNA export is linked to the initiation of translation in the cytoplasm or whether translation is truly compartmentalized and independent of the nuclear biogenesis and processing of mRNAs. In specialized cells, such as neurons, mRNAs that encode factors involved in guiding the growth and repair of axons or dentrites require transport prior to localized translation (Jung et al. 2012). Eukaryotic translation involves several coupled steps (Sonenberg and Hinnebusch 2009; Dever and Green 2012). The eukaryotic translation initiation factor eIF3 bridges the interactions between the 40S ribosome small subunit and the eIF4F factor, which is composed of the CAP-binding protein eIF4E, the DEAD box RNA helicase eIF4A and a scaffold protein eIF4G. This forms the 43S pre-initiation complex which allows scanning of the 5'-untranslated region (5'-UTR) of the mRNA until the initiating AUG codon is reached. In the 43S complex, the poly(A)-binding protein (PABP) also interacts with eIF4F to circularize the mRNA. Recruitment of the methionine-charged tRNA initiator in the large 60S ribosomal subunit by eIF5B leads to interactions with the 43S pre-initiation complex to form an 80S initiating complex. Linking of amino-acid moieties to the growing polypeptide chain occurs through eEF2 (eukaryotic translation elongation factor 2) dependent translocation of ribosomes onto the translating mRNA. Up to 5-10 elongating ribosomes are associated to each mRNA molecule composing actively translating polysomes. Eukaryotic release factor 1 (ERF1) is involved in the disassembly of the ribosome subunits by recognition of all 3 terminator (or stop) codons. Release of

the neo-synthesized protein anchored in the 60S subunit is assisted by factor ERF3. A simplified stepwise overview of the eukaryotic expression of mRNAs and protein-coding genes is represented in **Fig. 1**.

The physiological expression of genes also involves regulatory and proof-reading mechanisms such as binding of transcriptional activators or repressors on specific elements in promoters (Charoensawan et al. 2012), specific degradation of proteins by the ubiquitin/proteasome pathway (Schmidt and Finley 2014), RNA-interference mechanisms (Wilson and Doudna 2013) and RNA surveillance for the maturation/degradation of damaged/unprocessed RNAs by the nuclear exosome (Kilchert et al. 2016) or the degradation of RNAs by nonsense mediated decay in the cytoplasm (Trcek et al. 2013).

2 Nuclear export of RNAs: mechanisms and regulation

The nuclear export of eukaryotic RNAs involves functionally linked mechanistic steps ensuring that only mature transcripts are delivered to the cytoplasm. The process starts with the biogenesis of RNA molecules and the formation of ribonucleoprotein complexes. It follows on with the co-transcriptional processing of transcripts and the recruitment of adaptor proteins for selective and active transport. It ends with docking and transport through the nuclear pore prior to the release of transported RNA cargoes into the cytoplasm.

2.1 Formation of ribonucleoprotein complexes (mRNPs)

Within a cell, RNA molecules are never left naked but they dynamically associate with various sets of proteins to form ribonucleoprotein particles (RNPs) (Mitchell and Parker 2014). Most eukaryotic cells contain hundreds of RNA-binding proteins with diverse biological activities. Approximately 1,500 RNA-binding proteins are encoded from the human genome (Gerstberger et al. 2014). The changing composition of RNPs dictates the functionalities and fate of the RNA molecules. Formation of RNPs also protects against premature RNA degradation and is essential for driving forward the gene expression process in functionally linked steps. The biogenesis of messenger RNP complexes (mRNPs) has indeed been found to be orchestrated in separate but extensively coupled nuclear steps - transcription, processing (capping, splicing, cleavage/poly-adenylation) and nuclear

export - to allow correct expression of the chromatin into mature mRNA molecules which are translated into proteins in the cytoplasm (Luna et al. 2008).

2.2 Molecular mechanisms driving the human nuclear export of RNAs

2.2.1 The NXF1-dependent pathways mediate the nuclear export of bulk mRNAs

Evolutionary conserved Nuclear Export Factor 1 (NXF1) proteins form an essential family of nuclear export receptors with a modular structure which comprises an unstructured RNA-binding domain linked to a pseudo RNA Recognition Motif not required for RNA-binding (Hautbergue et al. 2008), a region containing Leucine Rich Repeats (LRRs), a stabilizing nuclear transport factor 2 (NTF2)-like domain that heterodimerizes with NTF2-related export protein 1 (NXT1 also known as p15) and a carboxyl terminal ubiquitin-associated (UBA) domain (Herold et al. 2000). Both the NTF2-like and UBA domains are implicated in the direct association of NXF1 with the phenylalanine-glycine (FG) repeat regions of nucleoporins embedded in the nuclear pore complex (NPC).

NXF1 proteins exhibit poor RNA-binding activity. They mediate the nuclear export of mRNA through direct interactions with nuclear export adaptors, which avidly bind RNA, and were initially thought to bridge the interactions between RNA molecules and NXF1. However, structural and functional studies have later shown that the nuclear export adaptor proteins Aly/REF (ALYREF) (Stutz et al. 2000) and some of the shuttling SR-rich splicing factors (SRSF1, 3, 7) (Huang et al. 2003) remodel NXF1 to increase *in vitro* its affinity for RNA thereby licensing the nuclear export (Hautbergue et al. 2008; Walsh et al. 2010). This process is orchestrated by the evolutionary conserved TREX complex which represents a major pathway for the eukaryotic transport of mRNA from the nucleus to the cytoplasm (Strässer et al. 2002). TREX interacts with the hyper-phosphorylated CTD of the RNAPII and associates with the CAP-binding complex, the exon-junction complex, 3'-end processing factors and nuclear export factors, thus harboring a pivotal role in functionally coupling co-transcriptional processing events to the nuclear export of mature transcripts (Wickramasinghe and Laskey 2015; Heath et al. 2016). In human, TREX is co-transcriptionally deposited at the 5'-end of the mRNA during splicing (Masuda et al. 2005). It forms a multimeric complex (Dufu et al. 2010) composed of THO, a stoichiometric and stable core comprising 6 subunits (THOC1, 2, 5-7 and TEX1/THOC3), the essential RNA/ATP-dependent DEAD-box RNA helicase 39B (DDX39B also known as UAP56 for U2AF65-associated protein

56), the nuclear export adaptor ALYREF (also known as THOC4) and a few other proteins including export adaptors UIF (UAP56-interacting factor) (Hautbergue et al. 2009), LUZP4 (leucine zipper protein 4) (Viphakone et al. 2015) and co-adaptor CHTOP (chromatin target of PRMT1) (Chang et al. 2013).

Adaptors ALYREF, UIF and LUZP4 contain a conserved UBM (UAP56-binding motif) sequence which is required and sufficient for the direct binding of DDX39B. Direct interactions between ALYREF and DDX39B, which promotes the assembly of spliceosomes on nascent pre-mRNAs, trigger the recruitment of ALYREF onto spliced mRNAs prior to recruitment of the nucleopore-interacting receptor NXF1 (Luo et al. 2001). DDX39B has also been implicated in the loading of ALYREF onto intronless vertebrate mRNAs in an ATP-dependent manner (Taniguchi and Ohno 2008). ALYREF-dependent activation of the ATPase and RNA helicase activities of RNA-bound DDX39B further lead to the handover of the RNA from the helicase to the nuclear export adaptor (Chang et al. 2013). Successive cycles of ATP hydrolysis by DDX39B are thought to facilitate TREX assembly by therefore loading nuclear export adaptors (ALYREF, UIF, LUZP4) and co-adaptor CHTOP along processing transcripts (Chang et al. 2013; Heath et al. 2016). Mutually exclusive interactions of ALYREF with DDX39B and NXF1 lead to further displacement of DDX39B and handover of the RNA from the export adaptor to NXF1 in concert with co-adaptors THOC5 and CHTOP (Hautbergue et al. 2008; Viphakone et al. 2012; Chang et al. 2013). Following transport of the mRNP through the nuclear pore, the remodeling of proteins and export adaptors from the mRNPs is predicted to revert NXF1 to a low RNA-binding affinity and promote the release of the transported mRNA in the cytoplasm.

TREX provides a binding platform for NXF1 through its direct association with nuclear export adaptors (ALYREF, UIF, LUZP4) which interact with the RNA-binding domain of NXF1 and nuclear export co-adaptors (THOC5, CHTOP) that bind the NTF2-like domain (Hautbergue et al. 2008; 2009; Katahira et al. 2009; Chang et al. 2013; Viphakone et al. 2015). In absence of interaction with ALYREF and THOC5, NXF1 adopts a closed conformation which silences its RNA-binding activity, whilst interactions with both ALYREF and THOC5 promote remodeling of NXF1 into an open conformation that exposes its RNA-binding domain (Viphakone et al. 2012) allowing efficient handover and binding to RNA. Coupling of the high RNA affinity remodeling of NXF1 with the co-transcriptional processing of transcripts offers in turn a retention mechanism to prevent the nuclear export of unprocessed transcripts in the cytoplasm (Hautbergue et al. 2008; Walsh et al. 2010). The nuclear export of human mRNAs is based upon successive RNA handover steps that link co-transcriptional splicing of pre-mRNAs and release from nuclear speckles to the

nuclear export of mature mRNAs. A simplified diagram of the RNA handover process is presented in **Fig. 2**.

Assembly of TREX and activation of DDX39B is more complex than described above and the handover of RNA from the export adaptor to NXF1 is also facilitated by dynamic rearrangement of TREX and post-translational regulation events. The interaction of THOC5 with the NTF2-like domain of NXF1 is key to the remodeling of NXF1 however both co-adaptors THOC5 and PRMT1 (protein arginine methyl transferase 1)-methylated CHTOP, which compete for the same binding domain of NXF1, are found in a single cellular protein complex (Viphakone et al. 2012; Chang et al. 2013) indicating that TREX undergoes substantial remodeling during processing and export of mRNA. In addition, the methylation of ALYREF by PRMT1 has also been shown to decrease its RNA-binding affinity but not its interaction with NXF1 promoting in turn the handover of RNA from the nuclear export adaptor to the nuclear export receptor (Hung et al. 2010).

In contrast to yeast, multiple nuclear export adaptors have been characterised in higher *Eukaryote* organisms. The depletion of ALYREF in human cells does not significantly affect the global nuclear export of mRNA (Katahira et al. 2009) and is dispensable to the development of *C. elegans* (Longman et al. 2003) and *D. melanogaster* (Gatfield and Izaurralde 2002). Consistent with this, several other nuclear export adaptors interact with the RNA-binding region of NXF1 including SRSF1, SRSF3, SRSF7 (Huang et al. 2003), UIF (Hautbergue et al. 2009) and LUZP4 (Viphakone et al. 2015). Different nuclear export adaptors can also associate with the same mRNA molecule (Hautbergue et al. 2009). Moreover, it has recently been reported that despite interacting with thousands of mRNAs, the individual depletion of the SRSF1-7 proteins only affect the nuclear export of a small proportion of transcripts (<0.5-2% mRNAs) clearly highlighting redundancy and/or cooperation in the NXF1-dependent nuclear export adaptor function (Müller-McNicoll et al. 2016). It is very likely that other adaptors remain to be characterised, particularly in cells with specialized functions in which the mechanisms of RNA nuclear export have not yet been thoroughly investigated. The presence of adaptors and co-adaptors in varied combinations may provide additional selectivity to the nuclear export process by producing several TREX complexes with alternative functionalities and/or specificities. For example, the adaptor UIF also interacts with the FACT (facilitates chromatin transcription) complex and may provide additional links between chromatin remodeling, transcription and mRNA nuclear export (Hautbergue et al. 2009). **Table 1** highlights various known NXF1 dependent pathways and their associated mRNA cargoes and illustrates that not all NXF1 pathways involve TREX.

2.2.2 Exportin-dependent pathways mediate the nuclear export of ncRNAs and some mRNAs

The nuclear export of rRNAs, in association with ribosomal subunits, of snRNAs and of a few subsets of mRNAs depends on the karyopherin CRM1 (chromosome region maintenance 1, also known as exportin1 or XPO1). This shuttling transport receptor associates to the GTP-bound form of the small GTPase Ran and plays a major role in the nuclear export of proteins containing nuclear export signals (NES). CRM1 does not interact with RNA but with nucleoporins and NES-containing adaptor proteins that bind RNA (Hutten and Kehlenbach 2007; Köhler and Hurt 2007). The nuclear-enriched form of GTP-bound Ran directly interacts with exportins to promote high affinity binding with protein/RNA cargoes, transport through the nuclear pore and release by GTP hydrolysis in the cytoplasm. The exportin is recycled back to the nucleus for additional rounds of Ran-GTP interactions and nucleocytoplasmic shuttling. Conversely, the GDP-bound form of Ran, which is predominant in the cytoplasm, directly binds importins to mediate the nuclear import of proteins with nuclear localization signal (NLS) and the release of transported proteins occur upon GTP exchange in the nucleus (**Fig. 3**). On the other hand, the nuclear export of tRNA and miRNA, which involves different exportins, is achieved through direct binding to exportin-t (Exp-t) and exportin-5 (Exp-5) respectively. **Table 2** summarizes known RNA export pathways mediated by exportins and their adaptor proteins.

2.2.3 Alternative RNA nuclear export pathways

It has recently been shown that mRNPs containing synaptic transcripts, too large to be transported through the channel of the nuclear pore, can exit the nucleus by budding through the nuclear membrane during the *Wnt* signaling development of synapses in *Drosophila* (Speese et al. 2012). The budding process involves phosphorylation of lamin A by an atypical protein kinase C which induces the invagination of the inner nuclear membrane prior to the fusion of a vesicle with the outer membrane. This allows cytoplasmic release in a similar mechanism used for the nuclear exit of *Herpes* viruses.

Some influenza type A viruses are also able to induce enlargement of the nuclear pore channel (by approximately a third) following viral activation of apoptotic cellular caspases 3/7 which trigger the degradation of nucleoporin 153 (Nup153) during the later stages of infection (Mühlbauer et al. 2015). At early stages, the viral RNA genome, which is replicated in the nucleus, is exported into the cytoplasm for translation of viral proteins and packaging of virus through the viral RNA-

binding nucleoprotein adaptor (NP) and the cellular CRM1-dependent pathway. However, switching the viral nuclear export pathway by viral induction of Nup153 degradation at the late stages of replication leads to increased production and high efficiency release of infectious virus progeny by passive diffusion through the enlarged nuclear pores. It remains unknown whether this mechanism is used for the nuclear export of cellular mRNAs either in health or other disease conditions.

2.3 The nucleocytoplasmic transport of RNPs

2.3.1 The nuclear pore complex

Vertebrate nuclear pore complexes form large 125-MDa protein assemblies stably embedded in circular holes created by fusion of the inner and outer nuclear membranes. They constitute aqueous transport channels which mediate and regulate the bidirectional exchange of macromolecules between the nucleus and cytoplasm (Knockenhauer and Schwartz 2016). Small molecules under 40 kDa (or approximately 5 nm in size) freely shuttle between the nucleus and cytoplasm by passive diffusion, while larger molecules and mRNPs require active transport mechanisms which depend on either NXF1 or exportins and importins.

The NPC has a symmetrical octagonal structure that contains a nuclear basket made of 8 rod-shaped filaments associated to a distal ring on the nucleoplasmic side and 8 extended flexible fibrils at the cytoplasmic side. Nucleoporin fibers from the central core of the NPC protrude inside the pore opening to form a selective semi-permeable barrier. The structure is well conserved among all metazoans. Approximately 30 different nucleoporins in multiple copies compose the NPC that contains between 500 and 1,000 protein molecules in the fully assembled state. An essential Y-shaped subcomplex composed of 6 to 10 nucleoporins constitute the basic scaffolding unit of the nuclear and cytoplasmic rings composing the central NPC core. Additional nucleoporins together with Y-shaped sub-complexes might be involved in the formation of the inner ring structure. Some of the scaffold nucleoproteins are the most long-lived proteins in cells with half-life of months and years, accounting for the very high structural stability of the NPC core. Other trans-membrane nucleoporins anchor the NPC to the fused nuclear membrane. The nuclear basket is made of specific nucleoporins and TPR (Translocated Promoter Region) while the cytoplasmic fibrils are composed of additional nucleoporins including GLE1 (RNA export mediator). Protruding channel fibres that make the semi-permeable barrier correspond to unstructured regions that extend from

about 10 nucleoporins. They are composed of approximately 50 interspersed phenylalanine-glycine (FG) repeats which form a size-selective hydrogel-like environment within the nuclear pore (**Fig. 4**).

2.3.2 Docking of mRNPs to the nuclear pore complex

The nuclear export of mRNPs appears to involve a specific mechanism for docking at the NPC. The transcription and export complex 2 (TREX-2), which is distinct from TREX, is required for the nuclear export of mRNAs. It was first isolated in yeast and suggested to dock mRNPs to nucleoporins at the nuclear side of the NPC (Fischer et al. 2002). The vast majority of mRNA transcripts are intronless and yeast TREX-2 was reported to interact with the RNA polymerase II via the mediator complex thus coupling the regulation of transcription initiation to the nuclear export of both coding and non-coding transcripts (Schneider et al. 2015). On the other hand, the human TREX-2 complex is recruited at the nuclear pore independently of RNA polymerase II transcription (Umlauf et al. 2013). It is composed of the scaffolding subunit GANP (germinal centre-associated nuclear protein), ENY2 (enhancer of yellow 2 transcription factor homologue), PCID2 (PCI domain-containing protein 2), the 26S Proteasome Complex Subunit DSS1 and either CETN2 or CETN3 (centrin 2 or 3). GANP was shown to be required for human mRNA nuclear export and for recruitment of NXF1 to the nuclear pore complex via direct interactions between the carboxyl terminal region of NXF1 and the amino terminal domain of GANP which contains a cluster of 6 nucleoporin-like FG repeats (Wickramasinghe et al. 2010). The ENYP subunit and carboxyl terminal region of GANP have been shown to associate with TPR and Nup153 at the nuclear basket of the NPC (Umlauf et al. 2013) allowing for docking of NXF1-containing mRNP complexes at the nuclear pore, potentially by association of GANP with NXF1-containing mRNP complexes downstream of TREX. TREX-2 might also play additional roles in coupling transcription or RNA processing to the nuclear export of specific subsets of transcripts. It is however unclear whether mRNAs can directly be transferred from TREX-2 to NXF1. Whether TREX and TREX-2 act on same or different transcripts is a long-standing question that remains yet to be answered.

2.3.3 Transport of RNPs through the nuclear pore

The nuclear transport receptors (exportins, importins and NXF1) have intrinsic affinity for the FG-repeat regions of the nucleoporins that protrude in the central channel of the NPC, however it is critical that these multiple fast-exchange interactions remain weak and transient to allow for rapid

passage through the nuclear pore (Hough et al. 2015; Milles et al. 2015). FG repeats are also able to interact with each other and might prevent entry or on the contrary "brush" transported complexes along the channel (Lim et al. 2007). In addition, several scaffold nucleoporins, which exhibit a structure related to nuclear transport receptors, have been reported to interact with FG repeats. Therefore, these free scaffold nucleoporins and unloaded nuclear transport receptors might confer an additional mobile layer that transiently interacts with FG-repeats to regulate the connectivity and permeability of the nuclear pore. Several thermodynamic models have been proposed and the biophysical basis for transport through the nuclear pore remains a very hot and debatable topic of research.

2.3.4 Cytoplasmic release

Unwinding of RNA and remodeling of mRNPs at the cytoplasmic side on the NPC has been investigated mainly in yeast. Yeast nucleoporins Gle1 and Nup159 (Nup214 in human) have been implicated in this process together with the yeast DEAD-box protein RNA helicase Dbp5 (DDX19 in human) in association with inositol hexakisphosphate (IP6), which spatially regulates the activity of Dbp5 at the cytoplasmic side of the NPC (Folkmann et al. 2011). IP6-bound Gle1 stabilizes the interaction with Dbp5 and stimulates ATP binding, leading therefore to the recruitment of Dbp5 onto mRNAs. Multiple rounds of ATP binding appear to be sufficient to promote unwinding of secondary RNA structures and remodeling of mRNPs by displacement of bound protein factors, while ATP hydrolysis is required for RNA release and recycling of Dbp5. Yeast Mex67p, the ortholog of NXF1, has been shown to be remodeled by Dbp5 (Lund and Guthrie 2005). Remodeling of nuclear export adaptors and Mex67p/NXF1 by Dbp5/DDX19 is predicted to revert NXF1 to a low affinity RNA-binding affinity mode that will promote mRNA release into the cytoplasm.

A summary diagram representing the molecular mechanisms involved in the co-transcriptional processing and NXF1-dependent nuclear export of bulk mRNAs is presented in **Fig. 5**.

3 Alteration of the nucleocytoplasmic transport of RNAs in cancer and neurodegeneration

Mutations disrupting RNP particles, whether they directly affect a protein, RNA or an assembly factor, are prone to cause disease. In particular, dysregulation of RNA-binding proteins and

formation of mRNPs is affected in cancer (Wurth 2012) and several neurological disorders (Ramaswami et al. 2013).

3.1 Alteration of the nucleocytoplasmic transport of mRNAs in cancer

Several forms of cancer have been linked to alterations of nucleoporins (Xu and Powers 2009; Köhler and Hurt 2010). Elevated levels of Nup88 have been reported in breast, hepatocellular, colorectal and ovarian tumors. Chromosomal translocations in TPR, Nup98 and Nup214 lead to the production of oncogenic chimeric nucleoporins which cause carcinogenesis, particularly leukemias, by up-regulating the expression levels of proto-oncogenes. Alternative splicing of Nup98 produce a transcript encoding a Nup98-Nup96 fusion protein that is subsequently processed into either Nup98, with a short carboxyl-terminal extension, or Nup98 and Nup96 proteins. Interestingly, expression levels of Nup96 contribute to cell cycle progression by regulating the differential nuclear export of mRNAs encoding key protein factors involved in the control of the cell cycle progression (Chakraborty et al. 2008). On the other hand, increased levels of GANP that shares some homology with nucleoporins have been reported in lymphomas (Fujimura et al. 2005) and in the malignant transformation of melanocytes (Kageshita et al. 2006).

CRM1 is overexpressed in glioma, cervical, ovarian and pancreatic cancers, as high levels of CRM1 are required for the survival of cancer cells. Conversely, the depletion of CRM1 leads to the inhibition of cervical cancer cell proliferation (van der Watt et al. 2009). A clear link has also been established between elevated levels of eIF4E, a CRM1 adaptor protein, and the *in vitro* and *in vivo* formation of tumors (Graff et al. 2008). The overexpression of eIF4E furthermore leads to oncogenic transformation (Culjkovic-Kraljacic et al. 2012) and expression levels of eIF4E are up regulated in approximately 30% of human cancers. The mRNA targets dependent on the eIF4E:CRM1 nuclear export pathway encode many known oncogenes and factors involved in cell proliferation (Culjkovic et al. 2006).

During transcription and co-transcriptional processing, the neo-synthesized RNA can interact with the melted complementary strand of DNA leading to the formation of DNA/RNA hybrids called R-loops. Specific factors such as the DNA/RNA helicase SETX (senataxin) are required to resolve these structures which otherwise leave the opposing strand of DNA susceptible to cleavage and varied forms of DNA damage (Wickramasinghe and Venkitaraman 2016). The co-transcriptional recruitment and direct interactions of TREX with processing transcripts is ideally

suited to prevent inappropriate annealing of nascent RNA with single-stranded DNA. Depletion of TREX subunits leads to increased R-loops and DNA damage both in yeast and human cells. TREX plays a pivotal role in the maintenance of the genome stability by regulating the selective nuclear export of mRNAs encoding proteins involved in genome duplication and DNA repair (including RAD51, CHK1, FANCD2). This selective nuclear export pathway involves differential regulation of the RNA-binding activity of ALYREF by the inositol polyphosphate multikinase (IPMK) (Wickramasinghe et al. 2013). Formation of R-loops, genome instability and alteration of TREX components have consistently been implicated in many types of cancer (Wickramasinghe and Venkitaraman 2016). The THOC1 subunit of TREX is up regulated in ovarian, colon and lung cancers but down regulated in testis and skin cancers (Domínguez-Sánchez et al. 2011). Phosphorylation of THOC5 by the leukemogenic protein tyrosine kinase (PTK) has been involved in chronic myeloid leukemia (Griaud et al. 2013). On the other hand, the interaction of the nuclear export co-adaptor CHTOP with the methylosome complex promotes PRMT1-dependent di-methylation of histone H4 on arginine 3 in gene promoters and intragenic regions, leading to the transcriptional activation of cancer-related genes involved in glioblastoma (Takai et al. 2014). Expression levels of nuclear export adaptor ALYREF are dysregulated in colon, stomach, pancreatic and testis tumors with down regulation observed in high-grade cancers (Domínguez-Sánchez et al. 2011). Expression of the adaptor LUZP4, which is normally restricted to testis, is up regulated in tumors from various tissues, and its expression is essential to the proliferation of melanoma cells (Viphakone et al. 2015). Depletion of ALYREF (Saito et al. 2013), THOC1 (Li et al. 2007) and LUZP4 (Viphakone et al. 2015) inhibit the proliferation of cancer cells and may therefore provide novel therapeutic strategies to treat some forms of cancer (Culjkovic-Kraljacic and Borden 2013).

3.2 Alteration of the nucleocytoplasmic transport of mRNAs in neurodegeneration

3.2.1 Neurological disorders caused by genetic mutations in TREX subunits

Mutations in the core THO subunits of the TREX complex affect neuronal development and cause rare diseases. A chromosomal translocation that produces a chimeric inactive PTK2-THOC2 protein fusion leads to cognitive impairment, cerebellar hypoplasia and congenital ataxia in childhood (Di Gregorio et al. 2013). The protein tyrosine kinase 2 (PTK2) is involved in axonal and neurite growth however depletion alone does not cause this phenotype, indicating that the disease is due to the loss-of-function of THOC2. In addition, THOC2 missense mutations that affect protein

stability have been implicated in X-linked intellectual disability (Kumar et al. 2015). In addition, the expression levels of THOC1, THOC3, THOC5 and THOC7 proteins, but not of ALYREF, DDX39B and CHTOP, were also decreased, supporting a loss-of-function mechanism by global alteration of the THO subcomplex. A homozygous missense mutation in THOC6 has also been reported in patients from two related Hutterite families, an isolated population of 40,000 individuals living in the North American prairies, which present with intellectual disability (Beaulieu et al. 2013). The mutation causes the THOC6 protein to mislocalize to the cytoplasm from the nucleus where it physiologically associates with THO in the TREX complex.

3.2.2 Mutations in Gle1 cause motor neuron diseases

Loss-of-function mutations in the mRNA export mediator Gle1 were shown to cause severe neurodegeneration that results in amyotrophic lateral sclerosis (ALS) (Kaneb et al. 2015) and fetal motor neuron disease (LCCS1, Lethal congenital contracture syndrome 1) (Nousiainen et al. 2008). ALS is an invariably fatal adult onset disease with an annual incidence rate of approximately 2 cases per 100,000 individuals. It leads to progressive loss of upper and lower motor neurons invariably resulting in progressive paralysis and death usually within 2-3 years from symptom onset. The autosomal recessive disease LCCS1 is caused by a *Fin major* mutation inherited in Finnish families with an incidence rate of 1 in 25,000 births. It leads to severe atrophy of spinal motor neurons and skeletal muscles and total immobility of the fetus, which lead to multiple joint contractures and pre-natal death. The *Fin major* mutation specifically impairs the nuclear export of bulk mRNAs in human cells, but not the Gle1 functions associated with translation, triggering loss of localization to the nuclear pore and nuclear accumulation of polyadenylated RNA (Folkmann et al. 2013). The mRNA targets and the pathophysiological mechanisms by which Gle1 loss-of-function leads to neurodegeneration remains uncharacterised due to the severity of the disease phenotype and the lack of experimental animal and cell models.

3.2.3 Altered nucleocytoplasmic transport of mRNAs in microsatellite repeat expansion disorders

The nucleocytoplasmic transport of RNA and proteins is affected in several neurodegenerative diseases caused by repetition of microsatellite expansions. These have been subdivided into poly-glutamine (poly-Q) and non-poly-glutamine disorders. Poly-Q sequences encoded by CAG trinucleotide-repeat expansions have been reported in Huntington disease (HD) and several

subtypes of spinocerebellar ataxia (SCA1-3, 6, 7, 17). SCA8 and myotonic dystrophy type 1 (DM1) further involve bi-directional transcription of CUG-sense and CAG-antisense trinucleotide-repeat transcripts. Non-poly-Q disorders are caused by expansions ranging from trinucleotide to hexanucleotide repeats including: (i) CGG repeats in Fragile X-associated mental retardations; (ii) pentanucleotide (ATTCT, TGGAA) and hexanucleotide (GGCCTG) expansions in SCA10, 31 and 36 respectively; (iii) bi-directionally transcribed GGGGCC-sense and GGCCCC-antisense hexanucleotide-repeat expansions in chromosome 9 open reading frame 72 (C9ORF72) related amyotrophic lateral sclerosis (ALS) (Orr and Zoghbi 2007; Walsh et al. 2015; Loureiro et al. 2016).

Repeat expansions are found both in non-coding (5'-UTR, intronic, 3'UTR) and coding regions of mRNAs. They cause neurodegeneration through a complex pathogenesis involving protein loss-of-function and protein/RNA toxic gain-of-function mechanisms through sequestration of proteins by RNA repeat sequences and production of toxic polymeric repeat proteins by repeat-associated non-ATG (RAN) translation (Walsh et al. 2015; Loureiro et al. 2016). This unconventional form of translation does not require a start codon and can be initiated in all frames at various positions within the length of the repeat expansion. The molecular mechanism(s) of RAN translation remain poorly characterised but are dependent on the formation of secondary RNA structures that may recruit ribosomes similarly to internal ribosome entry site elements used by virus. The RAN translation of CAG-repeat transcripts lacking start codons into homo-polymeric proteins in all frames (poly-glutamine, poly-serine and poly-alanine) was discovered in cell models and SCA8/DM1 human brain tissue (Zu et al. 2011). RAN translation of non-coding transcript regions was subsequently highlighted to occur from CGG repeats in the 5'-UTR of the *FMR1* (fragile X mental retardation 1) gene in fragile X–associated tremor and ataxia syndrome (FXTAS) (Todd et al. 2013) and from GGGGCC sense/antisense intronic repeats in C9ORF72-ALS (Mori et al. 2013; Zu et al. 2013). Moreover, RAN translation through coding CAG-repeat expansions in the Huntingtin (*HTT*) gene was reported in HD (Bañez-Coronel et al. 2015).

Exonic CAG-repeat expansions found in polymorphic glutamine regions in autosomal-dominant cerebellar ataxias and HD lead to the translation of abnormal proteins with extended poly-Q domains that promote misfolding/aggregation, abnormal binding to other protein factors and reduced interactions with protein partners. Strikingly, mechanisms of pathogenicity in microsatellite repeat expansion disorders are shared between the nuclear retention of repeat transcripts that form characteristic RNA foci which sequester RNA-processing proteins and the cytoplasmic production of aggregating repeat proteins by RAN translation. However the pathophysiological contribution of each of these alterations remains debatable and is to be precisely determined for each

neurodegenerative disease. The mechanisms driving and regulating the nuclear export of repeat transcripts are therefore expected to play a key role in delineating pathological outcomes induced by nuclear retention of repeat transcripts and RAN translation in the cytoplasm.

Indeed, manipulating the expression levels of mRNA nuclear export factors either exacerbates or suppresses neurodegeneration in cellular and animal models of DM1 (Garcia-Lopez et al. 2008), C9ORF72-ALS (Freibaum et al. 2015) and poly-Q diseases (Tsoi et al. 2011; Sun et al. 2015). Manipulating the expression of nuclear pore complex components is also a modifier of neurodegeneration in C9ORF72-ALS. On the other hand, nucleocytoplasmic alterations of protein trafficking have been extensively reported in HD, DM1, C9ORF72-ALS and upon expression of expanded poly-Q proteins (Boeynaems et al. 2016), but will not be reviewed in this chapter which focuses on RNA nuclear export.

The accumulation of intranuclear RNA foci in Poly-Q diseases, SCAs, DM1, HD and C9ORF72-ALS suggest resistance to the nuclear export of repeat transcripts however the occurrence of RAN translation in the cytoplasm implies that a fraction is at least transported through the nuclear pore. CAG-repeat transcripts were shown to directly bind U2AF65 which serves as a nuclear export adaptor by interacting with NXF1 (Tsoi et al. 2011). Consistent with this, the nuclear export of CAG-repeat transcripts is enhanced by overexpression of U2AF65 in HD models, while it is inhibited by overexpression of MBLN1 (muscleblind-like protein 1) which is sequestered on RNA repeats (Sun et al. 2015). Reduced levels of Ref1, the *Drosophila* orthologue of ALYREF in mammals, exacerbate neurodegeneration in a DM1 model of *Drosophila* (Garcia-Lopez et al. 2008) suggesting that ALYREF is involved in the nuclear export of CUG-repeat transcripts.

In ALS, GGGGCC-repeat expansions are found in intron 1 of the *C9ORF72* gene. Nuclear retention of pre-mRNA repeat transcripts would therefore be expected however RAN translation of dipeptide-repeat proteins indicates transport through the nuclear pore. The nuclear export adaptors ALYREF and SRSF1 were shown to directly interact with GGGGCC-repeat RNA and sequestration of adaptors was suggested to play a role in the abnormal nuclear export of *C9ORF72* repeat transcripts by triggering abnormal interactions with NXF1 (Cooper-Knock et al. 2014). Interestingly, depleting the non-essential ALYREF factor in a C9ORF72-ALS *Drosophila* model ameliorates the rough eye phenotype (Freibaum et al. 2015). Reduced levels of NXF1 and CHTOP intriguingly enhanced neurodegeneration in this model (Freibaum et al. 2015) however this might be caused by indirect effects due to global alterations of the nuclear export of mRNAs. CHTOP has also been implicated in multiple other cellular functions which would be altered upon depletion.

Widespread dysregulation of gene expression associated with these alterations of the nucleocytoplasmic transport in neurodegeneration present challenges but also opportunities for therapeutic intervention (Hautbergue 2016). Evaluating the pathogenic contributions between repeat protein production and RNA-mediated toxicity by nuclear retention of transcripts will be fundamental to the successful development of neuroprotective strategies. If expression of repeat proteins can kill cells *in vitro*, it is difficult to evaluate which levels are translated in patients and whether these are sufficient to trigger toxicity depending on the repeat expansion, disease and cell type. There are however growing evidences for a pathogenic role of RAN-translation. For example, FXTAS was initially thought to be caused by intranuclear retention of transcripts and sequestration of splicing factors (Jin et al. 2007; Sofola et al. 2007) however the discovery of RAN translation in the same model challenged this view (Todd et al. 2013). Similarly in C9ORF72-ALS, increasing ten fold the number of intranuclear RNA foci does not significantly alter cell survival or global RNA processing while expression of dipeptide repeat proteins causes neurodegeneration (Tran et al. 2015).

The nuclear export of repeat transcripts appears to involve the NXF1 pathway and various export adaptors such as ALYREF or U2AF65 in DM1 (Garcia-Lopez et al. 2008), poly-Q-related diseases (Tsoi et al. 2011; Sun et al. 2015) and C9ORF72-ALS (Freibaum et al. 2015). In FXTAS, PUR-alpha might play a role in the nuclear export of CGG-repeat transcripts (Jin et al. 2007) however the nuclear export receptor remains yet to be characterised. The partial depletion of individual RNA nuclear export adaptors does not appear to be essential to the functioning of higher eukaryote cells due to high level of redundancy. Therefore, they might constitute new therapeutic targets for inhibiting the nuclear export of repeat transcripts and the production of toxic repeat proteins, particularly in disease where RAN translation appears to have a prominent role over the nuclear retention of transcripts.

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Figure legends

Fig. 1 Simplified eukaryotic expression of mRNAs and proteins. The diagram represents stepwise expression of protein-coding genes. Nuclear steps are in fact functionally linked to each other in order to ensure directionality of the biogenesis and processing of mRNAs. Remodeling of chromatin stimulates the recruitment of transcription factors including the TATA box binding protein (TBP) and the hypo-phosphorylated RNA polymerase under form IIA upstream of the transcription start site (TSS). The capping enzyme (CE) is recruited through phosphorylation of the CTD on serine 5 residues shortly after the initiation of transcription. Addition of the CAP onto the 5'-end of the mRNA is required to stabilize the nascent mRNA and provide 5'-3' directionality to the nuclear export process. It is also essential to the recruitment of translation initiation factors. Processivity of transcription elongation is dependent upon phosphorylation of the CTD on serine 2 residues and is maintained through recruitment of the spliceosome for splicing and of the cleavage and polyadenylation machinery to end transcription. Nuclear export of bulk mRNAs is linked to splicing in humans via the TREX complex which controls for the timely recruitment and activation of the nuclear export receptor NXF1 for the transport of fully processed transcripts through the nuclear pore complex (NPC). Transported mRNAs are circularized and subject to translation in the cytoplasm.

Fig. 2 Diagrammatic process of RNA handover during splicing and nuclear export. In the nucleoplasm, interaction of the DEAD box RNA helicase DDX39B with RNA promotes the assembly of spliceosomes on the nascent pre-mRNA synthesized by the RNA polymerase II. Direct interactions with nuclear export adaptors (ALYREF, UIF, LUZP4) and co-adaptor (Co-ad.) CHTOP promote handover of the RNA from DDX39B and the loading of adaptor proteins onto pre-mRNAs. Successive cycles of ATP hydrolysis and recruitment of adaptors are thought to facilitate the assembly of TREX which is composed of the THO subcomplex, DDX39B, ALYREF and a few other proteins including the previously described adaptor proteins in various combinations. Direct interaction of NXF1 with the ternary RNA:ALYREF:DDX39B leads to the displacement of DDX39B and RNA handover onto NXF1. The high RNA affinity remodeling of NXF1 is achieved through direct interactions with both the adaptor (ALYREF and potentially UIF or LUZP4) and co-adaptors THOC5 or CHTOP within the TREX complex. On completion of transport through the nuclear pore, the mRNP is remodeled on the cytoplasmic side. Dissociation of adaptors are predicted to revert NXF1 to a low RNA binding activity that will promote the release of the transported mRNA in the cytoplasm.

Fig. 3 Schematic representation of the nucleocytoplasmic transport mediated by exportins and importins. This active transport is dependent on the small Ran GTPase. The GTP bound state of Ran is enriched in the nucleus while the Ran-GDP form is cytoplasmic. The nuclear Ran-GDP exchange factor (Ran-GEF) and the cytoplasmic Ran-GTPase-activating protein (Ran-GAP) maintain the Ran-GTP / Ran-GDP gradient across the nuclear membrane generating the driving force for the nucleocytoplasmic transport process. Binding of Ran-GTP to the exportin (CRM1, Exp-t or Exp-5 for respective nuclear export of some mRNAs/proteins, tRNAs or miRNAs) trigger conformational changes that induce high affinity of the exportin for NES-containing adaptor proteins that can either be transported across the nuclear pore or bind specific subsets of RNAs. The exportins are also able to directly bind nucleoporins and license the nuclear process. GTP hydrolysis by the cytoplasmic Ran-GAP protein will remodel the exportins to a low affinity for their NES-adaptor proteins releasing the transported proteins or adaptor:RNA complexes into the cytoplasm. The free exportins are recycled back to the nucleus using the importin-dependent pathway. Conversely, interactions of the Ran-GDP with importins induce high affinity for NLS-containing proteins. The Ran-GEF factor will promote exchange of GDP for GTP in the nucleus triggering the release of the transported proteins.

Fig. 4 Schematic representation of the nuclear pore complex (NPC). The NPC forms a stable symmetrical structure composed of a central core, a nuclear basket and 8 flexible fibrils on the cytoplasmic side built through interactions of approximately 30 different nucleoporins. The central core appears to contain three porous rings by electron microscopy: NR, nucleoplasmic ring; IR, inner ring; CR, cytoplasmic ring. The interspersed unstructured FG-repeat regions of some nucleoporins protrude within the channel of the pore to form a semi-permeable hydrogel-like environment that allows for selective transport. The NPC is anchored in pores created through fusion of the inner nuclear membrane (INM) and outer nuclear membrane (ONM).

Fig. 5 Model for the biogenesis, processing and nuclear export of bulk mRNAs in humans. The remodeling of compact heterochromatin into relaxed euchromatin is usually required for transcriptional activation by facilitating recruitment of transcription factors and the hypo-phosphorylated form of the RNA polymerase IIA upstream of the transcription start site (TSS). Phosphorylation on serine 5 residues of the CTD is associated with initiation of transcription and recruitment of the capping enzyme which lead to the addition of a cap onto the 5'end of the nascent transcript. Loading of the RNA helicase DDX39B onto the pre-mRNA promotes the assembly of

the spliceosome complex and splicing/processing occurs in the vicinity of nuclear speckles in the perispeckle space. The direct interaction of nuclear export adaptors (ALYREF, UIF, LUZP4) and co-adaptor CHTOP with DDX39B leads to RNA handover and deposition of adaptor proteins onto the spliced exon junction to promote the assembly of the TREX complex. TREX is composed of the stable hexameric core THO, DDX39B, ALYREF and a few other proteins that may produce different TREX complexes with various selectivity for various subsets of mRNAs. SR-rich splicing factors SRSF1, 3 and 7 proteins (SR-rich) also act as NXF1-dependent nuclear export adaptors however they have not been isolated in TREX. They are co-transcriptionally recruited and deposited along the processing transcript at the spliced exon junctions during alternative splicing. Export adaptors bound onto spliced transcripts further allow recruitment of NXF1, displacing in turn DDX39B upon mutually exclusive interactions with NXF1. Simultaneous interactions of a TREX nuclear export adaptor and co-adaptor THOC5 further remodel NXF1 into an open structural conformation exposing its RNA-binding domain which was silenced by intra-molecular interactions. The high RNA-binding affinity state of NXF1 triggers handover of the RNA from the nuclear export adaptor. The methylated CHTOP co-adaptor is also involved in this process by interacting with NXF1 in an exclusive manner with THOC5 promoting further events of remodeling within TREX. The SR-rich adaptors SRSF1, 3, 7 are also able to remodel NXF1 to directly induce its RNA-binding affinity in absence of TREX. The TREX-2 complex interacts with NXF1 and will allow docking of the mRNP at the nuclear pore complex (NPC) and interaction of NXF1 with the FG-repeats of the nucleoporins that protrude in the channel of the nuclear pore.

Table 1 Human NXF1-dependent nuclear export of mRNAs. NXF1 is either recruited by different nuclear export adaptors in the TREX/ AREX (alternative mRNA export) complexes or directly by the constitutive transport element (CTE) in type D retroviral mRNAs or by the signal sequence-coding region (SSCR) found in secretory proteins. The RNA transport element (RTE) was identified in some mouse transposons and is similar to the CTE element. The Intronless Transport Element (ITE) is a 22-nucleotide element in the histone H2A mRNA which is recognized by SR-rich splicing factors SRSF3 and SRSF7.

mRNA cargoes	**Nuclear export adaptor**	**NXF1-dependent export machinery**
mRNAs	ALYREF	TREX
mRNAs	UIF	TREX
mRNAs	LUZP4	TREX
mRNAs involved in genome duplication and repair	mRNA binding by ALYREF is regulated by inositol polyphosphate multi-kinase (IMPK)	TREX
mRNAs & ITE mRNA (Histone H2A)	SRSF1, SRSF3, SRSF7	Not identified in TREX
RTE mRNAs (Transposons)	RBM15	Not identified in TREX
mRNAs involved in cytokinesis	?	AREX composed of CIP29 and DDX39A (a paralog of DDX39B)
CTE mRNAs (Type D retrovirus)	None	Not identified in TREX
SSCR mRNAs (secretory proteins)	None	Not identified in TREX

Table 2 Exportin-mediated nuclear export of specific RNAs and associated adaptor proteins. Eukaryotic initiation translation factor 4E (eIF4E), the CAP-binding protein required for CAP-dependent translation of proteins, is also localized to the nucleus where it plays a role in the nuclear export of a subset of capped mRNAs that contain a 50-nucleotide 4E-sensitivity element (4ESE) in the 3'-untranslated region (3'-UTR). The 4ESE RNA element is bound by the leucine-rich pentatricopeptide repeat containing (LRPPRC) protein which also interacts with eIF4E and CRM1. Human antigen R (HuR) plays various roles in the processing and stability of RNA molecules through interactions with the AU-rich element (ARE) commonly found in the 3'-UTR of various transcripts. The NES-containing APRIL factor directly interacts with HuR and bridges the interactions between the HuR:RNA complex and CRM1. NXF3 belongs to the NXF1 family of proteins but has tissue specific expression with strong expression in testis. It lacks the carboxyl terminal nucleoporin-binding region and use CRM1 for translocation through the nuclear pore.

RNA cargoes	**Adaptor proteins**	**Exportin-dependent pathways**
5S rRNA	L5 in *Xenopus laevis*	CRM1
18S rRNA in pre-40S ribosomal subunit	RPS15 in metazoans?	CRM1
28S rRNA in pre-60S ribosomal subunit	NMD3 in human	CRM1
snRNAs	PHAX & CBC in *Xenopus laevis*	CRM1
4ESE mRNAs (including cyclin D1, BCL2, BCL6 and MYC)	eIF4E and LRPPRC in human	CRM1
ARE mRNAs (including c-Fos and CD83)	HuR and APRIL in human	CRM1
Unidentified polyA+ RNA in testis	NXF3	CRM1
mRNAs (human interferon alpha1, cyclooxygenase 2)	?	CRM1
tRNAs	None	Exp-t
miRNAs	None	Exp-5

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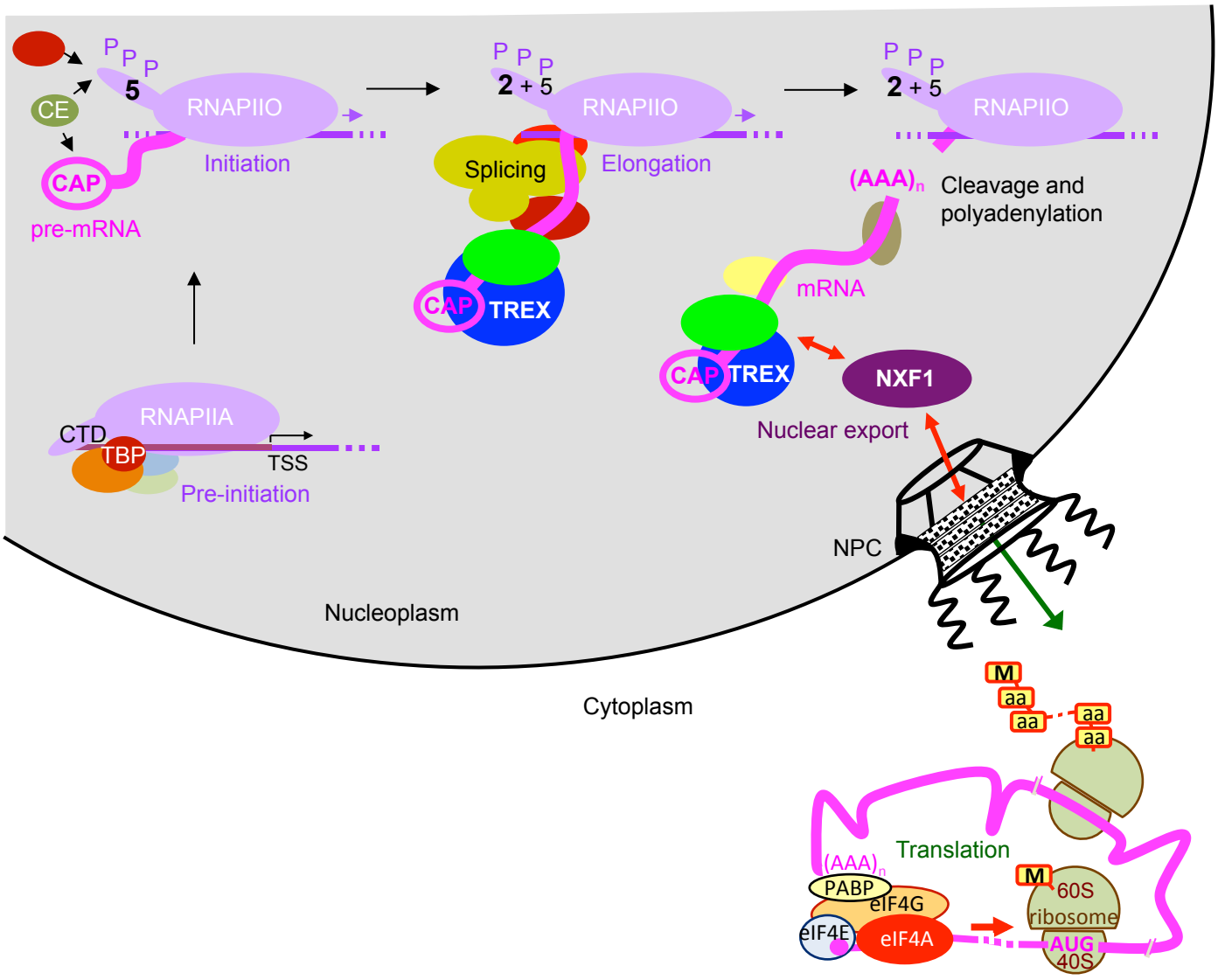
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Figure 1

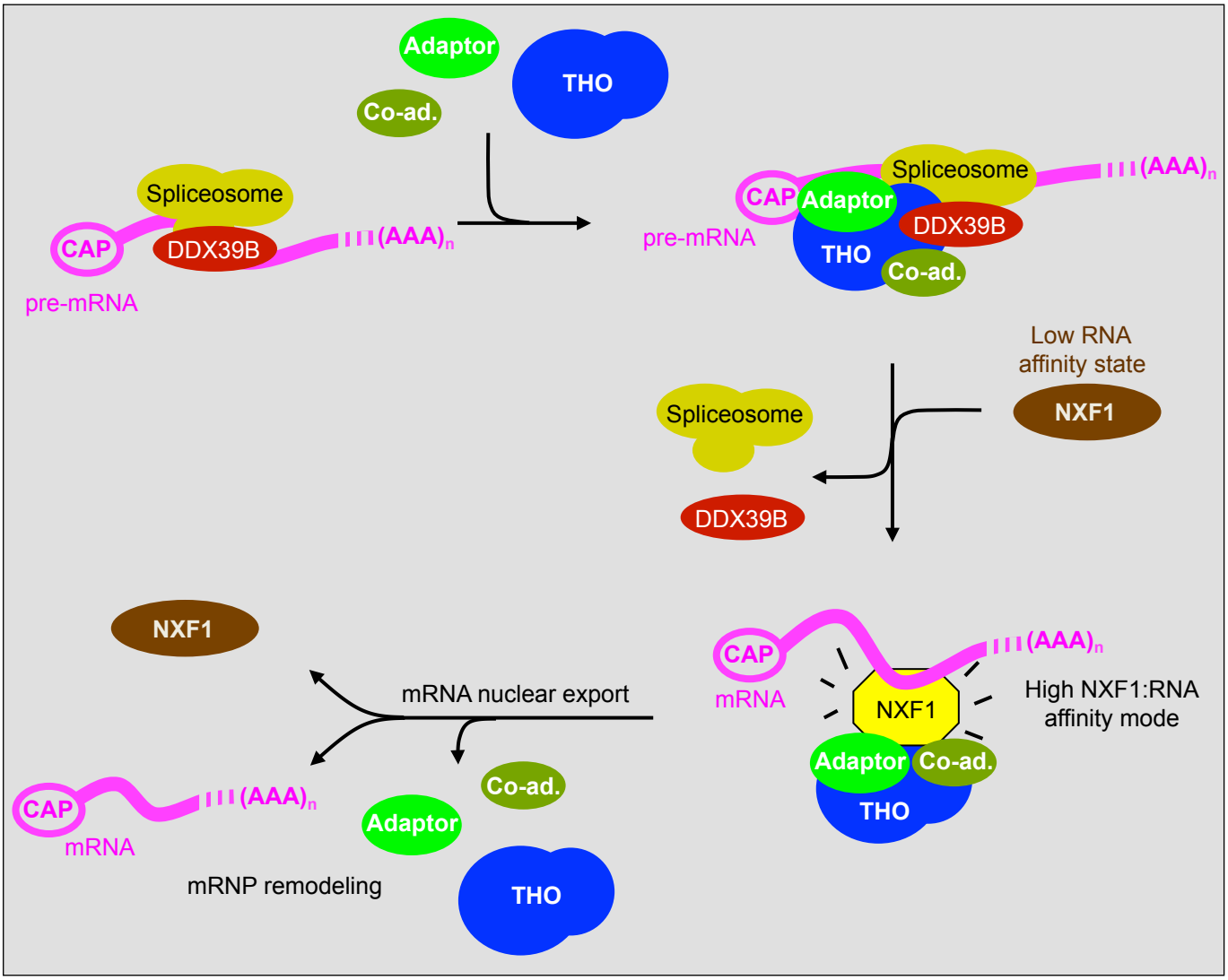
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Figure 2

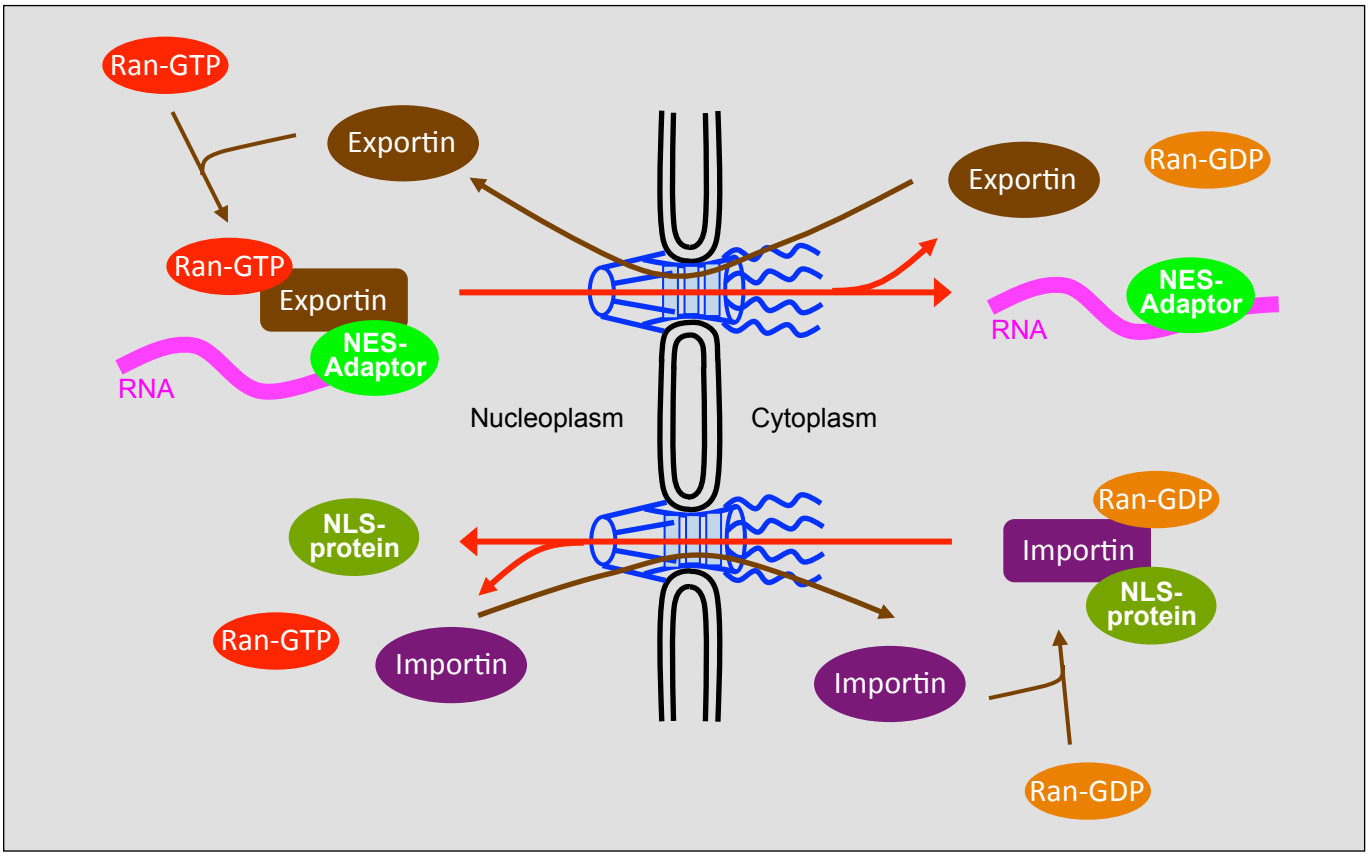
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Figure 3

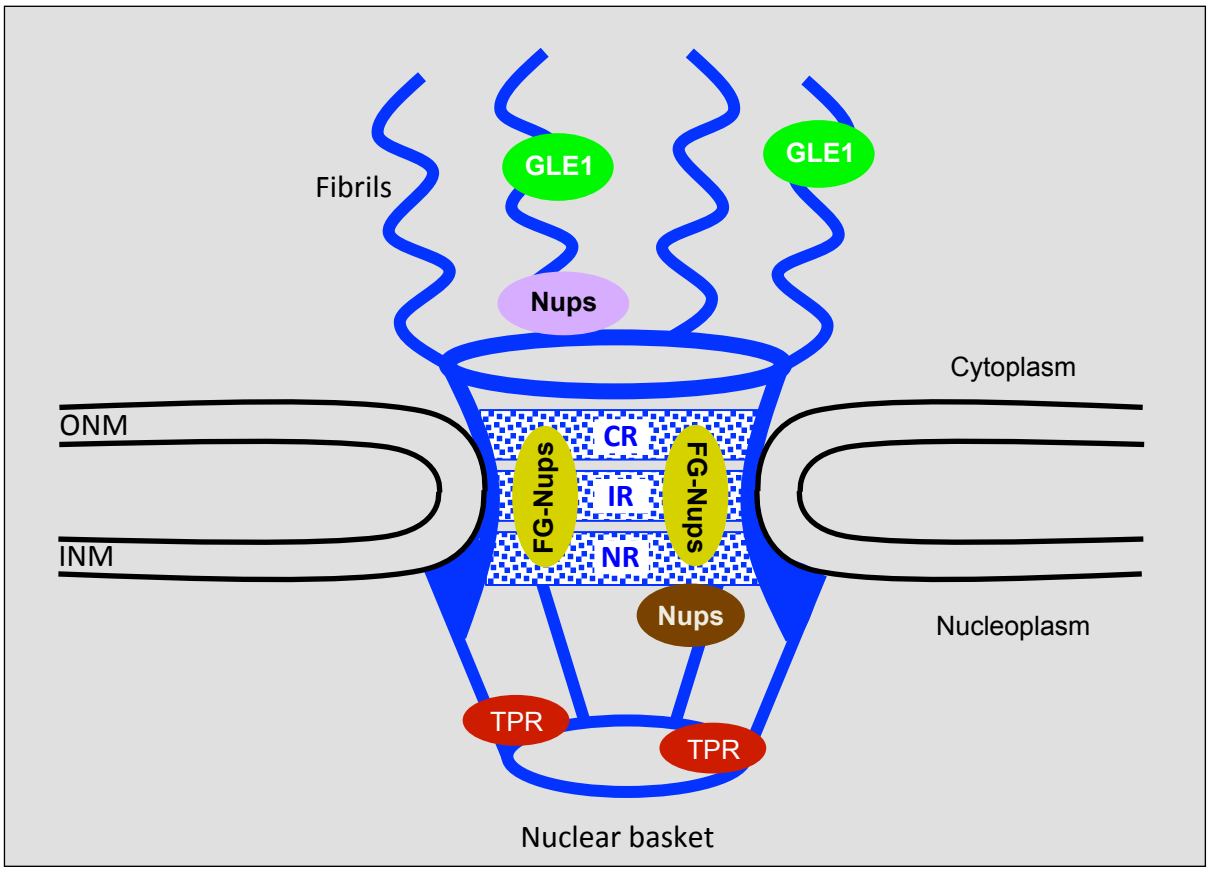
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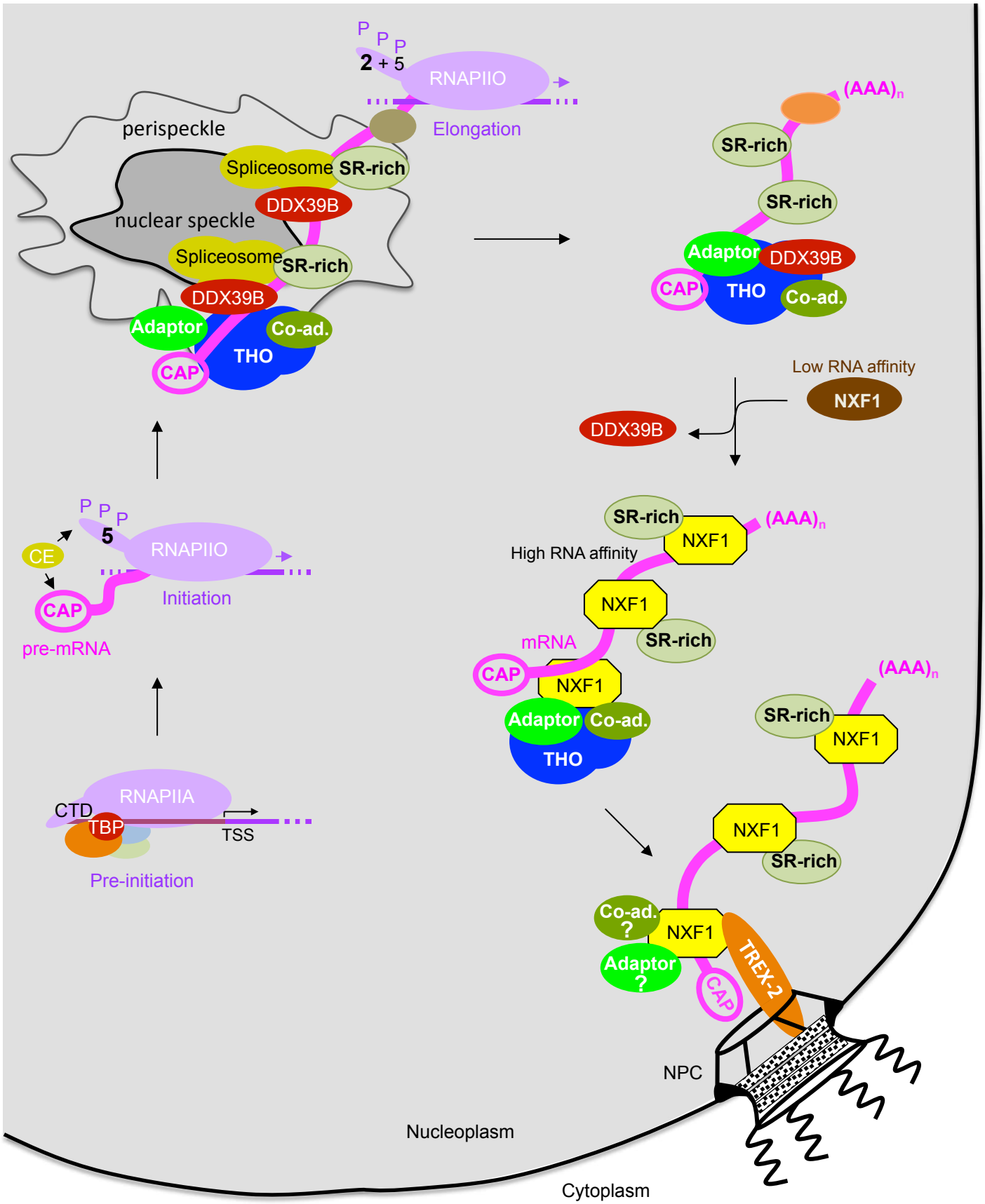
Figure 4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Cytoplasm

Figure 5