Exporting RNA from the nucleus to the cytoplasm

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Abstract | The transport of RNA molecules from the nucleus to the cytoplasm is fundamental for gene expression. The different RNA species that are produced in the nucleus are exported through the nuclear pore complexes via mobile export receptors. Small RNAs (such as tRNAs and microRNAs) follow relatively simple export routes by binding directly to export receptors. Large RNAs (such as ribosomal RNAs and mRNAs) assemble into complicated ribonucleoprotein (RNP) particles and recruit their exporters via class-specific adaptor proteins. Export of mRNAs is unique as it is extensively coupled to transcription (in yeast) and splicing (in metazoa). Understanding the mechanisms that connect RNP formation with export is a major challenge in the field.

The appearance of the cell nucleus marked a central evolutionary transition, but the sequence of events that led to this development and the immediate adaptive benefits it provided to primordial eukaryotic cells remain mysterious. Regardless of which selective pressure forced the nucleus–cytoplasm compartmentalization, it seems clear that by departing from the prokaryotic mechanism of co-transcriptional translation several immediate problems emerged. Following the physical separation of the transcription and translation processes, a strong selective pressure must have triggered the co-evolution of nuclear pore complexes (NPCs) and nucleocytoplasmic transport machineries to allow the transport of a myriad of molecules (proteins, RNAs and ribonucleoprotein (RNP) particles) between the nucleus and cytoplasm. Studies over the past 2 decades have revealed that nucleocytoplasmic transport occurs by various mechanisms that have in common the use of mobile transport receptors, which bind and move diverse cargoes. These transporters can pass through the highly specialized channels in the nuclear membrane that are formed by NPCs[1–3] (BOX 1).

A general paradigm for nucleocytoplasmic transport was established through the analysis of protein import into and export from the nucleus. These studies revealed that transport through NPCs requires a family of conserved nuclear transport receptors (also known as karyopherins or importin-β family members), which recognize a short peptide signal on a cargo protein — either a nuclear localization signal (NLS) or a nuclear export signal (NES)[2–4]. Moreover, karyopherins can recognize nucleotide motifs in RNA cargoes, which also enables them to export RNAs. Typically, karyopherins that import cargo are called importins and karyopherins that export cargo are called exportins.

A feature of karyopherins is their regulation by the small GTPase Ran[5]. Ran exists in a GTP-bound state in the nucleus and a GDP-bound state in the cytoplasm. The RanGTP–RanGDP gradient across the nuclear membrane is generated by the action of two regulators, RanGEF (Ran-GDP-exchange factor) in the nucleus and RanGAP (Ran-GTPase-activating protein) in the cytoplasm, and creates a driving force for directional nucleocytoplasmic transport processes[3]. Importins bind cargo in the cytoplasm and release it after transport into the nucleus upon binding of RanGTP. On the other hand, exportins bind nuclear cargo only together with RanGTP, and this ternary complex is translocated to the cytoplasm, where it dissociates upon hydrolysis of RanGTP by RanGAP.

Export of tRNA, microRNA (miRNA), small nuclear (sn)RNA and ribosomal (r)RNA follows this general paradigm that involves exportins of the karyopherin family and the Ran cycle[6]. However, general mRNA export is mechanistically different as it uses a transport receptor that is unrelated to karyopherins and does not directly depend on the RanGTP–RanGDP gradient[7,8]. Moreover, numerous additional export factors (for example, adaptors and release factors) cooperate with the mRNA export receptor[6,9–11].

In this review, we provide an overview of the major RNA export pathways (FIG. 1) starting with the simpler export routes of small RNAs and then discussing the sophisticated biogenesis and export mechanisms of

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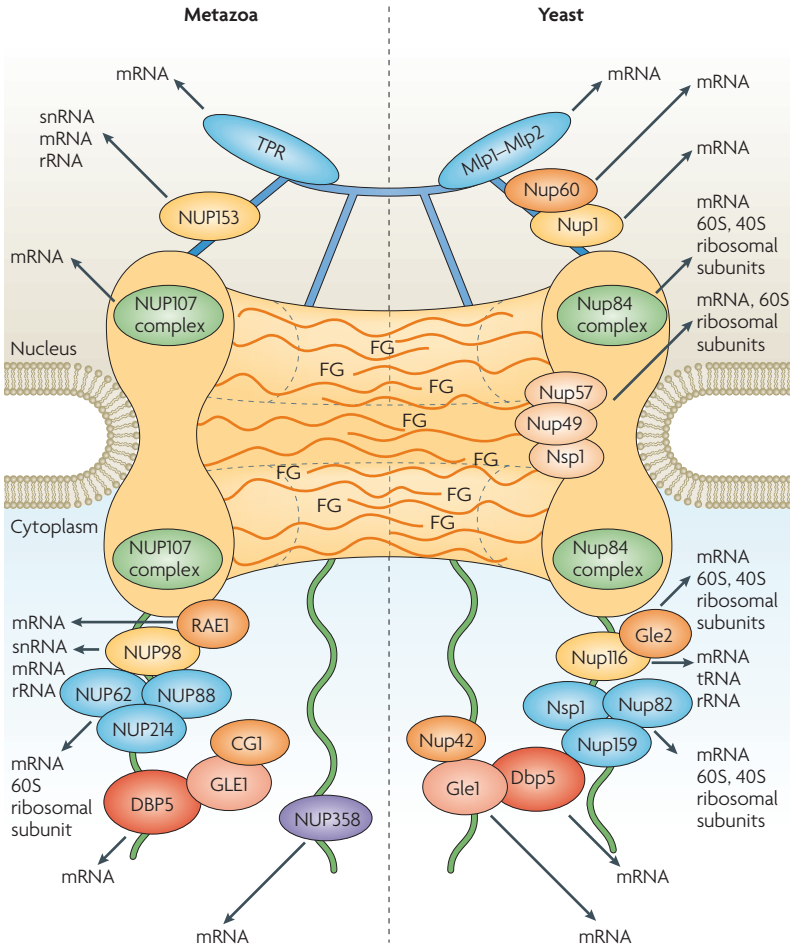
doi:10.1038/nrm2255
Published online 5 September 2007

Box 1 | **NPC organization and nucleoporins with a role in RNA export**



Nuclear pore complexes (NPCs) are large assemblies (~125 nm in diameter, with a mass of 125 MDa in metazoa and 60 MDa in yeast) that are embedded in the nuclear envelope. NPCs have an eightfold symmetrical core structure (called the spoke complex) that is sandwiched between a cytoplasmic and a nuclear ring[158]. The 8 spoke units surround the centre of the NPC through which active transport takes place. A structure called the nuclear basket and 8 short cytoplasmic fibrils (only 4 are shown) are attached, respectively, to the nuclear and cytoplasmic ring of the NPC (see figure).

The NPC is formed by ~30 different nuclear pore proteins (nucleoporins) that exist in 8 or 16 (or sometimes 32) copies per NPC[159,160]. Nucleoporins are grouped into three major classes. The first class are the FG nucleoporins, which contain Phe-Gly-rich repeat domains that fill up the active transport channel and function directly in nucleocytoplasmic transport by mediating the passage of the soluble transport receptors[161,162]. The second class are nucleoporins that are devoid of FG-repeat sequences; these are the predominant structural constituents of the NPC. The third class are Nups, which are integral membrane proteins and are thought to anchor the NPC in the nuclear membrane. FG nucleoporins can interact directly with the shuttling transport receptors[161]. Hydrophobic patches on the surface of these transporters bind transiently to the Phe residues that are part of the FG nucleoporin network in the active transport channel[161,163].

Most of the nucleoporins are located symmetrically on both sides of the NPC, but a few nucleoporins are found asymmetrically either on the cytoplasmic or nuclear side of the NPC. The asymmetrically located nucleoporins are thought to be involved in directional transport processes (initial receptor targeting or termination of transport) or to fulfil compartment-specific functions at the NPC (for example, interacting with chromatin or the transcription machinery, or as checkpoint proteins for quality-control steps during cargo export). Nucleoporins, which have been implicated in RNA export, are indicated (see main text for details). snRNA, small nuclear RNA; rRNA, ribosomal RNA. Orthologous proteins or complexes between yeast and metazoa are shown in the same colour.

much larger RNP particles. Minor export pathways of individual RNAs like the signal recognition particle 7S RNA will not be covered. We do not discuss the nuclear export of viral RNAs in depth (the reader is referred to REF. 12), but refer to them whenever they illuminate the function of a host system that has been exploited.

Export of tRNAs

Aminoacylated tRNAs are needed in the cytoplasm for ribosomal translation. About 40 different tRNAs exist in eukaryotic cells, and these tRNAs are short in length and contain single-stranded loops and double-stranded minihelix regions that fold into uniform cloverleaf structures. tRNAs are synthesized as larger precursors in the nucleus by RNA polymerase III (Pol III) (FIG. 2). Subsequent RNA-processing steps include removal of the 5′ and 3′ trailer sequences, CCA-nucleotide addition to the 3′ end to form the amino-acid acceptor stem, removal of introns (when present) by a specific tRNA-splicing endonuclease and numerous base modifications by tRNA-modifying enzymes[13]. After RNA processing and maturation, tRNAs are exported to the cytoplasm. Studies in several organisms established that fully processed, mature tRNAs are preferentially selected by the tRNA export machinery[14,15]. However, although it was initially suspected that tRNA splicing occurs in the nucleus before export, a recent study showed that the yeast tRNA-splicing endonuclease is associated with the outer mitochondrial membrane[16]. Unexpectedly, two other studies reported that mature, cytoplasmic tRNAs can be re-imported into the nucleus[17,18]. The implications of these findings are currently unclear, although a function in tRNA quality control has been suggested.

The classical tRNA export route. Export of tRNA follows the general paradigm of exportin-mediated protein export. The class-specific tRNA export receptor exportin-t, a member of the karyopherin superfamily, binds directly to tRNAs in a RanGTP-dependent manner[19,20] (FIG. 2; Supplementary information S1 (table)). The NESs in the tRNAs that are decoded by exportin-t are not linear motifs, but rather are coded in secondary and tertiary structural elements (such as minihelices) and properly processed 5′ and 3′ termini, which suggests that exportin-t can monitor the correct structural integrity of tRNAs before export[15,21]. However, exportin-t does not discriminate between intron-containing and spliced tRNAs[15,21]. After transport of the tRNA–exportin-t–RanGTP complex to the cytoplasm, RanGAP stimulates GTP hydrolysis on Ran, which induces release of the tRNA cargo from its receptor. Exportin-t is the principal tRNA exporter in vertebrate cells, but exportin-5, another member of the karyopherin family, is thought to be an auxiliary receptor[22,23]. The main role of exportin-5, however, is to export miRNAs (see below).

Additional tRNA export routes. Notably, the yeast tRNA export receptor Los1, an orthologue of exportin-t[24], is not essential for viability. This finding supported the idea that Los1-independent tRNA nuclear export pathways could exist. One of these alternative tRNA export routes

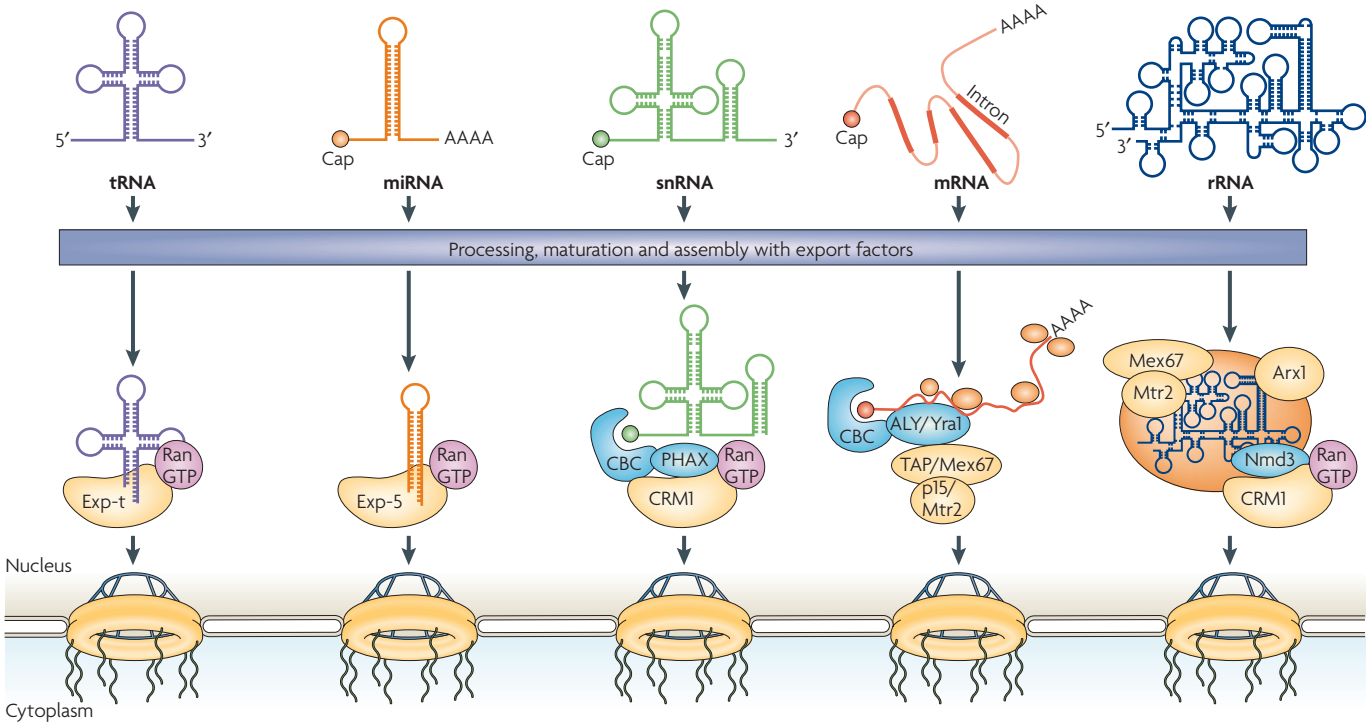


Figure 1 | **The different RNA export pathways.** The major RNA export routes are shown (tRNA, microRNA (miRNA), small nuclear (sn)RNA, mRNA, ribosomal (r)RNA). In each case, the primary RNA transcript is shown, as well as the transport-competent RNA after it has undergone processing, maturation and assembly with export factors (export adaptors are shown in blue, export receptors are shown in yellow). Prominent structural motifs in pre-RNAs are indicated (single/double-stranded RNA, loops, exons and introns, 5′ cap and 3′ poly(A) tail). For the mRNA export route, the names of both metazoan and yeast proteins are indicated, and mRNAs are shown with additional adaptor proteins and RNA-binding factors (orange ovals). In the case of rRNA, the general exporter in eukaryotes, CRM1, and two auxiliary exporters, Mex67–Mtr2 and Arx1, that have only been studied in yeast are depicted. CBC, cap-binding complex; Exp, exportin.

is linked to the aminoacylation machinery (FIG. 2). A study in yeast showed that the function of the Los1 tRNA export receptor became essential when the activity of an aminoacyl-tRNA synthetase cofactor (Arc1) was impaired[25]. Additional studies in *Xenopus laevis* oocytes and yeast demonstrated that aminoacylation of tRNAs can occur in the nucleus and, accordingly, aminoacylated tRNAs are exported from the nucleus to the cytoplasm[26–28]. Moreover, factors of the translational machinery were shown to be required for efficient tRNA export *in vivo*[13]. Taken together, these different findings point to the existence of another tRNA export pathway that overlaps with the Los1-dependent route. However, no clear export receptor candidate has as yet been identified[27,29]. Recently, novel auxiliary export factors that function upstream and downstream of the tRNA-transport step through the NPC were described. One of them, Cex1, was suggested to deliver the aminoacylated tRNAs from the nuclear export receptor at the cytoplasmic side of the NPC to the ribosomal elongation factor eEF1α[30].

Export of miRNAs

miRNAs, a class of non-coding RNAs that participate in gene regulation, are exported by the karyopherin exportin-5. miRNAs are produced as larger precursors in the nucleus and eventually mature in the cytoplasm to single-stranded RNA species that induce post-transcriptional gene silencing through base-pairing with their target mRNAs in the cytoplasm. miRNAs regulate a wide range of biological processes, including developmental timing, cell differentiation, apoptosis and immunity against viruses[31,32]. miRNA genes comprise an abundant class of regulatory molecules in higher eukaryotes, accounting for ~1% of the genome[31], and have been estimated to target a third of all human genes[33].

Biogenesis of miRNAs. The genes that encode miRNAs can be transcribed by either Pol II or Pol III[34–36] (FIG. 2). The primary transcript derived from Pol II (pri-miRNA) transiently receives a 5′ cap and a poly(A) tail similar to that of mRNAs. During subsequent miRNA processing, however, the cap and poly(A) tail are removed, whereas mRNAs keep these modifications. Some miRNAs can also be excised co-transcriptionally from the introns of coding genes. A characteristic metazoan miRNA primary transcript (pri-miRNA) contains a stem with a terminal loop and flanking segments. The stem–loop harbours a ~22-nucleotide miRNA motif that has to be excised, exported and finally assembled into the RNA interference (RNAi) effector complex (FIG. 2). The stem–loop is cut out (this is called cropping) in the nucleus by a type III RNase called Drosha[37], which cooperates

Signal recognition particle 7S RNA
The signal recognition particle is an evolutionarily conserved RNA–protein complex that contains a 7S RNA species and targets integral membrane and secretory proteins to the translocation machinery of the endoplasmic reticulum.

5′ cap
A structure at the 5′ end of eukaryotic mRNAs that consists of the m^7GpppN cap (in which m^7G represents 7-methylguanylate and ppp represents an unusual 5′→5′ triphosphate linkage from m^7G to N, which is the first regular nucleotide of the mRNA).

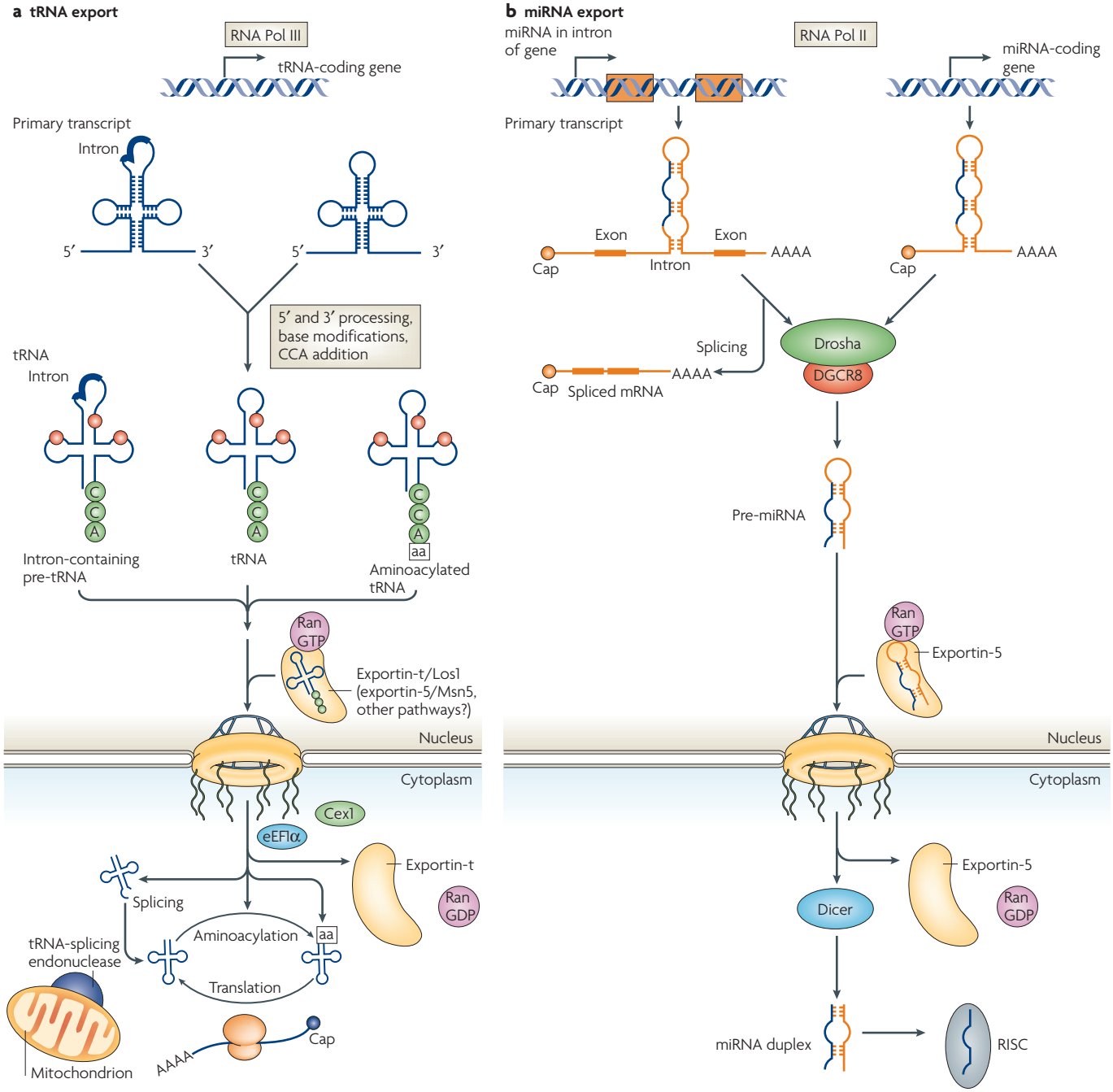


Figure 2 | **Nuclear export of tRNA and microRNA. a** | Transcription of a tRNA gene by RNA polymerase III (Pol III) generates a primary transcript, which in some cases contains an intron, with 5′ and 3′ extensions. After 5′ and 3′ processing, base modifications (red circles) and CCA-nucleotide addition at the 3′ end, the resulting tRNAs can follow different export routes. Intron-containing and intron-free tRNAs (aminoacylated or not) are exported either by the exportin-t/Los1-dependent pathway, or by other less well characterized routes. After export to the cytoplasm and dissociation of the tRNA cargo from its receptor, intron-containing tRNA is spliced by the tRNA-splicing machinery, which is bound to the outer mitochondrial membrane in yeast. Mature tRNA is then channelled to the translational machinery by release factors such as Cex1 and eEF1α. **b** | For microRNA (miRNA), only the Pol II-dependent generation of the primary mRNA (pri-mRNAs) is depicted. miRNAs can be encoded by an miRNA gene situated in the intron of a coding gene (left branch) or from a separate miRNA gene (right branch). In both cases, the primary transcript is cleaved by the Drosha–DGCR8 complex to generate a pre-miRNA with a stem–loop structure. Moreover, a spliced mRNA can be generated concomitantly with the excision of an intronic miRNA. The pre-miRNA with its typical ~2-nucleotide overhang at its 3′ end is specifically recognized by exportin-5 and is transported to the cytoplasm, where it dissociates from its receptor after RanGTP hydrolysis. Following release from exportin-5, Dicer further cleaves the pre-miRNA to finally generate a single-stranded miRNA species, which assembles into the RNA-induced silencing complex (RISC). Highlighted in blue is the RNA region that corresponds to the mature effector miRNA.

with the RNA-binding protein DGCR8[38]. DGCR8 is thought to function as a molecular ruler that measures the distance from the base of the stem and thus positions Drosha precisely for cleavage[38].

The released ~65-nucleotide stem–loop intermediate (now called pre-miRNA) is subsequently exported to the cytoplasm in a RanGTP-dependent manner by exportin-5, a member of the karyopherin family[39–41] (FIG. 2). After release in the cytoplasm upon GTP hydrolysis on Ran, the pre-miRNA hairpin is further cleaved by Dicer, another type III RNase that produces a ~22-nucleotide miRNA duplex[42] that contains imperfect base pairings. These mismatches cause one duplex strand to be less stably base paired at its 5′ end, which leads to its degradation, while the other strand is incorporated into an effector complex known as the RNA-induced silencing complex (RISC)[42–44]. RISC finally binds to its target mRNA through base pairing of the miRNA to the 3′ untranslated region (3′ UTR), thereby inducing mRNA cleavage, translational inhibition by sequestering the translational apparatus in P bodies or cleavage-independent mRNA degradation[45,46].

Coupling of miRNA processing with export. Biogenesis and nuclear export of miRNAs are coupled at several levels[32]. The key enzyme involved in this coupling is Drosha, which generates a double-stranded RNA minihelix with a ~2-nucleotide 3′ overhang, the unique structure of which is recognized both by exportin-5 and the downstream-acting processing enzyme Dicer. Thus, a strict linkage of all processing and export steps ensures the high specificity of miRNA production and function (FIG. 2).

Notably, many human miRNA genes are embedded in the intronic regions of coding genes and thus use an unusual biogenesis pathway, as their production is coupled to mRNA splicing[47–49] (FIG. 2). It seems that Drosha releases the pre-miRNA from the intron shortly before splicing, allowing the generation of both RNA species at the same time[49].

Nuclear export of miRNA has important implications for applied aspects of RNAi technology[50], as short hairpin RNAs use the same export pathway to produce small interfering (si)RNAs[51]. The expression of exportin-5 is low in most cell types, which suggests that the miRNA export receptor could limit the desired expression of siRNAs when used as a therapeutic tool in molecular medicine[51].

Export of snRNAs

The minimalist nuclear export device for RNA requires 'naked' RNA as cargo, an exportin and RanGTP. Although this model is sufficient to explain export of tRNAs and miRNAs, it does not account for the nuclear export of other cellular RNAs, which require more elaborate transport mechanisms. In terms of complexity, spliceosomal snRNAs lie between tRNAs/miRNAs and mRNAs/rRNAs. Their export requires adaptor proteins that recruit the export receptor (a mechanism that is extensively used in the case of mRNA and rRNA export). However, assembly into an RNP, another hallmark of mRNA and rRNA transport, is not needed for the nuclear export of snRNAs.

Spliceosomal snRNP biogenesis. The spliceosomal snRNAs participate in the removal of introns from pre-mRNAs. With the exception of U6 snRNA, which is produced by Pol III and does not exit from the nucleus, all the other spliceosomal snRNAs (U1, U2, U4 and U5) are synthesized as precursors (pre-snRNAs) by Pol II and acquire a 5′ cap that constitutes the signal for nuclear export (FIG. 1). However, pre-snRNAs do not become polyadenylated, although they exhibit specific 3′-end processing that requires factors related, but not identical, to the 3′-end processing machinery that acts on pre-mRNAs[52]. Why snRNA is exported from the nucleus before being imported again to function in splicing is still unknown, but it was proposed that the cytoplasmic phase of snRNA biogenesis might provide a proofreading step to prevent nuclear accumulation of non-functional snRNAs[53].

Phosphorylation-dependent snRNA export. The export receptor for snRNA is CRM1 (also known as exportin-1), the general protein exporter that recognizes the Leu-rich-type NES on proteins[54,55]. Consistently, CRM1 does not directly interact with the snRNA cargo, but requires the cap-binding complex (CBC) and a NES-containing adaptor protein called PHAX to be targeted to the 5′ cap of the snRNA[56–58]. Phosphorylation of PHAX in the nucleus is required for recruitment of CRM1 and RanGTP to the CBC-bound snRNA complex (FIG. 1). After export to the cytoplasm, GTP hydrolysis of Ran and dephosphorylation of PHAX are necessary to efficiently dissociate the export complex and release the snRNA[58]. Once in the cytoplasm, the survival of motor neurons (SMN) complex facilitates the assembly of the exported snRNA with a heteroheptameric ring of Sm proteins, which bind to a conserved Sm-binding site that is present on each snRNA[53]. Binding of Sm proteins induces trimethylation of the cap and exonucleolytic removal of the 3′ trailer sequences. The trimethylated cap and the associated Sm proteins provide a composite nuclear targeting signal for subsequent nuclear import of the mature snRNPs[59]. After re-import into the nucleus, snRNPs together with numerous other splicing factors assemble into the functional spliceosome[53].

Export of mRNAs

RNA classes with highly related structures (tRNAs and miRNAs) exhibit common identity elements that can be decoded by class-specific export receptors. mRNAs, however, differ substantially in length, sequence and structure, and so use other strategies to find their class-specific export receptors. mRNAs are channelled into the specific export pathway coordinately with their processing and assembly into messenger (m)RNPs. Typically, eukaryotic mRNAs are synthesized by Pol II as precursors (pre-mRNAs) that become capped at the 5′ end, spliced, cleaved at the 3′ end and polyadenylated (FIG. 1). During the successive steps of mRNP formation, many RNA-binding and -modifying proteins (for example, capping, splicing and processing factors) are recruited to the transcripts. Genome-wide analyses revealed a preferential association of certain RNA-binding proteins with distinct functional classes of mRNAs, which suggests

P bodies
(Processing bodies). Discrete cytoplasmic foci that are sites of degradation and surveillance of mRNAs and RNA-mediated gene silencing.

Short hairpin RNA
Artificially generated, usually vector-encoded, RNA that resembles pre-miRNA in structure and is used to experimentally induce RNA interference.

Small interfering RNA
(siRNA). Short double-stranded RNA fragment of ~22 nucleotides that is derived from longer double-stranded short hairpin RNA. siRNAs guide silencing complexes to their targets by base pairing with specific mRNA sequences.

Survival of motor neurons (SMN) complex
A large multiprotein complex that brings together the Sm proteins and small nuclear RNAs, thereby facilitating small nuclear ribonucleoprotein assembly.

Sm proteins
A set of seven proteins that are arranged as a ring structure on a specific small nuclear RNA-binding site to become part of spliceosomal small nuclear ribonucleoproteins.

that biogenesis, export and translation of mRNA subpopulations may be coordinated differently[60,61]. Among the factors bound to the pre-mRNAs are also export adaptors that serve to establish a physical bridge between the mRNA molecule and its export receptor. Studies carried out in a number of different model organisms revealed that the mRNA export pathway is conserved from yeast to humans, although every model organism has its own peculiarities[7,9,12,62].

Practically every step of mRNA biogenesis involves rigorous quality control to detect possible errors in transcription, mRNA processing or export[63]. Nuclear mRNA surveillance has been studied mostly in *Saccharomyces cerevisiae*. In analogy to the cytoplasmic machineries, nuclear pre-mRNAs can be degraded from either the 5′ or 3′ end. The actual pathway seems to depend on intrinsic features of the substrate (for example, the stage of biogenesis, intron-containing or not, and so on). mRNA decay in the 3′→5′ direction (for example, mRNAs with defective poly(A) tails) is mediated by the exosome, a complex of exonucleases that is functionally connected to various activating cofactors[63–66]. Apart from pre-mRNA turnover, the exosome is also responsible for degrading aberrant tRNAs, snRNAs, small nucleolar RNAs and rRNAs[67]. Another quality checkpoint is provided by the Mlp1–Mlp2 system, which consists of two filamentous proteins that are attached to the nuclear face of NPCs and prevent the exit of unspliced transcripts[63,68–70].

A general and a specific mRNA receptor. The yeast Mex67–Mtr2 complex and the homologous metazoan TAP–p15 complex (also known as NXF1–NXT1) function as general mRNA export receptors to transport mRNPs through the NPCs[71,72]. Although the mRNA exporter can bind directly to RNA, it operates together with adaptor RNA-binding proteins (FIG. 3). The conserved mRNA exporter is structurally unrelated to the karyopherins and is RanGTP independent; therefore, directionality of transport would have to be established by other mechanisms. Nevertheless, like karyopherins, the mRNA exporter can physically interact with the Phe-Gly-rich repeats of FG nucleoporins, which allows it to overcome the permeability barrier of the NPC that is formed by the FG-nucleoporin meshwork[7,10,62] (BOX 1). Besides its physiological role in cellular mRNA export, human TAP transports a set of viral pre-mRNAs to the cytoplasm by binding directly to specific viral RNA elements called constitutive transport elements (CTEs)[72].

The general RanGTP-dependent protein export receptor CRM1 does not have a major role in mRNA export[73,74]. However, CRM1 can be involved in the nuclear export of a subset of transcripts, such as mRNAs of several protooncogenes and cytokines, that contain AU-rich elements (AREs) in their 3′ UTRs[75]. These AREs can target NES-containing adaptor proteins and hence become connected to the CRM1-dependent export pathway. In yeast, Crm1 was recently reported to be required for export of the unspliced *YRA1* pre-mRNA (Yra1 is itself an mRNA export adaptor; see below)[76]. However, the mechanistic details of how Crm1 in conjunction with Mex67–Mtr2 can export an unspliced pre-mRNA are currently not clear.

Finally, it is well established that CRM1 acts in the nuclear export of a number of unspliced and partially spliced viral mRNAs. These viral mRNAs can bind adaptor proteins that contain NESs (for example, HIV Rev, adenovirus E1b 55 kDa), thereby targeting the transport receptor CRM1 (REF. 12). In fact, it was the identification of the NES in HIV Rev that led to the discovery of the CRM1-dependent nuclear protein export pathway[54].

The Yra1/ALY/REF adaptor. The mRNA export receptor is targeted to different transcripts by export adaptors that are typically mRNA-binding proteins. So far, only a few of these adaptor proteins have been identified, but it is likely that more will be uncovered among the huge number of predicted RNA-binding proteins in eukaryotic cells.

The Yra1 or ALY/REF adaptor (Yra1 in yeast and ALY or REF in metazoa) can associate directly with the general mRNA exporter[7,62] (FIG. 3). Moreover, Yra1 and ALY/REF physically interact with another conserved export factor — Sub2 in yeast and UAP56 in metazoa[77,78]. Sub2 and UAP56 are RNA helicases that can associate with several complexes involved in mRNP biogenesis (for example, the TREX (transcription-coupled export) complex and the spliceosome; see below). Thus, Yra1 and ALY/REF form a bridge between an upstream-acting RNA-binding protein and a downstream-acting mRNA export receptor. Consistent with this model, Yra1 is co-transcriptionally recruited to nascent transcripts and is therefore present at different steps during pre-mRNA formation and processing[79,80].

In addition to its role in export of cellular mRNAs, ALY/REF can function as an adaptor for the export of viral intronless mRNAs. For example, the herpes simplex virus protein ICP27 targets ALY/REF to access the TAP–p15-mediated export pathway of the host cell[81].

SR proteins can function as adaptors. The SR (Ser/Arg-rich) proteins have essential roles in splicing but also function as adaptors and regulators of multiple steps of mRNA metabolism including mRNA export, stability and translation[11]. SR proteins are abundant, evolutionarily conserved phosphoproteins. After splicing, several SR proteins remain bound to the spliced transcript and are exported to the cytoplasm, where they dissociate from the transcript and are re-imported[82]. Shuttling SR proteins (for example, SRP20 or 9G8) can recruit the general mRNA export receptor TAP–p15. TAP–p15 can bind directly to the SR motifs, which are sites of reversible phosphorylation[83,84]. SR proteins are recruited in a hyperphosphorylated form to the splicing machinery, but after splicing become hypophosphorylated, which favours the binding of TAP–p15 (REF. 83). Thus, the phosphorylation status of SR proteins could act as a switch to signal the export competence of the spliced mRNP.

A cycle of phosphorylation and dephosphorylation of an SR protein (Npl3) has also been implicated in a termination step during mRNA export in yeast[85]. Npl3 is first phosphorylated by the Sky1 kinase in the cytoplasm, which stimulates nuclear import of Npl3 (REF. 86). In the nucleus, Npl3 can associate with the nascent transcript in

AU-rich element
A motif that is located in the 3′-untranslated region of some mRNAs and that can induce rapid mRNA decay.

SR (Ser/Arg-rich) proteins
An abundant class of proteins that are involved in various aspects of mRNA metabolism. They contain one or two RNA-recognition motifs and an Arg/Ser-rich domain that can be phosphorylated at multiple positions.

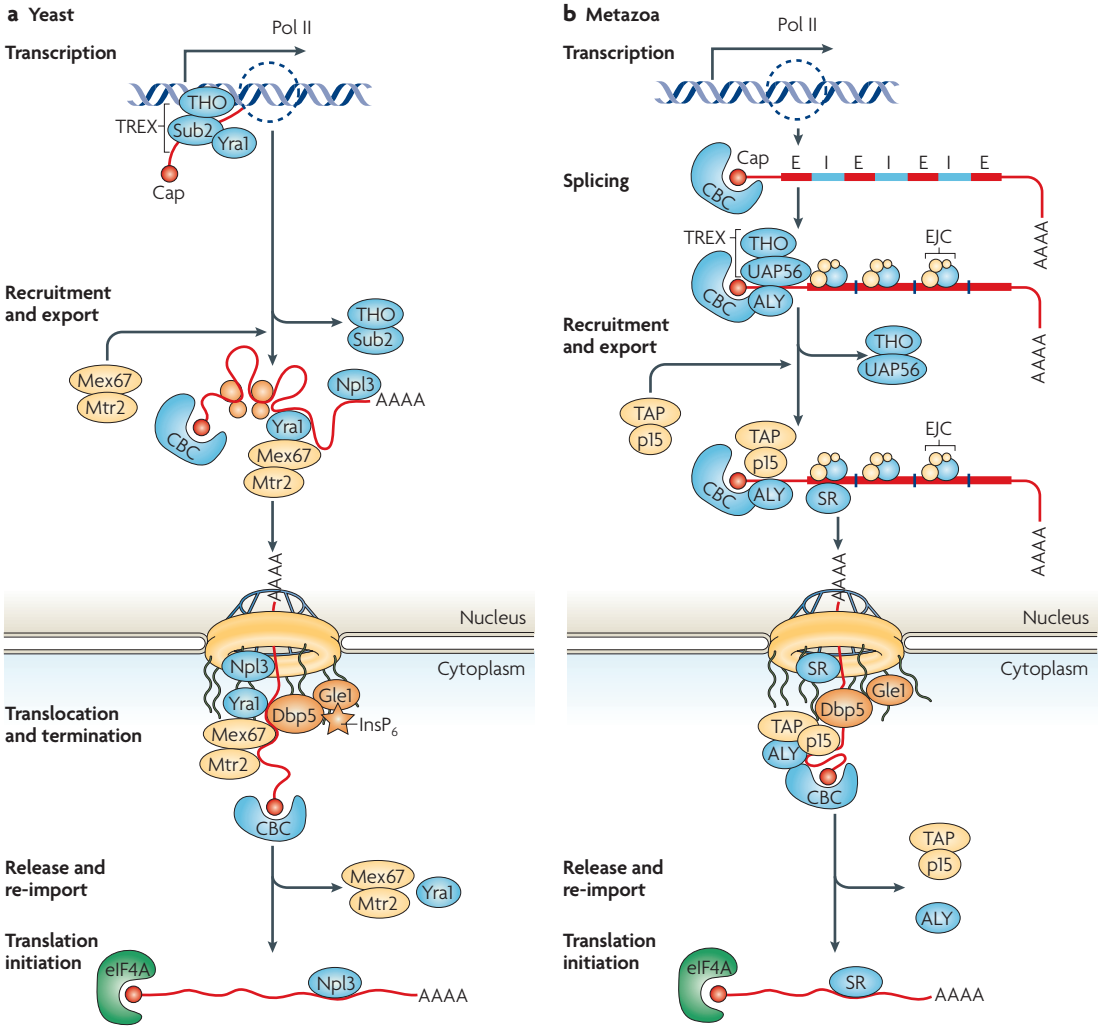


Figure 3 | **Transcription-coupled or splicing-coupled mRNA export. a** | In yeast, the nascent transcript generated by RNA polymerase II (Pol II) is co-transcriptionally folded and assembled into a pre-messenger ribonucleoprotein (pre-mRNP) by the THO/TREX complex (Yra1 and Sub2 are subunits of TREX, and THO is a subcomplex) that accompanies the elongating Pol II. After co-transcriptional association with additional pre-mRNA factors (for example, the cap-binding complex (CBC) and RNA-binding proteins, shown in orange), the Mex67–Mtr2 mRNA export receptor is recruited to the mRNP via adaptor proteins such as Yra1. When emerging at the cytoplasmic side of the nuclear pore complex (NPC), the mRNP encounters a remodelling machinery on the NPC fibrils that consists of the ATP-dependent RNA helicase Dbp5 and its activators Gle1 and the signalling molecule inositol hexakisphosphate ($InsP_6$). After dissociation of Mex67–Mtr2 and other export factors from the RNA cargo, the mRNA may still contain a few shuttling RNA-binding proteins, such as Npl3, that influence translation. mRNA is recruited to the translation-initiation machinery via its 5′ cap that is recognized by the initiation factor eIF4A and by additional initiation factors. Transport factors are re-imported, which in some cases requires phosphorylation. **b** | For metazoa, several mRNA export models have been described. Depicted are the splicing- and cap-dependent modes of human TREX recruitment to the mRNP. The downstream events in mRNA export, including the recruitment of the TAP–p15 mRNA export receptor, are similar for metazoa and yeast. Export receptors are indicated in yellow and adaptors in blue. For simplicity, the exon-junction complex (EJC) on the translocating and cytoplasmic mRNP is not shown. E, exon; I, intron; TREX, transcription-coupled export complex.

a transcription-dependent manner[87]. Subsequently, Npl3 becomes dephosphorylated by the nuclear phosphatase Glc7, allowing interaction with the Mex67–Mtr2 export receptor. After transport of the mRNP to the cytoplasm, Npl3 is rephosphorylated by Sky1, which destabilizes the interaction of Npl3 with the mRNA and Mex67–Mtr2 (REF. 86). Thus, successive phosphorylation and dephosphorylation of Npl3 could be a means to impose directionality on the mRNA export pathway[85].

TREX in transcription-coupled export. The conserved TREX complex integrates steps in mRNA biogenesis with nuclear export. TREX is found in yeast and higher eukaryotes including *Drosophila melanogaster* and humans[88–90]. Four of the yeast TREX subunits (Tho2, Hpr1, Mft1 and Thp2) form a robust subcomplex (THO)[91] that functions in various aspects of co-transcriptional mRNP formation and transcription-dependent recombination[92]. Two of the TREX subunits, Yra1 and Sub2, are export factors that

generate a physical link with the mRNA export receptor (FIG. 3). In yeast, TREX is continuously loaded onto emerging transcripts during transcription elongation, which facilitates folding of nascent transcripts into mRNPs and helps to recruit additional RNA-binding proteins[78,88,93–95].

When yeast TREX follows the elongating RNA polymerase along the activated gene, members of the THO subcomplex (for example, Hpr1) bind to chromatin, whereas Sub2 and Yra1 associate with nascent mRNA[93] (FIG. 3). RNAi studies in *D. melanogaster* and transcription profiling have led to the suggestion that the majority of mRNAs are transcribed and exported independently of TREX[89]. However, TREX mutants show nuclear accumulation of bulk mRNA and are synthetically lethal when combined with mutants of the mRNA export machinery, which suggests a broad role for TREX in transcription-coupled mRNA export. In the absence of a functional THO subcomplex, the formation of an RNA–DNA hybrid between a nascent unfolded transcript and the DNA template was observed, and this resulted in the inhibition of transcription and the generation of malformed transcripts that could not be exported[94]. However, by experimentally slowing down transcription, these defects can be partly compensated, which suggests that only a limited time window exists for co-transcriptional loading of RNA-binding proteins and export factors onto the nascent transcript[96].

Despite its conservation, the TREX complex is associated with the transcription apparatus in yeast and the splicing machinery in humans, possibly reflecting the higher prevalence of spliced mRNA in mammals than in yeast[9]. Initially, studies carried out in the Reed laboratory suggested a close link between splicing and mRNA export in higher eukaryotes with a key role for ALY/REF and UAP56 in this coupling[97,98]. Subsequent work from several laboratories reported that ALY/REF, UAP56 and TAP–p15 can associate with the exon-junction complex (EJC), which is deposited as a consequence of splicing ~20–24 nucleotides upstream of every exon–exon junction in the spliced mRNA[99,100]. The EJC operates in the nonsense-mediated decay (NMD) pathway. Additionally, due to its association with export factors, the EJC was suggested to recruit the mRNA export machinery and link splicing with export in metazoa. However, a recent study showed that the human TREX complex is recruited in a splicing- and cap-dependent manner only to the 5′ end of the mRNA, and this recruitment requires the cap-binding subunit CBP80, which interacts directly with the ALY/REF subunit of human TREX[101] (FIG. 3). This observation could explain why mRNA transcripts are exported in a 5′→3′ direction[102]. Notably, a function for the 5′ cap and the CBC in metazoan mRNA export was already proposed more than 15 years ago[56].

In addition, the metazoan mRNA export machinery can be loaded onto nascent transcripts by a splicing-independent mechanism similar to the one in yeast. In human cells, the transcription-elongation factor SPT6 recruits IWS1 to the nascent mRNA, thereby allowing IWS1 to act as a bridging protein for ALY/REF[103]. Thus, eukaryotic cells can exploit different mechanisms to load mRNA-processing and export factors onto newly forming mRNPs.

The TREX-2 mRNA export complex. Sac3 was identified as an additional mRNA export adaptor in yeast owing to its genetic interaction with the TREX complex and its ability to physically recruit the Mex67–Mtr2 export receptor[104,105]. Sac3, together with Thp1, Sus1 and Cdc31, constitute a complex (previously called the Sac3–Thp1–Sus1–Cdc31 complex)[104,106–108] that we henceforth call TREX-2 (FIG. 4). TREX-2 is tethered to the inner side of the NPC via the nucleoporins Nup1 and Nup60 (REF. 104). Interestingly, one TREX-2 component, the small protein Sus1, also interacts with SAGA, a large transcription-initiation complex that catalyses histone acetylation and deubiquitylation. In SAGA, Sus1 is part of a heterotrimeric deubiquitylation module together with Sgf11 and the protease Ubp8 (REFS 109,110). TREX-2 has therefore been proposed to functionally couple SAGA-dependent gene expression to mRNA export at the inner side of the NPC[106]. This model is supported by recent experiments that demonstrated a requirement for both SAGA and TREX-2 in the dynamic repositioning of gene loci from the nuclear interior to the nuclear periphery[111].

Gene gating and mRNA export. More than 20 years ago, Günter Blobel proposed in his provocative gene-gating hypothesis that every gene in the nucleus is physically connected (or gated) to a particular NPC in the nuclear membrane[112]. Several recent studies in yeast, *D. melanogaster* and mice indicated that gene gating indeed exists, although not in the strict sense as originally proposed[113]. In yeast and higher eukaryotes, the nuclear periphery was classically considered as a zone of transcriptional repression owing to the presence of silencing factors[113]. However, a number of genes are dynamically targeted to the nuclear envelope upon activation, and this positioning appears to facilitate transcription and subsequent nuclear mRNA export[111,114–119].

The precise molecular basis for the initial targeting and subsequent tethering of genes to the nuclear periphery is currently unclear, but can apparently involve multiple factors (FIG. 4). In support of the idea that gene gating and mRNA export are functionally coupled, the SAGA transcription-initiation complex was found to generate a physical contact with the NPC-associated TREX-2 mRNA export complex[106,111,120]. Additional gene–NPC interactions are mediated between Mex67 and the Mlp proteins, between the histone variant Htz1 and the nucleoporin Nup2 and through the Nup84 complex[119,121,122]. These factors might either operate synergistically or in a gene-specific manner.

Two general mechanisms of gene gating have emerged: either genes can interact directly with the NPC via adaptor proteins independently of mRNA production (including post-transcriptional tethering)[119,122–124], or transcript formation is necessary for gene gating to occur[111,115–118,125]. Taken together, gene gating could be a means to create a favourable environment for the recruitment of mRNA export factors and mRNA quality surveillance by the Mlp1–Mlp2 network, which in turn could increase the efficiency of mRNP entry into the transport channel of the NPC[106,117,120,126].

Exon-junction complex
(EJC). A complex of proteins that is deposited onto mRNA during pre-mRNA splicing (~20–24 nucleotides upstream of exon–exon junctions). The EJC remains bound to the mRNA during nuclear export and influences surveillance, translation and localization of mature mRNAs in the cytoplasm.

Nonsense-mediated mRNA decay
(NMD). A process by which a cell destroys mRNAs for which translation has been prematurely terminated owing to the presence of a nonsense codon in the coding region.

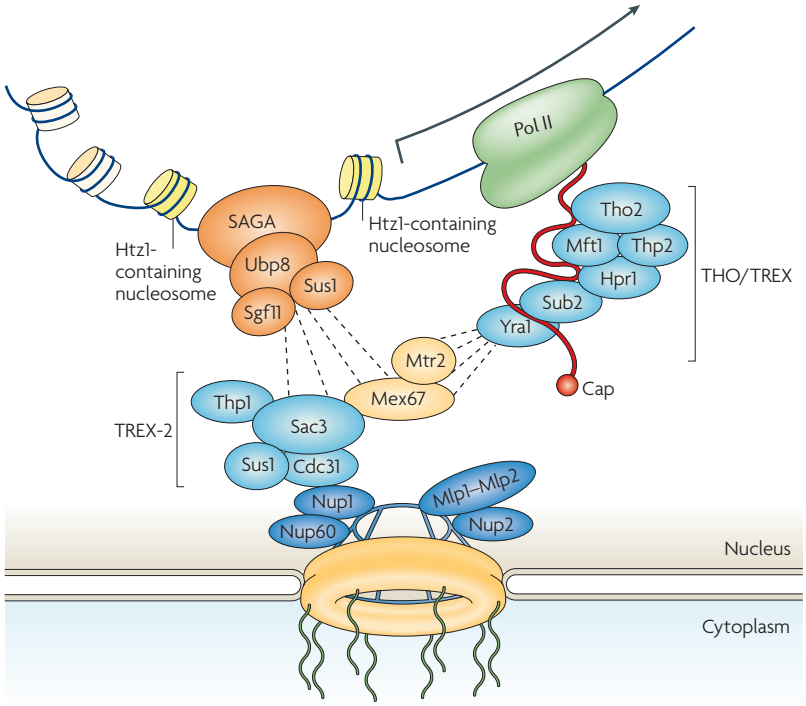


Figure 4 | **Gene gating and mRNA export.** Model of transcription-coupled mRNA export in yeast, which involves gating of the activated gene to the nuclear periphery via interaction with the nuclear pore complex (NPC). During transcription initiation, SAGA is recruited as part of a large transcription pre-initiation complex to the promoter of a gene, which at this point is located in the interior of the nucleus. Subsequently, the activated gene becomes tethered to the nuclear periphery via an interaction between SAGA and the nuclear pore-bound TREX-2 complex (which consists of the subunits Sac3, Thp1, Sus1 and Cdc31). Transcription of the tethered gene generates a nascent transcript that becomes assembled, with the aid of the THO/TREX complex, into an export-competent messenger ribonucleoprotein (mRNP). Thus, the mRNP is brought into the vicinity of the NPC-associated Mex67–Mtr2 mRNA export machinery. Mex67 can also directly contact the promoter region independently of the mRNA (not shown). Sus1 is a functional component of both TREX-2 and SAGA. Within SAGA, Sus1 is part of a heterotrimeric subcomplex together with the histone H2B deubiquitylating enzyme Ubp8 and Sgf11, a protein of unknown function. The nucleoporin Nup1 is part of the TREX-2 docking site at the nuclear basket. Nup2 was shown to associate with active promoters and might tether genes through the histone variant Htz1 in yeast. The Mlp1 and Mlp2 proteins contribute to RNA surveillance by retention of unspliced mRNA. Dotted lines represent predicted interactions between the complexes. TREX, transcription-coupled export complex. Proteins in the same complex have the same colour.

Directionality and termination of mRNA export. Unidirectional movement of mRNPs from the nucleus into the cytoplasm requires a termination step to release the mRNA from its receptor. In principle, remodelling of the mRNP upon its arrival in the cytoplasm could be a driving force for vectorial translocation. ATP-dependent RNA helicases such as Dbp5, which are involved in mRNA export, could trigger an irreversible ATP-driven mRNP rearrangement at the cytoplasmic side of the NPC[127,128]. Dbp5 is advantageously located to perform such a role as it is associated with the nucleoporin Nup159, which is asymmetrically positioned at the cytoplasmic side of the NPC[129,130]. However, Dbp5 shuttles between the nucleus and the cytoplasm and is already recruited to nascent mRNPs during transcription[128,129,131].

Inositol hexakisphosphate ($InsP_6$). One of many small messenger phosphoinositides that is found in cells. $InsP_6$ is synthesized by IPK1 from inositol 1,4,5-trisphosphate ($InsP_3$), a precursor that also regulates the release of intracellular calcium.

New evidence provides an explanation for how Dbp5 could dismantle the mRNP and release Mex67–Mtr2 only in the cytoplasmic compartment[132–134]. According to these studies, Dbp5 exhibits a very low ATP-dependent RNA-helicase activity, which can be stimulated by Gle1, an essential mRNA export factor that is also asymmetrically located at the cytoplasmic nuclear pore filaments[133,134] (FIG. 3). Maximal stimulation of the ATPase activity of Dbp5 requires the signalling molecule inositol hexakisphosphate ($InsP_6$), which is thought to regulate the interaction between Gle1 and Dbp5. Previously, it was also shown that the signalling pathway leading to the production of cytoplasmic $InsP_6$ is necessary for Gle1-dependent mRNA export in yeast[135]. Taken together, these data have reinforced a model of local mRNP remodelling by an RNA helicase that becomes activated at the cytoplasmic pore filaments. This local activation could generate directionality either by preventing backsliding of the mRNP (like a molecular ratchet) from the cytoplasm to the nucleus or by exerting a pulling force on the protruding 5′ end of the mRNA while in transit[10,136].

Export of rRNA

Ribosomes are the protein-synthesizing machines of the cell. They predominantly translate mRNAs in the cytoplasm, but mitochondria and chloroplasts have their own set of ribosomes. Ribosomes are composed of a large (60S) and a small (40S) subunit, which together contain 4 rRNA species (28S/25S rRNA, 5.8S rRNA, 5S rRNA and 18S rRNA) and more than 70 ribosomal proteins. The ribosomal subunits are assembled in the nucleolus and are transported to the cytoplasm by multiple export receptors.

Ribosome biogenesis. Ribosome biogenesis in eukaryotic cells is a highly regulated multistep process. It requires the transcription of precursor rRNAs (pre-rRNAs) from the ribosomal genes in the nucleolus as well as the synthesis of ribosomal proteins in the cytoplasm and the subsequent import of these ribosomal proteins into the nucleolus. In the nucleolus, the ribosomal proteins are then assembled with the nascent pre-rRNA. Ribosome biogenesis and export has mainly been analysed in *S. cerevisiae*. These studies revealed that more than 150 non-ribosomal factors associate transiently with the evolving pre-ribosomal particles on their way from the nucleolus through the nucleoplasm into the cytoplasm[137–139]. Notably, the nuclear export machinery is not recruited at early stages of pre-ribosome assembly (that is, during pre-rRNA synthesis, processing and modification), but only at a late stage, in the nucleoplasm, when the pre-ribosomal particles have already undergone a complicated cascade of processing, maturation and quality control.

Export of the ribosomal subunits. During the early stage of ribosome biogenesis, pre-60S and pre-40S particles are generated in the nucleolus, and these subsequently follow separate export routes. Currently, the mechanism of 40S subunit export is poorly understood. So far, it is only known that the Ran cycle and Crm1 play a role[140]. Several pre-40S assembly factors and ribosomal small

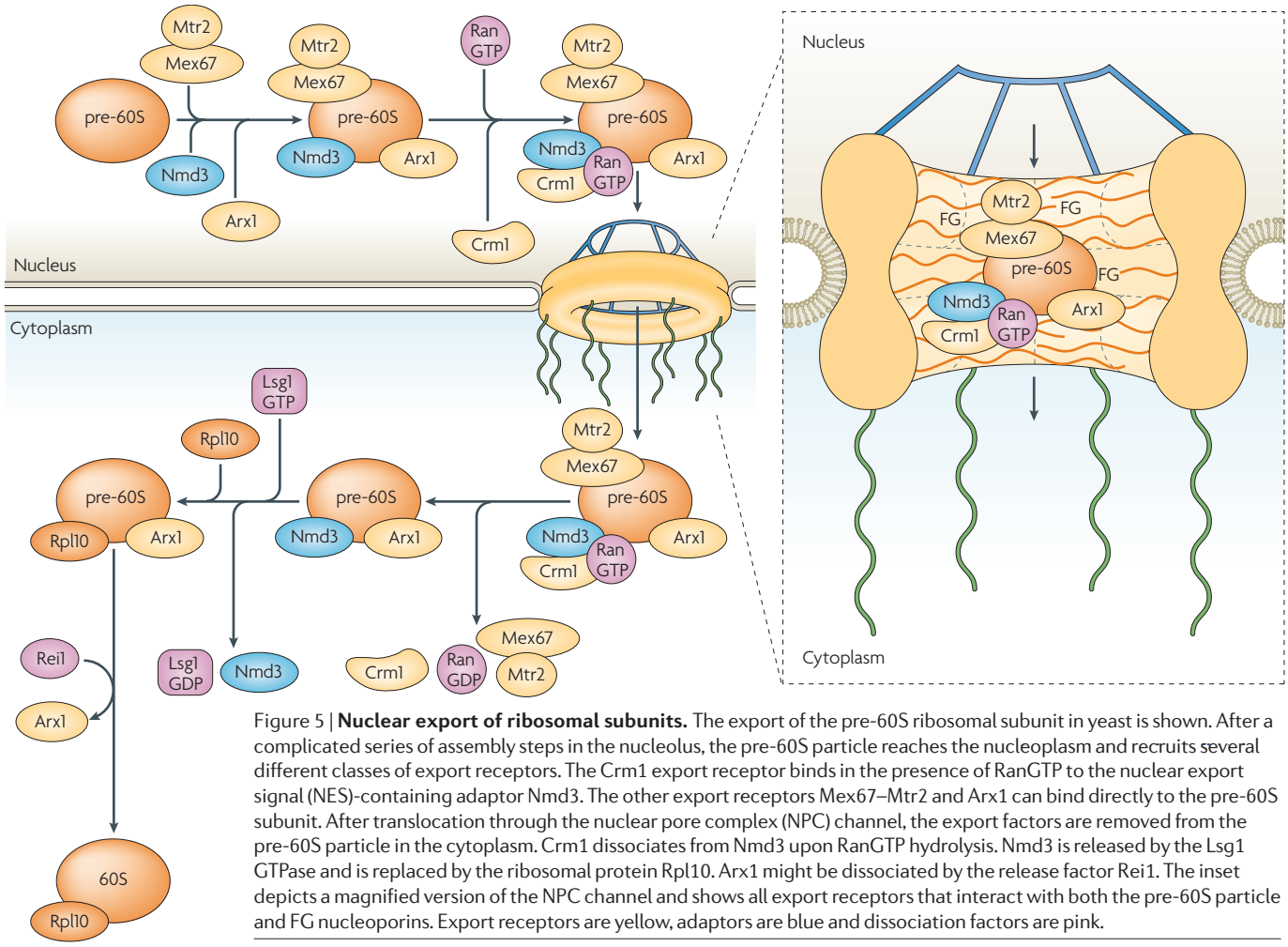


Figure 5 | **Nuclear export of ribosomal subunits.** The export of the pre-60S ribosomal subunit in yeast is shown. After a complicated series of assembly steps in the nucleolus, the pre-60S particle reaches the nucleoplasm and recruits several different classes of export receptors. The Crm1 export receptor binds in the presence of RanGTP to the nuclear export signal (NES)-containing adaptor Nmd3. The other export receptors Mex67–Mtr2 and Arx1 can bind directly to the pre-60S subunit. After translocation through the nuclear pore complex (NPC) channel, the export factors are removed from the pre-60S particle in the cytoplasm. Crm1 dissociates from Nmd3 upon RanGTP hydrolysis. Nmd3 is released by the Lsg1 GTPase and is replaced by the ribosomal protein Rpl10. Arx1 might be dissociated by the release factor Rei1. The inset depicts a magnified version of the NPC channel and shows all export receptors that interact with both the pre-60S particle and FG nucleoporins. Export receptors are yellow, adaptors are blue and dissociation factors are pink.

subunit proteins have been implicated in 40S export, but it is not clear whether they are *bona fide* export adaptors[141–143].

The mechanism of nuclear exit of 60S subunits has been elucidated in yeast and metazoa. These studies revealed that 60S subunit export is conserved and depends on the Ran system and the Crm1 export receptor[144–149] (FIG. 5). Nmd3, a conserved NES-containing protein that is recruited to a late export-competent pre-60S particle in the nucleoplasm, serves as the adaptor protein[146–151]. Following export to the cytoplasm, Crm1 is dissociated from the Nmd3 adaptor by RanGTP hydrolysis. Subsequently, Nmd3 is released from the 60S export cargo by a cytoplasmic GTPase (Lsg1), an event that is coupled to the loading of the ribosomal protein Rpl10 onto the 60S subunit[151,152].

Genetic studies in yeast recently indicated that Crm1 is not the sole export receptor that escorts the pre-60S particle through the NPC. Surprisingly, one of the additional export receptors for the pre-60S particle is Mex67–Mtr2, which uses a distinct interaction surface to bind to the pre-60S subunit[153] (FIG. 5). Thus, the Mex67–Mtr2 heterodimer provides a versatile molecular surface to bind to cargoes as diverse as mRNPs and pre-ribosomal particles.

Arx1 is another auxiliary shuttling export factor that is recruited to the late pre-60S particle concomitantly with Nmd3 and Mex67–Mtr2 (REFS 150,154–156) (FIG. 5). After nuclear export, Arx1 and its interacting partner Alb1 are released from the 60S subunit by Rei1, a cytoplasmic factor that operates at a terminal step during pre-60S biogenesis[154,155]. A recent study demonstrated that Arx1 has properties of shuttling transport receptors, which can directly interact with FG nucleoporins[157]. However, Arx1 is not related to typical nucleocytoplasmic transport receptors, but instead is homologous to Met aminopeptidases (MetAPs). It is speculated that Arx1, which does not have a MetAP activity, nevertheless uses its MetAP fold to bind to the Phe-Gly repeats of FG nucleoporins[157].

Thus, pre-60S subunits, in contrast to other RNA classes, can recruit several different export receptors to make their export more efficient. Notably, 60S subunits belong to the largest RNA-containing particles that have to pass the transport channels of the NPCs. The overall size of a 60S subunit is in the range of the functional diameter of the NPC, which is ~26 nm[2]. It is conceivable that the bulky pre-60S particle needs to mobilize several export receptors for an efficient passage through the NPC (FIG. 5).

Concluding remarks

Numerous studies from the past 20 years have shed light on how the major RNA species are exported from the nucleus into the cytoplasm. Today, we have detailed information about the factors that are involved in RNA export, and we are steadily gaining mechanistic insight into RNA-export processes. However, we do not have a profound structural knowledge of RNA export. Moreover, the lack of a comprehensive mechanistic understanding of RNA export is partly due to a lack of faithful *in vitro* systems, which are difficult to establish owing to inherent problems in coupling RNA transcription and processing to export. Although a few aspects of RNA export have been reconstituted, we have not yet developed the sophisticated *in vitro* export assays that are needed to recapitulate upstream events that occur during RNA generation, processing and RNP assembly.

Deciphering the functional connectivity of the different steps in RNA biogenesis in relation to nuclear export is therefore still a major challenge. Hypothetically, the combinatorial use of nuclear RNA-binding proteins might significantly expand the regulatory plasticity of gene expression at the level of NPC export. To fully understand the multifaceted behaviour of RNAs as they prepare to transit the nuclear pore will therefore require the integration of systems biology approaches with detailed mechanistic studies.

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Acknowledgements
We would like to acknowledge critical reading of the manuscript by the members of the Hurt laboratory and by S. Hauf.

Competing interests statement
The authors declare no competing financial interests.

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