POST-TRANSLATIONAL MODIFICATIONS SERIES — OPINION

Keeping mRNPs in check during assembly and nuclear export

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Abstract | The cell nucleus is an intricate organelle that coordinates multiple activities that are associated with DNA replication and gene expression. In all eukaryotes, it stores the genetic information and the machineries that control the production of mature and export-competent messenger ribonucleoproteins (mRNPs), a multistep process that is regulated in a spatial and temporal manner. Recent studies suggest that post-translational modifications play a part in coordinating the co-transcriptional assembly, remodelling and export of mRNP complexes through nuclear pores, adding a new level of regulation to the process of gene expression.

Eukaryotic protein-coding genes are transcribed by RNA polymerase II (RNAPII). Precursor RNAs undergo several co-transcriptional processing steps, including 5′ capping, splicing, 3′ cleavage and polyadenylation. The loading of key proteins to nascent mRNAs during this process participates in the formation of exportable messenger ribonucleoproteins (mRNPs), the quality of which is assessed before they can leave the nucleus. mRNPs acquire full translational competence through an additional remodelling step that occurs at the cytoplasmic face of the nuclear pore complex (NPC), leading to their release into the cytoplasm and the recycling of export factors[1,2].

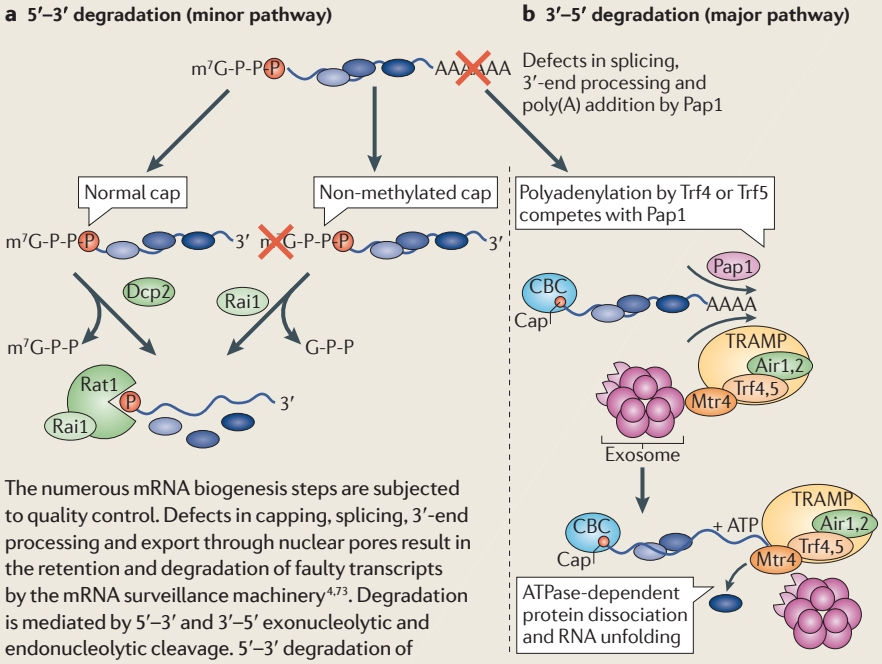
The carboxy-terminal domain (CTD) of the large subunit of RNAPII coordinates the sequential recruitment of processing factors to the nascent transcript as a function of its phosphorylation state or 'CTD code'. Export factors, such as the essential heterodimeric receptor mRNA export factor 67–mRNA transport regulator 2 (Mex67–Mtr2; TAP–p15 or NXF1–NXT1 in mammals) and its mRNA-binding adaptor proteins Yra1 (ALY or REF in mammals), nucleolar protein 3 (Npl3) and nuclear polyadenylated RNA-binding 2 (Nab2), are similarly recruited to transcribing genes through interactions with the transcription machinery, functionally coupling transcription and export. Although mRNP biogenesis and export steps are often described separately for simplicity, they are tightly coupled and interdependent, with some factors participating in multiple stages. Importantly, the early recruitment of proteins not only allows efficient pre-mRNA processing and mRNP assembly but also protects the mRNA from degradation through continuous competition with the nuclear surveillance machineries. These machineries include the exosome, which is an evolutionarily conserved complex that contains two 3′–5′ exoribonucleases, chromosome disjunction 3 (Dis3; also known as Rrp44) and ribosomal RNA-processing protein 6 (Rrp6). Stimulated by its nuclear cofactor TRAMP (a complex comprised of topoisomerase 1-related 4 (Trf4; also known as Pap2) or Trf5, Arg methyltransferase-interacting RING-finger 1 (Air1) or Air2, and mRNA-transport regulator 4 (Mtr4)), the exosome constitutes a quality control system that recognizes and degrades improperly formed mRNAs[3,4] (BOX 1).

> "mRNP biogenesis and export steps ... are tightly coupled and interdependent, with some factors participating in multiple stages."

The variety of gene-specific CTD codes, as well as specific sequences in the mRNA and the concentration of relevant mRNA-binding proteins in the nucleus, all contribute to the production of mRNPs with unique identities and destinies. Various studies within the past 10 years indicate that the cytoplasmic fate determination of mRNAs (that is, their translatability, stability and localization) begins in the nucleus, where the mRNP is formed. This has been shown in different biological processes: body axis determination in *Drosophila melanogaster* depends on the asymmetric distribution of *bicoid*, *oskar* and *nanos* mRNAs in the oocyte; plant flowering in *Arabidopsis thaliana* is triggered by the *FLOWERING LOCUS T* (*FT*) mRNA that is transcribed in leaves and transported to the shoot apical meristem, where it is translated into a protein that induces flowering; axon plasticity during development relies on β-actin mRNA localization and local translation in the growth cones of neurons driving cone navigation; and yeast budding is regulated by asymmetric distribution of specific transcripts such as, among others, the *ASH1* mRNA (reviewed in REFS 5,6).

How proteins are coordinately delivered to the transcription site and defined as shuttling factors that cross nuclear pores with the mRNA, or as proteins to be abandoned along the way, is still poorly understood. Another question is how cells control key transitions, including mRNP release from the transcription site or its delivery into the cytoplasm, and how the quality control system recognizes mRNPs that are irreversibly blocked at any of these stages (BOX 1). Growing evidence indicates that post-translational modifications (PTMs) play a part in this process. The advantages of using PTMs are multiple: they can increase the circuit complexity without an increase in genome size, modulate protein–protein interactions and mediate a fast response to environmental changes. PTMs involved in mRNP formation and shaping include phosphorylation, methylation, ubiquitylation and sumoylation (TABLE 1). These modifications are largely reversible and represent a dynamic mechanism of regulation, and their chemical properties and structural details have been reviewed elsewhere[7].

Box 1 | **Nuclear degradation of mRNA**



The numerous mRNA biogenesis steps are subjected to quality control. Defects in capping, splicing, 3′-end processing and export through nuclear pores result in the retention and degradation of faulty transcripts by the mRNA surveillance machinery[4,73]. Degradation is mediated by 5′–3′ and 3′–5′ exonucleolytic and endonucleolytic cleavage. 5′–3′ degradation of nuclear transcripts represents a minor pathway that contributes to the degradation of unprocessed or malformed messenger ribonucleoproteins (mRNPs). (see the figure, part **a**). It involves decapping by mRNA-decapping enzyme 2 (Dcp2), which results in the release of 7-methyl-GDP (m^7GDP; shown as m^7G-P-P) and 5′ monophosphate RNAs that are susceptible to 5′–3′ exonucleolytic degradation by ribonucleic acid-trafficking 1 (Rat1). Non-methylated G-cap structures, which cannot bind to the cap-binding complex (CBC) and are poor substrates for Dcp2, are removed by the Rat1 cofactor Rai1, producing monophosphate 5′ ends that are subsequently degraded by Rat1 (REF. 16). Under normal conditions, 3′-end cleavage, followed by polyadenylation by poly(A) polymerase 1 (Pap1), is linked to the recruitment of poly(A) binding proteins and export factors, resulting in stable and rapidly exported mRNAs. 3′-end formation undergoes surveillance by the nuclear exosome assisted by TRAMP (see the figure, part **b**). In *Saccharomyces cerevisiae*, TRAMP contains the non-canonical poly(A) polymerase topoisomerase 1-related 4 (Trf4; also known as Pap2) or Trf5, the RNA-binding protein Arg methyltransferase-interacting RING-finger 1 (Air1) or Air2 and the RNA helicase mRNA-transport regulator 4 (Mtr4)[74–76]. Inefficient polyadenylation by Pap1, which might occur as a result of defects in splicing and 3′-end processing, favours the recruitment of TRAMP. Trf4- or Trf5-mediated polyadenylation then occurs and forms a platform to recruit and activate the exosome, which leads to the rapid digestion of mRNA from the 3′ end[4]. The exosome is made of nine highly conserved core subunits. The catalytic activity is mediated by two additional factors: chromosome disjunction 3 (Dis3; also known as Rrp44), which harbours both endonucleolytic and 3′–5′ exonucleolytic activity, and ribosomal RNA-processing protein 6 (Rrp6), a 3′–5′ exonuclease that is specific to the nuclear exosome[73]. Recognition and elimination of defective mRNPs that are unable to proceed along the biogenesis pathway is likely to occur in association with the transcribing genes.

In this Opinion article, we discuss recent findings that strengthen the functional coupling between processes along the mRNP export pathway, and we suggest a role for PTMs in coordinating the remodelling and surveillance of mRNPs, or in regulating specific export pathways. mRNP quality control involves the nuclear exosome as well as other nucleases, and increasing evidence indicates that crucial aspects of this quality check occur in association with the NPC. We focus on studies performed in the yeast *Saccharomyces cerevisiae*, as many evolutionarily conserved factors and mechanisms have been identified through genetic studies in this organism.

Preparing mRNP for export

Formation of export-competent mRNPs involves several co-transcriptional maturation steps, including capping, splicing and the recruitment of export factors. These mRNP assembly events are orchestrated by the RNAPII CTD and factors associated with the transcription elongation complex.

CTD codes: influencing mRNP composition? The CTD of the large RNAPII subunit consists of heptad repeats (YSPTSPS) that are reversibly phosphorylated at Ser2, Ser5 or Ser7 (REFS 8,9). Earlier gene-specific studies indicated that the ratio of Ser5 to Ser2 phosphorylation decreases across the transcription unit from 5′ to 3′, defining the recruitment of mRNA-capping and 3′-processing machineries at the right time and place, whereas Ser7 was primarily implicated in promoting the transcription termination of short non-coding transcription units (that is, small nucleolar RNAs (snoRNAs)) by Nrd1 (REFS 9,10). Recent genome-wide analyses of yeast CTD phosphorylation states now reveal that both Ser5 and Ser7 phosphorylation are enriched at the 5′ end of protein-coding genes but Ser7 persists beyond Ser5 phosphorylation, consistent with a potential role for Ser7 phosphorylation in transcription elongation[11–13]. Notably, the phosphodynamics are not scaled to gene length and they present gene class-specific patterns, as well as variations according to the expression level, presence of introns and promoter structure of individual genes[11–13]. Thus, the unique composition of individual mRNP complexes could result, at least in part, from the variety of gene-specific CTD codes.

Capping and splicing: starting on the right foot. The acquisition of a 7-methylguanosine (m^7G) cap structure at the mRNA 5′ end is an early processing event that is important for mRNA stability and translation. In yeast, 5′-capping is mediated by three enzymes: the RNA triphosphatase Cet1, the guanylyltransferase Ceg1 and the 7-methyltransferase Abd1. These are recruited and activated by the CTD after transcription initiation, when it is phosphorylated at Ser5 (TABLE 1). The methylated cap is rapidly bound by the cap-binding complex (CBC), which accompanies the mRNP into the cytoplasm, where it is replaced by the cytoplasmic CBC eukaryotic translation initiation factor 4E (eIF4E) (reviewed in REFS 14,15). The Kiledjan laboratory identified a new quality control mechanism that is activated when a pre-mRNA cap fails to be methylated[16]. Here, the pyrophosphohydrolase Rai1 hydrolyses the unmethylated 5′ G-cap, releasing GDP and monophosphorylated nascent RNAs. These transcripts are rapidly degraded by ribonucleic acid-trafficking 1 (Rat1), inducing premature RNAPII transcription termination (BOX 1). The role of capping and CBC in the efficiency of downstream events, including

splicing[17,18], export[19] and translation, justifies the existence of a dedicated quality control step and further supports the notion that mRNP biogenesis steps are tightly coupled and not simply concomitant[14].

Earlier studies linked splicing to transcription by demonstrating the co-transcriptional loading of splicing factors[18,20] and established a role for the multifunctional Ser- and Arg-rich (SR)-like protein Npl3, which is involved in mRNP biogenesis and export, in this process[21] (BOX 2). However, it was still unclear whether the whole splicing reaction occurs on nascent pre-mRNAs, especially for genes with short terminal exons, at which transcripts may be released from RNAPII before the last splicing event. Using different approaches, the Neugebauer[22] and Beggs[23] laboratories recently showed that most transcripts are fully spliced while still attached to chromatin[22], and they provide evidence that spliceosome assembly induces pausing of RNAPII at the 3′ exon, thereby increasing the time window for the completion of splicing before transcript release[22,23]. The exact mechanism by which the spliceosome affects transcription elongation and whether this crosstalk is conserved in higher eukaryotes are interesting questions for the future.

Recruiting export factors during transcription. During elongation, the RNAPII machinery interacts with the evolutionarily conserved THO complex (composed of hyperrecombination protein 1 (Hpr1), mitochondrial fusion target 1 (Mft1), Thp2 and THO complex subunit 2 (Tho2)[24]), which has been implicated in transcription elongation, mRNA export and genome stability. THO co-purifies with the mRNA export adaptor Yra1, the ATP-dependent RNA-helicase Sub2 (UAP56 in mammals) that is involved in splicing and mRNA export, and the uncharacterized protein Tex1, to form the evolutionarily conserved TREX complex[25,26], which is involved in coupling transcription and mRNA export. Both Sub2 and Yra1 are recruited co-transcriptionally, Sub2 through direct interaction with the THO component Hpr1 (REF. 27), and Yra1 in a manner initially dependent on protein 1 of CFI (Pcf11), a 3′-end processing component of the cleavage factor IA (CFIA) complex that is associated with RNAPII early in transcription[28]. Yra1 probably joins Sub2 within TREX at the 3′ end, liberating Pcf11 so that it can interact with cleavage and polyadenylation factor (CPF) and CFIA (FIG. 1). In addition to Sub2, Hpr1 mediates co-transcriptional binding of the export receptor Mex67 (the presence of Mtr2 has not been addressed). This interaction involves the C-terminal ubiquitin-associated (UBA) domain of Mex67 (Mex67-UBA) and Hpr1 that has been ubiquitylated by the E3 ubiquitin ligase reverses SPT-phenotype 5 (Rsp5)[29,30]. It was proposed that Mex67-UBA protects ubiquitylated Hpr1 from proteasomal degradation during elongation. The dissociation of Mex67-UBA from Hpr1 at the gene 3′ end may induce Hpr1 degradation and promote THO recycling for a new round of transcription-dependent mRNP assembly. The stabilization of Hpr1 by Mex67 highlights one mechanism by which ubiquitin regulates protein–protein interactions in a reversible and temporal way. In this case, it ensures completion of elongation and release of mRNPs that are properly processed at their 3′ end[2] (FIG. 1 and see below). In metazoans, the recruitment of TREX is coupled to splicing, and recent studies are consistent with the view that TREX promotes export through binding and releasing spliced mRNPs from nuclear speckles[19,31–33].

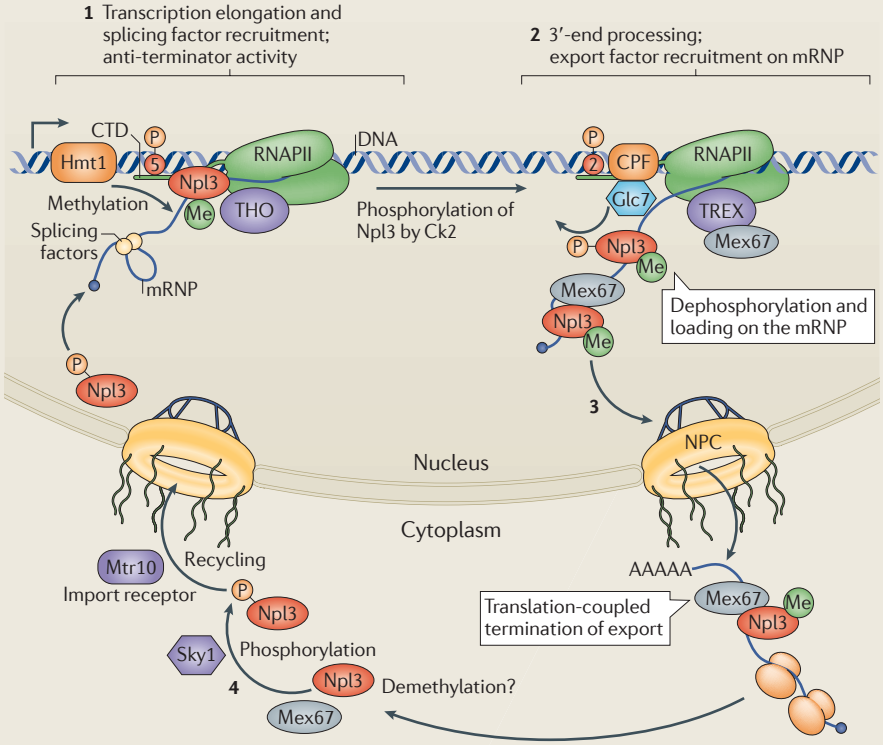
The interaction of genes with nuclear pores also contributes to the coupling of mRNP biogenesis and export. In yeast, a number of constitutively expressed and inducible genes (galactokinase 1 (*GAL1*)–*GAL10*, *GAL2*, inositol-3-phosphate synthase 1 (*INO1*), hexokinase 1 (*HXK1*) or heat shock protein 104 (*HSP104*)) relocate to nuclear pores upon activation[34,35]. Gene-to-pore association is mediated by multiple tethers and was proposed to contribute to efficient transcription and/or to facilitate processing and export[35]. Mex67 (presumably in complex with Mtr2) participates in the anchoring of genes to the NPC, possibly through its association both with transcribing genes and nuclear pore components[29].

Table 1 | **PTMs of proteins involved in mRNA export**

Target	PTM	Modifier	Proposed function	Refs
GpppN	Methylation	Abd1	Cap recognition by CBC, eIF4E and decapping enzymes Dcp2 and DcpS; mRNA stabilization and translation	16,96
Hpr1	Ubiquitylation	Rsp5	Recruitment of Mex67 via its UBA domain, protecting Hpr1 from proteasomal degradation during elongation	30
Hrp1 (Nab4)	Methylation	Hmt1	Hrp1 nuclear export	91
Mex67	Ubiquitylation	Unknown	Unknown	50
Nab2	Methylation	Hmt1	Nab2 nuclear export	97
	Phosphorylation	Slt2	Mediates block of poly(A) mRNA export on heat shock	49
	Ubiquitylation	Unknown	Unknown	50
Npl3	Methylation	Hmt1	Npl3 nuclear export	91
	Phosphorylation	Sky1	Dissociation of Npl3 from mRNA and its nuclear import	77
	Phosphorylation	Ck2	Reduces anti-termination activity; allows recruitment of CPF	83
	Dephosphorylation	Glc7	Promotes the association of Mex67 with poly(A) mRNAs	85
Yra1	Methylation	Hmt1	Unknown	90
	Ubiquitylation	Tom1	Promotes the dissociation of Yra1 from the mRNP	50
Nup1	Phosphorylation	Cdk1	Regulates the localization of genes to the nuclear pore complex through the cell cycle	41
Unknown	Desumoylation	Ulp1	Required for efficient retention of pre-mRNA in the nucleus	55

CBC, cap-binding complex; Cdk1, cyclin-dependent kinase 1; Ck2, casein kinase 2; CPF, cleavage and polyadenylation factor; Dcp, mRNA-decapping enzyme; eIF4E, eukaryotic translation initiation factor 4E; Hmt1, hnRNP Arg *N*-methyltransferase 1; Hpr1, hyperrecombination protein 1; Mex67, mRNA export factor 67; mRNP, messenger ribonucleoprotein; Nab, nuclear polyadenylated RNA-binding; Npl3, nucleolar protein 3; PTM, post-translational modification; Rsp5, reverses SPT-phenotype 5; UBA, ubiquitin-associated; Ulp1, ubiquitin-like-specific protease 1.

Box 2 | **The RNA-binding protein Npl3: a paradigm**



Nucleolar protein 3 (Npl3) is a paradigm for shuttling mRNA-binding proteins with a role in mRNA export as it is the best-characterized and most-abundant protein involved in this process. It is pivotal in multiple steps from transcription to translation, including splicing, 3′-end processing and messenger ribonucleoprotein (mRNP) remodelling and export. Npl3 belongs to the Ser- and Arg-rich (SR) and heterogeneous nuclear ribonucleoprotein (hnRNP) protein families, and multiple post-translational modifications (PTMs) affect its function[77–79]. It contains an amino-terminal RNA-binding domain and a carboxy terminus that consists of several RGG motifs and SR dipeptides that are subjected to Arg methylation and Ser phosphorylation, respectively[77,80]. Methylation may influence phosphorylation. Npl3 is recruited during transcription through interaction with RNA polymerase II (RNAPII) (see the figure, part **1**), where it promotes transcription elongation and the co-transcriptional recruitment of splicing factors[21] and also prevents early termination[81,82]. Phosphorylation of Npl3 by casein kinase 2 (Ck2) during transcription progressively reduces its anti-termination activity, allowing recruitment of cleavage and polyadenylation factor (CPF) at the 3′ end[83] (see the figure, part **2**). In addition, phosphorylation of Npl3 participates in auto-regulation by controlling poly(A) site choice within its own 3′ untranslated region[84]. During 3′-end formation, Npl3 is dephosphorylated by the CPF component Glc7, which promotes its interaction with mRNA export factor 67 (Mex67) on the mature export-competent mRNP and its nuclear export[85] (see the figure, part **3**). In higher eukaryotes, the dephosphorylation of SR proteins is similarly required for their interaction with export factors[86]. Following export, phosphorylation of Npl3 by Sky1 triggers its nuclear re-import by the mRNA transport regulator Mtr10 (REF. 77) (see the figure, part **4**). Both Npl3 phosphorylation and dephosphorylation are required for mRNA export. Like other mRNA export factors (such as Gle1 and DEAD-box protein 5 (Dbp5; also known as Rat8)), Npl3 is a multifunctional protein that also associates with polysomes and influences translation termination, a mechanism potentially linked to the efficiency of Npl3 recycling by Mtr10 (REFS 87,88).

Npl3 is also methylated by hnRNP Arg *N*-methyltransferase 1 (Hmt1), which is found in association with transcribing genes[80,89]. Although methylation by Hmt1 has been reported for other mRNA-binding proteins, including Yra1 (ALY or REF in mammals), nuclear polyadenylated RNA-binding 2 (Nab2) and Hrp1 (TABLE 1), it is not essential for growth or poly(A) RNA export[89,90]. However, it facilitates the release and export of specific transcripts, and increases the nuclear exit of Npl3 and other mRNA-binding proteins[79,91]. Gene-specific studies also indicate that Npl3 methylation promotes its elongation and anti-termination activities[92,93]. Generally, methylation of RNA-binding proteins reduces protein–protein or protein–RNA interactions, presumably facilitating mRNP dynamics and making the overall export process more fluid and efficient[94].

CTD, C-terminal domain; NPC, nuclear pore complex.

An additional connection between mRNP biogenesis and export was added upon discovery of the NPC-associated TREX2 complex, which is composed of Sac3, Thp1, Cdc31 and Sus1. Notably, Sus1 is also part of the SAGA co-activator complex, and it was proposed to link SAGA and TREX2, targeting activated genes to the nuclear periphery[36,37] (FIG. 1). Interestingly, TREX2 is evolutionarily conserved and may fulfil a similar function on some genes in higher eukaryotes[25,38,39]. Along these lines, the Brickner laboratory recently identified specific and evolutionarily conserved "ZIP code" DNA sequences, which are implicated in the association of certain transcribed genes with the nuclear periphery[40], and showed that phosphorylation of the nucleoporin Nup1 by cyclin-dependent kinase 1 (Cdk1) regulates gene localization through the cell cycle, indicating that PTMs also have an impact on pore function[41].

Checking mRNP quality before export

3′-end cleavage and polyadenylation is the last and most critical processing step for the acquisition of export competency. Completion of this reaction triggers mRNP release and probably coincides with the redistribution of export factors from the transcription machinery onto the transcripts. This crucial remodelling step may occur in association with nuclear pores and its accuracy is monitored by various quality control systems.

mRNP release: under tight exosomal control. The 3′-end processing complex comprises CPF, CFIA and Hrp1 (REF. 14). Its recruitment to the 3′ end of transcribing genes depends both on CTD Ser2 phosphorylation and on polyadenylation signals on the nascent mRNA. 3′-end cleavage followed by polyadenylation by poly(A) polymerase 1 (Pap1) is essential for export as it promotes the loading of poly(A) binding and export factors onto the mRNP. Recent studies implicate THO and associated Sub2 in the efficiency and accuracy of this process. *In vitro* experiments from the Jensen laboratory show that lack of THO or Sub2 results in defective polyadenylation by canonical Pap1, favouring polyadenylation by the TRAMP component Trf4 and degradation by the exosome 3′–5′ exonuclease Rrp6 (REF. 42) (BOX 1; FIG. 1). *In vivo* analyses from the Libri laboratory identified high molecular weight chromatin complexes forming at the 3′ end of some genes in THO/*sub2* mutants, which correspond to unreleased mRNPs. These complexes

contain CPF, mRNA export factors and nuclear pore proteins and only form in the presence of the nuclear exosome, in a process that is still poorly understood. Moreover, macromolecular complex formation is paralleled by exosome-dependent sequestration of both gene and nascent transcripts at the nuclear periphery, indicating that yeast 3′-end formation quality control may occur in close association with nuclear pores[43] (FIG. 1). Together, these observations indicate an important role for THO–Sub2 in coordinating a remodelling step at the 3′ end of genes that is critical for the dissociation of CPF, polyadenylation by Pap1 and transcript release. Interestingly, the mechanism of 3′-end formation quality control may be conserved, as improperly 3′-processed mRNPs are similarly retained at the transcription site in a process dependent on the exosome in higher eukaryotes[44].

Coincident with the 3′-processing reactions, Mex67 and its adaptors Yra1, Npl3 and the poly(A)-binding protein Nab2 may be transferred from the transcription machinery onto the mRNA, ensuring that only mature mRNPs access the NPC. This vast reorganization involves the dephosphorylation of Npl3 by Glc7 (BOX 2), as well as the ATPase and/or RNA helicase activity of Sub2. As these events may occur in the vicinity of the NPC, the low intrinsic activity of Sub2 could be stimulated by a pore component, ensuring that polyadenylation and transcript release occur in the right place and time (FIG. 1). Notably, mutations in Mex67 and Yra1 interfere with CPF dissociation and correct 3′-end formation, indicating that improper recruitment of export factors may have an impact on optimal THO–Sub2 function[45]. Thus, the whole process of co-transcriptional mRNP assembly converges to guarantee kinetically efficient 3′-end formation, in order to compete against mRNA retention and degradation by the TRAMP–exosome surveillance complex (BOX 1).

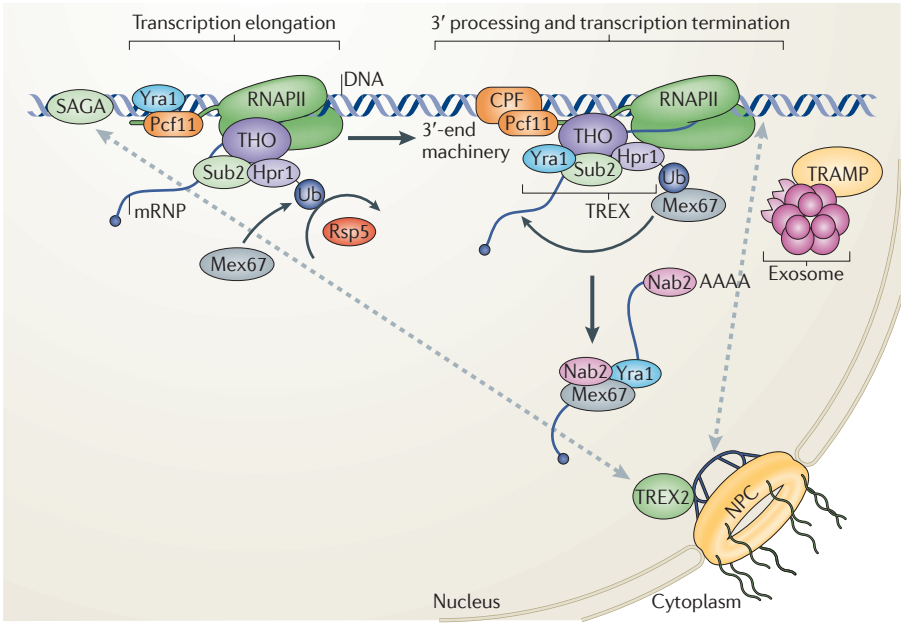


Figure 1 | **Co-transcriptional assembly of export-competent mRNPs.** The THO complex, which is implicated in transcription elongation and genome stability, also plays a pivotal part in messenger ribonucleoprotein (mRNP) export by promoting the co-transcriptional recruitment of export factors and stimulating 3′-end processing and the release of mature mRNP complexes. THO interacts with Sub2 (UAP56 in mammals) and Yra1 (ALY or REF in mammals) to form the TREX complex. Sub2 is recruited via the THO component hyperrecombination protein 1 (Hpr1), and Yra1 is recruited by protein 1 of CFI (Pcf11), a component of the 3′-end cleavage factor IA (CFIA; not shown). Yra1 probably joins Sub2 within TREX at the 3′ end, liberating Pcf11 for interaction with cleavage and polyadenylation factor (CPF) and CFIA. Ubiquitylation of Hpr1 by reverses SPT-phenotype 5 (Rsp5) recruits mRNA export factor 67 (Mex67), which protects Hpr1 from proteasomal degradation during elongation. THO–Sub2 stimulates 3′-end formation, resulting in CPF dissociation, polyadenylation by poly(A) polymerase 1 (Pap1; not shown) and mRNP release. This step is coupled to the loading of the mRNA export receptor Mex67 and its adaptors onto the mRNP, allowing access to the nuclear pore complex (NPC). The accuracy of this rearrangement is controlled by the nuclear TRAMP–exosome complex, which retains and degrades transcripts that have been inefficiently processed at their 3′ end by competing with Pap1 and favouring polyadenylation by topoisomerase 1-related 4 (Trf4; also known as Pap2) or Trf5, followed by exosomal 3′–5′ digestion. These reactions may be promoted by the NPC environment in the case of genes relocating to the nuclear periphery upon activation, such as highly expressed or inducible genes[35]. Both Mex67 and TREX2 have been implicated in the anchoring of genes to the nuclear pore complex. The Mex67 partner mRNA transport regulator 2 (Mtr2) has been omitted for simplicity. Dotted arrows indicate physical interactions identified between activated genes and the NPC at early and late stages of transcription[36,43]. Nab2, nuclear polyadenylated RNA-binding protein 2; RNAPII, RNA polymerase II; Ub, ubiquitin.

mRNP surveillance and remodelling at the NPC. Besides the nuclear exosome, several NPC proteins located at the nuclear basket have been implicated in mRNP surveillance before translocation through the pore. These include Nup60, which anchors NPC-associated myosin-like protein 1 (Mlp1) and Mlp2 (TPR in humans), pre-mRNA leakage 39 (Pml39), which is associated with myosin-like proteins, and the nuclear envelope protein Esc1 (establishes silent chromatin 1), which is involved in maintaining nuclear basket integrity. These proteins have no essential role in mRNA export but are implicated in the nuclear retention of unspliced pre-mRNAs, which is consistent with a role in mRNP quality control[46]. Unspliced transcripts are degraded by the nuclear exosome and by synthetically lethal with TREX 1 (Swt1), an endonuclease that is associated with nuclear pores and that functionally interacts with myosin-like proteins and Nup60 (REF. 47). Swt1 also presents genetic interactions with THO, suggesting that it may collaborate with Rrp6 to eliminate improperly processed transcripts retained in association with their gene at the nuclear periphery (FIG. 2).

Myosin-like proteins are also involved in the retention of malformed mRNPs produced in *yra1* or *nab2* mutants[48]. Nab2, a shuttling mRNA-binding protein that is involved in poly(A) tail length control and is required for general mRNA export, was proposed, like Npl3, to link 3′-end processing and export. Recent data show that Nab2 directly interacts with Mex67 and may act as an additional export adaptor[49,50]. Notably, Yra1 enhances the interaction between Nab2 and Mex67 *in vitro*, and becomes dispensable in cells overexpressing Nab2 or Mex67, suggesting that Yra1 acts as a cofactor stabilizing the adaptor–receptor interaction on mRNPs. Mex67 and Nab2 are shuttling proteins, whereas Yra1 remains nuclear[2].

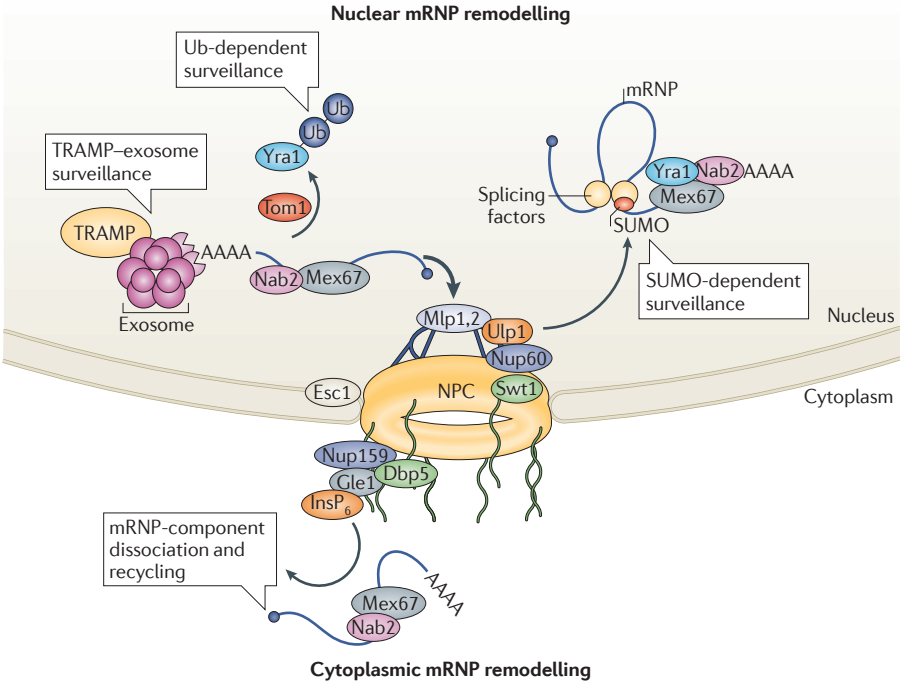


Figure 2 | **NPC-associated remodelling and surveillance.** Prior to translocation, messenger ribonucleoprotein (mRNP) composition is rearranged in a quality control step that removes non-shuttling proteins and may incorporate or reorganize proteins involved in mRNA localization. Ubiquitylation by the E3 ubiquitin ligase Tom1 promotes the dissociation of Yra1 (ALY or REF in mammals) from the mRNA. Tom1 genetically interacts not only with the exosome component ribosomal RNA-processing protein 6 (Rrp6) but also with the nuclear pore complex (NPC)-associated myosin-like protein 2 (Mlp2), and therefore Tom1 is likely to contribute to surveillance in the vicinity of the pore. Another nuclear quality control step involves the small ubiquitin-like modifier (SUMO) protease ubiquitin-like-specific protease 1 (Ulp1), which is tethered at the NPC by multiple factors, including the nucleoporin Nup60, Mlp1 and Mlp2 (REF. 46), and is involved in the detection and retention of unspliced mRNAs. Ulp1 targets may include sumoylated heterogeneous nuclear ribonucleoproteins (hnRNPs) that are loaded on unspliced mRNAs and induce mRNA retention when they are recognized by the SUMO protease. After translocation, the mRNP undergoes a remodelling step that occurs just before or concomitantly with translation initiation and depends on the RNA helicase DEAD box protein 5 (Dbp5; also known as Rat8). Dbp5 travels with the mRNP and interacts with Nup159 and Gle1 that are stably associated with the NPC cytoplasmic filaments. Binding of inositol hexakiphosphate ($InsP_6$) to Gle1 stimulates Dbp5 ATPase activity, inducing mRNA release and dissociation of export factors. The mechanism of Dbp5 action has recently been refined through structural studies of Dbp5 in association with Nup159, Gle1 and RNA[95]. The mRNA export factor 67 (Mex67) partner mRNA transport regulator 2 (Mtr2) has been omitted for simplicity. Esc1, establishes silent chromatin 1; Nab2, nuclear polyadenylated RNA-binding protein 2; Swt1, synthetically lethal with TREX 1; Ub, ubiquitin.

Our recent work shows that ubiquitylation of Yra1 by the E3 ubiquitin ligase Tom1 promotes the dissociation of Yra1 from Nab2 and nuclear mRNPs, allowing efficient mRNA export[50]. This finding fits earlier work indicating that Tom1 is required for the export of Nab2 containing mRNPs[51]. Moreover, Yra1 was found to accumulate on mRNPs in Δ*nup60* mutants, suggesting that its removal takes place at the pore[52]. Consistently, genetic interactions link Yra1, Tom1, and myosin-like proteins, supporting the view that ubiquitylation by Tom1 occurs in association with myosin-like proteins, facilitating mRNP release from this retention filter. Ubiquitylated Yra1 is not targeted for degradation and it is unclear whether this PTM weakens Yra1–mRNP interactions or is recognized by an ubiquitin-binding protein that actively dissociates modified Yra1 from the mRNP[53]. mRNP remodelling at the NPC may also define transcript fate in the cytoplasm. Indeed, Nup60 was recently implicated in the export and correct localization of the bud-specific *ASH1* transcript, suggesting that localized mRNPs acquire factors necessary for their proper destiny through rearrangements at the NPC nuclear basket[54].

Interestingly, Mlp1, Mlp2, Nup60 and Esc1 are also involved in anchoring the small ubiquitin-like modifier (SUMO) protease ubiquitin-like-specific protease 1 (Ulp1) at the NPC[46], and intact Ulp1 is required for the efficient retention of pre-mRNA in a mechanism that is linked to these proteins. Thus, the dynamics of sumoylation–desumoylation of pre-mRNA-bound proteins at the NPC may mark unspliced or malformed transcripts for retention[55]. The mRNA-binding factors that are targeted by Ulp1 are still unknown, although proteomic analysis of sumoylated proteins may provide some interesting candidates[56]. Another question for the future is whether the sumoylation–desumoylation and ubiquitylation reactions are coordinated in defining whether an mRNP is to be retained or released.

In summary, myosin-like proteins may provide a scaffold that concentrates remodelling activities. mRNPs might dock at the nuclear periphery via direct interaction of Nab2 with Mlp1 (REF. 57–59), and they might be maintained in this location until the required rearrangements have occurred. This step may be subjected to kinetic proofreading, resulting in mRNP retention and degradation when remodelling is too slow[4].

Recycling of mRNP components

Recent advancements in live imaging have laid the foundations for *in vivo* studies of single mRNP nucleocytoplasmic transport in mammalian cells[60,61]. Monitoring the passage of a single mRNP though the pore channel divided the kinetics of transport into three fundamental steps: docking, transport and release. Interestingly, the mRNP moves bi-directionally within the pore[60], suggesting that factors on the cytoplasmic side of the NPC may pull the mRNP to complete translocation. In yeast, the irreversible release of mRNAs into the cytoplasm is mediated by Gle1 and the RNA helicase DEAD-box protein 5 (Dbp5; also known as Rat8), which are strategically associated with the NPC cytoplasmic filaments. Dbp5 is a shuttling protein that is recruited to mRNPs during transcription. Its low intrinsic ATPase activity is greatly stimulated on the cytoplasmic face upon interaction with Gle1 that is bound to its activator inositol hexakiphosphate ($InsP_6$)[62,63]. ATP hydrolysis by Dbp5 is proposed to induce conformational changes that result in dissociation of export factors, such as Nab2 and Mex67, from mRNPs when they emerge from the NPC channel, and to facilitate their recycling back into the nucleus[2,52,64]. Notably, Dbp5 has a separate independent function during translation termination, which is similarly stimulated

by Gle1–$InsP_6$ (REF. 65). Recent genetic experiments demonstrate that Gle1 is a major target of $InsP_6$, as Gle1 mutations preventing $InsP_6$ interaction present phenotypes similar to those induced by mutations in inositol-pentakisphosphate 2-kinase ($InsP_5$2K; also known as Ipk1) that cause low levels of $InsP_6$ protein to be produced (REFS 66,67). Therefore, the regulation of $InsP_6$ levels by $InsP_5$2K may be one way to coordinately regulate mRNA export and protein synthesis.

Export pathways: one, two or more?

One question that remains to be addressed is whether mRNAs may be exported via non-redundant pathways that are differentially regulated in response to environmental changes. Mex67 is required for the export of all classes of mRNAs, and essential mRNA export adaptors, such as Npl3 or Nab2, appear to bind most, or a very broad set of, overlapping transcripts[68,69]. Whether they differentially regulate export of specific classes of transcripts is currently unclear; however, the combination of these general export factors with more specific players may create a variety of possibilities for regulation. Stress is the only well-described condition under which the export of specific transcript classes is regulated. Indeed, following heat or ethanol shock, bulk poly(A) RNA accumulates in the nucleus, whereas the transcription and export of heat shock mRNAs is rapidly induced. The block of poly(A) mRNA export during heat shock was recently shown to depend on the kinase Slt2 (REF. 49). Interestingly, Nab2 is a target of Slt2, and heat shock decreases Nab2 association with the general export receptor Mex67. By contrast, heat shock increases Nab2 interaction with Yra1 and Mlp1, resulting in the sequestration of these three proteins in nuclear foci, possibly in association with the blocked mRNAs. Notably, although Slt2 is required for poly(A) RNA retention, Nab2 phosphorylation is not sufficient for either poly(A) RNA export block or nuclear foci formation, indicating that another target of Slt2 implicated in poly(A) RNA retention remains to be identified. These results suggest that stress conditions trigger mRNP rearrangements that prevent bulk poly(A) RNA export, favouring nuclear exit of heat shock transcripts. Heat shock mRNA export depends on Mex67 but not on canonical export adaptors such as Yra1, Sub2 or Npl3 (REFS 70,71). A puzzling question is whether Mex67 binds these transcripts directly or via specific, unknown adaptors.

Conclusions

Early high-resolution electron-microscopy studies on Balbiani ring transcripts in insect cells[72], and recent live-imaging approaches in mammalian cells[61], revealed that mRNPs undergo important structural rearrangements along the biogenesis and export pathway, reflecting biochemical changes that occur within these complexes. 3′-end formation and NPC-associated remodelling have been identified as two crucial steps that are required for efficient mRNP engagement into the NPC channel. The conformational transitions undergone by the mRNP are driven, at least in part, by the ATPase and RNA helicase Sub2. These rearrangements are closely scrutinized by the nuclear exosome, which retains faulty mRNPs at the gene locus, sometimes in close association with the NPC, and triggers their degradation assisted by the TRAMP polyadenylation complex. It is still unclear how defective mRNPs are detected. One view is that the surveillance machinery associates and competes with the canonical processing apparatus, allowing prompt identification of maturation steps with suboptimal kinetic behaviours.

An increasing number of studies indicate that PTMs contribute to the coupling and coordination of mRNA export steps by regulating the dynamic association of proteins with maturing mRNPs. Indeed, from transcription to export, proteins signal their transition from one stage to the next through PTMs that inhibit or trigger interactions with sequential partners. Obtaining a global assessment of PTM function in mRNA export is a challenging task as the majority of PTMs are not essential and probably cooperate in a redundant way to create efficient and reproducible gene expression patterns. Many more proteins involved in mRNA export are likely to be modified post-translationally, and the elucidation of this higher level of regulation will deepen their functional characterization. Finally, how external cues may fine-tune PTMs in order to differentially regulate the export and fate of certain classes of transcripts is an additional important question for the future.

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