FEBS Letters 498 (2001) 150–156 FEBS 24907

Minireview

Nuclear export of mRNA

Daniel Zenklusen, Françoise Stutz*

Institute of Microbiology, Centre Hospitalier Universitaire Vaudois, 44, rue du Bugnon, 1011 Lausanne, Switzerland

Received 4 May 2001; accepted 4 May 2001

First published online 17 May 2001

Edited by Gunnar von Heijne

Abstract Export of mRNA through nuclear pore complexes (NPC) is preceded by multiple and well coordinated processing steps, resulting in the formation of an export competent ribonucleoprotein complex (mRNP). Numerous factors involved in the translocation of the mRNP through the NPC and its release into the cytoplasm have been isolated mainly through genetic approaches in yeast, and putative functional homologues have been identified in metazoan systems. Understanding the mechanism of mRNA export relies, in part, on the functional characterization of these factors and the establishment of a complete network of molecular interactions. Here we summarize recent progress in the characterization of yeast and mammalian components implicated in the export of an mRNA from the nucleus to the cytoplasm. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: mRNA export; Heterogeneous nuclear ribonucleoprotein complex; Nuclear pore complex; TAP/Mex67p; REF/Yra1p

1. Nucleo-cytoplasmic transport

The harmonious growth of eukaryotic cells relies on the constant exchange of macromolecules between the nucleus and the cytoplasm. Nucleo-cytoplasmic transport occurs through nuclear pore complexes (NPCs), huge macromolecular assemblies inserted within the nuclear envelope. Proteins termed nucleoporins (Nups) assemble to form the NPC, which ranges in size from 50 MDa in *Saccharomyces cerevisiae* to 125 MDa in higher eukaryotes and whose overall architecture is evolutionarily conserved [1]. The yeast NPC consists of about 30 different Nups, half of which contain degenerate FG repeats (FG nucleoporins) providing docking sites for transport complexes on the NPC [2,3]. Nuclear transport is energy-dependent and signal-mediated. Nuclear localization signals and nuclear export signals (NES) direct proteins for nuclear import or export through interactions with transport receptors of the importin-β family (termed karyopherins, or importins/exportins). Yeast contains 14 karyopherins, four of which are exportins. Karyopherins share an N-terminal domain involved in binding the small GTPase Ran, and presumably mediate movement of their cargoes through interactions with both Ran-GTP and FG nucleoporins. Directionality of transport is ensured by the asymmetric distribution of Ran-GTP (nuclear) and Ran-GDP (cytoplasmic), reflecting compartmentalization of the GDP/GTP exchange factor for Ran in the nucleus and its GTPase activating protein (GAP) in the cytoplasm. Ran-GTP binding to importins triggers the dissociation of cargo–import receptor complexes upon arrival in the nucleus. Conversely, Ran-GTP binding to exportins is required for the formation of cargo–export receptor complexes within the nucleus. These complexes disassemble and are released from the cytoplasmic side of the pore through GAP-induced Ran-GTP hydrolysis [4,5].

2. RNA export pathways

The different classes of RNAs, including tRNAs, rRNAs, UsnRNAs and mRNAs, are exported as ribonucleoprotein complexes (RNPs) through distinct pathways defined by specific signals present on the RNA and/or the RNA-bound proteins [6]. Export of most RNAs is dependent on Ran-GTP, suggesting a direct or indirect role of karyopherins [7].

A major contributor to our understanding of nuclear export pathways is the HIV-1 Rev protein. Binding of Rev to the RRE, a highly structured RNA sequence present on partially spliced or unspliced viral transcripts, promotes the nuclear exit of these RNAs normally retained within the nucleus. This results in the expression of viral structural proteins and packaging of new viral particles in the cytoplasm. Rev contains the first NES identified which directly interacts with the export receptor Crm1/Xpo1 in a Ran-GTP-dependent manner and Crm1 was shown to mediate the export of RRE-containing transcripts by interaction with the viral adapter protein Rev [8]. Crm1 was subsequently shown to be the receptor for cellular proteins containing a Rev-like NES. In addition, Crm1 directs the export of UsnRNAs and 5S rRNA in higher eukaryotes, and of the 60S ribosomal subunit in yeast, through interactions with specific cellular adapter proteins [9–11].

Substantial evidence supports that neither Crm1 nor other karyopherins are major contributors to mRNA export whether in yeast or metazoan systems [12–14]. Although Ran is important for mRNA export, its requirement is variable and dependent on the nature of the transcript. The role of Ran in this process may be indirect and reflect the recycling of mRNA binding proteins essential for mRNA export back to the nucleus [7,15,16]. More generally, nuclear export of mRNAs in the form of mRNPs is expected to be more complex than export of proteins or small RNA molecules and

*Corresponding author. Fax: (41)-21-314 40 95.
E-mail: fstutz@hola.hospvd.ch

PII: S0014-5793(01)02482-6

transport directionality may be achieved through distinct mechanisms.

A considerable number of factors have been linked to mRNA export. Many of them were initially identified in *S. cerevisiae*, by screening for conditional mutants that accumulate poly(A)$^+$ RNA in the nucleus at the restrictive temperature, or screens for genetic interactions with Nups or other transport-related factors [17,18]. The factors involved in mRNA export comprise RNA binding proteins (Npl3p, Nab2p, Hrp1, Yra1p, Mex67p), NPC or NPC-associated proteins (Mtr2p, Gle1p, Rip1p, Gle2p, Nup116p, the Nup84 and Nup159 NPC sub-complexes) and Dbp5p, a DEAD box ATPase/RNA helicase [19]. Putative metazoan orthologues have been identified for most of these proteins, strongly suggesting that the basic mechanism for mRNA export is conserved [20,21].

3. hnRNP proteins and mRNA export

Newly synthesized precursor RNAs become associated with numerous heterogeneous nuclear (hn) RNP proteins/processing factors and undergo a series of maturation steps, including 5′ capping, splicing, 3′ end cleavage and polyadenylation, resulting in the formation of mature and export-competent mRNP complexes [20]. Accumulating evidence suggests that processing reactions may generate signals on the mRNP necessary for the subsequent interaction with the export machinery, i.e. splicing and 3′ end processing defects lead to nuclear retention both in yeast and in mammalian systems [22–24]. This functional coupling may be part of quality control mechanisms ensuring that only fully matured transcripts reach the cytoplasm [25] (Fig. 1).

Because a subset of hnRNP proteins remain associated with the mRNA during export and shuttle between nucleus and cytoplasm, they were suggested to contain signals mediating nuclear exit. The abundant mammalian hnRNP proteins A1 and K contain signals conferring shuttling activity upon a passenger protein; however, no specific export receptor has been identified for these signals and it is unclear whether hnRNPA1 plays a direct role in mRNA export [20]. Similarly, no direct connection has been established between components of the export machinery and the yeast hnRNP-like proteins Npl3p, Hrp1p/Nab4p, Nab2p, all three essential for mRNA export. Since the shuttling of these RNA binding proteins is dependent on RNA synthesis, they may be passive cargoes of the mRNP [26,27]. Genetic and physical interac-

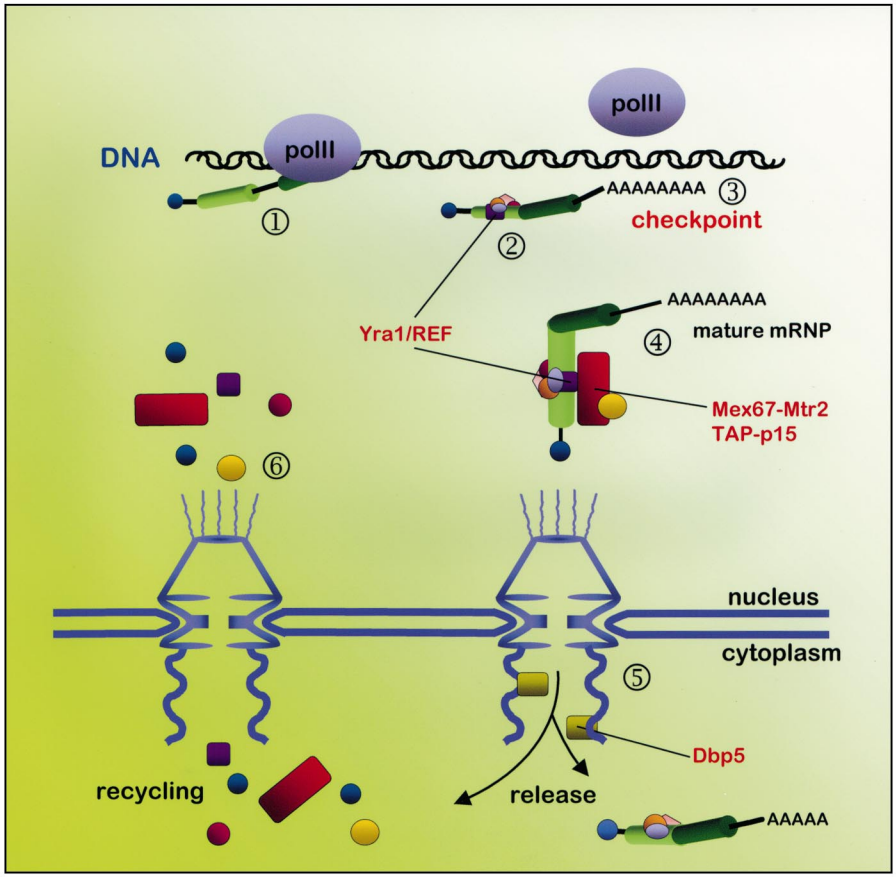

Fig. 1. Model for mRNA export. Nascent polymerase II transcripts (1) rapidly associate with hnRNP/processing factors for 5′ capping, splicing, 3′ cleavage and polyadenylation resulting in a mature and export-competent mRNP complex. In higher eukaryotes, splicing deposits a protein mark 20–24 nucleotides upstream of the exon–exon junction, which includes Aly/REF (2). In yeast, association of Yra1p with the mRNP may be different and independent of splicing, as only a small percentage of yeast genes contain introns. A putative surveillance mechanism ensures that only properly processed transcripts are released from their transcription site (3). After release from the transcription site and recruitment of the export receptor Mex67p/TAP (as Mex67p-Mtr2p or TAP-p15 heterodimers) by Yra1p/REF (4), the mRNP is directed towards the NPC. Mex67p/TAP subsequently mediates translocation of the mRNP through the pore by sequential interactions with FG nucleoporins. On the cytoplasmic side, the RNA helicase Dbp5 may trigger the release of the mRNP from the pore (5) and induce the recycling of mRNP components back to the nucleus for another round of export (6). For references see text.

tions functionally relate Npl3p to the cap binding protein Cbp80p and the 3′ end processing factors Hrp1p, Rna14p and Rna15p. It is possible that these proteins contribute to mRNA export indirectly, by ensuring proper processing and packaging of the mRNP [28,29].

4. The mRNA export receptor TAP/Mex67p

A key issue in mRNA export is the question of what factor(s) mediates the export-relevant interactions between the mRNP and the NPC. Currently, the most likely candidate is the essential yeast protein Mex67p and its human homologue TAP. Although not related in sequence to importin-β, Mex67p and TAP present the main features of mRNA export receptors. TAP and Mex67p shuttle between nucleus and cytoplasm, cross-links to poly(A)$^+$ RNA and interact directly with FG nucleoporins [30–33]. However, neither TAP nor Mex67p appears to interact with Ran. Consistent with a direct role in mRNA export, mutations in Mex67p induce a rapid and robust nuclear accumulation of poly(A)$^+$ RNA [34]. TAP was first identified as the cellular factor interacting with the constitutive transport element (CTE) present in RNAs from type D simple retroviruses and TAP promotes the export of CTE-containing transcripts [31,32,35]. As nuclear injection of CTE RNA into *Xenopus* oocytes competes with the export of cellular mRNAs, TAP was proposed to be implicated in the mRNA export pathway [36]. Consistently, over-expression of TAP in *Xenopus* oocytes or tissue cultured cells stimulates the export of transcripts that are otherwise inefficiently exported or retained within the nucleus, indicating a direct role in mRNA export [37,38].

5. TAP/Mex67p functional domains and partners

TAP and Mex67p are members of the NXF (nuclear export factor) family of putative mRNA export receptors, which has several members in most higher eukaryotes [38]. NXF knockouts in nematodes or *Drosophila* are lethal, underlining the functional importance of these proteins in different species ([38,39]; E. Izaurralde, personal communication). NXF proteins present a conserved modular architecture and considerable progress has recently been made in defining structural and functional domains of TAP/Mex67p. Crystal structures show that the N-terminal region of TAP includes a non-canonical RNP-type RNA binding domain (RBD) and four leucine-rich repeats (LRR). This region exhibits general RNA binding affinity and both the RBD and LRR are required for specific binding to the CTE [40]. The N-terminal domain also interacts with mRNP-associated proteins such as E1B-AP5 or REF (see below) and is probably involved in targeting TAP to cellular mRNPs [30] (Fig. 2).

The central domain of TAP interacts with p15/NXT1, a small protein related to the Ran-GDP binding nuclear transport factor NTF2 [33,41,42]. The p15 binding domain of TAP is itself related to NTF2 (NTF2-like), and a model for heterodimerization between TAP and p15 was inferred from the known homodimeric structure of NTF2 [43,44]. Interestingly, Mex67p similarly heterodimerizes with Mtr2p, a small NPC-associated protein essential for mRNA export in yeast [45]. Mtr2p is not related in sequence to p15/NXT1, but a *mex67*−/*mtr2*− double deletion is rescued by co-expression of TAP and p15, supporting that the TAP–p15 and Mex67p–Mtr2p heterodimers have similar functions [33].

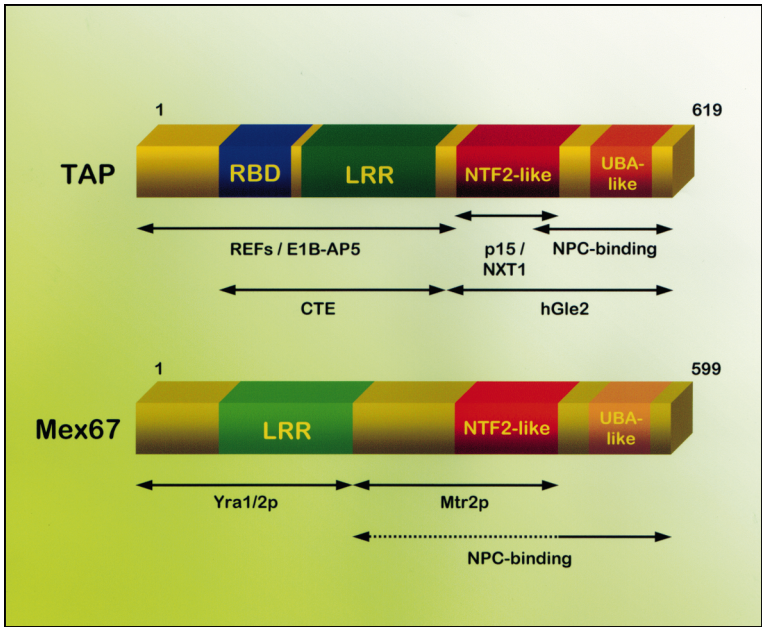

Fig. 2. Schematic diagram of TAP/Mex67p functional domains and partners. The domain organization of TAP and Mex67p is conserved. Based on crystal structure analysis, the N-terminal or substrate binding domain of TAP consists of a non-canonical RNP-like RBD and a LRR. The RBD and LRR are sufficient for CTE binding whereas the whole N-terminus mediates interaction with the RNP binding proteins REF and E1B-AP5 [30,40,53]. The yeast REF family members Yra1p and Yra2p similarly interact with the N-terminus of Mex67p, in which the LRR but not the RBD is conserved [54]. TAP and Mex67p also contain conserved NTF2-like and UBA-like domains involved respectively in p15/NXT1 and Mtr2p heterodimerization, and in association with the NPC. The minimal regions on Mex67p required for Mtr2p and NPC binding have not been determined. TAP interacts with hGle2 through its C-terminal region, suggesting a role for this interaction in the association of TAP with the NPC [30,44,48].

The C-terminal region of TAP contains a ubiquitin-associated domain (UBA-like), conserved in Mex67p, which mediates interaction with multiple FG nucleoporins in vitro. The UBA-like domain of TAP/Mex67p is necessary for stable association with the pore and for efficient export of CTE or mRNA in vivo [30–32,37,44,46].

The role of p15 or Mtr2p in mRNA export has been addressed in different ways. A recent report shows that Mex67p interacts with FG nucleoporins in vitro in the absence of Mtr2p [47]. Another study indicates, however, that the binding of Mex67p to FG nucleoporins occurs only in the presence of Mtr2p and is not dependent on the very C-terminal UBA-like domain of Mex67p. Consistently, over-expression of Mtr2p complements the temperature-sensitive phenotype of a C-terminal deletion of Mex67p which is not stably associated with the NPC. Mtr2p may therefore modulate the in vivo interaction of Mex67p with FG repeat sequences [48].

In contrast to Mtr2p, p15/NXT1 binding does not detectably contribute to the interaction of TAP with FG nucleoporins nor to NPC localization [30]. p15/NXT1 binding is not absolutely required for CTE-dependent export [49]. However, p15/NXT1 greatly enhances the stimulatory effect of TAP on the export of cellular mRNAs in tissue cultured cells, indicating that TAP–p15 heterodimerization is needed for efficient mRNA export and that mRNA and CTE export have distinct requirements [37,46]. Consistent with these data, stimulation of mRNA export by p15/NXT1 was also observed in a recently developed in vitro system [50]. In addition, p15/NXT1 was implicated in tRNA and in Crm1-dependent export of UsnRNAs and leucine-rich NESs suggesting a function in both CRM1-dependent and CRM1-independent pathways. NXT/p15 was proposed to enhance the export of a leucine-rich NES by stimulating its release from the cytoplasmic face of the NPC [51]. These effects of p15/NXT1 were suggested to depend on its ability to interact with Ran-GTP [50]. However, the interaction of p15/NXT1 with Ran is controversial [33,38]. The exact function of p15/NXT1 and Mtr2p is therefore still unclear. These proteins may be critical to stabilize respectively TAP and Mex67p, thereby enhancing the ability of the export receptors to interact with NPC components or perhaps with their target mRNPs.

6. Binding of TAP/Mex67p to mRNPs

TAP/Mex67p exhibits low affinity RNA binding and is likely to interact with cellular mRNPs through protein–protein rather than protein–RNA interactions [33,45]. Genetic and affinity purification methods in yeast identified Yra1p, an essential hnRNP-like protein that directly interacts with Mex67p. Yra1p belongs to the evolutionarily conserved REF (RNA and export factor binding) family of hnRNP-like proteins which has several members in most species. REF proteins contain a central putative RNP-type RBD and exhibit RNA binding activity in vitro. Surprisingly, it is the 5′ and 3′ flanking regions, and not the RBD, that mediate interaction with both RNA and Mex67p, and the RBD is not essential for viability in yeast. Depletion of Yra1p is paralleled by nuclear accumulation of poly(A)$^+$ RNA supporting that Yra1p has a direct role in mRNA export. [52–55]. Poly(A)$^+$ RNA export is also affected in strains expressing Yra1 mutant proteins that inefficiently interact with Mex67p in vitro. Affinity purification of mRNP complexes from these strains indicates reduced association of Mex67p with these complexes, consistent with the view that Yra1p recruits Mex67p–Mtr2p to promote mRNA export [54]. The mouse homologue Aly/REFI-I interacts with TAP and Mex67p, and partly complements the non-viable *YRA1* null mutant indicating functional conservation among the REF proteins. Consistent with a direct role in mRNA export, mouse Aly/REF shuttles and stimulates mRNA export in *Xenopus* oocytes [56,57].

7. mRNA splicing and export are coupled

The presence of an intron often enhances gene expression in metazoan systems. In *Xenopus* oocyte injection experiments, mRNAs generated through splicing are exported at higher rates than the corresponding synthetic non-intron-containing transcripts, suggesting that pre-mRNA splicing is biochemically coupled to mRNA export [58]. Aly/REF may functionally link these processes as this protein becomes tightly associated with mRNP complexes generated through splicing and may thereby facilitate the recruitment of TAP to these transcripts. Consistently, Aly/REF accumulates in splicing factor-containing nuclear speckles [57]. However, splicing may not be a necessary step for nuclear export of all mRNAs since only a small percentage of yeast genes contain introns and many mRNAs lacking introns can be efficiently expressed in vertebrates. Export-stimulating sequences have been mapped in some of these transcripts, which may enhance the recruitment of mRNA export factors [59]. Finally, *Xenopus* oocyte nuclear injection of anti-REF antibodies inhibits mRNA export irrespective of splicing, suggesting that spliced and unspliced mRNAs use common export factors to reach the cytoplasm, which include REF and TAP. Splicing may, however, confer a kinetic advantage to mRNA export by facilitating the recruitment of these factors to the mRNP [56].

As a result of splicing, Aly/REF becomes stably bound to mRNA in a complex with four other proteins, 20–24 bases upstream of the exon–exon junction, in a splicing-dependent and sequence-independent manner. These four proteins include the spliceosome-associated proteins DEK, SRM160, and RNPS1 and the small shuttling RNA binding protein Y14 [60,61]. Whereas Aly/REF rapidly dissociates from the mRNA after nuclear exit, Y14 remains bound to the transcripts within the cytoplasm. The protein mark deposited on the mRNA as a result of splicing is therefore likely to play a role in mRNA export as well as in cytoplasmic aspects of the life cycle of mRNA [62,63] (Fig. 1).

8. Translocation of mRNPs through the NPC

The intranuclear movement of an mRNP from its site of transcription to the NPC and its translocation through the pore are still poorly understood. Recent progress in defining the three-dimensional architecture and composition of NPCs gave important hints on how the NPC may guide transport complexes through its aqueous channel. The NPC consists of a central spoke assembly with an eight-fold symmetry, framed by nuclear and cytoplasmic rings. Filamentous structures extend from these rings into the nucleus and the cytoplasm, forming the nuclear basket and cytoplasmic fibrils [3]. Proteomic analysis combined with the localization of individual nucleoporins by immuno-electron microscopy (IEM) generated a low resolution map of the yeast NPC [1,2]. A number of pore

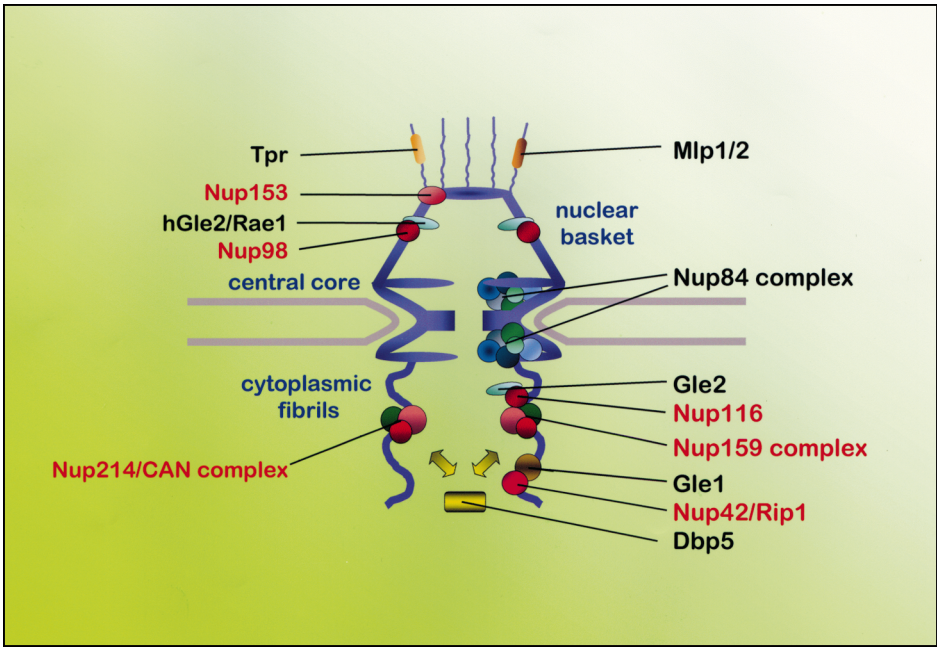

Fig. 3. Localization of Nups implicated in mRNA export within the NPC structure, as determined by immuno-electron microscopy (IEM) [1,2]. Yeast proteins and sub-complexes are shown on the right whereas metazoan nucleoporins with a proposed role in mRNA export are shown on the left. FG repeat-containing nucleoporins or sub-complexes are indicated in red. The Nup84 complex (C-Nup145p, Nup120p, Nup85p, Nup84p, Sec13p, and Seh1p) is placed symmetrically on both sides of the central core and its role may be primarily structural. Gle1p, Rip1p, and the Nup159 sub-complex (Nup159p, Nup82p, Nsp1p), in association with Nup116p, are cytoplasmically orientated and involved in a terminal step of mRNA export. This group of proteins provides binding sites for Mex67p–Mtr2p and the ATPase/RNA helicase Dbp5p. The mammalian CAN/Nup214 sub-complex (CAN/Nup214, Nup88, p62) is functionally equivalent to the Nup159 sub-complex. Gle2p and hGle2 associate with the pore through a conserved sequence in Nup116p and Nup98 respectively. Whereas Nup98 localizes at the basket, Nup116p and Gle2p are present on both the nuclear and cytoplasmic sides of the pore. These differences may reflect changes in steady-state localizations as Nup98 and hGle2 (like Nup153) are not stably associated with the NPC but shuttle between the nuclear and cytoplasmic compartments [20]. This raises the possibility that Nup116p and Gle2p may similarly be moving within the NPC. Finally mammalian Tpr and the yeast homologous proteins Mlp1p and Mlp2p are part of filamentous structures extending from the nuclear basket towards the nuclear interior. These structures were proposed to contribute to early steps in mRNA export by providing tracks guiding mRNP complexes from the nucleoplasm towards the NPC [84–86].

proteins are part of biochemically defined sub-complexes, some of which have primary roles in mRNA export (Fig. 3). Orthologues of most yeast nucleoporins exist in higher eukaryotes but the sequence identities are usually not very high. However, the location of these proteins within the NPC and their presence in specific sub-complexes indicate a high degree of functional conservation [21].

FG nucleoporins, which constitute about half of the yeast Nups, are located at the central core as well as on the basket and the fibrils. Mex67p–Mtr2p directly interacts with several FG repeat proteins, including Nup100, Nup116p, Nup42p, Nsp1p, and Nup159p, and genetic data support the functional relevance of these interactions [47,48]. A current model proposes that Mex67p–Mtr2p and, by analogy, TAP–p15 drive the mRNP through the pore by transient and sequential interactions with these FG repeat domains.

Two yeast NPC sub-complexes, Nup84 and Nup159, are essential for mRNA export. The Nup84 sub-complex (Nup145p-C, Nup120p, Nup85p, Nup84p, Sec13p, and Seh1p) contains no FG nucleoporins and is located on both the cytoplasmic and nuclear sides of the central core of the NPC [2,64]. Highly purified Nup84 sub-complexes analyzed by electron microscopy exhibit a Y-shaped morphology and were suggested to participate in the formation of the octagonal NPC internal rings. The Nup84 sub-complex was proposed earlier to provide binding sites for Mex67p–Mtr2p at the pore, but no direct interactions have been demonstrated yet, raising the possibility that this sub-complex influences Mex67p–Mtr2p targeting to the NPC indirectly, by affecting pore structure [45,48,65].

The components of the Nup159 sub-complex (Nup159p, Nup82p, Nsp1p, and more loosely bound Nup116p) are associated with the cytoplasmic fibrils, consistent with a role in a terminal step of export [1,2,66,67]. This complex contains several FG repeat domains able to interact with Mex67p–Mtr2p, and recruits the DEAD box RNA helicase Dbp5p through an evolutionarily conserved interaction with Nup159p [68,69]. Dbp5p is essential for mRNA export and its ATPase and RNA unwinding activities were proposed to mediate the remodelling of mRNP complexes emerging on the cytoplasmic face of the NPC. Dbp5p may contribute to the release of the mRNA into the cytoplasm and the recycling of mRNP proteins and export factors back into the nucleus (Fig. 1). The essential mRNA export factor Gle1p and the associated FG nucleoporin Rip1p/Nup42p are additional proteins on the cytoplasmic fibrils that bind Dbp5p and Mex67p–Mtr2p respectively [48,70]. Gle1p and Nup42p may therefore function in conjunction with Nup159p components by providing a platform from which the mRNP is released. There is evidence that Dbp5p shuttles and associates with Yra1p-containing complexes, suggesting a role in earlier, perhaps intranuclear, steps of mRNA export [47,68,69]. The extent to which Dbp5p may

contribute to export directionality is unclear, and a better understanding of the essential role of Dbp5p certainly awaits the identification of its specific substrates.

Gle2p is another pore-associated protein that plays a role in mRNA export. Gle2p and its human homologue hGle2/Rae1 bind the NPC via a conserved sequence in Nup116p and hNup98 respectively. There is evidence that hGle2/Rae1 shuttles and cross-links to poly(A)$^+$ RNA [71–73]. In *Schizosaccharomyces pombe*, Gle2p/Rae1p, but not Mex67p, is essential for mRNA export. This contrasts with *S. cerevisiae* where Mex67p, but not Gle2p, is essential, suggesting that these proteins have overlapping or redundant functions. Gle2p associates with Mex67p, directly or within a complex, and this interaction is conserved in human and *S. pombe* [30,54,74]. Gle2p/Rae1 may interact with mRNPs via a yet unidentified mRNP component and promote export in conjunction with Mex67p/TAP. Alternatively, Gle2p/Rae1 may contribute to the interaction of Mex67p/TAP with the nuclear pores.

9. mRNA export regulation and quality control

Diverse mechanisms regulate both protein nuclear import and export [75]. Increasing evidence suggests that mRNA export may be subjected to regulation as well. For example, the efficiency of yeast mRNA export is linked to the phospholipase C-dependent inositol phosphate kinase signalling pathway [76]. There is also evidence that mRNA export rates may be modulated by arginine methylation of hnRNP proteins [77]. Finally, the non-essential yeast E3 ubiquitin ligase Tom1p was proposed to contribute to the selective export of transcripts associated with the mRNA binding protein Nab2, but not to those bound to Npl3p, suggesting the existence of differentially regulated mRNA export pathways in yeast [78].

Regulation of mRNA export also occurs under stress, when heat-induced transcripts are efficiently exported but non-heat shock mRNAs exhibit a reduced ability to reach the cytoplasm [79–81,87]. In yeast, heat shock RNA export was initially proposed to occur through a specific pathway defined by the FG nucleoporin Rip1p/Nup42p [80,88]. However, this non-essential NPC protein also contributes to mRNA export under normal conditions, presumably by acting as a co-factor for the essential mRNA export factor Gle1p at the pore. This role of Rip1p is dispensable at low temperature, but becomes essential under stress [14,70]. More generally, nuclear export of heat shock and non-heat shock mRNA occurs via similar pathways, defined by common factors, i.e. Mex67p–Mtr2p, Dbp5p, and the NPC components specific for mRNA export [79]. Noteworthy, the hnRNP-like protein Npl3p is one of the few known proteins essential for normal mRNA but not heat shock mRNA export. The preferential export of heat-shock mRNAs under stress may be due, at least in part, to the dissociation of Npl3p from non heat-shock transcripts and the formation of abnormal mRNPs [80,81].

Interestingly, inhibition of heat shock mRNA export in a Δ*RIP* strain or in strains carrying mutations in other mRNA export factors, including Mex67p, Mtr2p, Gle1p, Nup159 and Dbp5p, leads to hyperadenylation of newly synthesized heat shock transcripts and to their accumulation at the site of transcription. Other 3′ end formation problems, i.e. absence of polyA tails, also lead to mRNA retention at the transcription site (T.H. Jensen and M. Rosbash, personal communication). These observations indicate the existence of checkpoint mechanisms, which ensure that only correctly processed transcripts are released from their site of synthesis and exported to the cytoplasm. The defect of transcript release may be indirect and due to the inability of these mutants to terminate export and recycle factors required for proper 3′ end formation. Hyperadenylation and retention apply also to non-heat shock mRNAs and may more generally explain why nucleoporin mutants deficient in mRNA export show an accumulation of poly(A)$^+$ RNA inside the nucleus, sometimes as a granular signal, and not at their site of action at the nuclear pore [82].

10. Concluding remarks

Main players involved in mRNA export have been identified in the few last years and start to get integrated into the network of interactions underlying this complex cellular process. Crystallizations and structure predictions have provided important tools for probing the function of some of these proteins and elucidating their mechanism of action [83]. Despite these advances, it is still unclear how an mRNP travels from its site of transcription towards the pore, when and where TAP/Mex67p is recruited to the mRNP and whether proteins distinct from REF/Yra1p are involved in this recruitment. Another question concerns the mechanism by which TAP/Mex67p mediates directional transport of the mRNP through the pore; how interactions between the export receptor and FG nucleoporins are modulated and whether enzymatic activities other than Dbp5 are involved in translocation and release of the mRNP from the pore. The diversity of NXF, REF and NXT family members in higher eukaryotes [38,53] probably reflects greater substrate complexity in metazoan systems and is consistent with the existence of multiple and possibly differentially regulated export pathways. mRNA export may therefore provide an additional level of gene expression control.

References

[1] Stoffler, D., Fahrenkrog, B. and Aebi, U. (1999) Curr. Opin. Cell Biol. 11, 391–401.
[2] Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y. and Chait, B.T. (2000) J. Cell Biol. 148, 635–652.
[3] Rout, M.P. and Aitchison, J.D. (2001) J. Biol. Chem. (in press).
[4] Mattaj, I.W. and Englmeier, L. (1998) Annu. Rev. Biochem. 67, 265–306.
[5] Görlich, D. and Kutay, U. (1999) Annu. Rev. Cell Dev. Biol. 15, 607–660.
[6] Jarmolowski, A., Boelens, W.C., Izaurralde, E. and Mattaj, I.W. (1994) J. Cell Biol. 124 (5), 627–635.
[7] Izaurralde, E., Kutay, U., von Kobbe, C., Mattaj, I.W. and Gorlich, D. (1997) EMBO J. 16, 6535–6547.
[8] Pollard, V.W. and Malim, M.H. (1998) Annu. Rev. Microbiol. 52, 491–532.
[9] Gadal, O., Strauß, D., Kessl, J., Trumpower, B., Tollervey, D. and Hurt, E. (2001) Mol. Cell. Biol. 21, 3405–3415.
[10] Ho, J.H., Kallstrom, G. and Johnson, A.W. (2000) J. Cell Biol. 151, 1057–1066.
[11] Ohno, M., Segref, A., Bachi, A., Wilm, M. and Mattaj, I.W. (2000) Cell 101, 187–198.
[12] Cullen, B.R. (2000) Mol. Cell. Biol. 20, 4181–4187.
[13] Neville, M. and Rosbash, M. (1999) EMBO J. 18, 3746–3756.
[14] Stutz, F. and Rosbash, M. (1998) Genes Dev. 12, 3303–3319.
[15] Clouse, K.N., Luo, Mj., Zhou, Z. and Reed, R. (2001) Nature Cell Biol. 3, 97–99.
[16] Dahlberg, J.E. and Lund, E. (1998) Curr. Opin. Cell Biol. 10, 400–408.
[17] Doye, V. and Hurt, E. (1997) Curr. Opin. Cell Biol. 9, 401–411.

[18] Fabre, E. and Hurt, E. (1997) Annu. Rev. Genet. 31, 277–313.
[19] Strasser, K. and Hurt, E. (1999) FEBS Lett. 452, 77–81.
[20] Nakielny, S. and Dreyfuss, G. (1999) Cell 99, 677–690.
[21] Ryan, K.J. and Wente, S.R. (2000) Curr. Opin. Cell Biol. 12, 361–371.
[22] Brodsky, A.S. and Silver, P.A. (2000) RNA 6, 1737–1749.
[23] Custodio, N., Carmo-Fonseca, M., Geraghty, F., Pereira, H.S., Grosveld, F. and Antoniou, M. (1999) EMBO J. 18, 2855–2866.
[24] Minvielle-Sebastia, L. and Keller, W. (1999) Curr. Opin. Cell Biol. 11, 352–357.
[25] Maquat, L.E. and Carmichael, G.G. (2001) Cell 104, 173–176.
[26] Kessler, M.M., Henry, M.F., Shen, E., Zhao, J., Gross, S., Silver, P.A. and Moore, C.L. (1997) Genes Dev. 11, 2545–2556.
[27] Lee, M.S., Henry, M. and Silver, P.A. (1996) Genes Dev. 10, 1233–1246.
[28] Shen, E.C., Stage-Zimmermann, T., Chui, P. and Silver, P.A. (2000) J. Biol. Chem. 275, 23718–23724.
[29] Henry, M., Borland, C.Z., Bossie, M. and Silver, P.A. (1996) Genetics 142, 103–115.
[30] Bachi, A., Braun, I.C., Rodrigues, J.P., Pante, N., Ribbeck, K., von Kobbe, C., Kutay, U., Wilm, M., Gorlich, D., Carmo-Fonseca, M. and Izaurralde, E. (2000) RNA 6, 136–158.
[31] Bear, J., Tan, W., Zolotukhin, A.S., Tabernero, C., Hudson, E.A. and Felber, B.K. (1999) Mol. Cell. Biol. 19, 6306–6317.
[32] Kang, Y. and Cullen, B.R. (1999) Genes Dev. 13, 1126–1139.
[33] Katahira, J., Strässer, K., Podtelejnikov, A., Mann, M., Jung, J.U. and Hurt, E. (1999) EMBO J. 18, 2593–2609.
[34] Segref, A., Sharma, K., Doye, V., Hellwig, A., Huber, J., Luhrmann, R. and Hurt, E. (1997) EMBO J. 16, 3256–3271.
[35] Grüter, P., Tabernero, C., von Kobbe, C., Schmitt, C., Saavedra, C., Bachi, A., Wilm, M., Felber, B.K. and Izaurralde, E. (1998) Mol. Cell 1, 649–659.
[36] Saavedra, C., Felber, B. and Izaurralde, E. (1997) Curr. Biol. 7, 619–628.
[37] Braun, I.C., Herold, A., Rode, M., Conti, E. and Izaurralde, E. (2001) J. Biol. Chem. (in press).
[38] Herold, A., Suyama, M., Rodrigues, J.P., Braun, I.C., Kutay, U., Carmo-Fonseca, M., Bork, P. and Izaurralde, E. (2000) Mol. Cell. Biol. 20, 8996–9008.
[39] Tan, W., Zolotukhin, A.S., Bear, J., Patenaude, D.J. and Felber, B.K. (2000) RNA 6, 1762–1772.
[40] Liker, E., Fernandez, E., Izaurralde, E. and Conti, E. (2000) EMBO J. 19, 5587–5598.
[41] Ribbeck, K., Lipowsky, G., Kent, H.M., Stewart, M. and Gorlich, D. (1998) EMBO J. 17, 6587–6598.
[42] Black, B.E., Levesque, L., Holaska, J.M., Wood, T.C. and Paschal, B.M. (1999) Mol. Cell. Biol. 19, 8616–8624.
[43] Bullock, T.L., Clarkson, W.D., Kent, H.M. and Stewart, M. (1996) J. Mol. Biol. 260, 422–431.
[44] Suyama, M., Doerks, T., Braun, I.C., Sattler, M., Izaurralde, E. and Bork, P. (2000) EMBO Rep. 1, 53–58.
[45] Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Panté, N. and Hurt, E. (1998) Mol. Cell. Biol. 18, 6826–6838.
[46] Guzik, B.W., Levesque, L., Prasad, S., Bor, Y.C., Black, B.E., Paschal, B.M., Rekosh, D. and Hammarskjold, M.L. (2001) Mol. Cell. Biol. 21, 2545–2554.
[47] Strawn, L.A., Shen, T. and Wente, S.R. (2001) J. Biol. Chem. 276, 6445–6452.
[48] Strasser, K., Bassler, J. and Hurt, E. (2000) J. Cell Biol. 150, 695–706.
[49] Kang, Y., Bogerd, H.P. and Cullen, B.R. (2000) J. Virol. 74, 5863–5871.
[50] Ossareh-Nazari, B., Maison, C., Black, B.E., Levesque, L., Paschal, B.M. and Dargemont, C. (2000) Mol. Cell. Biol. 20, 4562–4571.
[51] Black, B.E., Holaska, J.M., Levesque, L., Ossareh-Nazari, B., Gwizdek, C., Dargemont, C. and Paschal, B.M. (2001) J. Cell Biol. 152, 141–155.
[52] Strässer, K. and Hurt, E. (2000) EMBO J. 19, 410–420.
[53] Stutz, F., Bachi, A., Doerks, T., Braun, I.C., Séraphin, B., Wilm, M., Bork, P. and Izaurralde, I. (2000) RNA 6, 638–650.
[54] Zenklusen, D., Vinciguerra, P., Strahm, Y. and Stutz, F. (2001) Mol. Cell. Biol. (in press).
[55] Cole, C.N. (2000) Nature Cell Biol. 2, E55–E58.
[56] Rodrigues, J.P., Rode, M., Gatfield, D., Blencowe, B.J., Carmo-Fonseca, M. and Izaurralde, E. (2001) Proc. Natl. Acad. Sci. USA 98, 1030–1035.
[57] Zhou, H., Luo, M.-J., Straesser, K., Katahira, J., Hurt, E. and Reed, R. (2000) Nature 407, 401–405.
[58] Luo, M.J. and Reed, R. (1999) Proc. Natl. Acad. Sci. USA 96, 14937–14942.
[59] Huang, Y. and Steitz, J.A. (2001) Mol. Cell 7, 899–905.
[60] Le Hir, H., Izaurralde, E., Maquat, L.E. and Moore, M.J. (2000) EMBO J. 19, 6860–6869.
[61] Kataoka, N., Yong, H., Kim, N.V., Velazquez, F., Perkinson, R.A., Wang, F. and Dreyfuss, G. (2000) Mol. Cell 6, 673–682.
[62] Kim, V.N., Yong, J., Kataoka, N., Abel, L., Diem, M.D. and Dreyfuss, G. (2001) EMBO J. 20, 2062–2068.
[63] Lykke-Andersen, J. (2001) Curr. Biol. 11, R88–R91.
[64] Siniossoglou, S., Wimmer, C., Rieger, M., Doye, V., Tekotte, H., Weise, C., Emig, S., Segref, A. and Hurt, E.C. (1996) Cell 84, 265–275.
[65] Siniossoglou, S., Lutzmann, M., Santos-Rosa, H., Leonard, K., Mueller, S., Aebi, U. and Hurt, E. (2000) J. Cell Biol. 149, 41–54.
[66] Bailer, S.M., Balduf, C., Katahira, J., Podtelejnikov, A., Rollenhagen, C., Mann, M., Pante, N. and Hurt, E. (2000) J. Biol. Chem. 275, 23540–23548.
[67] Ho, A.K., Shen, T.X., Ryan, K.J., Kiseleva, E., Levy, M.A., Allen, T.D. and Wente, S.R. (2000) Mol. Cell. Biol. 20, 5736–5748.
[68] Hodge, C.A., Colot, H.V., Stafford, P. and Cole, C.N. (1999) EMBO J. 18, 5778–5788.
[69] Schmitt, C., von Kobbe, C., Bachi, A., Panté, N., Rodrigues, J.P., Boscheron, C., Rigaut, G., Séraphin, B., Wilm, M., Fonseca, M.C. and Izaurralde, E. (1999) EMBO J. 18, 4332–4347.
[70] Strahm, Y., Fahrenkrog, B., Zenklusen, D., Rychner, E., Kantor, J., Rosbash, M. and Stutz, F. (1999) EMBO J. 18, 5761–5777.
[71] Bailer, S.M., Siniossoglou, S., Podtelejnikov, A., Hellwig, A., Mann, M. and Hurt, E. (1998) EMBO J. 17, 1107–1119.
[72] Kraemer, D. and Blobel, G. (1997) Proc. Natl. Acad. Sci. USA 94, 9119–9124.
[73] Pritchard, C.E., Fornerod, M., Kasper, L.H. and van Deursen, J.M. (1999) J. Cell Biol. 145, 237–254.
[74] Yoon, J.H., Love, D.C., Guhathakurta, A., Hanover, J.A. and Dhar, R. (2000) Mol. Cell. Biol. 20, 8767–8782.
[75] Komeili, A. and O'Shea, E.K. (2000) Curr. Opin. Cell Biol. 12, 355–360.
[76] York, J.D., Odom, A.R., Murphy, R., Ives, E.B. and Wente, S.R. (1999) Science 285, 96–100.
[77] Shen, E.C., Henry, M.F., Weiss, V.H., Valentini, S.R., Silver, P.A. and Lee, M.S. (1998) Genes Dev. 12, 679–691.
[78] Duncan, K., Umen, J.G. and Guthrie, C. (2000) Curr. Biol. 10, 687–696.
[79] Vainberg, I.E., Dower, K. and Rosbash, M. (2000) Mol. Cell. Biol. 20, 3996–4005.
[80] Saavedra, C.A., Hammell, C.M., Heath, C.V. and Cole, C.N. (1997) Genes Dev. 11, 2845–2856.
[81] Krebber, H., Taura, T., Lee, M.S. and Silver, P.A. (1999) Genes Dev. 13, 1994–2004.
[82] Jensen, T.H., Patricio, K., McCarthy, T. and Rosbash, M. (2001) Mol. Cell 7, 887–898.
[83] Conti, E. and Izaurralde, E. (2001) Curr. Opin. Cell Biol. (in press).
[84] Bangs, P., Burke, B., Powers, C., Craig, R., Purohit, A. and Doxsey, S. (1998) J. Cell Biol. 143, 1801–1812.
[85] Kosova, B., Pante, N., Rollenhagen, C., Podtelejnikov, A., Mann, M., Aebi, U. and Hurt, E. (2000) J. Biol. Chem. 275, 343–350.
[86] Strambio-de-Castillia, C., Blobel, G. and Rout, M.P. (1999) J. Cell Biol. 144, 839–855.
[87] Gallouzi, I.-E., Brennan, C.M., Stenberg, M.G., Swanson, M.S., Eversole, A., Maizels, N. and Steitz, J.A. (2000) Proc. Natl. Acad. Sci. 97, 3073–3078.
[88] Stutz, F., Kantor, J., Zhang, D., McCarthy, T., Neville, M. and Rosbash, M. (1997) Genes Dev. 11, 2857–2868.