
REVIEW

Transport of macromolecules between the nucleus and the cytoplasm

ELISA IZAURRALDE¹ and STEPHEN ADAM²

¹ University of Geneva, 30 quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

² Department of Cell and Molecular Biology, Northwestern University Medical School, Chicago, Illinois 60611, USA

ABSTRACT

Nuclear transport is an energy-dependent process mediated by saturable receptors. Import and export receptors are thought to recognize and bind to nuclear localization signals or nuclear export signals, respectively, in the transported molecules. The receptor–substrate interaction can be direct or mediated by an additional adapter protein. The transport receptors dock their cargoes to the nuclear pore complexes (NPC) and facilitate their translocation through the NPC. After delivering their cargoes, the receptors are recycled to initiate additional rounds of transport. Because a transport event for a cargo molecule is unidirectional, the transport receptors engage in asymmetric cycles of translocation across the NPC. The GTPase Ran acts as a molecular switch for receptor–cargo interaction and imparts directionality to the transport process.

Recently, the combined use of different *in vitro* and *in vivo* approaches has led to the characterization of novel import and export signals and to the identification of the first nuclear import and export receptors.

Keywords: nuclear transport; protein import; RNA export; Ran GTPase; importins; exportins

NUCLEAR IMPORT

The nuclear localization sequences

In the mid 1980s, identification of specific sequences required for the nuclear localization of several proteins opened the way for much of the experimental progress made since. Because these sequences were necessary and sufficient to target a protein to the nucleus, they were called nuclear localization sequences or NLSs (Kalderon et al., 1984; Lanford & Butel, 1984). These classical NLSs fall into two categories, a simple sequence of 3–5 basic amino acid residues, sometimes associated with a proline or glycine, and a bipartite signal comprised of the simple sequence 10 residues downstream from a basic dipeptide (reviewed by Dingwall & Laskey, 1991). Classical NLSs are also referred to as basic NLSs or canonical NLSs.

Recently, it was found that nuclear protein localization could be specified by different signals. For instance, several ribosomal proteins and hnRNP proteins do not contain classical NLSs (Moreland et al., 1985;

Underwood & Fried, 1990; Schaap et al., 1991; Schmidt et al., 1995; Siomi & Dreyfuss, 1995; Michael et al., 1997; Russo et al., 1997). This is also the case for several small nuclear RNA-binding proteins, such as U1 A (Kambach & Mattaj, 1992) and U170K (Romac et al., 1994). Among these nonclassical NLSs, the best characterized is the NLS of hnRNP A1 and related proteins. Called the M9 domain, the sequence consists of a glycine-rich sequence of 38 amino acids and is required for both import and export of A1 (Michael et al., 1995; Siomi & Dreyfuss, 1995). A second nonclassical NLS was identified recently in the hnRNP K protein and named the KNS (Michael et al., 1997). Certainly one of the most complex NLSs is the NLS present on small nuclear U snRNPs formed by the Sm core proteins binding to the U snRNA and trimethylation of the 5'-guanosine cap. Depending on the cell type or the U snRNA, the trimethyl guanosine cap is either essential or required for optimal U snRNP import efficiency (Fischer & Lührmann, 1990; Hamm et al., 1990; Fischer et al., 1993).

The NLS receptors

The basic paradigm of nuclear-cytoplasmic transport is that the signals for import or export are recognized by

Reprint requests to: Stephen Adam, Department of Cell and Molecular Biology, Northwestern University Medical School, W129 303 East Chicago Ave., Chicago, Illinois 60611, USA; e-mail: s-adam@nwu.edu.

saturable transport receptors that shuttle between nucleus and cytoplasm (reviewed by Görlich & Mattaj, 1996; Görlich, 1997; Nigg, 1997). According to this model, depicted in Figure 1, an import receptor binds its cargo in the cytoplasm and carries it through the NPC into the nucleus. On the nuclear side, the imported cargo is released and the receptor returns to the cytoplasm without the cargo. Conversely, an export receptor binds its export cargo in the nucleus and releases it in the cytoplasm. This model predicts that the different environments of the nucleus and cytoplasm regulate the binding of receptors to their cargoes. Several lines of evidence suggest that the small GTPase Ran is a major determinant of compartment identity by regulating cargo binding to and release from the receptors. In the following sections, we will review the NLS receptors characterized thus far and we will discuss how the GTPase Ran modulates the interaction of these receptors with their cargoes.

The heterodimeric receptor, Importin- α/β , mediates nuclear import of classical NLSs

In vitro analysis of protein import initially identified two distinct steps in the import pathway (Newmeyer & Forbes, 1988; Richardson et al., 1988). The first step is the association of the NLS-containing protein with the

cytoplasmic side of the pore complex, or docking. Docking activity could be reconstituted with two factors: a pair of 54/56-kDa proteins that bound directly to the NLS, and a 97-kDa protein that linked the 54/56-kDa proteins to the nuclear pore (Adam & Adam, 1994; Görlich et al., 1995; Radu et al., 1995). The second step, accumulation of the NLS protein in the nucleoplasm, was found to require the small GTPase Ran as well as the docking proteins (Melchior et al., 1993; Moore & Blobel, 1993). The cDNA sequences for the 54/56-kDa and 97-kDa proteins have been determined subsequently (reviewed in Görlich & Mattaj, 1996; Nigg, 1997). Because several different groups simultaneously reported the cDNA sequences of the proteins, they have come to be known by several names. The 54/56-kDa proteins are variously known as the NLS receptor/Importin- α /karyopherin- α /PTAC 58 in vertebrates and SRP1 in yeast. The 97-kDa protein is known as p97/Importin- β /karyopherin- β /PTAC 97 in vertebrates and Kap95p in yeast. Hereafter, we will refer to these proteins as Importin- α and Importin- β .

How do these components interact to achieve protein localization to the nucleus? Importin- α and - β dimerize in solution, and this dimer is sometimes referred to as the NLS receptor. It is not clear if the complex of Importin- α , Importin- β , and the NLS protein forms in the cytoplasm and subsequently docks at the pore, or if assembly occurs on the pore complex. Docking of

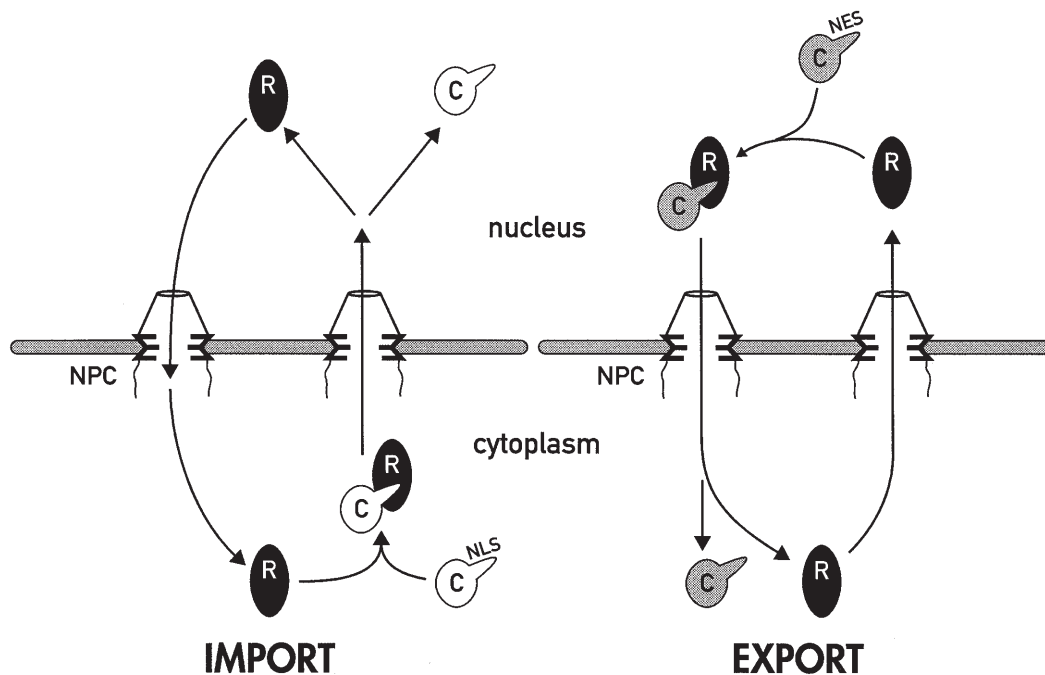


FIGURE 1. Shuttling receptor model. Nuclear import and export are mediated by shuttling receptors (R), which recognize and bind to nuclear localization sequences (NLS) or nuclear export sequences (NES), respectively. An import receptor binds its cargo (C) in the cytoplasm, translocates through the nuclear pore complex (NPC), and releases the cargo into the nucleoplasm. The receptor is recycled back to the cytoplasm to initiate another round of import. Conversely, an export receptor binds its cargo in the nucleoplasm and releases it in the cytoplasm.

this trimeric complex is mediated by the binding of Importin- β to components of the pore. In solution binding assays, Importin- β binds a family of nuclear pore complex proteins (nucleoporins) containing FG peptide repeats (Iovine et al., 1995; Moroianu et al., 1995; Radu et al., 1995). A number of nucleoporins contain these peptide repeats, creating the potential for multiple docking sites on the pore. One protein in particular, Nup358, also known as RanBP2 (Ran Binding Protein 2), is a prime candidate for initial docking. Localized to the distal ends of the cytoplasmic pore filaments, Nup358 binds Importin- β and Ran-GTP (Melchior et al., 1995; Wu et al., 1995; Yokoyama et al., 1995). This arrangement would place Nup358 in an ideal position to nucleate the assembly of a cytoplasmic import complex and allow its commitment to the import machinery.

Importin- β mediates import of different substrates by interacting with specific adaptors

Multicellular organisms encode a family of Importin α -like proteins sharing several structural features. On the other hand, yeast possess only a single Importin- α protein. Although sequence identity between members of the Importin- α family varies from 45% to 85%, the overall structure of the proteins is shared. Each is comprised of approximately 540 amino acids divided into a large central domain of eight armadillo-like repeats flanked by short amino and carboxyl termini. Within the amino-terminal region lies an NLS-like region of positively charged amino acids, the Importin- β binding or IBB domain, that interacts with Importin- β . The IBB domain is sufficient to bind Importin- β and direct a fused carrier protein to the nucleus (Görlich et al., 1996a; Weis et al., 1996b). In this respect, Importin- α can be considered an adaptor between the NLS protein and Importin- β ; the NLS protein is then a passive passenger. The role of the different Importin α -like proteins in nuclear import is not yet clear. Specificity for different NLSs has been demonstrated for two of the human Importin- α proteins and, in *Drosophila*, at least one Importin- α homologue is expressed in a tissue-specific manner (reviewed by Görlich, 1997). This suggests that Importin- β may mediate nuclear import of a variety of nuclear proteins by interacting with a multiplicity of adaptors with different specificities.

Snurportin, an α -like protein, is the adaptor for U snRNPs import

U snRNPs represent a distinct class of macromolecules imported into the nucleus. As described above, nuclear targeting relies on the Sm core proteins and the trimethyl-guanosine cap (m3G-cap). Recently, a 46-kDa protein that could be specifically crosslinked to

m3GpppAmpUmpCp was identified as an m3G-cap-binding protein (J. Huber, C. Marshallsay, U. Crontz-hagen, M. Sekine, & R. Lührmann, in prep.). This protein, Snurportin, stimulates import of microinjected U snRNPs in *Xenopus* oocytes, providing strong support for a direct role in this process. Interestingly, Snurportin is an Importin α -like protein, containing an IBB domain.

Importin- β may also mediate U snRNP import (Palacios et al., 1997). The Importin- α IBB domain specifically inhibited both protein and U snRNP nuclear uptake in vitro. In contrast, classical NLS peptides do not inhibit U snRNP import, indicating that U snRNPs do not require Importin- α for their import (Palacios et al., 1997 and references therein). Depletion of Importin- β from cytoplasmic extracts, which resulted in co-depletion of Importin- α , abolished both protein and U snRNP import. Restoration of U snRNP import required only the re-addition of recombinant Importin- β . Although it is likely that upon binding to the m3G cap on the U snRNPs Snurportin recruits Importin- β via the IBB domain, it is not yet clear how the Sm core proteins, carrying the essential import signal, are recognized. Snurportin may also recognize the Sm core or at least one additional essential adaptor may be missing from the picture.

A family of β -like proteins mediates import of karyophiles bearing nonclassical NLSs

One of the most exciting recent revelations in the transport field has been the identification of specific receptors for the import of different classes of nuclear proteins. Unlike the import of proteins containing a classical NLS, these proteins bind directly to an Importin β -like protein and do not require a separate adaptor or Importin α -like protein. The β -like proteins are members of a large family of Ran-GTP binding proteins exhibiting limited sequence similarity with the Ran binding domain of Importin- β (Fornerod et al., 1997b; Görlich et al., 1997).

The import receptor for the M9 sequence of hnRNP A1 is a β -like protein called Transportin in humans (Fig. 2; Nakielny et al., 1996; Pollard et al., 1996; Fridell et al., 1997). A similar protein in yeast, Kap104p, was co-isolated biochemically with the hnRNP proteins Nab2p and Hrp1p/Nab4p (Aitchison et al., 1996). A Kap104p mutant strain mislocalizes Nab2p at the non-permissive temperature. The import signal recognized by Kap104p has not been identified, but analysis of the Nab2p and Nab4p sequences does not identify a sequence conforming to the human M9 domain. Kap121p/Pse1p and Kap123p are receptors for the import of ribosomal proteins in yeast (Rout et al., 1997; Schlenstedt et al., 1997). A human putative homologue, RanBP7, has been described, but its role in any import process has not been demonstrated (Görlich et al., 1997).

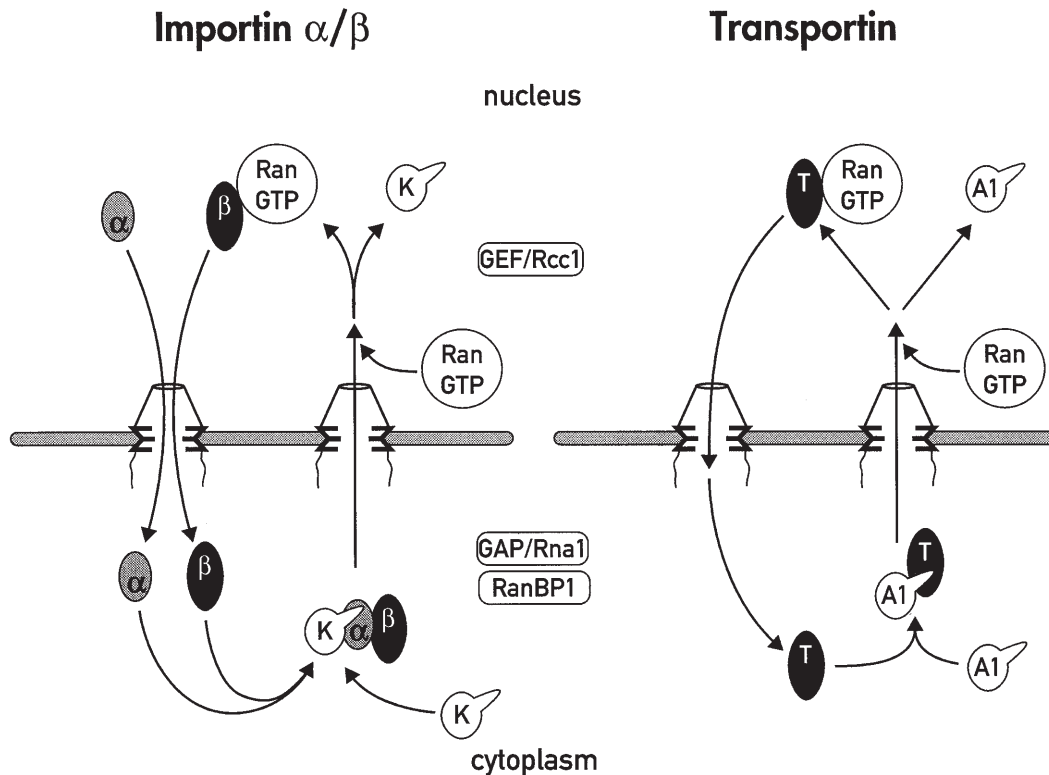


FIGURE 2. Importin and Transportin import cycles. Karyophilic proteins (K) bearing a canonical NLS are imported by the Importin- α/β complex. Importin- α directly binds the NLS and interacts with Importin- β via the IBB domain. Importin- β docks the complex to the NPC. Translocation of the trimeric complex (K/ α/β) requires Ran. After translocation, the high levels of Ran-GTP in the nucleoplasm promote the dissociation of the complex by direct binding to Importin- β . Importin- β and α are then recycled separately to the cytoplasm. CAS/Cse1p (see text) mediates export of Importin- α . Transportin mediates import of hnRNP A1 and related proteins having an NLS referred to as the M9 domain. Transportin binds directly to the M9 domain and does not require an adapter or an α -like protein. Ran regulates the Transportin-A1 interaction.

ROLE OF THE SMALL GTPase RAN AND ITS COFACTORS IN NUCLEAR TRANSPORT

The small GTPase, Ran, has been implicated in most nuclear import and export processes described thus far (reviewed by Koepp & Silver, 1996). Like other small GTPases, Ran is regulated by several cofactors that modulate its nucleotide-bound state (see Fig. 3). The Ran GTPase-activating protein-1 (RanGAP1) stimulates the intrinsic GTPase activity of Ran by up to five orders of magnitude and triggers the conversion of Ran-GTP into Ran-GDP (Bischoff et al., 1994, 1995a). The action of RanGAP1 is potentiated by Ran binding protein 1 (RanBP1, Coutavas et al., 1993), which co-stimulates the activity of RanGAP1 by a factor of 10 (Bischoff et al., 1995b). RanGAP1 and RanBP1 are predominantly cytoplasmic (Hopper et al., 1990; Melchior et al., 1993; Bischoff et al., 1995a; Richards et al., 1996). RanGAP1 modified by the ubiquitin-like molecule SUMO-1 is also found in association with the NPC (Matunis et al., 1996; Mahajan et al., 1997; Saitoh et al., 1997). In the nucleus, the chromatin-bound guanine nucleotide exchange factor, or GEF (RCC1), promotes the exchange of Ran-bound nucleotide. Because

the concentration of GTP in the cell is higher than that of GDP, RanGEF activity results in a net conversion of Ran-GDP into Ran-GTP (Ohtsubo et al., 1989; Bischoff & Ponstingl, 1991). Therefore, nuclear Ran is likely to be GTP-bound, whereas the small amounts of Ran found in the cytoplasm are likely to be in the GDP-bound form. The nucleotide-bound state of Ran can be thought of as a determinant of compartment identity.

How is Ran utilized during transport? Ran is required for the accumulation of NLS-containing cargoes in the nucleus in digitonin-permeabilized cells. In intact cells, Ran must cycle through the GTP- and GDP-bound forms during protein import (reviewed by Koepp & Silver, 1996). The mechanism of Ran action during import or export is still unknown. The interaction of the β -like receptors with their cargoes or with nucleoporins is regulated by the binding of Ran to the receptor (reviewed by Görlich, 1997). During export, the binding of Ran-GTP to the receptor is required for interaction of the receptor with its cargo (Fornerod et al., 1997a; Kutay et al., 1997; Stade et al., 1997). It is not clear if Ran-GTP binding is required for the binding of the receptor to the pore. The opposite situation exists during import. The binding of Ran-GTP to the import receptor pre-

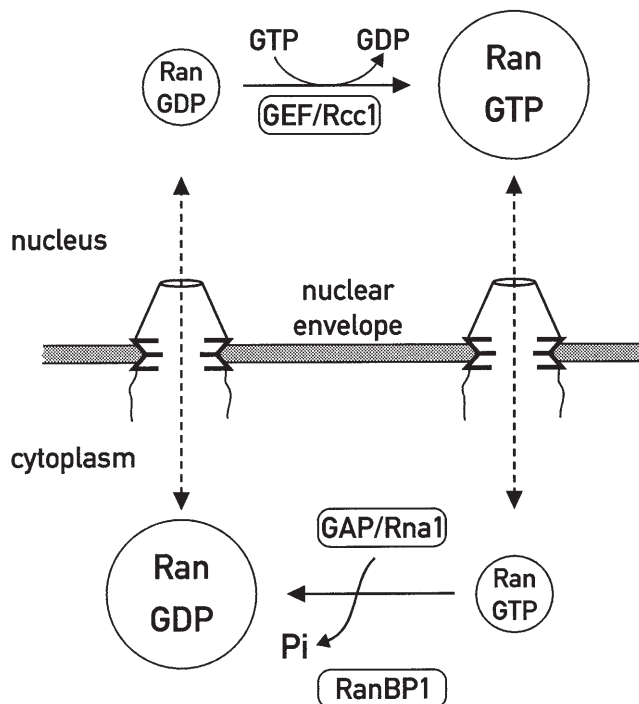


FIGURE 3. The nucleotide-bound state of Ran as a determinant of compartment identity. The small GTPase Ran can bind either GDP or GTP. The intrinsic GTPase activity of Ran is activated by the concerted action of the GTPase-activating protein (GAP, or Rna1p in yeast) and the Ran binding protein 1 (RanBP1). Because both of these proteins are localized in the cytoplasm, Ran would be mainly in the GDP-bound form in this compartment. The conversion of Ran-GDP into Ran-GTP requires the guanine nucleotide exchange factor (GEF or RCC1). Because RanGEF is tightly associated with chromatin, nuclear Ran is likely to be bound to GTP.

vents the interaction of the receptor with its cargo and the nuclear pore (Rexach & Blobel, 1995; Chi et al., 1996; Görlich et al., 1996c). Such an interaction of Ran-GTP with the import receptor would be useful for terminal dissociation of the import complex on the nuclear side of the pore, but would be clearly detrimental in the cytoplasm. A mutant form of Importin- β that cannot bind Ran cannot release its cargo on the nuclear side of the pore, supporting this suggestion (Görlich et al., 1996c). Importin- β also forms a tetrameric complex with Ran-GDP, RanBP1, and Importin- α in solution, and RanBP1 enhances docking and nuclear accumulation of proteins suggesting that such a complex might form in the cytoplasm during import (Chi et al., 1996). A mutant form of Importin- β that binds Ran-GTP but not Ran-GDP/RanBP1 docks at the pore normally, but cannot accumulate cargo within the nucleus (Chi et al., 1996). Such experiments suggest that both the GTP-bound and GDP-bound forms of Ran may participate in import of cargoes mediated by Importin- β .

During translocation through the pore, the import or export complex likely interacts with several different nucleoporins. During import, the initial docking of the receptor complex has been suggested to occur on a

single nucleoporin, RanBP2/Nup358 (Melchior et al., 1995). Nup358 binds Importin- β , Ran-GTP, and the SUMO-1-modified form of RanGAP1 (Wu et al., 1995; Yokoyama et al., 1995; Mahajan et al., 1997; Saitoh et al., 1997). This assemblage of factors suggests that the initial docking at the pore may involve a commitment step requiring the hydrolysis of GTP by Ran. Less is known about possible sites of association for the transport complex more internally in the pore structure. A more internally disposed nucleoporin complex, the p62 complex, localizes to the central core regions of the pore. This complex is noteworthy because of its association with the Ran-GDP binding protein, p10/NTF2 (Paschal & Gerace, 1995). NTF2 stimulates Importin- β -mediated import in vitro, but its role in import is less well characterized than other factors. In solution binding assays, NTF2 has been suggested to nucleate the assembly of several transport factors (Nehrbass & Blobel, 1996). However, the identity of the NPC component(s) that would translate Ran's GTP cycle into a directed movement through the NPC is not yet known. Note that there is some evidence that Ran may not be the only GTPase required for import in permeabilized cells (Sweet & Gerace, 1996), although other studies suggest that Ran accounts for the sole utilization of GTP (Weis et al., 1996a). It remains to be seen if another requirement for GTP or ATP has been overlooked in the in vitro assays.

NUCLEAR EXPORT

Although the export of monomeric proteins from the nucleus is likely to be an important activity, the vast majority of export substrates are complex ribonucleoproteins (RNPs), such as messenger RNPs and ribosomal subunits, each composed of RNA and multiple distinct proteins. Recent work has identified two export receptors that mediate export of monomeric proteins. One of the two is also involved in export of one class of RNA substrate, the U snRNAs.

In the next sections, we will focus on the RNA-binding proteins involved in export, the nature of their export signals and of their putative receptors, and how the small GTPase Ran and its cofactors determine the directionality of the export process.

RNA-binding proteins involved in nuclear export of cellular RNAs

More than a decade ago, Zasloff (1983) and coworkers showed that export of tRNA was temperature dependent and saturable, thus an active receptor-mediated process. Similar conclusions were drawn on the export of ribosomal subunits (Bataillé et al., 1990; Pokrywka & Goldfarb, 1995) and of polymerase II-transcribed RNAs (mRNAs and UsnRNAs) (Jarmolowski et al., 1994). The major classes of nuclear RNAs do not compete with

each other for export, suggesting that each class uses a distinct limiting export mediator.

During the last few years, progress has been made in the identification of saturable, class-specific, RNA export mediators. Paradoxically, although the export of tRNA and ribosomal subunits were the first mechanisms known to be receptor mediated, little is known about the factors involved. The ribosomal protein L5 and the transcription factor TFIIIA have been suggested to mediate 5S rRNA export (Guddat et al., 1990), but direct evidence is lacking.

U snRNA nuclear export: The role of the nuclear cap binding complex (CBC)

A heterodimeric nuclear CBC consisting of a cap binding protein of 80 kDa (CBP80) and a cap binding protein of 20 kDa (CBP20) (Izaurralde et al., 1994, 1995; Kataoka et al., 1994) is a limiting factor for the export of U snRNAs (Jarmolowski et al., 1994). Microinjection of antibodies to CBP20 that prevent CBC binding to the cap structure blocked U snRNA export in *Xenopus* oocytes (Izaurralde et al., 1995), providing direct evidence for a role of CBC in U snRNA export. The genes coding for the yeast homologues of CBP80 and CBP20 are not essential, although their disruption severely impairs vegetative growth (Colot et al., 1996; Görlich et al., 1996b and references therein; P. Fortes & I.W. Mattaj, pers. comm.). However, because export of U snRNAs has not been demonstrated to occur in yeast, it is not possible to ascertain if an essential role of CBC in U snRNA export is evolutionarily conserved.

mRNA export is likely to be mediated by hnRNP proteins

Messenger RNAs, like all polymerase II-transcribed RNAs, have a modified 5' cap structure. Electron microscopy studies on the Balbiani ring RNP particle of *Chironomus tentans* indicated that mRNA export occurs with the 5' end of the RNA interacting with the pore first and leading the way as the RNA translocates into the cytoplasm (reviewed by Daneholt, 1997). As with U snRNAs, the CBC binds to the 5' end of mRNA transcripts and remains bound to the cap during translocation of the RNP (Visa et al., 1996). Thus, the CBC could be involved in the association of the RNP particle with the NPC or in the translocation process itself. However, several lines of evidence suggest that the cap structure and CBC do not play an essential role in mRNA export (Jarmolowski et al., 1994). First, microinjection of anti-CBP20 antibodies into *Xenopus* oocyte nuclei did not affect mRNA export, whereas, as mentioned above, they completely blocked U snRNA export (Izaurralde et al., 1995). Moreover, yeast strains carrying conditional alleles of the gene encoding the guanylyl transferase subunit (CEG1) of the yeast capping en-

zyme under restrictive conditions (Fresco & Buratowski, 1996), or having a disruption of the genes encoding yCBP80 and yCBP20, do not show a defect in mRNA nuclear export as assessed by oligo(dT) in situ hybridization (P. Fortes & I.W. Mattaj, pers. comm.).

A second common feature of nuclear mRNP particles is the association of numerous RNA-binding proteins (hnRNP proteins) with each RNA. The shuttling of several hnRNP proteins between the nucleus and the cytoplasm suggests that they may be involved in the nucleocytoplasmic trafficking of mRNA (reviewed by Nakielny & Dreyfuss, 1997). Of the characterized hnRNP proteins, the shuttling of hnRNP A1 is the best understood. The M9 domain of A1 is required for both Transportin-dependent import (Nakielny et al., 1996; Pollard et al., 1996; Fridell et al., 1997) and nuclear export of the protein (Michael et al., 1995). Because A1 remains bound to the mRNA during translocation through the pore, it was suggested that A1 promotes export of the mRNA via the M9 domain. Nuclear injection of a large excess of hnRNP A1, but not of a mutant protein lacking the M9 domain, inhibits mRNA export (Izaurralde et al., 1997a). However, the M9 domain alone does not inhibit mRNA export, although it binds Transportin and saturates hnRNP A1 import both in vivo and in vitro (Pollard et al., 1996; Izaurralde et al., 1997a). These results indicate that A1 is involved in mRNA export, but this export is likely not to be mediated by Transportin (Izaurralde et al., 1997a, 1997b). In addition, export of some mRNAs is not inhibited by an excess of hnRNP A1 (Saavedra et al., 1997a), implying that mRNA export may not occur by a single mechanism. Export of an individual mRNA might depend on the pattern of associated hnRNP proteins and the type of export signals they carry. Consistent with this is the recent finding of a signal that confers shuttling on the hnRNP K protein (Michael et al., 1997). Interestingly, the K shuttling signal (KNS) is not conserved in *Xenopus* hnRNP K. It is not known which RNA, if any, utilizes the KNS for export.

Several yeast proteins structurally related to vertebrate hnRNP proteins have been described (Wilson et al., 1994). Npl3p/Nop3p is structurally and functionally related to hnRNP A1 (Flach et al., 1994; Wilson et al., 1994) and mutations in Npl3 that affect the export of polyadenylated mRNA from the nucleus have been described (Russel & Tollervey, 1995; Singleton et al., 1995). In spite of the structural and functional similarities, there are critical differences between the two proteins. The export of Npl3p/Nop3p from the nucleus requires continuous transcription of RNA, consistent with the idea that Npl3p/Nop3p leaves the nucleus only in association with RNA (Lee et al., 1996). HnRNP A1, on the other hand, leaves the nucleus independently of ongoing transcription, but its nuclear import is transcription dependent (Piñol-Roma & Dreyfuss, 1991). These data may suggest that hnRNP-like proteins

present in the yeast cell nucleus, in contrast with their vertebrate counterparts, do not directly provide the nuclear export signal (NES) to the mRNP export substrate, but may be involved in recruiting proteins that do have an NES to the mRNP (see below).

The NESs

One of the best characterized export factors is the HIV protein Rev. Rev promotes export of viral RNAs by binding to a *cis*-acting element, the Rev response element (RRE), through an RNA-binding domain. A second domain, called the Rev activation domain, functions as an NES (for a review see Cullen & Malim, 1991 and references therein). Peptides comprising the NES trigger rapid export of large reporter proteins that would otherwise be restricted to the nucleus (Fischer et al., 1995; Wen et al., 1995). The Rev NES consist of a short leucine-rich stretch of amino acids with the consensus: Leu-X₂₋₃-Y-X₂₋₃-Leu-X-Leu/Ile, where X represents any amino acid and Y represents Leu/Ile/Phe/Val or Met (Bogerd et al., 1996). To avoid confusion, this type of NES is referred to as the Rev-like NES or leucine-rich NES. The leucine-rich NES is present in several proteins, including the transcription factor TFIIIA (Fischer et al., 1995; Fridell et al., 1996), involved in 5S rRNA export, and in two yeast nuclear pore-associated proteins, Gle1p (Murphy & Wenthe, 1996) and Mex67p (Segref et al., 1997). A functional Rev-like NES is also present in proteins that are not known to be involved in RNA export, such as PKI (Wen et al., 1995), IκB (Wen et al., 1995; Fritz & Green, 1996), RanBP1 (Richards et al., 1996; Zolotukhin & Felber, 1997), and MAPKK (Fukuda et al., 1996).

The U snRNP and 5S rRNP export substrates carry a Rev-like NES

Rev NES peptides have been used to identify cellular RNAs that are likely to be exported by factors carrying a Rev-like NES. In *Xenopus* oocytes, Rev-NES conjugates saturate U snRNA and 5S rRNA export without affecting mRNA or tRNA export (Fischer et al., 1995). The saturation of 5S rRNA export could be explained in part by the observation that TFIIIA has a Rev-like NES (Fischer et al., 1995; Fridell et al., 1996). Export of 5S rRNA can be mediated by either TFIIIA or ribosomal protein L5, which does not contain a Rev-like NES. Therefore, one would predict that, if TFIIIA-mediated export is blocked, 5S rRNA molecules should access the cytoplasm by the L5 pathway. However, this is not the case, suggesting the involvement of an additional leucine-rich NES-bearing protein in 5S rRNA export. In that respect, it would be interesting to know if export of mutant 5S rRNA molecules, that cannot longer bind TFIIIA, can still be inhibited by an excess of Rev NES peptides.

In the case of U snRNAs, it is not yet clear whether the export signal is carried directly by CBC or if CBC binding allows addition of other NES-bearing proteins, because U snRNAs form sizeable particles before export (Terns et al., 1993).

The mRNP NESs

The export mediators for mRNA and tRNA export are not saturated by the Rev NES and probably recognize different signals. This is supported by the observation that hnRNP A1, which does not contain a leucine-rich NES, may be involved in the export of some mRNAs (Izaurralde et al., 1997a). Two yeast proteins involved in mRNA export, Gle1p/Rss1p (Del Priore et al., 1996; Murphy & Wenthe, 1996) and Mex67p (Segref et al., 1997), contain Rev-like NESs. Mutations in the NESs of Gle1p and Mex67p result in nuclear accumulation of polyadenylated RNA (Murphy & Wenthe, 1996; Segref et al., 1997). The Mex67p NES saturates Rev-mediated export and export of U1 snRNA, but does not affect mRNA export in vertebrate cells (Segref et al., 1997). Furthermore, the NESs are not conserved in the human homologues of Mex67p, TAP (Segref et al., 1997), or Gle1p (S. Wenthe, pers. comm.). These observations raise the possibility that the NESs in Mex67p and Gle1p are not acting as export signals for mRNA. Thus, mRNA export in yeast as well as vertebrates would not be using a Rev-like NES pathway. Alternatively, it is possible that in yeast the major mRNA export pathway is the Rev-like NES pathway. During evolution, the need to specifically regulate export of some mRNAs may have led to the appearance of alternative pathways, such that the NES pathway is essentially used by U snRNAs and 5S rRNA, but not by mRNAs. Although we cannot exclude that some mRNAs would still use the Rev-NES pathway in higher eukaryotes, it is tempting to speculate that the large number and diversity of hnRNP proteins in these organisms versus yeast may correlate with the diversity of export pathways available for mRNAs.

How many different NESs exist and how many different pathways from the nucleus are available to mRNAs? In spite of the limited number of mRNAs and proteins that have been analyzed for export, we know already three different classes of export signals: the Rev-like NES, the M9 domain, and the recently described shuttling signal in hnRNP K (KNS). Taking into account that about 20 different hnRNP proteins have been characterized in vertebrate cells (reviewed by Dreyfuss et al., 1993) and that many also shuttle, one can predict that additional export signals will be described.

Most nuclear export processes require Ran-GTP in the nucleus

A number of studies indicate that Ran and its regulatory proteins are required for the export of mRNAs and

snRNAs. However, these studies did not distinguish whether Ran was directly or indirectly involved in export. Depletion of Ran-GTP from the nuclear compartment by injection of either RanGAP1, RanBP1, or a Ran mutant that cannot stably bind GTP (RanT24N) blocks all major export pathways across the NPC (Izaurralde et al., 1997b), including export of mRNA, U snRNA, tRNA, Importin- α , and Rev protein. Export of Importin- β and Transportin was also severely compromised. The export block was not a secondary consequence of import inhibition because, under these conditions, nuclear uptake of either NLS or M9 reporter proteins was not affected. In contrast, introduction of a Ran mutant deficient in GTP hydrolysis (RanQ69L, Klebe et al., 1995) did not affect export of several substrates, including Importin- α and - β , Transportin, Rev-like NESs, and tRNA. These results suggest that the export of most molecules from the nucleus requires nuclear Ran-GTP, but may be independent of GTP hydrolysis by Ran (Izaurralde et al., 1997b; Richards et al., 1997). Earlier studies with the tsBN2 cell line, which contains a temperature-sensitive RCC1, showed that at the nonpermissive temperature tRNA was exported, whereas both mRNA and U snRNA accumulated in the nucleus. This result was interpreted as suggesting that tRNA export occurs independently of the Ran system (Cheng et al., 1995; see Goldfarb, 1997). An alternative explanation for this observation could be that the levels of nuclear Ran-GTP are depleted slowly upon inactivation of RCC1. Consistent with this second suggestion, Richards et al. (1997) have shown that tsBN2 cells at the nonpermissive temperature export the Rev-NES, indicating that sufficient Ran-GTP remains in the nucleoplasm to support some export. Rev-NES export could only be inhibited after the shift to the nonpermissive temperature by microinjection of RanGAP1 into the nucleus.

In summary, most export processes analyzed so far require Ran-GTP in the nucleus, however, not all depend on GTP hydrolysis by Ran. This would, in turn, suggest that the energy required for translocation is provided by another source, which remains to be identified.

The nuclear export receptors or exportins

In the Importin-mediated nuclear protein import pathway, free nuclear Ran-GTP is thought to trigger the release of the cargo on the nucleoplasmic side of the pore by direct binding to Importin- β (Rexach et al., 1995; Chi et al., 1996; Görlich et al., 1996c). Conversely, it is the presence of Ran-GTP in the nucleus that allows the formation of the receptor-cargo complexes for export (Fornerod et al., 1997a; Kutay et al., 1997; Stade et al., 1997). These observations provide a rationale for the requirement for nuclear Ran-GTP in most export processes described.

Chromosomal region maintenance (CRM1) is the export receptor for Rev-like NESs

Recently, the Rev-like NES receptor was identified as a previously described protein CRM1 (Fornerod et al., 1997a; Stade et al., 1997). CRM1 was originally identified in *Schizosaccharomyces pombe* as a gene that is required for normal chromosome morphology (Adachi & Yanagida, 1989). CRM1 belongs to the family of Ran binding proteins that share similarities with the Ran binding domain of Importin- β (Fornerod et al., 1997b; Görlich et al., 1997). CRM1 also interacts with the nucleoporin Nup214/CAN and was therefore predicted to be a transport receptor. The cytotoxin Leptomycin B provided two keys to the identification of CRM1 as the NES receptor. First, in *S. pombe*, resistance to Leptomycin B was mapped to the CRM1 gene (Nishi et al., 1994). Second, Leptomycin B inhibits Rev-mediated export in mammalian cells (Wolff et al., 1997) and *Xenopus* oocytes (Fornerod et al., 1997a).

Leptomycin B-mediated inhibition of U snRNA and Rev protein export could be rescued by overexpression of CRM1. Direct interaction of recombinant CRM1 with Rev-NES peptide conjugates could be reconstituted in vitro in the presence of Ran-GTP. Furthermore, this interaction could be competed by Leptomycin B. Independently, Stade et al. (1997) isolated a conditional mutant of CRM1 that blocks Rev NES export at the nonpermissive temperature. Together, these results provide strong evidence for a direct role of CRM1 in mediating Rev-like NES nuclear export. Thus, the export of NES-containing proteins fits to a simple model in which the NES binds to CRM1 in the nucleus due to the high concentration of Ran-GTP there. This complex is translocated through the NPC. On the cytoplasmic side, the presence of RanGAP and RanBP1 leads to hydrolysis of the Ran-bound GTP and the release of the NES-protein (Fig. 4). Although this model is a plausible description of NES-protein export, it is, however, not clear how CRM1 is involved in the export of more complex RNP substrates.

Two-hybrid screenings using Rev-NES and yeast or human libraries led to the isolation of human or yeast NES interacting proteins (hRip/Rab, yRip; Bogerd et al., 1995; Fritz et al., 1995; Stutz et al., 1995). The human and the yeast Rips contain repeated motifs present in several nucleoporins, but do not share any other common motif. Both are localized at the nuclear pore, although cytoplasmic staining is observed also for the human proteins. Rip is a nonessential protein, and yeast strains carrying a disruption of this gene show a partial reduction of Rev-mediated export (Stutz et al., 1995). However, because a direct interaction between Rev-NES and Rip was difficult to obtain with recombinant proteins and Rev-NES interacts with several nucleoporins, including CAN/Nup214, in the two-hybrid assay (Fritz & Green, 1996; Stutz et al., 1996), it

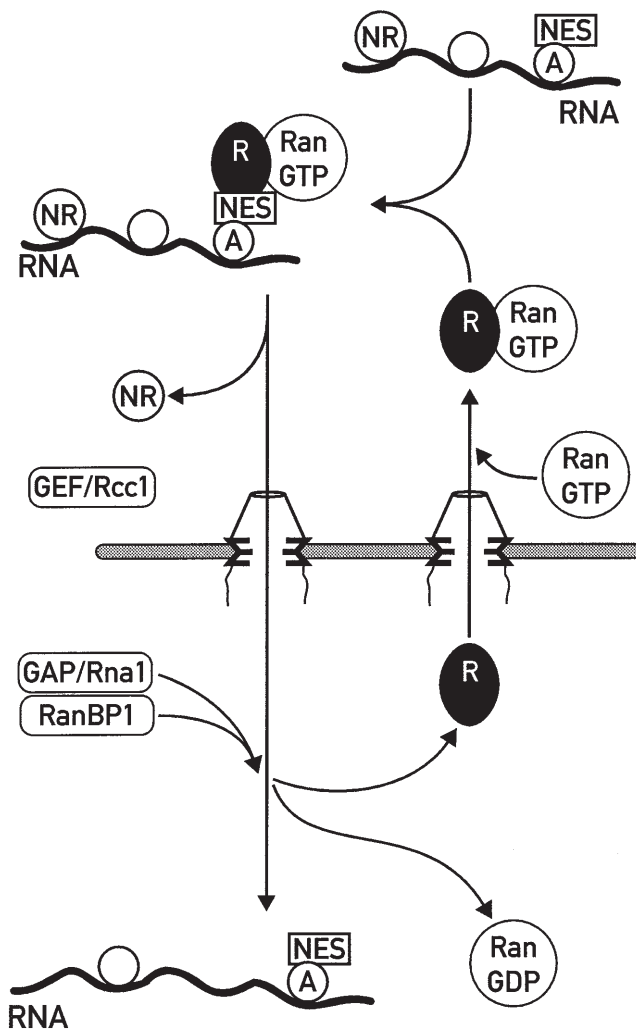


FIGURE 4. Steps in RNA nuclear export. RNAs are exported in association with proteins as large RNP complexes. The RNP NESs are likely to be provided by some of the RNP-associated proteins. RNA-binding proteins bearing an NES are defined as adapters (A). The presence of high concentrations of Ran-GTP in the nucleus facilitates the binding of the export receptors (R) with the NES. Prior to translocation, some nuclear retention (NR) factors are stripped from the RNA. In the cytoplasm, RanGAP and RanBP1 promote GTP hydrolysis by Ran and thus the dissociation of the receptor RNP complexes.

is likely that these interactions were mediated by the endogenous yeast CRM1 protein. Indeed, a recent report demonstrated that, in three CRM1 mutant strains, Rev-NES/nucleoporin interactions are no longer observed, but can be reestablished by introducing the wild-type CRM1 gene (Neville et al., 1997). In these strains, mRNA export was not affected. Thus, although it is clear that CRM1 is an export receptor for Rev-like NESs that interact with both nucleoporins and Ran, its role in mRNA export in yeast needs further investigation. In contrast, in higher eukaryotes, the lack of specific inhibition of mRNA export with BSA-NES conjugates (see above) and with Leptomycin B (Fornerod et al., 1997a) strongly suggests that mRNAs are not exported via the Rev-NES pathway.

Cellular apoptosis susceptibility protein (CAS) is the nuclear export receptor of Importin- α

The receptor for the classical NLS-mediated import pathway is comprised of Importin- α and Importin- β . After translocation of the receptor through the pore and release of the cargo in the nucleus, the two subunits are returned separately to the cytoplasm. The protein responsible for the export of Importin- α was identified recently as the CAS protein (Kutay et al., 1997). CAS was isolated originally as a protein implicated in apoptosis and cell proliferation (Brinkmann et al., 1996). The observation that CAS belongs to the family of Ran binding proteins sharing similarities with the Ran binding domain of Importin- β (Görlich et al., 1997) suggested that CAS was a potential transport receptor whose substrate binding could be modulated by Ran. Indeed, CAS binds Importin- α preferentially in the presence of Ran-GTP, forming a trimeric complex. Dissociation of this complex requires the action of RanBP1 and is therefore likely to occur immediately after translocation to the cytoplasm.

Nuclear retention versus export

Binding of the RNA class-specific export factors is probably the first step in the commitment of an RNA to a transport pathway. However, binding of hnRNP proteins to a primary transcript is not sufficient for export. For instance, unspliced pre-mRNAs associated with hnRNP A1 are not exported. These observations lead to the suggestion that factors mediating nuclear retention, such as the spliceosome, must be removed from the RNA before export can take place (Legrain & Rosbash, 1989). Little is known about the mechanism underlying nuclear retention, but the recent identification of a dominant nuclear retention signal (NRS) in hnRNP C suggests that the selection of RNAs for export may involve removal of specific retention factors (Nakielny & Dreyfuss, 1996). Indeed, it has been reported that several proteins that are associated with the mRNP in the nucleoplasm are selectively removed prior to or during translocation through the pore (reviewed by Daneholt, 1997).

Viral RNA elements can override nuclear retention

Retroviruses have evolved a mechanism that allows the nuclear export of unspliced forms of viral RNAs necessary for viral replication. In complex retroviruses such as HIV-1, this occurs by the interaction of the viral Rev protein with an RNA sequence, the RRE (Malim et al., 1989). The complex is then exported by virtue of the Rev-NES (Fischer et al., 1995). Simple retroviruses do not encode a Rev-like *trans*-acting protein and export of their unspliced RNA relies on the interaction of

an RNA element with cellular factors. In simian type D retrovirus, this sequence element is referred to as CTE, constitutive transport element, because its interaction with cellular factors results in constitutive export of unspliced viral RNAs (Taberner et al., 1996; Ernst et al., 1997). Cellular factor(s) then may play a role analogous to that of Rev protein. Substitution of the RRE by the SRV-1 CTE renders HIV-1 replication Rev-independent (Zolotukhin et al., 1994), reinforcing the idea that the CTE directs unspliced RNAs to the same export pathway used by Rev protein. Surprisingly, this is not the case. Microinjection experiments in *Xenopus* oocytes (Pasquinelli et al., 1997; Saavedra et al., 1997a) and transfection experiments in mammalian cells (Zolotukhin & Felber, 1997) demonstrate that the CTE promotes export of intron-containing RNAs by a pathway distinct from that used by the RRE-Rev system. Furthermore, although CRM1 mediates Rev and U snRNAs export, the CTE interacts with factors required for the export of cellular mRNAs (Pasquinelli et al., 1997; Saavedra et al., 1997a). Thus, it seems that different retroviruses have recruited different cellular factors to accomplish the same function: overcoming nuclear retention and promoting export of their RNAs. Although it is likely that Rev exports RRE-containing RNAs by interacting with CRM1, it is not obvious how Rev, or the CTE binding factors, overcome retention. The study of viral elements that bypass nuclear retention may shed light on this poorly understood intranuclear mechanism.

Regulated nuclear export of cellular RNAs

Only two examples of regulation of cellular nuclear RNA export are known. Maternal histone mRNAs in sea urchin oocytes are retained in the nucleus till the onset of oocyte maturation (Showman et al., 1982; De Leon et al., 1983). In the absence of stress, the cellular pathway for mRNAs exports yeast heat-shock mRNAs. Following heat or ethanol stress, the bulk of polyadenylated RNAs is retained in the nucleus, whereas heat-shock mRNAs are exported by a specific pathway that does not require Npl3p and seems to be independent of the RanGTPase cycle (Saavedra et al., 1996, 1997b). Disruption of the nonessential RIP1 gene blocks the heat-shock pathway following stress (Saavedra et al., 1997b; Stutz et al., 1997). Rip1p previously has been proposed to mediate Rev function in yeast (Stutz et al., 1995). Consistent with this proposal, overexpression of the HIV-1 Rev protein leads to a partial block of heat shock mRNA export following stress (Saavedra et al., 1997b). Furthermore, Rip1p interacts genetically with Gle1p/Rss1p (Murphy & Wentz, 1996; Stutz et al., 1997). Because Gle1p exhibits a Rev-like NES and is essential for mRNA export under normal growth conditions, these observations provide further support to the hypothesis that, in yeast, mRNA and heat shock mRNA export pathways are somehow linked to the Rev-like

NESs export pathway (see above, Murphy & Wentz, 1996; Segref et al., 1997; Stade et al., 1997).

Recycling of export factors: The link between export and import

One important consequence of the shuttling receptor concept is that, once the cargo is delivered in one compartment, the receptors need to be recycled to the original compartment to initiate further rounds of transport. Shuttling, the cyclic movement of a molecule between the nucleus and cytoplasm, seems to be an intrinsic property of the transport receptors and mediators. CBC provides a clear example of the shuttling of a transport mediator and the link between import and export. In *Xenopus* oocytes, CBC exists in complex with a fraction of Importin- α . Binding of Importin- α to CBC is mediated by a bipartite NLS present on CBP80. The heterotrimer, CBP80/CBP20/Importin- α , binds capped RNAs. However, when Importin- β interacts with the CBC-bound Importin- α , capped RNA is released (Görllich et al., 1996b). As discussed above, the asymmetric distribution of Ran-GTP only allows the Importin- α/β interaction to occur in the cytoplasm. Thus, in the cytoplasm, binding of Importin- β leads to the formation of β/α /CBC complexes that will promote the release of the export substrate and the commitment of CBC to nuclear re-entry. Regardless of whether Importin- α binding occurs before or after translocation, the end result of Importin- β addition will be the release of the RNA in the cytoplasm and the recycling of CBC to the nucleus. This provides an economical and efficient mechanism to accomplish two goals: RNA release and recycling of an RNA-export mediator to the nucleus. It remains to be elucidated how other RNA-binding proteins that accompany the export substrate to the cytoplasm are removed from the RNA and returned to the nucleus.

Different export pathways converge at the NPC

The existence of specific RNA export factors does not exclude that some nonlimiting factors may be common and play a role in the export of all RNA classes. Evidence for common steps in RNA export came from the use of inhibitors that affect more than one RNA class, such as WGA (Neuman de Vegvar & Dahlberg, 1990), anti-nucleoporin antibodies (Featherstone et al., 1988; Terns & Dahlberg, 1994), and Rev-NES peptide conjugates (Fischer et al., 1995). It is not surprising that WGA and the monoclonal antibody mab414 block several export processes because they each bind to multiple nucleoporins. Powers et al. (1997) have reported that microinjection of antibodies raised against the nucleoporin Nup98p inhibited U snRNA and mRNA export without affecting tRNA export. In another study, overexpression of Nup153 in mammalian cells resulted in nuclear accumulation of poly(A)⁺ RNA (Bastos et al.,

1996). Interestingly, the overexpressed C-terminal domain of Nup153 accumulated in the cytoplasm and inhibited mRNA export, suggesting that this domain may be titrating a soluble factor required for export of cellular mRNAs. In both studies, protein import was not affected, suggesting that Nup98p and Nup153p have a function only in export. This is consistent with their localization at the nucleoplasmic side of the pore complex. Analysis of the role of specific nucleoporins on transport is far more advanced in yeast (for a recent review, see Doye & Hurt, 1997). Although some nucleoporins may affect nuclear export indirectly, either by blocking protein import or by affecting the structure of the NPC, a direct role of some nucleoporins in mRNA export has been suggested by the existence of conditional mutants that led to a rapid accumulation of poly(A)⁺ RNA in the nucleus, at the nonpermissive temperature, without affecting protein import. Nucleoporins of these class are Nup133p/Rat3p, Nup159/Rat7p, Nup120p/Rat2p, Nup84p, Nup85p/Rat9p, Gle2p/Nup40p/Rae1p, Gle1p/Brr3/Rss1p, and Mex67p (see Doye & Hurt, 1997 and references therein).

PERSPECTIVES

The past years have seen great progress in the characterization of import and export signals and the identification of the receptors that recognize them. We are also close to understanding how the receptor-cargo interactions are regulated and how these interactions impart directionality to the transport process. However, the mechanism for movement of macromolecules through the pore remains a challenge for the foreseeable future. Two working models suggested by structural and biochemical analyses of the pore complex have been proposed. The first model proposes that transport is a stochastic process, mediated by repeated assembly and disassembly of transport complexes on an array of nucleoporin binding sites through the pore (Rexach & Blobel, 1995). The second model proposes that a discrete number of binding sites mediate movement of the transport complex, with involvement of the cytoplasmic pore filaments (Panté & Aebi, 1996). Whether there are few or many binding sites for the transport complex as it moves through the pore, the physical interactions of all of the transport factors will need to be known to fully understand this process.

The question of movement of transport complexes through the pore will be addressed more readily when we know more about the structure of the pore. The recent sequencing of the yeast genome and the identification of nucleoporins in purified yeast nuclear pores means that we will soon have in hand many of the components of the pore, at least in yeast (Rout & Blobel, 1993). One difficulty in defining nucleoporin functions has been the lack of clear counterparts between yeast and vertebrate nucleoporins. Much of the de-

tailed pore structure and localization of pore proteins has been done on *Xenopus* oocyte nuclear pores (Panté & Aebi, 1993). It would be extremely useful if the sequence information and genetic interactions of nucleoporins derived from yeast could be tied to the *Xenopus* structural work.

Probably the least clear aspect of transport is the role of energy in the process. Is the energy released upon GTP hydrolysis by Ran utilized for a mechanical process or is GTP hydrolysis merely needed to maintain the proper ratio of Ran-GDP to Ran-GTP? Are there other GTPase or ATPases involved and how are they utilized? Does movement through the pore require a motor molecule or are guided diffusion pathways sufficient to explain the transport kinetics observed? If these questions are answered as quickly as the receptors have been identified, we should very soon have a clearer picture of the mechanism of nucleo-cytoplasmic transport.

ACKNOWLEDGMENTS

We thank the many colleagues who helped in the preparation of this review by providing us with unpublished information. We also thank J.E. Dahlberg, D. Görlich, A. Jarmolowski, and I.W. Mattaj for critical comments on the manuscript. We apologize to those authors whose work was not cited owing to space limitations.

Received October 7, 1997; returned for revision October 24, 1997; revised manuscript received January 12, 1998

REFERENCES

- Adachi Y, Yanagida M. 1989. Higher order chromosome structure is affected by cold-sensitive mutations in a *Schizosaccharomyces pombe* gene CRM1+ which encodes a 115-kD protein preferentially localized in the nucleus and its periphery. *J Cell Biol* 108: 1195–1207.
- Adam EJH, Adam SA. 1994. Identification of cytosolic factors required for nuclear location sequence-mediated binding to the nuclear envelope. *J Cell Biol* 125:547–555.
- Aitchison JD, Blobel G, Rout MP. 1996. Kap104p: A karyopherin involved in the nuclear transport of messenger RNA binding proteins. *Science* 274:624–627.
- Bastos R, Lin A, Enarson M, Burke B. 1996. Targeting and function in mRNA nuclear export of nuclear pore complex protein Nup153. *J Cell Biol* 134:1141–1156.
- Bataillé N, Helser T, Fried HM. 1990. Cytoplasmic transport of ribosomal subunits microinjected into the *Xenopus laevis* oocyte nucleus: A generalized, facilitated process. *J Cell Biol* 111:1571–1582.
- Bischoff FR, Klebe C, Kretschmer J, Wittinghofer A, Ponstingl H. 1994. RanGAP1 induces GTPase activity of nuclear ras-related Ran. *Proc Natl Acad Sci USA* 91:2587–2591.
- Bischoff FR, Krebber H, Kempf T, Hermes I, Ponstingl H. 1995a. Human RanGTPase activating protein RanGAP1 is a homolog of yeast RNA1p involved in messenger RNA processing and transport. *Proc Natl Acad Sci USA* 92:1749–1753.
- Bischoff FR, Krebber H, Smirnova E, Dong WH, Ponstingl H. 1995b. Coactivation of RanGTPase and inhibition of GTP dissociation by Ran GTP binding protein RanBP1. *EMBO J* 14:705–715.
- Bischoff FR, Ponstingl H. 1991. Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. *Nature* 354:480–482.

- Bogerd HP, Fridell RA, Benson RE, Hua J, Cullen BR. 1996. Protein sequence requirements for function of the human T-cell leukemia virus type I Rex nuclear export signal delineated by a novel in vivo randomization-selection assay. *Mol Cell Biol* 16:4207–4214.
- Bogerd HP, Fridell RA, Madore S, Cullen BR. 1995. Identification of a novel cellular cofactor for the Rev/Rex class of retroviral regulatory proteins. *Cell* 82:485–494.
- Brinkmann U, Brinkmann E, Gallo M, Scherf U, Pastan I. 1996. Role of CAS, a human homologue of the yeast chromosome segregation gene CSE1, in toxin and tumor necrosis factor mediated apoptosis. *Biochemistry* 35:6891–6899.
- Cheng Y, Dahlberg JE, Lund E. 1995. Diverse effects of the guanine nucleotide exchange factor RCC1 on RNA transport. *Science* 267:1807–1810.
- Chi NC, Adam EJH, Visser GD, Adam SA. 1996. RanBP1 stabilizes the interaction of Ran with p97 in nuclear protein import. *J Cell Biol* 135:559–569.
- Colot H, Stutz F, Rosbash M. 1996. The yeast splicing factor Mud13p is a commitment complex component corresponding to CBP20, the small subunit of the nuclear cap binding complex. *Genes & Dev* 10:1699–1708.
- Coutavas E, Ren M, Oppenheim JD, D'Eustachio P, Rush MG. 1993. Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature* 366:585–587.
- Cullen BR, Malim MH. 1991. The HIV-1 Rev protein: Prototype of a novel class of eukaryotic post-transcriptional regulators. *Trends Biochem Sci* 16:346–350.
- Daneholt B. 1997. A look at messenger RNP moving through the nuclear pore. *Cell* 88:585–588.
- De Leon DV, Cox KH, Angerer LM, Angerer RC. 1983. Most early-variant histone mRNA is contained in the pronucleus of sea urchin eggs. *Dev Biol* 100:197–206.
- Del Priore V, Snay CA, Bahr A, Cole CN. 1996. The product of the *Saccharomyces cerevisiae* RSS1 gene, identified as a high-copy suppressor of the rat7-1 temperature-sensitive allele of the RAT7/NUP159 nucleoporin, is required for efficient mRNA export. *Mol Biol Cell* 7:1601–1621.
- Dingwall C, Laskey RA. 1991. Nuclear targeting sequences—A consensus. *Trends Biochem Sci* 16:478–481.
- Doye V, Hurt E. 1997. From nucleoporins to nuclear pore complexes. *Curr Opin Cell Biol* 9:401–411.
- Dreyfuss G, Matunis MJ, Piñol-Roma S, Burd CG. 1993. hnRNP proteins and the biogenesis of mRNA. *Annu Rev Biochem* 62:289–321.
- Ernst RK, Bray M, Rekosh D, Hammarskjöld ML. 1997. A structured retroviral RNA element that mediates nucleocytoplasmic export of intron-containing RNA. *Mol Cell Biol* 17:135–144.
- Featherstone C, Darby MK, Gerace L. 1988. A monoclonal antibody against the nuclear pore complex inhibits nucleocytoplasmic transport of protein and RNA in vivo. *J Cell Biol* 107:1289–1297.
- Fischer U, Huber J, Boelens WC, Mattaj IW, Lührmann R. 1995. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell* 82:475–483.
- Fischer U, Lührmann R. 1990. An essential signaling role for the m3G cap in the transport of U1 snRNP to the nucleus. *Science* 249:786–790.
- Fischer U, Sumpter V, Sekine M, Satoh T, Lührmann R. 1993. Nucleocytoplasmic transport of U snRNPs: Definition of a nuclear location signal in the Sm core domain that binds a transport receptor independently of the m3G cap. *EMBO J* 12:573–583.
- Flach J, Bossie M, Vogel J, Corbett A, Jinks T, Willins DA, Silver PA. 1994. A yeast RNA-binding protein shuttles between nucleus and cytoplasm. *Mol Cell Biol* 14:8399–8407.
- Fornerod M, Ohno M, Yoshida M, Mattaj IW. 1997a. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* 90:1051–1060.
- Fornerod M, van Deursen J, van Baal S, Reynolds A, Davis D, Murti KG, Fransen J, Grosveld G. 1997b. The human homologue of yeast Crm1 is in a dynamic subcomplex with Can/Nup214 and a novel nuclear pore component Nup 88. *EMBO J* 16:807–816.
- Fresco LD, Buratowski S. 1996. Defects in the yeast capping enzyme cause an inhibition of mRNA splicing. *RNA* 2:584–596.
- Fridell RA, Fischer U, Lührmann R, Meyer, BE, Meinkoth J, Malim MH, Cullen BR. 1996. Amphibian transcription factor IIIA proteins contain a sequence element functionally equivalent to the nuclear export signal of human immunodeficiency virus type 1 Rev. *Proc Natl Acad Sci USA* 93:2936–2940.
- Fridell RA, Truant R, Thorne L, Benson RE, Cullen BR. 1997. Nuclear import of hnRNP A1 is mediated by a novel cellular cofactor related to karyopherin- β . *J Cell Sci* 110:1325–1331.
- Fritz CC, Green MR. 1996. HIV Rev uses a conserved cellular protein export pathway for the nucleocytoplasmic transport of viral RNAs. *Curr Biol* 6:848–854.
- Fritz CC, Zapp ML, Green MR. 1995. A human nucleoporin-like protein that specifically interacts with HIV Rev. *Nature* 376:530–533.
- Fukuda M, Gotoh I, Gotoh Y, Nishida E. 1996. Cytoplasmic localization of MAP kinase kinase directed by its N-terminal, leucine rich short amino acid sequence, which acts as a nuclear export signal. *J Biol Chem* 271:20024–20028.
- Goldfarb DS. 1997. Whose finger is on the switch? *Science* 276:1814–1816.
- Görlich D. 1997. Nuclear protein import. *Curr Opin Cell Biol* 9:412–419.
- Görlich D, Dabrowski M, Bischoff FR, Kutay U, Bork P, Hartmann E, Prehn S, Izaurralde E. 1997. A novel class of Ran-GTP binding proteins. *J Cell Biol* 138:65–80.
- Görlich D, Henklein P, Laskey RA, Hartmann E. 1996a. A 41 amino acid motif in Importin- α confers binding to Importin- β and hence transit into the nucleus. *EMBO J* 15:1810–1817.
- Görlich D, Kostka S, Kraft R, Laskey RA, Hartmann E. 1995. Two different subunits of Importin cooperate to recognize nuclear localization signals and bind them to the nuclear envelope. *Curr Biol* 5:383–392.
- Görlich D, Kraft R, Kostka S, Vogel F, Hartmann E, Laskey RA, Mattaj IW, Izaurralde E. 1996b. Importin provides a link between nuclear protein import and U snRNA export. *Cell* 87:21–32.
- Görlich D, Mattaj IW. 1996. Nucleocytoplasmic transport. *Science* 271:1513–1518.
- Görlich D, Panté N, Kutay U, Aebi U, Bischoff FR. 1996c. Identification of different roles for Ran-GDP and Ran-GTP in nuclear protein import. *EMBO J* 15:5584–5594.
- Guddat U, Bakken AH, Pieler T. 1990. Protein-mediated nuclear export of RNA: 5S rRNA containing small RNPs in *Xenopus* oocytes. *Cell* 60:619–628.
- Hamm J, Darzynkiewicz E, Tahara SM, Mattaj IW. 1990. The trimethylguanosine cap structure of U1 snRNA is a component of a bipartite nuclear targeting signal. *Cell* 62:569–577.
- Hopper AK, Traglia HM, Dunst RW. 1990. The yeast RNA1 gene product necessary for RNA processing is located in the cytosol and apparently excluded from the nucleus. *J Cell Biol* 111:309–321.
- Iovine MK, Watkins JL, Wente SR. 1995. The GLFG repetitive region of the nucleoporin Nup116p interacts with Kap95p, an essential yeast nuclear import factor. *J Cell Biol* 131:1699–1713.
- Izaurralde E, Jarmolowski A, Beisel C, Mattaj IW, Dreyfuss G, Fischer U. 1997a. A role for the M9 transport signal of hnRNP A1 in mRNA nuclear export. *J Cell Biol* 137:27–35.
- Izaurralde E, Kutay U, von Kobbe C, Mattaj IW, Görlich D. 1997b. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J* 16:6535–6547.
- Izaurralde E, Lewis J, Gamberi C, Jarmolowski A, McGuigan C, Mattaj IW. 1995. A cap-binding protein complex mediating U snRNA export. *Nature* 376:709–712.
- Izaurralde E, Lewis J, McGuigan C, Jankowska M, Darzynkiewicz E, Mattaj IW. 1994. A nuclear cap binding protein complex involved in pre-mRNA splicing. *Cell* 78:657–668.
- Jarmolowski A, Boelens WC, Izaurralde E, Mattaj IW. 1994. Nuclear export of different classes of RNA is mediated by specific factors. *J Cell Biol* 124:627–635.
- Kalderon D, Roberts BL, Richardson WD, Smith AE. 1984. A short amino acid sequence able to specify nuclear location. *Cell* 39:499–509.
- Kambach C, Mattaj IW. 1992. Intracellular distribution of the U1A protein depends on active transport and nuclear binding to U1 snRNA. *J Cell Biol* 118:11–21.
- Kataoka N, Ohno M, Kangawa K, Tokoro Y, Shimura Y. 1994. Cloning of a complementary DNA encoding an 80 kilodalton nuclear cap binding protein. *Nucleic Acids Res* 22:3861–3865.

- Klebe C, Bischoff FR, Ponstingl H, Wittinghofer A. 1995. Interaction of the nuclear GTP-binding protein Ran with its regulatory proteins RCC1 and RanGAP1. *Biochemistry* 34:639–647.
- Koepp DM, Silver PA. 1996. A GTPase controlling nuclear trafficking: Running the right way or walking randomly? *Cell* 87:1–4.
- Kutay U, Bischoff FR, Kostka S, Kraft R, Görlich D. 1997. Export of Importin- α from the nucleus is mediated by a specific nuclear transport factor. *Cell* 90:1061–1071.
- Lanfior R, Butel JS. 1984. Construction and characterization of an SV40 mutant defective in nuclear transport of T antigen. *Cell* 37:801–813.
- Lee MS, Henry M, Silver PA. 1996. A protein that shuttles between the nucleus and the cytoplasm is an important mediator of RNA export. *Genes & Dev* 10:1233–1246.
- Legrain P, Rosbash M. 1989. Some *cis*- and *trans*-acting mutants for splicing target pre-mRNA to the cytoplasm. *Cell* 57:573–583.
- Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 88:97–107.
- Malim MH, Hauber J, Le SY, Maizel J, Cullen BR. 1989. The HIV-*rev* trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature* 338:254–257.
- Matunis MJ, Coutavas E, Blobel G. 1996. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J Cell Biol* 135:1457–1470.
- Melchior F, Guan T, Yokoyama N, Nishimoto T, Gerace L. 1995. GTP hydrolysis by Ran occurs at the nuclear pore complex in an early step of protein import. *J Cell Biol* 131:571–581.
- Melchior F, Weber K, Gerke V. 1993. A functional homologue of the RNA1 gene product in *Schizosaccharomyces pombe*: Purification, biochemical characterization, and identification of a leucine-rich repeat motif. *Mol Biol Cell* 4:569–581.
- Michael WM, Choi M, Dreyfuss G. 1995. A nuclear export signal in hnRNP A1: A signal-mediated, temperature-dependent nuclear protein export pathway. *Cell* 83:415–422.
- Michael WM, Eder PS, Dreyfuss G. 1997. The K nuclear shuttling domain: A novel signal for nuclear import and nuclear export in the hnRNP K protein. *EMBO J* 12:3587–3598.
- Moore MS, Blobel G. 1993. The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. *Nature* 365:661–663.
- Moreland RB, Nam HG, Herford LM, Fried HM. 1985. Identification of a nuclear localization signal of a yeast ribosomal protein. *Proc Natl Acad Sci USA* 82:6561–6565.
- Moroianu J, Higikata M, Blobel G, Radu A. 1995. Mammalian karyopherin α and β heterodimers: α 1 and α 2 subunits binds nuclear localization signal and β subunit interacts with peptide repeat-containing nucleoporins. *Proc Natl Acad Sci USA* 92:6532–6536.
- Murphy R, Wentz SR. 1996. An RNA-export mediator with an essential nuclear export signal. *Nature* 383:357–360.
- Nakielny S, Dreyfuss G. 1996. The hnRNP C proteins contain a nuclear retention sequence that can override nuclear export signals. *J Cell Biol* 134:1365–1373.
- Nakielny S, Dreyfuss G. 1997. Nuclear export of proteins and RNAs. *Curr Opin Cell Biol* 9:420–429.
- Nakielny S, Siomi MC, Siomi H, Michael WM, Pollard V, Dreyfuss G. 1996. Transportin: Nuclear transport receptor of a novel nuclear protein import pathway. *Exp Cell Res* 229:261–266.
- Nehrbass U, Blobel G. 1996. Role of the nuclear transport factor p10 in nuclear import. *Science* 272:120–122.
- Neville M, Lee L, Stutz F, Davis LI, Rosbash M. 1997. Evidence that the Importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export in *S. cerevisiae*. *Curr Biol* 7:767–775.
- Newmeyer DD, Forbes DJ. 1988. Nuclear import can be separated into distinct steps in vitro: Nuclear pore binding and translocation. *Cell* 52:641–653.
- Nigg EA. 1997. Nucleocytoplasmic transport: Signals, mechanism and regulation. *Nature* 386:779–787.
- Nishi K, Yoshida M, Fujiwara D, Nishikawa M, Horinouchi S, Beppu T. 1994. Leptomycin B targets a regulatory cascade of CRM1, a fission yeast nuclear protein, involved in control of higher order chromosome structure and gene expression. *J Biol Chem* 269:6320–6324.
- Neuman de Vegvar HE, Dahlberg JE. 1990. Nucleocytoplasmic transport and processing of small nuclear RNA precursors. *Mol Cell Biol* 10:3365–3375.
- Ohtsubo M, Okazaki H, Nishimoto T. 1989. The RCC1 protein, a regulator for the onset of chromosome condensation locates in the nucleus and binds to DNA. *J Cell Biol* 109:1389–1397.
- Palacios I, Hetzer M, Adam SA, Mattaj JW. 1997. Nuclear import of U snRNPs requires Importin- β . *EMBO J* 16:6783–6792.
- Panté N, Aebi U. 1993. The nuclear pore complex. *J Cell Biol* 122:977–984.
- Panté N, Aebi U. 1996. Sequential binding of import ligands to distinct nucleopore regions during their nuclear import. *Science* 273:1729–1732.
- Paschal BM, Gerace L. 1995. Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. *J Cell Biol* 129:925–937.
- Pasquinelli AE, Ernst RK, Lund E, Grimm C, Zapp ML, Rekosh D, Hammarskjöld ML, Dahlberg JE. 1997. The constitutive transport element (CTE) of Mason-Pfizer monkey virus (MPMV) accesses an RNA export pathway utilized by cellular messenger RNAs. *EMBO J* 16:7500–7510.
- Piñol-Roma S, Dreyfuss G. 1991. Transcription-dependent and transcription-independent nuclear transport of hnRNP proteins. *Science* 253:312–314.
- Pokrywka NJ, Goldfarb DS. 1995. Nuclear export pathways of tRNA and 40S ribosomes include both common and specific intermediates. *J Biol Chem* 270:3619–3624.
- Pollard VW, Michael WM, Nakielny S, Siomi MC, Wang P, Dreyfuss G. 1996. A novel receptor mediated nuclear protein import pathway. *Cell* 88:985–994.
- Powers MA, Forbes DJ, Dahlberg JE, Lund E. 1997. The vertebrate GLFG nucleoporin, Nup98, is an essential component of multiple RNA export pathways. *J Cell Biol* 136:241–250.
- Radu A, Moore MS, Blobel G. 1995. The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. *Cell* 81:215–222.
- Rexach M, Blobel G. 1995. Protein import into nuclei: Association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell* 83:683–692.
- Richards SA, Carey KL, Macara IG. 1997. Requirement of guanosine triphosphate-bound Ran for signal-mediated nuclear protein export. *Science* 276:1842–1844.
- Richards SA, Lounsbury KM, Carey KL, Macara IG. 1996. A nuclear export signal is essential for the cytosolic localization of the Ran binding protein, RanBP1. *J Cell Biol* 134:1157–1168.
- Richardson WD, Mills AD, Dilworth SM, Laskey RA, Dingwall C. 1988. Nuclear protein migration involves two steps: Rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. *Cell* 52:655–664.
- Romac JM, Graff DH, Keene JD. 1994. The U1 small nuclear ribonucleoprotein (snRNP) 70K protein is transported independently of U1 snRNP particles via a nuclear localization signal in the RNA-binding domain. *Mol Cell Biol* 14:462–70.
- Rout MP, Blobel G. 1993. Isolation of the yeast nuclear pore complex. *J Cell Biol* 123:771–783.
- Rout MP, Blobel G, Aitchison JD. 1997. A distinct nuclear import pathway used by ribosomal proteins. *Cell* 89:715–725.
- Russell I, Tollervey D. 1995. Yeast Nop3p has structural and functional similarities to mammalian pre-mRNA binding proteins. *Eur J Cell Biol* 66:293–301.
- Russo G, Ricciardelli G, Pietropaolo C. 1997. Different domains cooperate to target the human ribosomal L7a protein to the nucleus and to the nucleoli. *J Biol Chem* 272:5229–5235.
- Saavedra C, Felber BK, Izaurralde E. 1997a. The simian retrovirus-1 constitutive transport element, unlike the HIV-1 RRE, utilizes factors required for the export of cellular mRNAs. *Curr Biol* 7:619–628.
- Saavedra C, Hammell CM, Heath CV, Cole CN. 1997b. Export of heat shock mRNAs following stress in *Saccharomyces cerevisiae* employs a distinct pathway defined by Rip1p and also requires a subset of factors essential for export of poly(A)⁺ mRNA. *Genes & Dev* 11:2845–2856.
- Saavedra C, Tung KS, Amberg DC, Hopper AK, Cole CN. 1996. Regulation of mRNA export in response to stress in *Saccharomyces cerevisiae*. *Genes & Dev* 10:1608–1620.

- Saitoh H, Pu R, Cavenagh M, Dasso M. 1997. RanBP2 associates with Ubc9p and a modified form of RanGAP1. 1997. *Proc Natl Acad Sci USA* 94:3736–3741.
- Schaap PJ, van't Riet J, Woldringh CL, Raue HA. 1991. Identification and functional analysis of the nuclear localization signals of ribosomal protein L25 from *Saccharomyces cerevisiae*. *J Mol Biol* 221:225–237.
- Schlenstedt G, Smirnova E, Deane R, Solsbacher J, Kutay U, et al. 1997. Yrb4p, a yeast Ran-GTP-binding protein involved in import of ribosomal protein L25 into the nucleus. *EMBO J* 16:6237–6249.
- Schmidt C, Lipsius E, Kruppa J. 1995. Nuclear and nucleolar targeting of human ribosomal protein S6. *Mol Biol Cell* 6:1875–1885.
- Segref A, Sharma K, Doye V, Hellwig A, Huber J, Lührmann R, Hurt E. 1997. Mex67p, a novel factor for nuclear mRNA reexport binds to both poly(A)+ RNA and nuclear pores. *EMBO J* 16:3256–3271.
- Showman RM, Wells DE, Anstrom J, Hursh DA, Raff RA. 1982. Message-specific sequestration of maternal histone mRNA in the sea urchin egg. *Proc Natl Acad Sci USA* 79:5944–5947.
- Singleton DR, Shaoping C, Hitomi C, Kumagai C, Tartakoff A. 1995. A yeast protein that bidirectionally affects nucleocytoplasmic transport. *J Cell Sci* 108:265–272.
- Siomi H, Dreyfuss G. 1995. A nuclear localization domain in the hnRNP A1 protein. *J Cell Biol* 129:551–560.
- Stade K, Ford CS, Guthrie C, Weis K. 1997. Exportin 1(Crm1p) is an essential nuclear export factor. *Cell* 90:1041–1050.
- Stutz F, Izaurralde E, Mattaj IW, Rosbash M. 1996. A role for nucleoporin FG repeat domains in export of human immunodeficiency virus type 1 Rev protein and RNA from the nucleus. *Mol Cell Biol* 16:7144–7150.
- Stutz F, Kantor J, Zhang D, McCarthy T, Neville M, Rosbash M. 1997. The yeast nucleoporin Rip1p contributes to multiple export pathways with no essential role for its FG-repeat region. *Genes & Dev* 11:2857–2868.
- Stutz F, Neville M, Rosbash M. 1995. Identification of a novel nuclear pore-associated protein as a functional target of the HIV-1 Rev protein in yeast. *Cell* 82:495–506.
- Sweet DJ, Gerace L. 1996. A GTPase distinct from Ran is involved in nuclear protein import. *J Cell Biol* 133:971–983.
- Taberner C, Zolotukhin AS, Valentin A, Pavlakis GN, Felber BK. 1996. The posttranscriptional control element of the simian retrovirus type 1 forms an extensive RNA secondary structure necessary for its function. *J Virol* 70:5998–6011.
- Terns MP, Dahlberg JE. 1994. Retention and 5' cap trimethylation of U3 snRNA in the nucleus. *Science* 264:959–961.
- Terns MP, Lund E, Dahlberg JE. 1993. A pre-export U1 snRNP in *Xenopus laevis* oocyte nuclei. *Nucleic Acids Res* 21:4569–4573.
- Underwood MR, Fried HM. 1990. Characterization of nuclear localizing sequences derived from yeast ribosomal protein L29. *EMBO J* 9:91–99.
- Visa N, Izaurralde E, Ferreira J, Daneholt B, Mattaj IW. 1996. A nuclear cap-binding complex binds Balbiani ring pre-mRNA cotranscriptionally and accompanies the ribonucleoprotein particle during nuclear export. *J Cell Biol* 133:4–14.
- Wen W, Meinkoth JL, Tsien RY, Taylor SS. 1995. Identification of a signal for rapid export of proteins from the nucleus. *Cell* 82:463–473.
- Weis K, Dingwall C, Lamond AI. 1996a. Characterization of the nuclear protein import mechanism using Ran mutants with altered nucleotide binding specificities. *EMBO J* 15:7120–7128.
- Weis K, Ryder U, Lamond A. 1996b. The conserved amino-terminal domain of hSRP1 alpha is essential for nuclear protein import. *EMBO J* 15:1818–1825.
- Wilson SM, Datar KV, Paddy MR, Swedlow JR, Swanson M. 1994. Characterization of nuclear polyadenylated RNA-binding proteins in *Saccharomyces cerevisiae*. *J Cell Biol* 127:1173–1184.
- Wolff B, Sanglier JJ, Wang Y. 1997. Leptomycin B is an inhibitor of nuclear export: Inhibition of nucleocytoplasmic translocation of the human immunodeficiency virus type 1 (HIV-1) Rev protein and Rev-dependent mRNA. *Chem Biol* 4:139–147.
- Wu J, Matunis MJ, Kraemer D, Blobel G, Coutavas E. 1995. Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. *J Biol Chem* 270:14209–14213.
- Yokoyama N, Hayashi N, Seki T, Pante N, Ohba T, et al. 1995. A giant nucleopore protein that binds Ran/TC4. *Nature* 376:184–188.
- Zaslouf M. 1983. tRNA transport from the nucleus in a eukaryotic cell: Carrier-mediated translocation process. *Proc Natl Acad Sci USA* 80:6436–6440.
- Zolotukhin AS, Valentin A, Pavlakis GN, Felber BK. 1994. Continuous propagation of RRE(–) and Rev(–)RRE(–) Human immunodeficiency virus type 1 molecular clones containing a cis-acting element of simian retrovirus type 1 in human peripheral blood lymphocytes. *J Virol* 68:7944–7952.
- Zolotukhin AS, Felber BK. 1997. Mutations in the nuclear export signal of human Ran-binding protein RanBP1 block the Rev-mediated posttranscriptional regulation of human immunodeficiency virus type 1. *J Biol Chem* 272:11356–11360.