

# Transport of Proteins and RNAs in and out of the Nucleus

## Review

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Eukaryotic cells are equipped with a machinery charged with the responsibility of transporting a vast number of molecules in and out of the nucleus in a rapid, accurate, and often regulated manner. The cargos for this machinery are diverse, comprising proteins and more elaborate RNA–protein complexes (RNPs). Although the extent and complexity of trafficking between the nucleus and the cytoplasm have long been appreciated, it is only relatively recently that some of the molecular mechanisms involved have been uncovered.

Proteins and some RNPs that need to move between the nucleus and cytoplasm in order to perform their normal cell functions are most often recognized by soluble proteins, the nuclear transport receptors. These receptors mediate translocation of cargos through the nuclear pore complex (NPC), the route for all macromolecular traffic across the nuclear membrane. After depositing their cargos in the appropriate compartment, the unloaded receptors shuttle back through the NPC to pick up more cargo. Like many vectorial cellular processes, directionality is imposed on nucleocytoplasmic traffic (at least in part) by a nucleotidase, the small GTPase Ran.

Several experimental approaches, including *in vitro* import and export assays in higher eukaryotic cells, *in vivo* transport studies in frog oocytes and yeast genetics, have been instrumental in the discovery of molecules and mechanisms of nucleocytoplasmic transport. In this review, we attempt to bring together the large body of information in a manner accessible to the general reader interested in basic cell function. We cover cargos, transporters, route, direction, fuel and translocation mechanisms, and regulation, emphasizing general principles along the way.

### Cargos and Transporters

Cargos identify themselves to the nucleocytoplasmic transport machinery by signals (nuclear localization signals, NLSs, and nuclear export signals, NESs) that can be protein- or RNA-based, or a composite of the two. The signals in many protein cargos, and in some RNP cargos, are recognized by one or more members of the nuclear transport receptor family (Tables 1–3). Members of this protein family have been given various names, including karyopherins (Radu et al., 1995), p97 (Chi et al., 1995), PTACs (Imamoto et al., 1995), importins (Gorlich et al., 1995), transportins (Pollard et al., 1996), exportins (Stade et al., 1997), and Ran-binding proteins (reviewed in Corbett and Silver, 1997; Dingwall and

Laskey, 1998; Gorlich, 1998; Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998; Pemberton et al., 1998). Here, we refer to the individual nuclear transport receptors by the specific names used in their original descriptions. These receptors are generally large (90–130 kDa) acidic proteins sharing 15%–25% sequence identity within a given organism. They all have an N-terminal RanGTP-binding domain, a C-terminal cargo-binding domain, and the capacity to bind components of the NPC (Figure 1A).

Many classes of cargo contain signals that bind directly to a cognate receptor, but three types have signals that bind to the importin  $\beta$  receptor indirectly, via adaptor proteins. These are proteins with classical basic NLSs (either simple or bipartite) that bind the importin  $\alpha$  family of adaptors (Dingwall and Laskey, 1998; Gorlich, 1998; Weis, 1998), U snRNAs that bind the snurportin adaptor (Huber et al., 1998; Palacios et al., 1997), and replication protein A that binds the RIP $\alpha$  adaptor (Jullien et al., 1999). As all adaptor-utilizing cargos identified so far are imported by the importin  $\beta$  receptor, they share an N-terminal importin  $\beta$ -binding (IBB) domain (Figure 1B).

A variation of the simple receptor–import cargo mechanism is illustrated by the import of histone H1. This is mediated by a dimeric complex of two receptors, importin  $\beta$  and importin 7, in which importin 7 behaves like an adaptor or coreceptor (Jakel et al., 1999). Importin 7 can exist in either coreceptor or receptor mode, and as a receptor it functions in ribosomal protein import (Jakel and Gorlich, 1998). This phenomenon of receptors functioning in pairs might not be a quirk of importin 7: another receptor family member, importin 8, heterodimerizes with importin  $\beta$ , although a cargo for this complex has yet to be identified (Jakel et al., 1999).

The nuclear transport receptor family is therefore central to much of the traffic through the NPC. There are, however, some proteins that are imported into the nucleus apparently without the aid of a soluble receptor family member (Tables 1–3). Furthermore, there are two classes of RNA cargo, mRNA and ribosomal subunits, whose export to the cytoplasm might not be mediated by any member of the receptor protein family (see Export of RNPs section).

### Simple Signals, Complex Signals

Transport signals show considerable variation in complexity, from short peptide motifs like the classical monopartite basic NLS and the leucine-rich NES, up to large protein domains, and even multicomponent signals such as those involved in U snRNA transport (Tables 1–3). Multicomponent signals are so far specific to RNP transport, and are therefore readily rationalized: linking recognition by transport receptors to features of a mature, fully processed RNP provides a way to ensure that only functional RNPs are transported. Why some proteins have short peptide signals and others have extended domains is more difficult to explain. However, it has been suggested that receptors can have a second function—to bind to and hide protein domains that have

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Table 1. Import Signals and Receptors

Import Cargo	Cargo Function	Import Signal	Import Receptor	Comments (References)
Proteins with classical monopartite or bipartite basic NLS	Wide ranging	Monopartite PKKKRKV  Bipartite KRPAAIKKAGQAKKKK Signals are lysine-rich	Imp $\alpha$ /imp $\beta$ complex	1 imp $\beta$ and ~6 imp $\alpha$ 1 imp $\beta$ and 1 imp $\alpha$ [Sc] See text for references.
U snRNA	Splicing	m3G cap  Sm core domain	Snurportin/imp $\beta$ complex  N/K	Export and import of U snRNA may be specific to multicellular eukaryotes. See Table 2. (1, 2)
Replication protein A 70 kDa subunit	Replication	Not delineated	RIP $\alpha$ /imp $\beta$ complex	No RIP $\alpha$ homologue in Sc. (3)
Histone H1	Chromosome structure and function	Extended basic-rich domain	Imp7/imp $\beta$ complex	Imp7/imp $\beta$ complex can bind IBB and H1 simultaneously, but 2-cargo complex may not be efficiently imported. H1 binds, but is not imported by, imp $\alpha$ /imp $\beta$ . (4)
HIV-1 Rev HTLV-1 Rex HIV-1 Tat	Viral RNA export factors Viral transcription factor	RQARRNRRRRWR (Rev) Signals are arginine-rich	Imp $\beta$	Transcription-sensitive NLS. NLS overlaps RNA-binding domain. NLS and IBB bind overlapping sites on imp $\beta$ . Rev and Rex shuttle, using leucine-rich NESs. (5, 6)
Ribosomal proteins (including [Sc])	Translation	Extended arginine-rich domains	Kap123p/Yrb4p [Sc]  Kap121p/Pse1p [Sc]/imp5  imp $\beta$ , imp7, TRN1	TRN1 can bind M9 and ribosomal protein simultaneously, but 2-cargo complex may not be efficiently imported. (7-9)
Cyclin B1	Cell cycle	Within aa 121-373  Within aa 1-161	Imp $\beta$  Import <i>in vitro</i> does not need soluble factors	Cyclin B1 and IBB bind different regions of imp $\beta$ . Cyclin B1/CDC2 shuttles (B1 has leucine-rich NES). (10, 11)
5S rRNA	Translation	Ribosomal protein L5	Possibly imp $\beta$	Export and import of 5S rRNA is specific to oocyte. See Table 2. (12, 13)
SR proteins	Splicing	SR domains	TRN-SR	Transcription-sensitive NLS. Some SR proteins shuttle; see Table 2. (14, 15)
Lhp1p [Sc]	Biogenesis of RNA pol III transcripts	113 aa spanning RNA-binding domain	Sxm1p/Kap108p [Sc] N/K if adaptor required	Lhp1p is the [Sc] homolog of La protein, but human La is imported by imp $\alpha$ /imp $\beta$ . (16)
Npl3p [Sc]	Yeast hnRNP protein	120 aa spanning RGG box RNA-binding domain	Mtr10p/Kap111p [Sc]	Npl3p shuttles. (17-19)
Hog1p [Sc]	Signal transduction MAP kinase.	Uncharacterized	Nmd5p/Kap119p [Sc]	(20, 21)
TFIIS [Sc]	RNA pol II transcription elongation factor.	Uncharacterized		
TBP [Sc]	Transcription	Uncharacterized	Kap114p [Sc]	Kap121p and Kap123p may play minor role in TBP import. Dissociation of TBP-receptor is stimulated by dsDNA. (22, 23)
TFIIA [Sc]	Transcription	Uncharacterized	Pdr6p/Kap122p [Sc]	(24)
$\beta$ -catenin	Transcription regulation in signal transduction (Wnt pathway)	Not delineated, spans ARM repeats	Import <i>in vitro</i> does not need soluble factors	Shuttles. (25, 26)

a propensity to make inappropriate or premature interactions (Jakel et al., 1999).

### Many Signals, Many Receptors

There are many signals and many receptors, and the actual numbers depend on the organism. The genome of *S. cerevisiae* encodes 14 receptors, which can be recognized by sequence homology, and one importin  $\alpha$  adaptor (Corbett and Silver, 1997; Pemberton et al., 1998; Wozniak et al., 1998). Nine of these are import receptors, four are export receptors, and one is uncharacterized. Only four of the yeast receptors are essential proteins, but several of those that are not essential do nevertheless transport essential cargos. The explanation for this apparent contradiction is that each cargo is not necessarily dedicated to one receptor, and each receptor is not necessarily dedicated to one type of cargo. Not surprisingly, multicellular eukaryotes are equipped with more receptors and adaptors than *S. cerevisiae*. Nuclear transport receptor genes identified in higher organisms are currently similar in number to *S. cerevisiae*, but this situation is likely to change. However many more receptor genes there are in higher eukaryotes compared to yeast, it is likely that yet further receptor complexity is generated by the presence of variants with peptide inserts, which could transport different cargos (Siomi et al., 1997). At least six importin  $\alpha$  family members exist in higher eukaryotes. They are anywhere between 45% and 85% identical to one another, and have different expression patterns and signal preferences (Mattaj and Englmeier, 1998). Snurportin and RIP $\alpha$  adaptors are also unique to multicellular eukaryotes (Huber et al., 1998; Jullien et al., 1999).

The overall number of signals appears to be approximately similar to the number of receptors (Tables 1–3). The bidirectional class of signal is intriguing. Whether by coincidence or reflecting a biological significance, virtually all bidirectional signals, i.e., those whose NLS and NES activities cannot be separated, are found in mRNA-binding proteins, and no export receptor has been identified for this type of signal.

### Route

Macromolecules cross the nuclear membrane through aqueous channels formed by nuclear pore complexes (NPCs) (Figure 2). These elaborate protein structures of some 66 MDa (*S. cerevisiae*) or 125 MDa (higher eukaryotes) are composed of up to about 50 (*S. cerevisiae*) or 100 (higher eukaryotes) different proteins termed

nucleoporins (Nups) that assemble in multiple copies to form a baroque structure, the precise architecture of which, studied by various forms of electron microscopy, is still not clear (Ohno et al., 1998; Yang et al., 1998; Stoffler et al., 1999). The membrane-spanning portion of the NPC is symmetrical, but the extremities of the structure that extend into the cytoplasm and nucleoplasm are quite different in form and protein composition (Figure 2). The longitudinal center of the NPC is perforated by an aqueous, possibly gated, channel through which translocation occurs. The diameter of the channel appears to be flexible, and can expand from  $\sim 10$  nm to  $\sim 25$  nm to translocate large cargos (Dworetzky et al., 1988). The characterization of both yeast and higher eukaryotic Nups allows some general features to be ascribed to this family of proteins (Stoffler et al., 1999). They are often large proteins with generic protein-protein interaction domains of the coiled-coiled and leucine zipper type. Significantly, about half of the Nups described so far contain multiply repeated (at least 6) phenylalanine-glycine (FG) motifs. The FG repeats might not be diagnostic for Nups, because not all proteins containing FG repeats are localized to the NPC. However, as the other proteins identified so far as having this motif also have features suggestive of roles in nucleocytoplasmic trafficking, the FG motif could be predictive of a role in transport. The perception of the NPC as a static structure has recently been questioned by reports that some of its components are mobile within the NPC, a point we return to when considering models of translocation below (Nakielny et al., 1999; Zolotukhin and Felber, 1999).

### Direction

The Ran GTPase cycle is key to promoting the directionality of transport. Ran is localized predominantly inside the nucleus at steady state, and, like all small GTPases, it has very low nucleotide hydrolysis and exchange activities on its own (Melchior and Gerace, 1998; Moore, 1998). These are strongly stimulated by a GTPase-activating protein (RanGAP) and by a guanine nucleotide exchange factor (RanGEF). Central to the issue of directionality, these Ran regulators have restricted subcellular localizations: RanGAP and its coactivator, Ran-binding protein 1 (RanBP1), are largely cytoplasmic, and are also partially located at the cytoplasmic filaments of the NPC, where they bind to Nup358/RanBP2 (Mahajan et al., 1998; Matunis et al., 1998; and references therein).

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Proteins are higher eukaryotic unless indicated otherwise. [Sc], *Saccharomyces cerevisiae*. N/K, not known.

References: (1) Palacios et al. (1997) EMBO J. 16, 6783–6792; (2) Huber et al. (1998) EMBO J. 17, 4114–4126; (3) Jullien et al. (1999) EMBO J. 18, 4348–4358; (4) Jakel et al. (1999) EMBO J. 18, 2411–2423; (5) Palmeri and Malim (1999) Mol. Cell. Biol. 19, 1218–1225; (6) Truant and Cullen (1999) Mol. Cell. Biol. 19, 1210–1217; (7) Rout et al. (1997) Cell 89, 715–725; (8) Schlenstedt et al. (1997) EMBO J. 16, 6237–6249; (9) Jakel and Gorlich (1998) EMBO J. 17, 4491–4502; (10) Moore et al. (1999) J. Cell Biol. 144, 213–224; (11) Hagting et al. (1999) Curr. Biol. 9, 680–689; (12) Murdoch and Allison (1996) Exp. Cell Res. 227, 332–343; (13) Rudt and Pieler (1996) EMBO J. 15, 1383–1391; (14) Caceres et al. (1997) J. Cell Biol. 138, 225–238; (15) Kataoka et al. (1999) J. Cell Biol. 145, 1145–1152; (16) Rosenblum et al. (1998) J. Cell Biol. 143, 887–899; (17) Pemberton et al. (1997) J. Cell Biol. 139, 1645–1653; (18) Senger et al. (1998) EMBO J. 17, 2196–2207; (19) Lee et al. (1996) Genes Dev. 10, 1233–1246; (20) Ferrigno et al. (1998) EMBO J. 17, 5606–5614; (21) Albertini et al. (1998) J. Cell Biol. 143, 1447–1455; (22) Pemberton et al. (1999) J. Cell Biol. 145, 1407–1417; (23) Morehouse et al. (1999) Proc. Natl. Acad. Sci. USA 96, 12542–12547; (24) Titov and Blobel (1999) J. Cell Biol. 147, 235–246; (25) Fagotto et al. (1998) Curr. Biol. 8, 181–190; (26) Yokoya et al. (1999) Mol. Biol. Cell 10, 1119–1131.

Table 2. Export Signals and Receptors

Export Cargo	Cargo Function	Export Signal	Export Receptor	Comments (References)
Proteins with leucine-rich NES (including [Sc])	Wide-ranging	LALKLAGLDI (NES in PKI, the inhibitor of cAMP-dependent protein kinase). Signals are generally leucine-rich.	CRM1/exportin 1	Cell-permeable fungal metabolite leptomycin B specifically inhibits CRM1 (including [Sp]). NES has no clear consensus sequence. (1–8)
U snRNA	Splicing	m7G cap binds CBC CBC or proteins that interact with it contain NES.	CRM1/exportin 1	Export of U snRNA may be specific to multicellular eukaryotes. (1, 5, 9, 10)
5S rRNA	Translation	Possibly mediated by TFIIIA or ribosomal protein L5	CRM1/exportin 1	Export of 5S rRNA outside context of ribosomal subunits is unique to oocytes. (1, 11)
Snurportin 1	Adaptor protein for U snRNA import	Large region (~150 aa)	CRM1/exportin 1	The snurportin-CRM1 interaction is the strongest cargo-CRM1 interaction so far. (12)
tRNA	Translation	Mainly acceptor and TΨC arms. Mature 5' and 3' termini also important.	Exportin t/ los1p [Sc]	Sole case so far of transport receptor binding directly to RNA cargo. (13–16)
Imp $\alpha$ (including [Sc])	Adaptor for imp $\beta$ receptor	Large region (~140 aa)	CAS/Cse1p [Sc]	(17–21)
Pho4p [Sc]	Transcription factor	Uncharacterized	Msn5p [Sc]	Export is regulated, Msn5p binds only to phosphorylated forms of Pho4p and Far1p. (22, 23)
Far1p [Sc]	Regulates cell cycle and cytoskeleton during mating			
mRNA	Gene expression	mRNA export factors include: Shuttling hnRNP proteins (and Sc) Rae1p (and [Sp]/Gle2p [Sc]/mrnp41 Mex67p [Sc]/TAP Dbp5p/Rat8p (and [Sc])	N/K N/K N/K CRM1/exportin 1	See bidirectional table and text for further information.
SR proteins ASF/SF2, SRp20, 9G8	Splicing	SR domain and RNA-binding activity	N/K	(24)

Proteins are higher eukaryotic unless indicated otherwise. [Sc], *Saccharomyces cerevisiae*. [Sp], *Schizosaccharomyces pombe*. N/K, not known.

References: (1) Fischer et al. (1995) *Cell* 82, 475–483; (2) Wen et al. (1995) *Cell* 82, 463–473; (3) Bogerd et al. (1996) *Mol. Cell. Biol.* 16, 4207–4214; (4) Wolff et al. (1997) *Chem Biol.* 4, 139–147; (5) Fornerod et al. (1997) *Cell* 90, 1051–1060; (6) Stade et al. (1997) *Cell* 90, 1041–1050; (7) Fukuda et al. (1997) *Nature* 390, 308–311; (8) Ossareh-Nazari et al. (1997) *Science* 278, 141–144; (9) Jarmolowski et al. (1994) *J. Cell Biol.* 124, 627–635; (10) Izaurralde et al. (1995) *Nature* 376, 709–712; (11) Guddat et al. (1990) *Cell* 60, 619–628; (12) Paraskeva et al. (1999) *J. Cell Biol.* 145, 255–264; (13) Zasloff (1983) *Proc. Natl. Acad. Sci. USA* 80, 6436–6440; (14) Arts et al. (1998) *EMBO J.* 17, 7430–7441; (15) Kutay et al. (1998) *Mol. Cell* 1, 359–369; (16) Lund and Dahlberg, (1998) *Science* 282, 2082–2085; (17) Kutay et al. (1997) *Cell* 90, 1061–1071; (18) Herold et al. (1998) *J. Cell Biol.* 143, 309–318; (19) Hood and Silver (1998) *J. Biol. Chem.* 273, 35142–35146; (20) Kunzler and Hurt (1998) *FEBS Lett.* 433, 185–190; (21) Solsbacher et al. (1998) *Mol. Cell. Biol.* 18, 6805–6815; (22) Kaffman et al. (1998) *Nature* 396, 482–486; (23) Pines (1999) *Nat. Cell Biol.* 1, E73–E79; (24) Caceres et al. (1998) *Genes Dev.* 12, 55–66.

RanGEF, on the other hand, is a nuclear protein associated with chromatin (Ohtsubo et al., 1989). This distribution predicts that nuclear Ran is mostly GTP-loaded and cytoplasmic Ran is GDP-loaded (Figure 3). It should be noted, however, that the amounts of RanGTP and RanGDP in the nucleus and the cytoplasm have not been determined, so the RanGTP gradient is currently predicted rather than established. The RanGTP-binding domain of the transport receptors provides them with a sensor that informs them of their subcellular location. In the case of import receptors, RanGTP binding in the nucleus triggers dissociation of the import receptor-cargo complex, thus releasing cargo into the nucleus,

and the import receptors return to the cytoplasm as RanGTP-receptor complexes (Rexach and Blobel, 1995; Gorlich et al., 1996; Izaurralde and Adam, 1998; Hieda et al., 1999). Export receptors respond in the opposite way. They bind cargo with much higher affinity in the presence of RanGTP (Fornerod et al., 1997; Kutay et al., 1997; Arts et al., 1998). When this trimeric complex encounters RanGAP and RanBP1 on the cytoplasmic side of the NPC, it disassembles, releasing export cargo into the cytoplasm (Kutay et al., 1997; Gorlich, 1998; Kehlenbach et al., 1999) (Figure 4). This cycle would result in a nucleus depleted of Ran. However, the NTF2 protein binds RanGDP in the cytoplasm, and, by virtue

Table 3. Shuttling Signals and Receptors

Shuttling cargo	Cargo Function	Bidirectional Signal	Receptors	Comments (References)
hnRNP A1, A2, F Nab2p, and Nab4p/Hrp1p [Sc]	Role in mRNA export	M9 (~40 aa domain rich in glycine and aromatic residues)	Import receptor TRN1 Kap104p [Sc]  Export receptor N/K	NLS and NES functions of M9 cannot be separated by extensive mutagenesis (1). M9 not well conserved at primary sequence level (2, 3). M9 is transcription sensitive NLS (4).
Nup153	NPC protein and role in mRNA export	M9	Import receptor TRN1 Export receptor N/K	(5, 6)
hnRNP K	Role in mRNA export	KNS (~40 aa)	KNS import in vitro does not need soluble factors. Export receptor N/K	KNS is transcription-sensitive NLS. HnRNP K also contains classical basic NLS (7).
Mex67p [Sc]/TAP	Role in mRNA export	~80 aa at C terminus of TAP	N/K	(8–10)
HuR	RNA-binding protein with role in mRNA stability	HNS (~30 aa)	N/K	HNS shows sequence similarity to M9, and is transcription sensitive NLS (11)

This table lists shuttling cargos that contain a bidirectional signal that confers both import and export. Proteins are higher eukaryotic unless indicated otherwise. [Sc], *Saccharomyces cerevisiae*. N/K, not known.

References: (1) Bogerd et al. (1999) *J. Biol. Chem.* 274, 9771–9777; (2) Siomi and Dreyfuss (1995) *J. Cell Biol.* 129, 551–560; (3) Siomi et al. (1998) *Mol. Cell. Biol.* 18, 4141–4148; (4) Siomi et al. (1997) *J. Cell Biol.* 138, 1181–1192; (5) Enarson et al. (1998) *Chromosoma* 107, 228–236; (6) Nakielny et al. (1999) *EMBO J.* 18, 1982–1995; (7) Michael et al. (1997) *EMBO J.* 16, 3587–3598; (8) Bear et al. (1999) *Mol. Cell. Biol.* 19, 6306–6317; (9) Braun et al. (1999) *EMBO J.* 18, 1953–1965; (10) Kang and Cullen (1999) *Genes Dev.* 13, 1126–1139; (11) Fan and Steitz (1998) *Proc. Natl. Acad. Sci. USA* 95, 15293–15298.

of its ability to interact with Nups, delivers Ran back into the nucleus (Ribbeck et al., 1998; Smith et al., 1998).

In some cases, Ran apparently acts cooperatively with other factors to promote cargo unloading in the nucleus. For example, dissociation of import receptor–cargo complexes containing some hnRNP protein cargos or TBP, can be stimulated by their target nucleic acids (Senger et al., 1998; Lee and Aitchison, 1999; Pemberton et al., 1999). Cargo loading and unloading in the correct cellular compartment solves only part of the directionality problem. Receptors loaded with cargo still need to get to the NPC and travel the length of the NPC, a distance of some 200 nm. Ran may also function in NPC targeting and release of transport complexes, as RanGTP facilitates export complex–NPC interactions, and, in general, destabilizes import complex–NPC interactions (Rexach and Blobel, 1995; Delphin et al., 1997; Shah and Forbes, 1998; Askjaer et al., 1999; Kehlenbach et al., 1999; Nakielny et al., 1999). The asymmetric structure of the NPC at its extremities (Figure 2) is thought to contribute to directionality of translocation (Keminer et al., 1999). However, a role for the NPC in directionality is questioned by a report that inverting the RanGTP gradient across the nuclear membrane in vitro allows the direction of traffic to be reversed (Nachury and Weis, 1999).

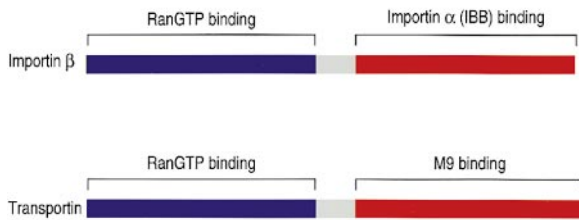
### Fueling and Mechanics

The fueling of nuclear translocation is a controversial issue that has been a recent focus of experimental scrutiny. In the following consideration of translocation energetics, we distinguish between events in the cytoplasm/nucleus and events within the body of the NPC.

Early studies of nuclear import established that cargo binds to the cytoplasmic face of the NPC in a temperature-independent manner, but accumulation of cargo in

the nucleus is strictly temperature-dependent (Newmeyer et al., 1986; Newmeyer and Forbes, 1988; Richardson et al., 1988). Identification of the Ran GTPase as an essential protein import factor suggested that the temperature dependence reflected a RanGTP hydrolysis-driven translocation mechanism (Melchior and Gerace, 1998; Moore, 1998). However, recent persuasive evidence shows that this is not the case. First, some export cargo–receptor complexes are exported in cells in which RanGTP has been depleted and replaced by a Ran mutant that binds GTP, but cannot hydrolyze it (Izaurralde et al., 1997b; Richards et al., 1997). Second, unloaded receptors can translocate in and out of the nucleus by a mechanism that shows no requirement for RanGTP hydrolysis or indeed any other nucleotide triphosphate (NTP) hydrolysis (Kose et al., 1997; Nakielny and Dreyfuss, 1998). Finally, cargo–receptor complexes, like unloaded receptors, can translocate in both directions through the NPC without the use of NTP hydrolysis (Schwoebel et al., 1998; Englmeier et al., 1999; Nachury and Weis, 1999; Ribbeck et al., 1999). The conclusion that the transport machinery has the capacity to move protein cargos through the NPC without energy input in the form of NTP hydrolysis should perhaps be greeted not with surprise, but as a reflection of our general ignorance of translocation mechanisms across cellular membranes through aqueous channels. The energy needed in vivo to effect cargo concentration in either the nucleus or cytoplasm is provided in the form of GTP hydrolysis by Ran in the cytoplasm, which is essential for the release of exported cargo and Ran from export receptors, and for release of Ran from import receptors, that are exported as RanGTP–receptor complexes. Thus, GTP hydrolysis outside the NPC provides the energy for cargo accumulation against a concentration gradient, but there is no evidence that it, or any

A. RECEPTOR FAMILY



B. ADAPTERS

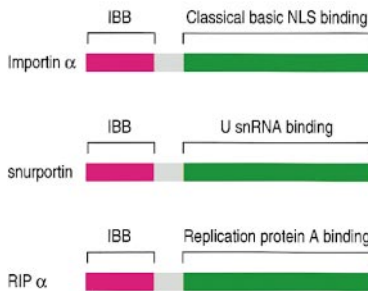


Figure 1. Nuclear Transport Receptors and Adaptors

Most known soluble transport receptors are large (90–130 kDa), acidic proteins that are related by amino acid sequence. They constitute a protein family referred to here as the nuclear transport receptor family. Importin  $\beta$  and transportin, the first receptors to be described, mediate the import of basic NLS-bearing cargos and M9-bearing cargos, respectively. The figure depicts the domain structure of the receptors (A), and of the adaptor proteins that mediate the interaction of several cargos with importin  $\beta$  (B). IBB is the importin  $\beta$ -binding domain.

The structures of an adaptor, importin  $\alpha$ , and two import receptors, importin  $\beta$  and transportin, were recently reported (Mattaj and Conti, 1999). The ARM repeats of importin  $\alpha$  and the HEAT repeats of receptor proteins form nonglobular, superhelical structures, presenting extended surfaces that are perfectly designed for making multiple contacts with cargos and regulatory molecules.

other NTP hydrolysis, is needed to propel transport complexes through the channel of the NPC.

We are left with temperature dependence as the single undisputed property relating to the energetics of movement through the NPC. Based on recent observations, we suggest one model, and refer the reader to excellent discussions of alternatives/variations to this (Kiseleva et al., 1998; Ohno et al., 1998; Pemberton et al., 1998; Nachury and Weis, 1999; Talcott and Moore, 1999). Interactions between members of the receptor family and the FG-rich domains of Nups have been reported, leading to the expectation that these are central to the translocation process (Ohno et al., 1998). Although hydrophobic, at least some Nup FG domains are exposed in the NPC. It is possible that temperature-sensitive receptor interactions with these FG domains, and temperature-sensitive conformational changes of the NPC, together facilitate movement through the NPC. This translocation mechanism assumes mobile receptor–cargo complexes and stationary Nups. However, since some Nups are mobile within the body of the NPC, it is possible that

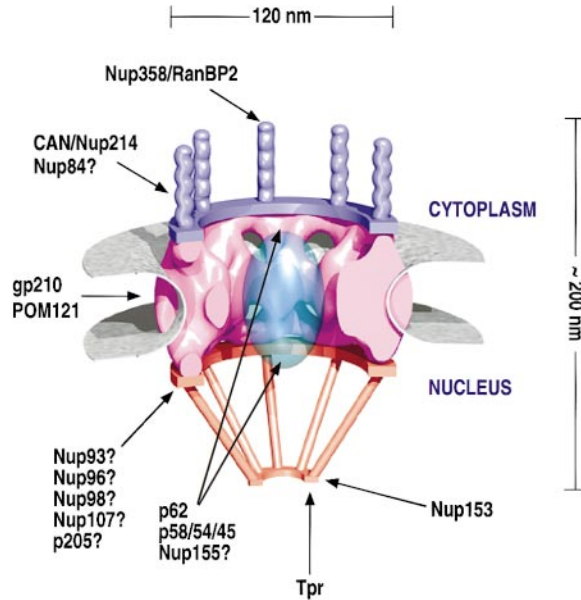


Figure 2. The Nuclear Pore Complex (NPC)

A model of the higher eukaryotic NPC, based on electron microscopic structural studies. The symmetrical membrane-spanning portion (pink) contains an aqueous, possibly gated, central channel through which translocation occurs, and a central plug (blue) of unclear composition and function. The cytoplasmic filaments (purple) and nuclear basket (orange) are involved in the initial and terminal stages of translocation. The locations of some nucleoporins (Nups), as determined by immuno-electron microscopy, are indicated. The gray portion represents the double membrane of the nuclear envelope. (This figure was adapted from Pante and Aebi, 1996.)

association of receptor–cargo complexes with particular Nups triggers dissociation of a Nup–receptor–cargo complex that moves through the pore as a unit (Nakielny et al., 1999; Zolotukhin and Felber, 1999).

It is unclear whether cargos move through an individual NPC in both directions at the same time, or whether each NPC is endowed with an internal communication system such that it knows to accept transport complexes at one entrance only. We favor a mechanism in which there is no one-way system within the pore (Feldherr and Akin, 1994). Rather, the NPC can accommodate cargos moving in both directions at the same time, so that they can pass each other en route. Clearly, exceptions to this are very large cargos such as ribosomal subunits and mRNPs (Daneshmandi, 1997), and these might translocate to the exclusion of all else. The many more contacts made by such large cargos with the channel of the NPC could prevent smaller cargos from making productive interactions with Nups, resulting in a bulldozer effect. But what keeps cargos moving forward, and stops them moving randomly over the FG platform? It is possible that the simple effect of cargos coming in behind them and thus covering the FG surface, giving the leading cargo no option but to move forward, is sufficient to prevent random movement within the pore. Ran-mediated termination mechanisms at the nuclear and cytoplasmic faces of the NPC would expose FG surfaces, providing the opportunity for forward movement onto the free surface. This mechanism assumes

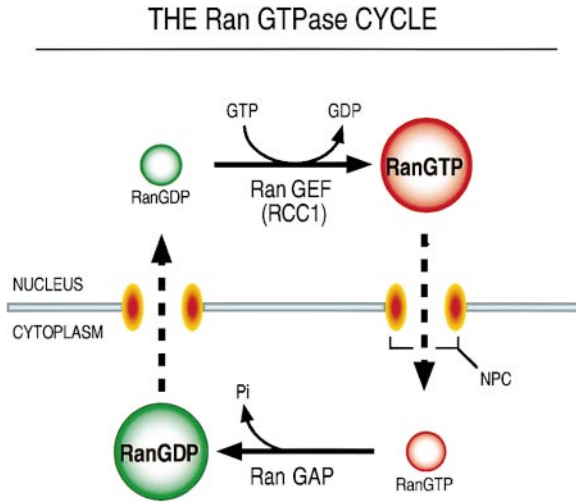


Figure 3. The Ran GTPase Cycle

Ran is maintained as RanGTP in the nucleus by the activity of the Ran GTP-GDP exchange factor (RanGEF or RCC1) and as RanGDP in the cytoplasm by the RanGTPase activating protein (RanGAP). RanBP1 in the cytoplasm and RanBP1-like domains on the cytoplasmic fibrils of the NPC are coactivators of RanGAP (not shown). The structures of RanGDP, RanGEF, RanGAP and a RanGTP-RanBP1 domain complex have been solved, providing structural explanations for some of the biochemical properties of Ran and its regulators, and highlighting the importance of the C-terminal extension of Ran, which is unique among small GTPases. This C-terminal region functions as a novel molecular switch (Macara, 1999).

that receptor-NPC contacts made by import complexes are different from those made by export complexes, at least at the extremities of the NPC. Support for this is provided by reports that import and export receptors do indeed make distinct contacts with Nups *in vitro* (Shah and Forbes, 1998; Askjaer et al., 1999; Kehlenbach et al., 1999), anti-Nup antibodies block some transport pathways while others are immune (Powers et al., 1997; Ullman et al., 1999), and some Nup mutations specifically inhibit import or export (Corbett and Silver, 1997; Segref et al., 1997).

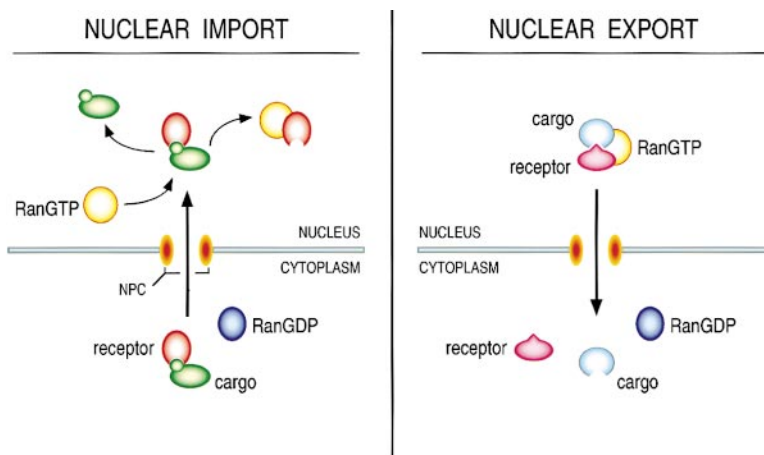


Figure 4. The Differential Effects of RanGTP on Nuclear Import and Nuclear Export Receptor-Cargo Complexes

Import receptors bind their cargos in a RanGTP-independent manner and RanGTP causes dissociation of these complexes. They are thus permitted to form in the cytoplasm and dissociate in the RanGTP-rich nucleus. Export receptors form stable complexes with their cargos only in the presence of RanGTP. These ternary complexes are thought to be the export unit, and dissociate in the cytoplasm and/or on the cytoplasmic filaments of the pore where RanGAP activity converts the RanGTP to RanGDP.

Crystal structures of import receptors bound to cargo (importin  $\beta$ -IBB complex) and bound to Ran (transportin-RanGppNHp and importin  $\beta$ -RanGppNHp complexes) suggest how Ran

may mediate cargo unloading (Macara, 1999; Mattaj and Conti, 1999). Ran contacts an acidic loop in the central region of the receptor molecules, and this interaction is probably responsible, at least in part, for cargo displacement. Structures of export receptors have not yet been reported.

Although this vision of the mechanics, fueling, and directionality of translocation through the NPC does not involve NTP hydrolysis, it should be emphasized that it is largely based on studies of simple protein cargo-receptor complexes. As we move on to describe the transport of RNPs, it will become evident that the mechanism of their movement is even less clear than that of protein cargos, and could have different energy requirements.

### Export of RNPs

The vast majority of cellular RNA molecules are synthesized in the nucleus, and most of these need to move into the cytoplasm in order to perform their functions. These exported RNAs can be grouped into four major classes: mRNA, rRNA (in the form of ribosomal subunits and 5S rRNA), U snRNA, and tRNA. All classes of RNA exist as, and are exported as RNPs, and these can be many-fold the mass of simple protein cargos (Nakiely et al., 1997; Mattaj and Englmeier, 1998). RNA export involves movement from the site of transcription to the NPC, and translocation through the NPC. Although not without controversy, current evidence favors models in which RNA diffuses from point of synthesis through the nucleus to point of exit, at least in the case of mRNPs (Daneholt, 1999). Little is known about mechanisms of intranuclear movement of the other classes of RNP complex, but a role for Ran has been proposed (Dahlberg and Lund, 1998).

A property of the export of all classes of RNA cargo is that of selective saturability. Each type of RNA blocks its own export when present in excess, but not that of the others, indicating the existence of class-specific export factors (Bataille et al., 1990; Jarmolowski et al., 1994; Pokrywka and Goldfarb, 1995). Another general feature of RNP transport is the ability of transport machineries to discriminate between immature and mature RNPs. This ensures that nonfunctional RNPs do not get into the cytoplasm, where they would have potentially deleterious effects. The mechanisms involved in this aspect of transport fidelity have been most closely studied for tRNA, as described below, but similar principles could apply to the export of other RNPs.

### *U snRNA, rRNA, and tRNA*

Export of U snRNA, 5S rRNA, and tRNA are all mediated by members of the nuclear transport receptor family of proteins (Table 2). How ribosomal subunits exit the nucleus is largely unexplored territory, although the Ran-GTPase cycle and certain Nups are involved (Hurt et al., 1999; Moy and Silver, 1999).

For U snRNA export, the 5' m7G cap structure is essential, and some of the molecules involved are now known. Export is mediated by CRM1, together with the cap binding complex (CBC, comprising a heterodimer of CBP80 and CBP20) and possibly other factors (Jarmolowski et al., 1994; Izaurralde et al., 1995; Fornerod et al., 1997). Following export and dissociation of the export factors, U snRNAs undergo several maturation steps in the cytoplasm, and are then reimported to the nucleus by snurportin/importin  $\beta$  (see Cargos and Transporters section).

5S rRNA export outside the context of ribosomal subunits is a phenomenon that is specific to oocytes, where it is exported and stored in the cytoplasm until vitellogenesis, when there is an increased demand for ribosomes. At this time it is reimported and incorporated into ribosomal subunits (reviewed in Nakielny et al., 1997). Export of 5S rRNA can involve either of two 5S rRNA-binding proteins, TFIIIA and L5. Export is probably mediated by CRM1, because excess leucine-rich NES blocks transport (Table 2). TFIIIA is proposed to be the protein that bridges receptor and RNA, but this has yet to be demonstrated directly (Mattaj and Englmeier, 1998 and references therein). Other proteins must be involved, because 5S rRNA mutants that no longer bind TFIIIA are nevertheless exported.

The tRNA export pathway is so far unique among RNP transport pathways in that no bridging RNA-binding protein is involved. Instead, a member of the receptor family, termed exportin-t in higher eukaryotes and los1p in *S. cerevisiae*, binds directly to tRNA (Arts et al., 1998; Kutay et al., 1998; Sarkar and Hopper, 1998). An obligate order of nuclear tRNA processing events, involving splicing, end trimming and modification, and aminoacylation, appears to be part of the mechanism that ensures nonfunctional tRNAs do not get into the cytoplasm, in higher eukaryotes at least. The last step, aminoacylation, produces charged tRNAs that are exported more efficiently than uncharged ones (Arts et al., 1998; Lund and Dahlberg, 1998). Exportin-t participates in the fidelity of export, because it can discriminate between tRNAs with immature and mature termini, binding only to the latter (Arts et al., 1998). Charging could also affect the affinity of the tRNA-exportin interaction.

### *mRNA*

**Visualizing mRNP Export.** Translocation of very large mRNPs, such as the Balbiani ring mRNP of the insect *Chironomus tentans*, can be visualized by electron microscopy (Daneshmandi et al., 1997). This 50 nm diameter mRNP is dramatically reorganized during translocation to produce an elongated particle of about 20 nm in diameter, an acceptable size for the NPC channel. The NPC basket structure also undergoes large structural changes, including a widening of the distal ring aperture, similar to the opening of an iris (Kiseleva et al., 1998; Stoffer et al., 1999).

**RNA Elements.** Both the 5' m7G cap and 3' poly(A) tail

(or histone 3' end) stimulate mRNA export, but neither structure appears to be essential for transport (reviewed in Nakielny et al., 1997). However, the observation that Balbiani ring transcripts invariably move through the NPC with 5' end in the lead indicates that the cap structure and the CBC play some role in translocation, perhaps to initiate association with the NPC, and thereby increase the efficiency of translocation of large mRNPs (Visa et al., 1996). This inessential role for the CBC in mRNA export contrasts with its critical role in U snRNA export (Izaurralde et al., 1995, 1997b). The other RNA element that affects mRNA transport, the intron, is considerably more influential. If an RNA molecule contains introns, it does not normally exit the nucleus, due to a saturable retention mechanism (Nakielny et al., 1997).

**Insights from Viruses.** Efforts to understand how retroviruses export their intron-containing RNA genomes and mRNAs led to the identification of a bona fide RNA export factor. The complex retrovirus, human immunodeficiency virus (HIV) employs a small viral protein termed Rev to accomplish this (reviewed in Pollard and Malim, 1998). Rev is an RNA-binding protein that interacts with the Rev response element in the viral RNA. A leucine-rich NES in Rev links the viral RNA to the cellular CRM1 export pathway. Rev is therefore an RNA export factor that mediates export of viral RNA that in its absence is retained in the nucleus (Pollard and Malim, 1998). Simple retroviruses, although also containing special RNA elements to mediate export of intron-containing RNA, do not encode RNA export factors, and instead use cellular proteins. The components of such a pathway in type D retroviruses, such as Mason-Pfizer monkey virus, have recently been characterized (Bear et al., 1999 and references therein). They employ a viral RNA export element called the constitutive transport element (CTE). The cellular protein TAP binds specifically to the CTE and mediates export of the RNP (Gruter et al., 1998). Another protein, RNA helicase A, has also been suggested to be an effector of CTE function (Tang et al., 1997).

**Soluble mRNA Export Factors.** A large number of candidate molecules that exhibit properties suggestive of a role in exporting mRNA have been described. Many of these putative mRNA export factors were uncovered in yeast in searches for mutants in which poly(A) RNA accumulates in the nucleus or in screens for genetic interactions with Nups. The former approach to identifying mRNA export factors, although valuable, is slightly tempered by the fact that poly(A) RNA includes other RNAs in addition to mRNA, for example pre-mRNA and other transcripts that might never leave the nucleus. It can also be difficult to ascribe a direct transport function to genes unearthed in such screens, since proteins that function in other cellular processes, such as RNA metabolism, impinge on export without being components of the machinery. Here, we describe the stronger candidates from higher eukaryotes and yeast. Significantly, they are all RNA-binding proteins of one sort or another.

In higher eukaryotes, the hnRNP protein family was the first group of proteins to be implicated in mRNA export, and this suggested the concept that RNA-binding proteins hold the key to mRNA export. These pre-mRNA/mRNA-binding proteins are very abundant components of the nucleus, and a large number of the 20

or so major hnRNP proteins, although predominantly nuclear, shuttle rapidly in and out of the nucleus (Pinol-Roma and Dreyfuss, 1991, 1992; Krecic and Swanson, 1999). Several lines of evidence support the idea that these proteins participate in mRNA export: they are bound to mRNA in both compartments (Pinol-Roma and Dreyfuss, 1992); their export is mediated by NESs that are not part of the RNA-binding domain (Michael et al., 1995; Pollard et al., 1996); excess of the shuttling hnRNP A1 protein specifically blocks mRNA export (Izaurralde et al., 1997a); an A1-like hnRNP protein is associated with translocating Balbiani ring transcripts all the way through the NPC and into the cytoplasm (Daneshmandi, 1997) and mutants in yeast shuttling hnRNP proteins have an mRNA export block (Lee et al., 1996). They therefore remain good contenders as mRNA export factors and certainly figure in this process.

The role of shuttling hnRNP proteins in mRNA export cannot be considered in isolation from the remaining hnRNP proteins, which are restricted to the nucleus of interphase cells (Pinol-Roma and Dreyfuss, 1992). These proteins are retained in the nucleus not because they lack an NES, but rather because they carry a nuclear retention signal (NRS). The NRS overrides the function of hnRNP protein NESs (Nakielnny and Dreyfuss, 1996). Consequently, mRNPs approaching the NPC associated with both nonshuttling and shuttling hnRNP proteins cannot exit the nucleus. This might be one part of the mechanism by which cells prevent export of unprocessed or partially processed transcripts. The removal of the NRS-bearing nonshuttlers from the mRNA must be essential, and carefully regulated, to allow functional mRNA to be exported. This removal process has been visualized: dissociation of several hnRNP proteins from mRNPs prior to or during export can be discerned by electron microscopy of Balbiani ring transcripts (Daneshmandi, 1997; Sun et al., 1998).

Three other candidate mRNA export factors appear to be associated with mRNA, Gle2p, TAP, and Dbp5p. Gle2p (also known as Rae1p or mRNP41), was first identified in a screen for mRNA export mutants in *S. pombe* (Brown et al., 1995; Murphy et al., 1996; Pritchard et al., 1999 and references therein). Gle2p localizes to NPCs and contains several copies of the WD motif, which is involved in targeting it to the NPC via a specific interaction with Nups that contain a Gle2p-binding sequence (GLEBS) domain (Bailer et al., 1998; Pritchard et al., 1999). Importantly, overexpression of the GLEBS domain in *Xenopus* oocytes inhibits association of Gle2p with the NPC and inhibits mRNA export, and mRNA export is restored by overexpression of Gle2p (Pritchard et al., 1999). Two other properties of this protein are relevant in considering its role in mRNA export: although it does not contain an obvious RNA-binding motif, Gle2p can be cross-linked to poly(A) RNA (Kraemer and Blobel, 1997), and it shuttles in and out of the nucleus (Pritchard et al., 1999).

TAP originally came to light as a candidate mRNA export factor in *S. cerevisiae*. Temperature-sensitive mutations in the essential MEX67 gene cause a rapid and specific mRNA export defect at the restrictive temperature (Segref et al., 1997). Mex67p is the yeast homolog of TAP, the higher eukaryotic protein that mediates export of CTE-containing viral RNPs (above). TAP also

appears to participate in cellular mRNA transport, because excess CTE inhibits export of nonviral mRNAs, and this effect can be overcome by TAP (Pasquinelli et al., 1997; Gruter et al., 1998). How TAP/Mex67p functions in cellular mRNA export is not yet clear, but several of its properties seem relevant. First, TAP/Mex67p has a novel type of RNA-binding domain, a leucine-rich repeat domain, that mediates its high-affinity interaction with the CTE (Braun et al., 1999; Kang and Cullen, 1999). This domain has been proposed to bind cellular mRNA indirectly, acting as a protein interaction motif to target TAP to mRNPs (Braun et al., 1999). Second, TAP shuttles between the nucleus and the cytoplasm (Bear et al., 1999; Braun et al., 1999; Kang and Cullen, 1999; Katahira et al., 1999). Finally, it has the ability to interact with Nups (Segref et al., 1997; Katahira et al., 1999). Taken together, this information suggests that TAP mediates export by virtue of its capacity to interact with both mRNPs and the NPC, and to shuttle in and out of the nucleus. Two small proteins, which are unrelated at the primary structure level, also need to be incorporated into a model of TAP/Mex67p function. In *S. cerevisiae*, Mtr2p forms a complex with Mex67p and mediates NPC association of Mex67p (Santos-Rosa et al., 1998 and references therein), and in higher eukaryotes TAP binds to p15, a protein with similarity to NTF2 (Katahira et al., 1999).

The last protein that we describe, Dbp5p (also known as Rat8p), is particularly interesting because it is one of very few enzymes to be implicated in nucleocytoplasmic transport. Dbp5p was also identified in screens for mRNA export factors in yeast (Snay-Hodge et al., 1998; Tseng et al., 1998). The gene encodes a DEAD box protein, and, like several other members of this family, Dbp5p is an ATP-dependent RNA helicase (Tseng et al., 1998; Schmitt et al., 1999). Helicase activity appears to be important for its mRNA export function, because mutants of human Dbp5p lacking ATPase and RNA unwinding activities specifically block mRNA export when injected into *Xenopus* oocytes (Schmitt et al., 1999). Dbp5p localizes in the cytoplasm and on the cytoplasmic fibrils of the NPC, and it can shuttle in and out of the nucleus (Snay-Hodge et al., 1998; Tseng et al., 1998; Hodge et al., 1999; Schmitt et al., 1999). The Dbp5p-NPC interaction is inhibited by ATP, suggesting that it could be regulated by nucleotide binding and hydrolysis (Schmitt et al., 1999). This information suggests appealing mechanisms for Dbp5p in mRNA export. First, by mediating changes in both RNA-RNA and RNA-protein interactions, helicase activity might unfold this class of cargo, which can be quite massive, to create mRNPs of dimensions compatible with the maximum aperture of the NPC. In this capacity, helicases would function in NPC translocation in an analogous way to hsp ATPases in translocation across the ER and mitochondrial membranes, during which protein cargos must be unfolded to fit through the translocation channels of these membranes. Second, Dbp5p might terminate mRNP transport, by facilitating release of mRNPs from the cytoplasmic side of the NPC. In this model, the helicase would play a role similar to that of RanGTP hydrolysis that triggers release of simpler export cargo

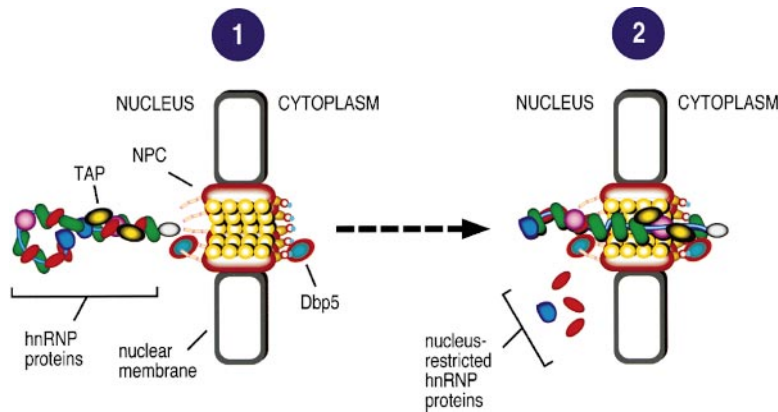


Figure 5. A Model for mRNA Export

Role of hnRNP proteins, TAP and Dbp5. mRNPs approach the nuclear pore complex (NPC) associated with both nuclear export signal (NES)-bearing shuttling and nuclear retention signal (NRS)-bearing nonshuttling hnRNP proteins and TAP (1). Since NRSs override NESs, the mRNA cannot be exported until the nonshuttlers have been removed. When this happens, NESs are activated, and participate in propelling the mRNP through the NPC (2), either via yet to be identified soluble NES receptors, or by direct interaction with FG domains (yellow balls) within the NPC. The RNA helicase Dbp5 may function to remodel the mRNP in the nucleus so that it can fit into the NPC, and also in the cytoplasm, to release the mRNA from the NPC, to dissociate shuttling export factors, and to produce an mRNA that is functional for translation.

complexes from the NPC. Finally, Dbp5p could restructure the mRNP when it emerges in the cytoplasm, concomitantly releasing export factors such as shuttling hnRNP proteins, allowing them to return to the nucleus for further rounds of mRNP export, and creating an mRNP that is functional for translation.

**A Model for mRNA Export.** A concept of where, when, and how these potential mRNA export factors could function in mRNA export is shown in Figure 5. Notably absent from this perusal of mRNA export factors is any member of the nuclear transport receptor family. Some evidence has implicated CRM1 in mRNA export, but this could reflect an indirect role (Hodge et al., 1999; Neville and Rosbash, 1999). NESs have been described in hnRNP proteins and in TAP/Mex67p, and Gle2p and Dbp5p are shuttling proteins. Perhaps soluble proteins with different properties to those of the nuclear transport receptor family interact with these signals, or the NESs on the exported mRNPs interact directly with components of the NPC during the translocation. Although the Dbp5p helicase might promote reorganization of mRNPs, either in the nucleus at the beginning of translocation or in the cytoplasm following translocation, it is probably not solely responsible. It is quite likely that posttranslational modifications of mRNA-binding proteins such as hnRNP proteins, also facilitate the structural changes that accompany mRNP export (Pinol-Roma and Dreyfuss, 1993; Soulard et al., 1993; Shen et al., 1998).

The large structural changes in the NPC and in the translocating cargo that are evident during translocation of mRNPs such as the Balbiani ring transcripts, might seem difficult to reconcile with a mechanism of transport that does not directly involve NTP hydrolysis. NTP hydrolysis could well be necessary to effect their reorganization in the nucleoplasm prior to translocation, and for restructuring them into a functional form in the cytoplasm. It also remains possible that mRNP translocation is different from simple protein translocation, and uses NTP hydrolysis to bring about NPC deformations and movement within the body of the NPC. That is, the transport machinery might be able to vary its energy consumption according to cargo load.

### Regulating Transport

A number of cellular processes are regulated at the level of nucleocytoplasmic transport. The specific cargos, mechanisms and physiological rationale for controlling their localization and trafficking have been the focus of several recent reviews (Mattaj and Englmeier, 1998; Hood and Silver, 1999; Pines, 1999). Here, we will outline the emerging principles of regulation and highlight some unresolved aspects of traffic control.

Global regulation is suggested by observations that the rate of transport and the maximum aperture of the NPC translocation channel vary according to the physiological state of the cell (Feldherr and Akin, 1994; Feldherr et al., 1998). An increase in both of these parameters is seen as cells pass from quiescence to proliferating to transformed (Feldherr and Akin, 1994), and during oogenesis (Feldherr et al., 1998). Changes in NPC capacity during oogenesis appear to be mediated in part by NTF2 (Feldherr et al., 1998), the protein that mediates import of Ran (see Direction section). Beyond this, the mechanisms involved in global regulation have not been investigated, but might involve changes to the Ran system and/or components of the NPC, and regulated expression of receptors could also contribute.

The second type of control involves highly specific regulatory mechanisms that affect the localization of individual protein cargos at particular stages of both mitotic and meiotic cell cycles (Ohno and Mattaj, 1999; Pines, 1999), in signaling pathways and during development (Hood and Silver, 1999). Phosphorylation plays a major role, and it is the cargos and their binding proteins that are the targets of kinases and phosphatases. The outcome of these changes in phosphorylation is altered cargo-receptor affinity. This can be a direct effect, for example, the yeast transcription factor Pho4p must be phosphorylated to be recognized by its export receptor and dephosphorylated to bind its import receptor, or indirect, as when phosphorylation of the cytoplasmic retention factor I $\kappa$ B disrupts its interaction with the transcription factor NF $\kappa$ B, allowing the NLS of NF $\kappa$ B to be recognized by import receptors (Mattaj and Englmeier, 1998). Although phosphorylation is predominant, it might not be the only posttranslational modification involved in traffic control. Methylation of arginine residues

has also been found to have an effect on the export of some yeast hnRNP proteins (Shen et al., 1998).

Traffic control is not restricted to protein cargos. Several cases of regulated RNA transport have been uncovered (Mattaj and Englmeier, 1998). For example, in yeast cells stressed by elevated temperature and other cellular insults, export of most mRNAs is blocked, but mRNAs encoding heat shock (hs) proteins continue to be transported into the cytoplasm (Saavedra et al., 1997 and references therein). Selective export of hs mRNA is dependent on *cis*-acting signals in the hs mRNA molecules that can confer export on heterologous mRNAs, which would otherwise be retained in the nucleus. The export pathway taken by hs mRNAs under stress conditions appears to be distinct from the general mRNA export pathway. General and hs mRNA export show differential requirements for two proteins, the hnRNP protein Npl3p and an FG-containing protein, Rip1p. Npl3p is essential for general but not hs mRNA export, while Rip1p is essential for transport of hs but not general mRNA (Lee et al., 1996; Saavedra et al., 1997; Stutz et al., 1997). Identifying the stress-activated signaling pathways that ultimately result in selective export of hs mRNAs, and the molecules that directly hinder general mRNA transport and facilitate hs mRNA export will be very interesting. On the way to these goals, Npl3p dissociates from mRNA in stressed cells, although whether this phenomenon is the effector of the mRNA export block is unclear (Krebber et al., 1999).

Finally, one particularly intriguing form of transport regulation, the molecular mechanism of which remains elusive, ties the nuclear localization of some of the major nuclear mRNA binding proteins to the transcriptional activity of RNA polymerase II (Pinol-Roma and Dreyfuss, 1991). In cells treated with pol II inhibitors, many of the abundant mRNA-binding proteins, e.g., hnRNP A1, redistribute and accumulate in the cytoplasm. The coupling of the output of newly synthesized mRNA to the nuclear content of the RNA-binding proteins that participate in its processing and transport to the cytoplasm is an ingenious regulatory circuit. This control appears to operate at the level of nuclear transport, most likely via import but possibly also via export. It is conceivable that either the cargos themselves and/or their cognate NTRs become modified such that high affinity cargo-receptor interactions are prevented. At least for the M9-bearing proteins, such as hnRNP A1 and Nup153, the M9 nuclear transport signal is sufficient to confer transcription-dependent nuclear localization, indicating that M9 itself is the sensor (Siomi et al., 1997; Nakielny et al., 1999). It is likely that specific posttranslational modifications play a role in transducing the green light/red light signal from the mRNA production machinery. Unraveling this mechanism should illuminate some fundamental aspects of how cells utilize the dynamic transport machinery to so precisely regulate the level of each component in the nucleus, and of how they deliver specific RNAs to the cytoplasm.

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