Nuclear export of proteins and RNAs

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Our understanding of protein export from the nucleus to the cytoplasm has been advanced recently by the discovery of active, signal-mediated export pathways. Nuclear export signals have been identified in several proteins, the majority of which are RNA-binding proteins. Nuclear export of RNA molecules is likely to be driven by protein-based nuclear export signals.

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Abbreviations

BR	Balbiani Ring
CBC	cap-binding complex
CBP	cap-binding protein
FG	phenylalanine–glycine
hnRNP protein	heterogeneous nuclear RNA-binding protein
hRIP	human RIP
KNS	hnRNP K nuclear shuttling domain
NES	nuclear export sequence
NLS	nuclear localization sequence
NPC	nuclear pore complex
NRS	nuclear retention sequence
PKI	protein kinase inhibitor of protein kinase A
Rab	Rev/Rex activation domain binding protein
RCC1	regulator of chromosome condensation 1
Rip	Rev-interacting protein
RNP	RNA–protein
RRE	Rev response element
sn	small nuclear

Introduction

Compartmentalization of the eukaryotic cell into nucleus and cytoplasm necessitates the transport of a large variety of RNA and protein molecules between their sites of synthesis, maturation and function. It also allows for the regulation of cellular processes by control of the nucleocytoplasmic distribution of proteins and RNA. The molecular mechanisms of transport across the nuclear envelope are therefore of fundamental interest in cell biology.

The nuclear envelope is punctuated by large proteinaceous structures termed nuclear pore complexes (NPCs) that provide the means for proteins and RNA to enter and exit an otherwise impermeable capsule. Comprising upwards of 100 different polypeptides that assemble to form a complex that is about thirty times the size of a ribosome, each NPC allows the passive diffusion of ions, small molecules and most macromolecules of less than ~60 kDa, while larger molecules are transported by facilitated, energy-dependent pathways ([1,2]; see Doye and Hurt, this issue, pp 401–411). Like all other intracellular transport mechanisms characterized to date, active transport of proteins in both directions across the nuclear envelope requires the presence of specific targeting sequences within the proteins [3–5]. Although this review will focus on protein export and protein-mediated RNA export, it is relevant to outline our current knowledge of the mechanisms of import to the nucleus, as import and export are inextricably linked.

Overview of nuclear protein import

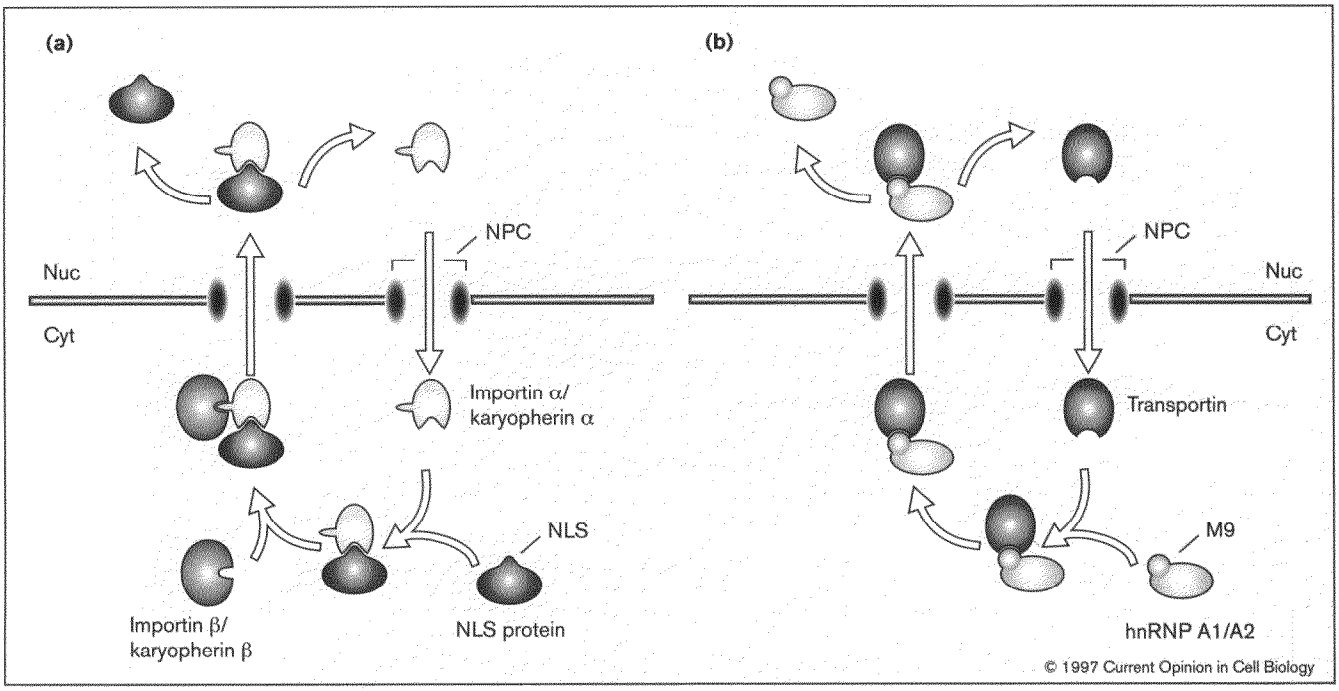
Many nuclear proteins are imported by virtue of a nuclear localization sequence (NLS) that is rich in basic amino acids [6,7]. Quite recently, the import machinery that recognizes basic NLSs, and mediates the translocation of the NLS-bearing protein into the nucleoplasm, has been identified and characterized ([4,8]; see Görlich, this issue, pp 412–419). An *in vitro* nuclear import assay has been instrumental in identifying three essential proteins, along with several potential regulators, that mediate import. A heterodimeric complex of importin α and importin β (also known as karyopherin α and β) recognizes and binds basic NLSs via the NLS-binding site of the α subunit, and importin β mediates docking of the cargo–importin complex with the NPC, in a temperature-independent manner (Fig. 1). Translocation into the nucleoplasm is mediated by the Ran GTPase cycle, and requires energy in the form of GTP hydrolysis.

A second pathway for import of proteins to the nucleus has very recently been identified [9••,10••,11,12]. A subset of hnRNP proteins (heterogeneous nuclear RNA-binding proteins), typified by hnRNP A1, is imported by virtue of a signal of 38 amino acids [13•,14]. This sequence, termed M9, is different in character from basic NLSs, being rich in glycine and aromatic residues. M9 is recognized by a specific M9 receptor termed transportin, which is distantly related to importin β, and translocation of the cargo–transportin complex is mediated by the Ran GTPase [11] (Fig. 1). Although currently only two pathways for importing proteins to the nucleus have been described at the molecular level, it seems likely that the cell employs several more receptor-mediated pathways in order to import the diverse RNA and protein species that function in the nucleus.

Protein export

Nearly 40 years ago, Goldstein [15] reported that proteins are capable of moving out of the cell nucleus. When an amoeba nucleus containing labelled proteins was transplanted into an unlabelled amoeba, labelled proteins

Figure 1



Nuclear protein import. Two nuclear import pathways have been characterized at the molecular level. **(a)** The classical NLS import pathway. Proteins containing classical basic NLSs are imported by the importin α/β (also termed karyopherin α/β) pathway. The basic-NLS-bearing protein is recognized in the cytoplasm by importin α, and importin β mediates docking (not shown) of the complex with the NPC. Ran GTPase (not shown) is required for translocation of the cargo–importin complex through the NPC. Importin α and the cargo are released into the nucleoplasm. The importins are recycled back to the cytoplasm for another round of import. **(b)** The M9 protein import pathway. Proteins containing an M9 NLS, for example hnRNP A1/A2, are imported by the transportin pathway. In the cytoplasm, M9 is recognized by a specific receptor termed transportin, which is distantly related to importin β. Translocation of the transportin–cargo complex into the nucleus is mediated by Ran GTPase (not shown). Following release of the cargo into the nucleoplasm, it is likely that transportin is exported to the cytoplasm for further import of M9-bearing proteins. See text and Görlich, this issue, pp 412–419, for details. Nuc, nucleus; Cyt, cytoplasm.

appeared in the nucleus of the recipient cell [15]. The more recent use of the heterokaryon assay, microinjection of recombinant proteins into somatic cell or *Xenopus* oocyte nuclei, and other methods for assaying protein export (Table 1) has demonstrated that this import/export, or 'shuttling', is undergone by diverse cellular and also viral proteins, for example some hnRNP proteins [16,17], transcription factors [18,19], protein kinases [20••,21] and the retrovirus Rev/Rex proteins [22,23]. Nuclear protein export is therefore important for a number of cellular and viral processes. Here, we focus largely on the role of protein export in the transport of RNA molecules from the nucleus to the cytoplasm.

Protein nuclear export sequences

The techniques outlined in Table 1 allowed a search for protein nuclear export sequences (NESs) that, by analogy with NLSs, promote the export of heterologous proteins that would otherwise be restricted to the nucleus. The search has so far yielded three types of NES, namely the ~10 amino acid leucine-rich NES initially identified in PKI (protein kinase inhibitor, the cellular inhibitor of protein kinase A [20••]) and in HIV-1 Rev protein [20••,24••], the M9 sequence of hnRNP A1 [25••], and a 24 amino acid signal found in hnRNP K that is termed KNS for hnRNP K nuclear shuttling domain [26•] (Fig. 2). When fused to a nuclear-restricted protein, these NESs are capable of exporting the protein in a rapid, temperature-dependent manner. Quite a number of proteins have now been reported to contain a leucine-rich NES (see, for example, [27]), and, although these sequences may function as NESs when isolated, it will be important to demonstrate that they are functional in their native protein context. Database searches with this motif identify a large number of proteins. It is likely that other constraints on this type of NES have yet to be defined [28].

The NESs identified in hnRNP proteins distinguish themselves from leucine-rich NESs not only in primary sequence, but also in their ability to target proteins both into and out of the nucleus [13•,25••,26•]. This suggests that the leucine-rich NES pathway and the hnRNP protein NES pathways are mechanistically distinct.

Leucine-rich NES-mediated export is saturable [24••]. This property of NES-mediated protein export indicates that specific receptors recognize NESs and mediate the rapid export of NES-bearing proteins to the cytoplasm.

Table 1

Protein nuclear export assays*.

	Heterokaryons	*Xenopus* oocyte nuclear injection	Somatic cell nuclear injection
Test protein	Endogenous Expressed from transfected DNA encoding NPc–test-protein fusion†	Recombinant *In vitro* translated	Recombinant
Detection	Immunofluorescence	Dissect oocyte into N and C‡, analyze N and C fractions by SDS-PAGE and Western blotting/autoradiography	Immunofluorescence/fluorescence
Advantages	Can delineate NES, even if it is coincident with NLS No need for purified recombinant protein Identifies shuttling proteins	Allows analyses of export pathways by competition experiments Can study RNP export	Allows analyses of export pathways by competition experiments Can study RNP export
Disadvantages	Requires reasonably high transfection efficiency	Difficult to identify shuttling proteins	Difficult to identify shuttling proteins Requires purified recombinant proteins
References	[16,25••]	[24••,32]	[20••,81]

*Methods for assaying nuclear protein export. The three most general methods for identifying shuttling proteins and delineating NESs are compared. †NPc, nucleoplasmin core domain. ‡N, nucleus; C, cytoplasm. See selected references for further details.

Progress in the identification and characterization of NES receptors is covered below.

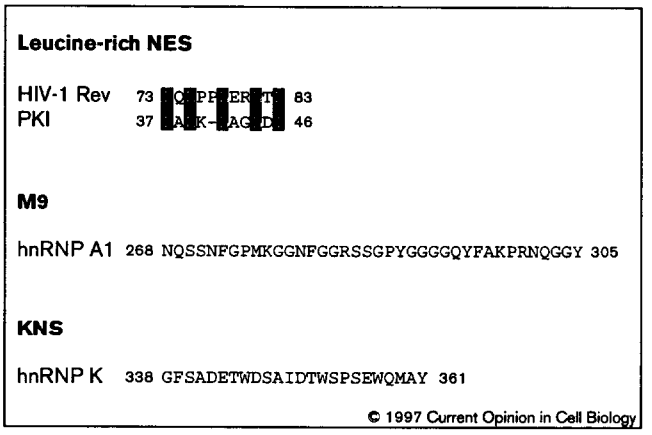
RNA export

The major classes of cellular RNA that are synthesized in the nucleus and that either function in the cytoplasm (i.e. mRNA, rRNA and tRNA) or undergo maturation in the cytoplasm prior to being re-imported to the nucleus (e.g. spliceosomal U sn [small nuclear] RNA) are exported as RNA–protein (RNP) complexes [29,30]. The substrates for the export machinery are therefore complex structures comprising the RNA molecule and, in many cases, a large retinue of specific RNA-binding proteins, and probably proteins associated with them.

Although the export pathways are likely to converge at some point, it is clear that the pathway taken by each class of RNA is in some way unique, as microinjection of large amounts of mRNA, U snRNA, or tRNA into *Xenopus* oocyte nuclei inhibits the export of only the injected RNA species [31–33]. Beyond this, relatively little is understood of the molecular mechanisms of cellular RNA export. The complexity of the exported moiety, our as yet rudimentary understanding of the NPC (less than 20 of the 100 or more polypeptides that assemble into NPCs have been identified), and the absence of a reliable *in vitro* export assay are the main reasons for the current dearth of knowledge.

RNA sequence elements that influence export have been identified in RNA polymerase II transcripts. Splicing signals and introns generally prevent pre-mRNA export [34–36], and the 5′ m^7G cap structure appears to be essential for U snRNA export [36,37•] and plays a stimulatory role in the transport of mRNA [32,36]. The poly(A)+ tail and the histone 3′ stem loop also stimulate mRNA export [32,38,39]. Here, we emphasize the role of RNA-binding proteins in RNA transport (see [30,40] for more detailed reviews of RNA sequence elements that modulate export).

Figure 2



Nuclear protein export signals. Three types of NES have been identified, namely, the leucine-rich NES, the M9 signal and the KNS. Examples of proteins containing each type of NES are shown. The numbers flanking each sequence refer to the amino acid (aa) position of the NES within the 116 aa Rev protein, the 75 aa PKI protein, the 320 aa hnRNP A1 and the 463 aa hnRNP K protein. Residues that are important for function and/or are conserved in leucine-rich NESs are boxed in black.

Viral RNA transport

As has often been the case for other fundamental cellular processes, studies of viral RNA transport have provided much insight into cellular RNA export mechanisms. The HIV-1 Rev protein, and its functional homologues in

other retroviruses, are the best characterized RNA export factors. Rev is essential for the nuclear export of partially spliced and unspliced viral transcripts encoding structural proteins, and for the export of the HIV genome. Without Rev, these RNAs are retained in the nucleus until they are either spliced or degraded [41]. Rev contains two functional domains that are necessary for its transport activity, namely an arginine-rich RNA-binding domain that binds specifically to an RNA element found in all HIV partially spliced/unspliced transcripts, termed the Rev response element (RRE), and an effector domain that harbours a leucine-rich NES (Fig. 2; [20••,24••]). Thus, Rev binds to RRE-containing RNA, and, by virtue of the Rev NES, the RNP complex is exported to the cytoplasm. Rev appears to direct intron-containing viral transcripts to an export pathway that can override nuclear retention of intron-containing RNAs. Rev protein mediated RNA export provides a clear demonstration that RNA-binding proteins mediate RNA export.

Rev can function in *Xenopus* oocytes [24••] and in yeast [42•], in addition to mammalian cells, and all of these systems are being used to address the molecular mechanism of Rev-mediated transport. A number of proteins bearing a phenylalanine–glycine (FG) repeat motif, a characteristic of a group of NPC proteins, can interact with Rev in a yeast two-hybrid protein–protein interaction assay [42•–44•,45]. Some of these are NPC proteins, while others may be nucleoplasmic or associated with the NPC. Overexpression of a human FG-containing Rev-interacting protein (hRIP, also termed Rab for Rev/Rex activation domain binding protein) enhances Rev-mediated RNA export [43•,44•]. A direct interaction between Rev and any of these FG-motif proteins has not yet been demonstrated. As Rev can still function in yeast that have deletions in Rip1p [42•], it seems that the viral RNP complex–FG protein interactions are redundant with one another to some extent. Cellular leucine-rich NES-containing proteins that are not involved in RNA transport, for example PKI, also interact with hRIP in the yeast two-hybrid assay [46,47], suggesting that Rev uses an existing cellular export pathway rather than forging its own route out of the nucleus (see also below). The translation factor eIF5A (eukaryotic initiation factor 5A) has been implicated in Rev-mediated export [48]. However, as Rev functions in *Xenopus* oocytes that apparently do not contain eIF5A, it is unlikely to perform an essential role in transport.

The intriguing question of whether the cell contains a Rev-like factor has been brought into sharp relief with the identification of RNA elements in two simple retroviruses, Mason-Pfizer monkey virus (MPMV) and Rous sarcoma virus, that mediate the export of unspliced viral transcripts in the absence of any viral protein [49,50]. Termed constitutive transport elements (CTEs), these RNA elements can functionally replace Rev and the RRE to promote the export of intron-bearing HIV RNA, and the MPMV CTE can promote the export of a cellular intron-containing RNA that is normally retained in the nucleus. It is therefore believed that a cellular protein with Rev-like properties binds to the viral CTE and could thereby mediate RNA export. The identification of this factor may shed light on cellular mRNA export mechanisms.

Several other viruses, for example adenovirus and influenza virus, encode proteins that control viral, and sometimes cellular, RNA export (reviewed in [51,52]). In the case of adenovirus, a complex of two proteins, E1B 55 kDa and E4 34 kDa, facilitates the cytoplasmic accumulation of late viral transcripts, and at the same time blocks the cytoplasmic accumulation of host cell mRNAs [53,54]. Although the molecular mechanisms involved are currently unknown, further analysis promises to reveal the tricks that this and other viruses employ to export their transcripts in a timely, efficient fashion, and how these mechanisms relate to, or subvert, cellular RNA export pathways.

Cellular RNA transport

The definition of the Rev-mediated viral RNA export pathway invited the question of whether this pathway overlaps with cellular RNA export pathways. In *Xenopus* oocytes, microinjection of excess Rev NES blocks export of U snRNA and 5S rRNA, but has no effect on the export of mRNA, tRNA or ribosomal subunits [24••]. These competition experiments suggest that Rev-mediated viral RNA, U snRNA and 5S rRNA export pathways share at least one component. The 5S rRNA binding protein TFIIIA has been implicated in 5S rRNA transport [55], and, like Rev, it contains a leucine-rich NES [56]. Rev and TFIIIA may therefore compete for the same NES receptor. As yet, no U snRNA associated protein has been reported to contain an NES.

Protein nuclear export sequences, nuclear retention sequences and mRNA export

Export of mRNP complexes can in some cases be visualized by electron microscopy (EM). For example, EM has been employed to observe the transport of Balbiani Ring (BR) transcripts expressed in the salivary gland cells of the insect *Chironomus tentans* [57]. These exceptionally large (~30 kb) transcripts undergo a dramatic and ordered reorganization just prior to, and during, translocation through the NPC [58]. Proteins are lost from the RNP complex during translocation; several of these proteins have been identified [59]. In addition, some of the proteins that accompany the RNA into the cytoplasm have been identified ([60••,61•]; see below).

All three types of protein NES identified so far have been implicated in cellular mRNA export. The M9 and KNS signals (Fig. 2) are found in hnRNP proteins, a group of some 20 or more very abundant, predominantly nuclear RNA-binding proteins that are associated specifically with

pre-mRNA and mRNA molecules [62,63]. Several lines of evidence have implicated hnRNP proteins in mRNA export. First, a subset of them, exemplified by hnRNP A1, rapidly and continuously shuttles in and out of the nucleus, and whilst in the cytoplasm they are associated with mRNA [16,17]. There are ~70 million molecules of each hnRNP protein per cell nucleus [64], so shuttling of these proteins constitutes a major nucleocytoplasmic trafficking pathway. Second, an hnRNP A1 like protein in *Chironomus tentans* exits the nucleus bound to BR mRNA [60••]. Finally, as mentioned, several of the shuttling hnRNP proteins bear NESs that are capable of rapidly exporting proteins that would otherwise be restricted to the nucleus, in a temperature-dependent manner [25••,26•]. As these NESs are devoid of RNA-binding activity, the shuttling hnRNP proteins that harbour them cannot simply be hitching a ride out of the nucleus on an RNA molecule. Rather, it is likely that shuttling hnRNP proteins are part of the mRNA export machinery (Fig. 3).

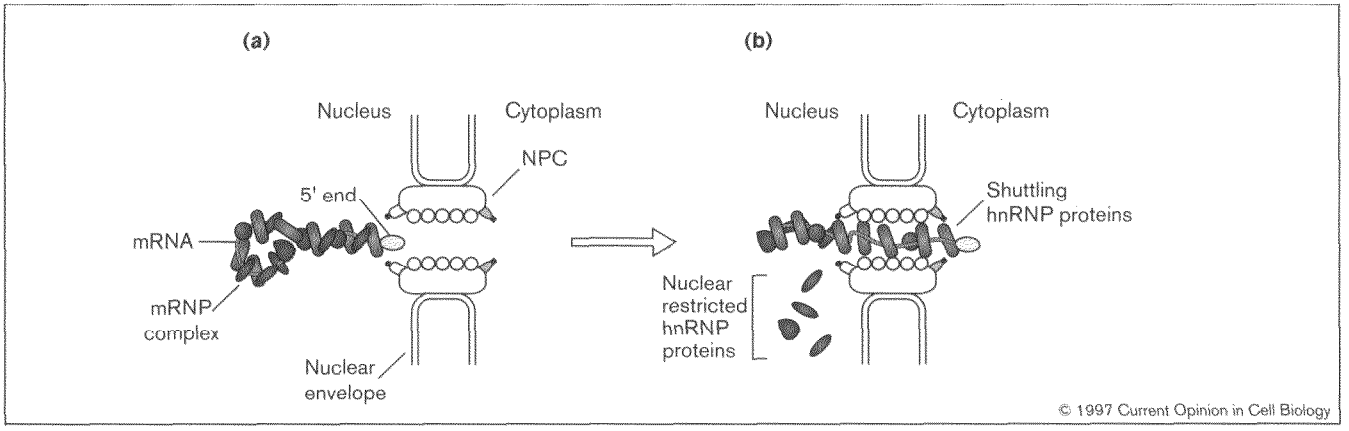
Nonshuttling, nucleus-restricted hnRNP proteins cannot be considered as bystanders in this model of hnRNP protein mediated mRNA export. The nonshuttling hnRNP C proteins contain a nuclear retention sequence (NRS) that, when fused to a shuttling hnRNP protein such as A1, blocks the export of the shuttling hnRNP protein to the cytoplasm [65•]. As this NRS overrides NESs, transcripts that are associated with both NRS- and NES-bearing hnRNP proteins presumably cannot leave the nucleus. Perhaps the NRSs of hnRNP proteins retain transcripts in the nucleus until they are fully processed, thus preventing export of partially processed or unprocessed RNAs, whose appearance in the cytoplasm would be deleterious to the cell. The removal of NRS-containing hnRNP proteins from mRNA prior to export may represent an important regulatory step in RNA transport. Selective release of proteins from BR transcripts prior to or during translocation has been observed [59], and these released proteins may be the insect homologues of NRS-containing hnRNP proteins (Fig. 3).

The leucine-rich NES has also been linked with cellular mRNA export, at least in yeast. An essential yeast protein, termed Gle1, is associated with the NPC, and appears to contain a functional leucine-rich NES [66•]. Yeast with a deletion in the Gle1 NES rapidly accumulate poly(A)$^+$ RNA in their nuclei. Furthermore, Gle1 interacts with some of the FG-containing proteins that are involved in Rev-mediated export, for example Rip1p [66•]. Although the mechanism by which Gle1 operates is unknown, it appears that, at least in yeast, Rev can mimic this element of the cellular mRNA export machinery. This may seem difficult to reconcile with the lack of competition between Rev export and mRNA export in *Xenopus* oocytes (see above). It is possible that yeast mRNA export is different in some ways from export in higher eukaryotes; this difference may be related to the fact that, in *Saccharomyces cerevisiae*, less than 5% of genes contain introns, while in higher eukaryotes most transcripts undergo splicing.

The cap-binding complex

The mandatory role of the 5′ m^{7}G cap in U snRNA export appears to be mediated by a cap-binding complex (CBC), comprising two cap-binding proteins, CBP80 and CBP20 [67,68]. Anti-CBP20 antibodies specifically inhibit U snRNA transport [37•]. In yeast, the genes encoding CBP80 and CBP20 are not essential [69,70••], suggesting that there is not an absolute requirement for these proteins

Figure 3



Model for hnRNP protein mediated mRNA export. **(a)** The 5′ end of the mRNA–protein (mRNP) complex may provide for recognition of the export-competent mRNA by the NPC. **(b)** The mRNP complex undergoes a dramatic reorganization prior to, and during, translocation through the NPC. This has been visualized by electron microscopy of large transcripts in insect cells. The mRNP sheds nucleus-restricted NRS-bearing hnRNP proteins, for example hnRNP C proteins, to release the RNA from nuclear retention. Shuttling NES-containing hnRNP proteins, for example hnRNP A1, accompany the mRNA through the pore and into the cytoplasm, and most likely actively participate in the process of mRNA export. The different shapes and shadings at the nuclear and cytoplasmic faces of the NPC reflect the well documented asymmetry of the NPC at these faces.

in transport of U snRNA or mRNA. However, it is interesting that BR transcripts invariably orient themselves at the NPC with the 5′ end in the lead [57,58], and that CBP20 accompanies this mRNA into the cytoplasm [61•], suggesting that the CBC, or proteins associated with it, provide for the recognition of an export-competent mRNA by the NPC.

The Ran GTPase cycle and RNA transport

The Ran GTPase, an abundant, largely nuclear protein, has been implicated, together with its regulators, in mRNA, U snRNA and 5S rRNA export (reviewed in [71,72]). It does not appear to be necessary for tRNA transport. As mammalian cells with a temperature-sensitive mutation in a guanine nucleotide exchange factor for Ran, RCC1 (regulator of chromosome condensation 1), are defective in the transport of U3 snRNA from the nucleoplasm to the nucleolus, as well as in mRNA and U snRNA export, it has been suggested that the Ran GTPase cycle is necessary for the intranuclear transport of RNA from its site of synthesis to the NPC or to the nucleolus [73]. However, mutations in components of the Ran GTPase cycle have a wide variety of effects. These include defects in nuclear structure, transcription initiation and cell cycle progression [71,72]. Ran is clearly a component of the protein import machinery (Fig. 1), and expression of Ran mutants in mammalian cells has revealed that Ran functions directly in at least one other cellular process, possibly protein and RNA export [74].

RNA transport and the nuclear pore complex

RNP complexes translocating through the NPC must undoubtedly interact with components of the pore complex. Certain NPC proteins have been implicated in Rev-mediated mRNP export (see above), and numerous yeast NPC protein mutants accumulate poly(A)+ RNA in their nuclei, indicating a block in mRNA export (reviewed in [75]). However, these mutations are frequently associated with other phenotypes, such as defects in protein import, RNA processing, and NPC structure, and fragmentation of nucleoli. It has therefore been difficult to ascertain whether the mutant NPC proteins are directly involved in RNA trafficking. In higher eukaryotic cells, overexpression of the FG-repeat portions of various nucleoporins causes nuclear poly(A)+ RNA accumulation, without any apparent effects on protein import [76]. Further study may reveal genetic or biochemical interactions between nucleoporins and RNP complexes that may be unique to, and essential for, the export of particular classes of RNA.

Protein–protein interactions between the NPC and the export substrate may not be the only important interactions during translocation of RNP complexes. One of the yeast nucleoporins, Nup145, whose disruption causes inhibition of mRNA export, appears to contain an RNA-binding motif [77]. This region of the protein can bind RNA homopolymers *in vitro*, and may be a site of interaction between translocating RNA and the NPC. Alternatively, RNA may play a structural role within the NPC, or maybe an RNA component of the NPC mediates important interactions between the NPC and the RNA-binding proteins of translocating RNP complexes.

Regulation of protein and RNA export

It has become abundantly clear that nuclear protein import is subject to regulation (reviewed in [78]). It is therefore anticipated that nuclear export can also be controlled as part of the cells' response to extracellular signals, or during different phases of the cell division cycle. An interesting demonstration of regulated cellular mRNA export was recently reported. Heat-shocked yeast cells respond to this stress by shutting down general mRNA export. However, transcripts encoding heat shock proteins are not subject to this transport inhibition, allowing the yeast cell to synthesize heat shock proteins [79••].

Proteins that are not involved in RNA transport may also be subject to regulated export. For example, in response to signals that increase intracellular calcium levels, the transcription factor NF-AT (nuclear factor of activated T cells) becomes dephosphorylated in the cytoplasm, allowing it to translocate to the nucleus and activate transcription [18,19]. Upon termination of the signal, NF-AT becomes phosphorylated again and is apparently rapidly exported from the nucleus. It will be of interest to know the identity of the NES of NF-AT, and how it may be regulated in response to phosphorylation.

Integrating import and export

That import and export pathways are inextricably linked is likely on consideration of the machineries that mediate transport into and out of the nucleus. The import machinery, having deposited its cargo in the nucleus, has to be re-exported to the cytoplasm to pick up its next cargo; likewise, the molecules mediating export, once they have released their substrate in the cytoplasm, have to be re-imported to the nucleus for another round of export. The continuous cycling of transport machineries through the NPC raises the important question of whether their cargo-carrying properties are restricted to one direction, or whether, at least in some instances, import and export machineries can be one and the same. Interactions between protein import and RNA export machineries have recently been revealed through studies of U snRNA transport. It has been reported that importin α can bind CBC–U snRNA in the nucleus and accompany the RNA into the cytoplasm [70••]. Following export, the importin α–CBC–U snRNA complex can be dissociated by importin β. With this single activity, importin β functions dually to release the export cargo into the cytoplasm, and to mediate recycling, that is, re-import of the export factor CBC [70••]. This is a fascinating mechanism to provide directionality to the transport process and it is the first example of a likely prevalence of interactions between import and export machineries.

Conclusions

It has recently been established that active, receptor-mediated mechanisms exist that mediate protein export from the nucleus to the cytoplasm. Several proteins that function in signal transduction and growth control pathways bear NESs that target them for receptor-mediated export. Nuclear protein export is therefore likely to be a critical point of regulation in signalling and cell cycle control. The cell also exports diverse RNA species from the nucleus to their site of function or maturation in the cytoplasm. Proteins of the CBC are export factors for U snRNAs; the HIV-1 Rev protein is the exporter of viral transcripts; and there is increasingly compelling evidence for the involvement of hnRNP proteins in cellular mRNA transport. Therefore, the reduction of any discussion of RNA export to one of protein export becomes more and more reasonable.

From this condensed survey of our current understanding of protein export and protein-mediated RNA export from the nucleus, several tractable questions are immediately apparent. First, the range of NESs (and NRSs) is unclear. It is possible that all proteins that are actively exported bear NESs that fall into one of the three types of NES already identified (Fig. 2). Perhaps the leucine-rich NES is the export equivalent of the classical basic NLS, in that it is found in a great number of actively exported proteins. The assays outlined in Table 1 will allow the range of cellular and viral NESs to be established. Second, conspicuous by their absence from this discussion are bona fide NES receptors, whose existence is inferred from the saturable property of most export pathways. By analogy with NLS receptors such as importins and transportin (Fig. 1), NES receptors are expected to target NES-containing proteins to, and through, the NPC. As the M9 NLS of hnRNP A1 also functions as an NES, the NLS receptor, transportin, is also a candidate NES receptor. The identification of NES receptors is eagerly anticipated. Third, the molecular mechanisms of regulation of NESs have yet to be uncovered. Most likely, post-translational modifications that modulate NES–receptor interactions will be a common means of regulation.

The identification of NES receptors will accelerate studies of nuclear export to a point where the major current preoccupations in studies of import will also be those for the export field, namely the molecular mechanisms of translocation through the NPC, and the imposition of directionality. For protein import, the Ran GTPase cycle has been hailed as the mediator of both translocation and directionality. However, the mechanism of translocation through the NPC and the role of Ran in this process are quite mysterious. Current knowledge suggests that the Ran GTPase cycle may dictate the direction of movement of the cargo–machinery complex, by virtue of the subcellular locations of Ran regulators: its guanine nucleotide exchange factor (RCC1) is exclusively nuclear, while its GTPase-activating protein (Rna 1) is largely cytoplasmic. Ran-GDP, the predominant form of Ran in the cytoplasm, appears to mediate translocation through the NPC, whereas the nuclear form of Ran, Ran-GTP, mediates release of the cargo into the nucleoplasm by dissociating the cargo–machinery complex (see Görlich, this issue, pp 412–419). There appears to be precedent for such a mechanism for imposing directionality on a transport process. The ARF (ADP-ribosylation factor) GTPase cycle that mediates vesicular transport facilitates transport in one direction only, apparently because its guanine nucleotide exchange factor is restricted to the donor membrane, and its GTPase-activating protein is restricted to the target membrane [80]. It is possible that the Ran GTPase cycle can impose directionality on protein and RNA export, as well as import, and that GTPase cycle mediated directionality will be a common mechanism in diverse intracellular transport pathways.

A more detailed appreciation of NPC structure will of course be essential for dissecting the translocation process, and will allow the important question of NPC communication to be addressed, namely how import and export pathways communicate to avoid collisions or traffic jams within an individual NPC.

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- •• of outstanding interest

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dent of the importin-mediated classical NLS pathway. The gene encoding an M9-binding protein is cloned by the use of yeast two-hybrid screening. Termed transportin, this M9-binding protein is distantly related to importin β. Transportin is shown to mediate import of M9-containing proteins.

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Describes the characterization of a protein, termed Kap104p, that was identified from a search of the yeast genome database for proteins with similarity to importin β. Recombinant Kap104p binds two nuclear RNA-binding proteins, Nab2 and Nab4, in cell extracts. A temperature-sensitive mutant of Kap104p accumulated Nab2 in the cytoplasm, whereas the localization of a classical NLS-containing protein was unaffected. Kap104p therefore defines a novel import pathway in yeast. Kap104p appears to be the yeast homologue of human transportin (see [9••]).

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Identifies the first protein NESs, within the cellular inhibitor of protein kinase A, PKI, and within the HIV-1 Rev protein (see also [24••]). By microinjecting into the nucleus fluorescently labeled proteins comprising portions of PKI fused to heterologous proteins and monitoring their subcellular localization, a 10 amino acid leucine-rich sequence of PKI is found to be capable of promoting the rapid export of proteins that would otherwise remain nuclear. Rev is shown to contain a similar leucine-rich NES.

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The first NES within an RNA-binding protein is identified. The HIV-1 Rev protein, which binds to viral RNAs in the nucleus and mediates their export to the cytoplasm, is shown to contain a 10 amino acid leucine-rich sequence within its previously defined 'activation domain' that functions as an NES. Peptides comprising this sequence cross-linked to BSA (bovine serum albumin) promote rapid export of BSA from the nucleus, in a temperature-dependent manner. By microinjection of saturating amounts of BSA–Rev-NES conjugate into the nucleus of *Xenopus* oocytes, such that Rev-mediated RNA export is blocked, it is shown that U snRNA export and 5S rRNA export are also inhibited, while mRNA, tRNA and ribosomal subunit export are unaffected. Rev therefore directs viral mRNAs into an export pathway that shares at least one component with the cellular U snRNA and 5S rRNA export pathways.

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The previously identified novel NLS within the shuttling pre-mRNA/mRNA-binding protein hnRNP A1 (see [13•]) is here shown to also function as an NES. When fused to a nuclear-restricted heterologous protein, the nucleoplasmin core domain (NPc), the M9 segment of A1 promotes nucleocytoplasmic shuttling of NPc in a heterokaryon assay. The NLS and NES activities of M9 are found, by limited mutagenesis of this sequence, to be inseparable, suggesting that the two signals may be one and the same. The M9 portion of A1 is devoid of RNA-binding activity, strengthening the possibility that A1 is carrying (rather than being carried by) mRNA to the cytoplasm.

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Using a heterokaryon assay, the hnRNP K protein is shown to contain a 24 amino acid NES, thus defining a third type of NES. Although different in primary sequence, the hnRNP K protein NES (termed KNS) shows striking similarities to the M9 signal of hnRNP A1: both KNS and M9 confer bidirectional transport across the NPC, and the NLS activity of both signals is inhibited by inhibitors of RNA polymerase II transcription. By competition experiments in an *in vitro* nuclear import assay, KNS is shown to mediate import by a novel pathway, which is distinct from the classical basic NLS and M9 import pathways.

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Identifies a putative Rev NES receptor, termed human Rev-interacting protein (hRIP), that has properties of an NPC protein (see also [42•,44•]). A yeast two-hybrid screen is used to clone the gene encoding hRIP, which is found to interact in this assay with the Rev effector domain, or Rev NES. hRIP contains FG repeats, a characteristic of nucleoporins. hRIP is localized primarily in the nucleoplasm, and a small amount is associated with the NPC. Overexpression of hRIP in mammalian cells increases the activity of Rev.

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Immunoelectron microscopy demonstrates that an hnRNP protein of *Chironomus tentans* that is similar to human hnRNP A1 translocates through the NPC in association with an mRNA. Although A1 was known to shuttle between the nucleus and the cytoplasm, and to be associated with mRNA in both compartments, the observation that a similar insect hnRNP protein translocates through the pore bound to mRNA demonstrates that A1 is part of the export mRNP.

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Immunoelectron microscopy reveals that Balbiani Ring mRNA molecules translocate through the NPC in association with CBP20. This observation suggests that CBP20 mediates the previously reported stimulatory effect of the cap structure on mRNA export. During translocation through the pore, Balbiani Ring mRNA invariably orients itself with the 5′ end in the lead, suggesting that the 5′ cap structure, and its associated proteins, may be involved in an mRNA–NPC recognition event during mRNA export.

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to mediate the export of U snRNA. Most interestingly, when this complex reaches the cytoplasm, importin β dissociates CBC from U snRNA. The released CBC, associated with importin α and β, is thereby re-imported to the nucleus for another round of U snRNA export. The importin β mediated dissociation of cargo and export factors is suggested to be restricted to the cytoplasm, because the high nuclear concentration of Ran-GTP prevents any importin α–importin β interaction in the nucleus.

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