

Nucleocytoplasmic Transport

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Active transport of proteins and RNAs between the nucleus and cytoplasm is a major process in eukaryotic cells. Recently, factors that recognize transport substrates and mediate nuclear import or export have been characterized, revealing interactions that target substrates to the nuclear pore complexes, through which translocation occurs. Translocation requires energy, and for the import process this energy is at least partly consumed by the action of the small guanosine triphosphatase Ran. In the first half of the review, some of the well-established general background information on nucleocytoplasmic transport is discussed. The second half describes recent information on the mechanistic details of nuclear import and export as well as major unresolved issues such as how directionality is conferred on either import or export. The whole review is slanted toward discussion of metazoan cells.

to the cytoplasm (12). The biogenesis of ribosomes impressively demonstrates that nuclear transport is a major activity. For example, a HeLa cell contains 10 million ribosomes and divides every 24 hours. This means a total of 560,000 ribosomal proteins must be imported and 14,000 ribosomal subunits exported every minute, meaning that 100 ribosomal proteins and 3 ribosomal subunits travel through each pore each minute.

Signals for Transport Across the Pore

The defining feature of a eukaryotic cell is its nucleus. It is enclosed by the nuclear envelope, a double membrane that is continuous with the endoplasmic reticulum, which separates nucleoplasm from cytoplasm and thereby offers a means of regulation of gene expression that is unavailable to prokaryotes. Nuclear pore complexes (NPCs) are the sites of exchange of macromolecules between the two compartments (1). The NPCs have a mass of about 125 megadaltons in higher eukaryotes and are estimated to contain roughly 100 different polypeptides [for review, see (2, 3)]. Characteristic features of many, but not all, vertebrate nuclear pore proteins (nucleoporins) are the modification with O-linked N-acetyl glucosamine and the presence of short degenerate repeats such as the FXFG (4) repeats in Nup153p and p62 or GLFG (4) repeats in Nup98p. The NPC constitutes a passive diffusion channel about 9 nm in diameter [for review, see (5)]. Small proteins such as cytochrome c (13 kD) can diffuse freely through the pore, whereas diffusion of ovalbumin (43 kD) is delayed and that of bovine serum albumin (BSA) (66 kD) is virtually prevented. Proteins above the size limit for passive diffusion can enter the nucleus only in an active way. However, even small nuclear proteins, such as histones, generally enter the nucleus actively rather than by diffusion (6).

Active transport across the NPC can accommodate particles up to 25 nm in diameter. This process is characterized by energy (7, 8) and signal dependence (9) and saturability (8, 10) and is thus carrier-me-

diated. Transport across the pore occurs in both directions and involves various substrates. For example, all nuclear proteins must be imported from the cytoplasm, their site of synthesis. Transfer RNAs (tRNAs) and messenger RNAs (mRNAs), on the other hand, are exported from the nucleus to the cytoplasm, their site of function.

The biogenesis of other ribonucleoproteins (RNPs) is more complex. For example, some of the small nuclear RNAs (snRNAs) involved in precursor mRNA (pre-mRNA) processing (U1, U2, U4, U5, or U7, for instance) are rapidly exported out of the nucleus after transcription. In the cytoplasm, after their assembly with a group of common proteins called the Sm proteins, they undergo a number of modifications such as cap hypermethylation, where their m⁷GpppN 5' ends are modified to m^{2,2,7}GpppN. These partly mature U small nuclear ribonuclear proteins (snRNPs) reenter the nucleus to complete their assembly by association with U snRNP specific proteins or, in the case of U4, with U6 snRNA [reviewed in (11)].

The assembly of ribosomes in the nucleolus requires the initial import of ribosomal proteins from the cytoplasm, their incorporation into ribosomal subunits, and reexport

Transport of proteins and RNPs across the NPC is generally selective and signal-dependent. "Classical" nuclear localization sequences (NLSs) are generally characterized by one or more clusters of basic amino acids, but they do not fit a tight consensus. For example, nucleoplasmin, the first protein in which a nuclear targeting signal was experimentally demonstrated, contains a bipartite NLS (13) (see Table 1). The NLS of the large T antigen of simian virus 40 (SV40) was initially found by point mutations that mislocalized the protein to the cytoplasm (14) and was later defined as a seven-amino acid sequence (see Table 1) sufficient to confer nuclear localization even when conjugated as a synthetic peptide to, for example, serum albumin (10, 15). Microinjection of NLS peptide conjugates at high concentration led to saturation of the protein import pathway (10), providing strong evidence for the existence of a saturable NLS receptor. Kinetic competition studies (16) and later, direct import and binding experiments (17, 18), have shown that the SV40 and nucleoplasmin NLSs use the same receptor, whereas U snRNPs do not compete with karyophilic proteins for import (16, 19) and presumably have distinct receptors.

Table 1. Signals involved in protein transport across the pore.

Signal (length)	Sequence (4)	Function
SV40 large T antigen NLS (7)	PKKKRKV	Nuclear import
Nucleoplasmin bipartite NLS (16)	KRPAAIKKAGQAKKKK	Nuclear import
IBB domain from importin- α (41)	RMRKFNKGKDTAELRRRRV-EVSVELRKAKKDEQILKRRNV	Targets the nuclear import receptor to the nucleus
hRNP A1 M9 (38)	NQSSNFGPMKGGNFGGRSSGP-YGGGGQYFAKPRNQGGY	Confers rapid shuttling
HIV-1 Rev NES (9)	LPPLERLTL	Rapid nuclear export
PKI NES (10)	LALKLAGLDI	Rapid nuclear export
TFIIIA NES (19)	QPDASKADPLPVLENLTlk	Rapid nuclear export

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By strategies similar to those used for the NLS, signals for rapid nuclear export [nuclear export signal (NES)] (see Table 1) have been found thus far in the human immunodeficiency virus (HIV) Rev protein, transcription factor IIIA (TFIIIA), and PKI, an inhibitor of protein kinase A (20–22) [reviewed in (23)]. The one signal defined thus far that functions to direct both import and export, or shuttling between the nucleus and cytoplasm, is the M9 domain of hnRNP A1 (Table 1) (24).

The question of what signals the export of cellular RNAs from the nucleus is more difficult to answer. RNA export is dependent on the completion of posttranscriptional processing events. The activities responsible for these maturation steps bind to the precursor RNAs and retain them in the nucleus (25), making release from retention essential for RNA export. Second, not naked RNAs, but RNPs of complex composition are the export substrates, and the composition of these RNPs can change during export [reviewed in (26)]. Proteins that leave the nucleus bound to RNA, such as the NES-carrying Rev or TFIIIA proteins mentioned above, are thought to carry the actual export signals. Third, it is very likely that many (redundant) signals contribute to the export of large RNPs, making identification of export mediators difficult. Perhaps because of these problems, the only well-defined RNA export signal is the cap structure of RNA polymerase II transcripts, which binds to proteins involved in export. These cap-binding proteins are most critical for the export of U snRNAs (27), whose cap becomes trimethylated when they reach the cytoplasm. The resulting structure, $m^2,2,7\text{GpppN}$, is part of the signal that targets U snRNPs to the nucleus (11).

Nuclear Protein Import

Most knowledge of nuclear protein import originates from studies in mammalian and *Xenopus* systems. Experiments based on microinjection into *Xenopus* oocytes or in vitro transport into nuclei assembled in egg extract first allowed the import process to be divided into two steps: binding to the NPC, probably to fibrils extending from the cytoplasmic side of the pore, followed by energy-dependent translocation through the pore (28). A critical technical breakthrough was the development of an in vitro system based on cultured mammalian cells treated with digitonin to selectively permeabilize the plasma membrane (29). A fluorescein-labeled import substrate, such as a BSA-NLS-peptide conjugate or nucleoplasmin, can be introduced into the cells through the leaky plasma membrane, and its uptake into the nucleus can be followed by fluorescence microscopy. Digitonin treatment of the cells

has a second consequence: It depletes the cells of their soluble contents. The observation that active import was dependent on the readdition of crude or fractionated cytosol (29, 30) led to the purification of the four soluble factors presently known to be required for nuclear protein import: (i) importin- α (17, 18, 31, 32), (ii) importin- β (33, 34), (iii) the small guanosine triphosphatase (GTPase) Ran (35, 36), and (iv) pp15 (37) [for nomenclature, see (38)].

It seems certain that additional factors will be involved in nuclear protein import. Factors that are essential will only be detected if they are sufficiently depleted upon cell permeabilization. Also, additional activities might be found that are needed to recycle the components thus far identified. Furthermore, regulatory components have yet to be identified which are, for example, responsible for the higher import capacity of proliferating cells as compared with that of quiescent cells (39).

Mechanism of Protein Import

Several stages of nuclear protein import have been distinguished experimentally (Fig. 1). First, the import substrate binds via its NLS to the importin α - β heterodimer in the cytoplasm (34, 40). The heterodimer therefore corresponds to the entity originally defined as the NLS receptor (10), with the α subunit providing the NLS-binding

site (17, 18, 31). This NLS protein-receptor complex docks to the nuclear pore complex via importin- β (32, 41) and is subsequently translocated through the pore by an energy-dependent mechanism (28) that also requires Ran and pp15 (36, 37). The constituents of the NLS recognition complex become separated as a result of this process. The import substrate and importin- α reach the nucleoplasm, whereas importin- β accumulates at the nuclear envelope (32, 41). Immunoelectron microscopy detects importin- β at both sides of the NPC (41), which suggests that it does not remain at its initial docking site but moves with importin- α and the import substrate through the pore. No importin- β accumulation is seen in the nucleoplasm, presumably because its recycling to the cytoplasm is too rapid. In the nucleus, importin- α has to dissociate from the NLS-containing import substrate. Given the high concentration of NLSs in the nucleus, which would tend to keep the NLS-binding site on importin- α occupied, it is likely that this involves conversion of importin- α to a form with a low affinity for the NLS. The different rate of reexport of the α and β receptor subunits indicates that they return to the cytoplasm separately, possibly by different routes. We will now discuss the steps of this process in more detail.

Both importin- α and importin- β consist largely of repeated domains, the so-called

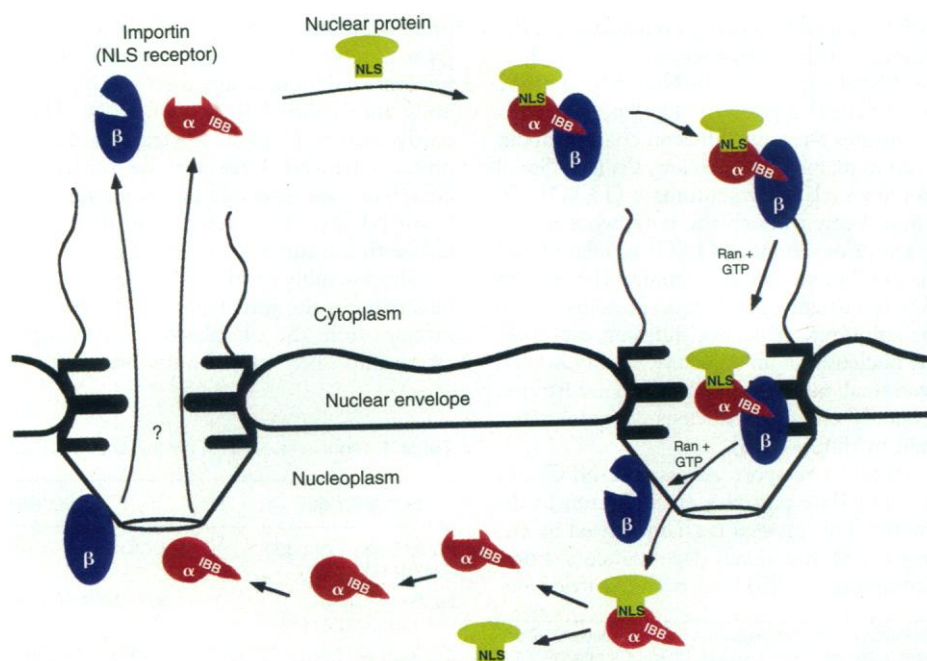


Fig. 1. Steps of the nuclear protein import cycle. NLS-containing proteins bind to the importin heterodimer (NLS receptor) in the cytosol. The NLS interacts primarily with the α subunit; the IBB domain of α mediates heterodimerization. NLS binding to α can precede α - β interaction. The β subunit mediates docking of the complex at the NPC. Translocation involves GTP hydrolysis by Ran and is probably a multistep process. The α - β heterodimer dissociates, and α enters the nucleoplasm with the substrate. Dissociation of α from the nuclear protein must then occur. For a further round of import, the subunits of importin are returned to the cytoplasm, possibly separately.



arm repeats that are found in several functionally unrelated proteins, including the founder member of the family, the *Drosophila* armadillo protein [for review, see (42)]. Importin- α has 8 repeats and importin- β has 11 similar but more divergent repeats, although the precise number depends on how the domains are defined (17, 34, 43). The NLS binds directly to the arm repeat region of importin- α . The exact binding site is not well defined, but a limited number of repeats have been shown to be sufficient for NLS binding (44, 45). Open questions about this interaction include how many NLSs can bind simultaneously to a single importin- α monomer and whether all NLS-containing proteins interact similarly with importin- α . It is important to note that *Saccharomyces cerevisiae* has a single α subunit, SRP1p (46), but higher eukaryotes express a family of related but divergent genes, even within the same cell; for example, Rch1p and hSRP1p in human HeLa cells (44, 47). Studies in *Drosophila* indicate that different family members are not fully equivalent (48). The importin- α -related *oho31* gene is not essential, thus other family members must suffice to import most proteins. However, *oho31* inactivation causes malignant transformation of hematopoietic cells, which strongly suggests that the functions of the different family members are not identical.

The importin- β binding domain (IBB domain) (Table 1) is located at the NH₂-terminus of importin- α (49, 50). Remarkably, this region of the protein has all the features of an NLS, being characterized by clusters of basic amino acids. Nevertheless, the IBB domain does not detectably bind to full-length importin- α , but instead interacts strongly with the β subunit. The similarity between an NLS and the IBB domain points to the armlike repeat regions of importin- β as a likely site of interaction with α .

When fused to a heterologous protein, the IBB domain confers not only binding to importin- β but also transit into the nucleus, bypassing the requirement for importin- α (49, 50). Interaction with the NPC and the mechanism that drives translocation must therefore target importin- β . Consistent with this, importin- β has been shown to bind to the GLFG or FXFG (4) repeat domains of several nucleoporins in vitro (32, 51–53), although the function of this binding in either docking or translocation has not yet been established.

One can consider the IBB domain as the archetypal nuclear targeting signal in the sense that it is sufficient to target a protein to which it is attached to the nucleus, and its structural similarity to an NLS suggests that the two may be evolutionarily related. In this scenario, importin- β would have been the original NLS receptor and nuclear

proteins would have had an IBB-like NLS. Addition of the α subunits at a later time would likely have been favored by their capacity to allow recognition of a greater variety of transport substrates.

Ran and Translocation

After the nuclear protein–NLS receptor complex has been assembled and docked to the outer face of the NPC through the interactions described above, the next event in import is energy-driven translocation. It has been clearly established that at least part of the energy requirement is due to guanosine triphosphate (GTP) hydrolysis by Ran. We will now discuss some models of translocation, but lest anyone take them too seriously, we should say now that it is by no means certain that GTP hydrolysis by Ran is the only source of energy for nuclear import. Further, no detailed function has yet been ascribed to p15.

Because the import substrate has to move over a rather long distance, it is likely that translocation is actually a succession of consecutive energy-dependent steps, perhaps driven by the same mechanism. To get a rough idea of how many nucleotides might need to be hydrolyzed for a single nuclear pore passage, a comparison can be made with the energy consumption of other directed movements. The motor protein kinesin moves one 8-nm step per hydrolyzed adenosine triphosphate (ATP) (54). A single ATP-driven myosin powerstroke is about 10 nm (55). If these analogies hold, and movement of the importin-substrate complex occurs in steps across a support formed by nuclear pore proteins, one could expect the consumption of about 10 molecules of GTP, given the roughly 100-nm distance through the NPC.

Ran, like other GTPases, requires effectors to help it hydrolyze GTP and exchange the formed guanosine diphosphate (GDP) for GTP. The only known guanine nucleotide exchange factor for Ran is the nuclear protein RCC1 (56); the only known Ran GTPase activating protein (57) is cytoplasmic. Does this mean that GTP exchange happens only in the nucleus and GTP hydrolysis only in the cytoplasm? Ran itself is small enough to enter and leave the nucleus by diffusion. If we assume that Ran-catalyzed GTP hydrolysis provides the energy required for a translocation event, our rough calculation suggests that 10 molecules of Ran would have to diffuse through the pore in each direction to achieve the active transport of a single molecule—certainly not a very attractive model given the huge numbers of transported molecules, even if Ran is an extremely abundant protein.

A fraction of Ran can be visualized bound to NPCs (41, 58), and the nuclear

pore proteins RanBP2 and Nup2p bind Ran with high affinity (58, 59). When Ran association with the NPC was measured with an antibody raised against a COOH-terminal peptide (58), it was reported that only Ran-GTP could bind. However, it is possible that binding of Ran-GDP was not detected because antibodies raised against this peptide preferentially recognize Ran-GTP (60). In another study, Ran-GDP and Ran-GTP were found to bind equally well to the nuclear envelope when examined by immunofluorescence with antibodies raised against recombinant Ran (61). If NPC-bound Ran can be in the GDP or GTP state, nucleotide exchange could also occur at the pore.

Translocation involves movement through the NPC for a considerable distance, and importin- β has been shown to bind to several nucleoporins containing FG (4) repeats (see above). One model to explain movement of the NLS-receptor complex through the pore involves docking of the complex, via importin- β binding, to one set of repeats, followed by Ran-dependent undocking, diffusion, and docking to the next binding site and so forth (52). In vitro studies with recombinant yeast proteins led to the discovery of several interactions that cause dissociation of complexes that are important for nuclear protein import (53). Binding of the FXFG (4) repeat region from the nucleoporin Nup1p to an importin heterodimer–NLS protein complex caused dissociation of the NLS protein. In addition, Ran-GTP addition was found to dissociate either the Nup1p FXFG repeat or an NLS protein from the importin heterodimer and even to cause dissociation of importin- α from - β (53). Ran-GTP itself was found to bind importin- β .

The sum of these reactions would make perfect biological sense for the terminal step of translocation, when the NLS-receptor complex has to be disassembled before the importin subunits are recycled to the cytoplasm. Nevertheless, they have been interpreted in terms of the above translocation model by the suggestions that each step in the translocation process is a round of dissociation and reassociation of all the components of the complex and that movement through the pore might take place when these reactions occur stochastically (53).

A difficulty with this model is that translocation is a directional event that occurs against a concentration gradient. For a protein such as importin- β that binds directly to the NPC, directionality could be achieved (in one direction) by an organized array of binding sites through the NPC, going from lower to higher affinity. It is more difficult to imagine how the other components of the dissociated NLS-receptor complex would be targeted to reassoci-

ate with the molecule of importin- β that has moved one step forward rather than with the one nearer the cytoplasmic face of the NPC. A further problem with the model is to imagine how large particles can be moved. Gold particles 25 nm in diameter coated with many molecules of nucleoplasmin, or export substrates such as large RNPs, have multiple NLSs or NESs and thus are likely to contact the NPC at several places simultaneously. All contacts would need to be released at the same time if they were to move at all. The transport of a large particle such as a ribosome or a 25-nm gold particle requires substantial conformational change in the pore, and it is hard to imagine how this can be achieved without force.

The translocation model we would favor has some analogy to motor proteins in that movement would be proposed to occur in a processive way in discrete steps along a stationary phase. The importin-substrate complex would move from one binding site on the pore complex to the next without detaching entirely from the NPC and without complete dissociation of the complex components. Ran is the best candidate to provide energy for the movement. A translocation driven by multiple Ran-GTP cycles could occur by a single NLS-receptor complex interacting with different Ran molecules along its journey, or Ran might move with the complex, undergoing several GTPase cycles while bound to importin- β . Processive movement from one site on the NPC to the next without detachment implies the existence of more than one NPC binding site within the Ran-NLS receptor complex. These putative distinct binding sites would be used alternately to allow stepwise movement. Their affinity for the NPC might be regulated by the GTP state of Ran.

Receptor Recycling and Shuttling

After the transport of the import substrate into the nucleus has been completed, importin- α and - β have to return to the cytoplasm without the import substrate. Reexport of the NLS-receptor subunits cannot therefore be the simple reversal of the import reaction. The observation that importin- β is recycled much faster than importin- α (41) could indicate that the two subunits are returned to the cytoplasm separately.

The reexport of importin- α appears to be so rapid that most cells show a higher cytoplasmic than nuclear concentration. Importin- α enters the nucleus by its IBB domain. When this IBB domain is fused to a heterologous protein, the fusion, in contrast to native importin- α , accumulates in the nucleus to a very high concentration, with no signs of reexport (49). It again

follows that reexport of importin- α involves different interactions from those needed for its entry. The different domains of importin- α that are responsible for its nuclear entry and return to the cytoplasm might cross-talk to its NLS-binding site in such a way that importin- α adopts a high-NLS affinity conformation on import but a low-affinity form on reexport.

RNA Export

Most aspects of RNA export have been comprehensively reviewed elsewhere (3, 23, 26), and we will concentrate on recent results that provide mechanistic insight into the process. The best understood mediator of RNA export is the HIV-1 Rev protein. Like many other retroviruses, HIV-1 produces a variety of proteins from alternatively spliced mRNAs. However, this raises a problem because some of the essential mRNAs, as well as the full-length genomic RNA, contain introns, and intron-containing pre-mRNAs would not normally leave the nucleus. One intron in the unspliced HIV-1 RNAs contains the RRE, or Rev response element. This is a binding site for several Rev molecules. When bound to the RRE, Rev acts to allow export of the unspliced RNA. This export function requires not only the RNA binding domain of Rev but an additional "activation" domain [reviewed in (62)]. Recent work has shown that the activation domain is an NES (Table 1). When coupled to a heterologous protein, the Rev NES directs the rapid export of the fusion product from the nucleus to the cytoplasm. Thus the NES signals export of the protein, with bound RNA being exported as a consequence (20, 21). In comparison to nuclear import, it is perhaps easiest to think of Rev as an adaptor between the RNA and the export machinery, with the NES being equivalent to an NLS.

So what is the "NES receptor"? Through use of the two-hybrid method, three studies have identified proteins that interact with the Rev NES. Two groups identified the same human protein, called hRip (63) (Rev-interacting protein) or Rab (64) (Rev activation domain-binding protein; note that this protein is unrelated to the many Rab GTPases involved in vesicle transport), whereas the third, after showing that Rev functions in yeast (65), found a yeast protein called Rip1p that is distantly related in sequence to the human protein (66).

Both the yeast and the human Rip bear a resemblance to certain nucleoporins. They contain degenerate repeats characterized by FG (4) dipeptides and are most similar to the human CAN (67) and the yeast Rat7p-Nup159p (68) nucleoporins. Additional evidence that Rip might be functionally related to nucleoporins came

from the finding that FG-containing repeat segments from two bona fide nucleoporins also showed positive interaction with the Rev NES in the two-hybrid test (66). The data on Rip localization are still contradictory (63, 64, 66), thus it is not yet clear whether the Rev-Rip interaction is restricted to the NPC or whether Rip binds Rev initially in the nucleoplasm and targets Rev to the pore.

At least two models for Rip involvement in Rev-mediated translocation through the pore seem possible (Fig. 2). Rev-containing RNPs might be translocated by stepwise interaction of the NES with Rip, then with other nucleoporins. Alternatively, Rip and Rev might be moved together as a complex. At this stage, more detailed models are premature because Rip localization is not yet resolved and a convincing demonstration of direct, specific Rev-Rip interaction has not yet been provided.

What about cellular RNAs? It has been shown by competition studies that the export of different classes of cellular RNA such as mRNA, tRNA, 5S ribosomal RNA (rRNA), U snRNA, and rRNA (as ribosomal subunits) is mediated, at least in part, by class-specific factors (27, 69). These specific factors are likely to be RNA binding proteins (26), and it is unclear whether the pathways of export of the different classes of RNA will converge at some point. It has been reported that when Rev NES peptides conjugated to BSA are microinjected into *Xenopus* oocyte nuclei at high concentration, they saturate not only the Rev-mediated export of RRE-containing RNAs but also that of cellular 5S rRNA and U snRNAs, whereas they have no effect on mRNA, tRNA, or ribosome export (20). This suggests that export of 5S rRNA and of U snRNAs might be mediated by proteins carrying an NES that is similar to that of Rev.

At least in *Xenopus* oocytes, 5S rRNA leaves the nucleus shortly after transcription and is reimported at a later stage for incorporation into ribosomes. Two proteins, TFIIIA and ribosomal protein L5, have been implicated as redundant mediators of the export step, because mutant 5S RNAs that bind to neither remain in the nucleus, whereas RNAs that bind to one or the other can be exported (70). Although this evidence is indirect, the observation that vertebrate TFIIIA's contain a sequence similar to the Rev NES (20) was provocative. Indeed, this sequence can functionally substitute for the Rev activation domain in the context of HIV-1 growth and, if conjugated to BSA, acts as an NES (22). However, no interaction between Rip and TFIIIA was detected in the two-hybrid assay (22). To proceed further with this analysis, it is essential to establish a TFIIIA-

dependent 5S rRNA export system and then to ask whether the NES-like sequence is required for TFIID action.

A nuclear monomethyl cap-binding protein complex (CBC) is involved in the export of U snRNAs (71). Cap structures are added cotranscriptionally to all RNA polymerase II transcripts, including mRNAs and most U snRNAs (72). The CBC consists of two proteins, CBP80 and CBP20, both of which are required for cap-specific binding (71, 73). Although CBP80 does contain sequences that somewhat resemble the hydrophobic NESs in Table 1, there is no evidence that they are required for CBC function in export. It is also plausible that CBC might work indirectly, for example, by the recruitment of another NES-containing polypeptide to the RNP. In HeLa cells, CBP80 and CBP20 are nucleoplasmic proteins that show a relatively uniform distribution, with no detectable enrichment at NPCs (73, 74). In the salivary glands of the insect *Chironomus tentans*, which provide excellent material for

the morphological study of RNP synthesis, assembly, splicing, and transport, CBP20 is seen to bind to nascent Balbiani Ring (BR) transcripts early in synthesis and to remain part of the BR RNP while splicing, transit through the nucleoplasm, and translocation through the NPC occur (74). The direct observation of CBC on translocating RNPs is consistent with a role in either docking or movement through the NPC.

Another human protein whose role in (m)RNA export is strongly suspected, if not definitively proven, is hnRNP A1. It shuttles rapidly between the nucleus and cytoplasm, and in both compartments is bound to polyadenylated RNA (75). A short region of hnRNP A1, called M9 (Table 1), is sufficient to confer shuttling behavior on a heterologous protein. Thus, M9 is both an NES and an NLS, and in fact both signals are clearly interdigitated, because several point mutations in M9 abolish both functions (24). These data make it highly plausible that hnRNP A1 is involved in mRNA export,

and recent indirect evidence also suggests a role for a second hnRNP protein, L, in the export of a subclass of mRNAs (76). In vivo, however, A1 and L are two of a considerable number of abundant hnRNP proteins that bind to both pre-mRNAs and mRNAs in the nucleus. Most mRNAs are therefore coated with a mixture of hnRNP proteins, as well as with members of other families of abundant RNA binding proteins such as the SR proteins [reviewed in (26)].

Several of the hnRNP proteins, which are the only family to have been tested, are shuttling proteins, whereas others appear to be retained in the nucleus, presumably by dissociation from the mRNA before or during transport (75). Although a few of the shuttling proteins have signals similar to M9, others do not (24). The complexity of these messenger RNP export substrates will continue to make it extremely difficult to test the contribution of individual proteins to the mRNA export process in vivo.

Questions for the Future

Many questions remain in the field of nuclear transport. For protein import, we know little about the disassembly of the NLS-receptor complex after its arrival at the nuclear face of the pore and nothing about recycling of the importin subunits. Similarly, exported RNA must be dissociated from the "export receptor" in the cytoplasm. In both cases, recycling of the transport receptor to its original location must occur, and this process cannot be a simple reversal of the first transport event if directionality of transport is to be achieved. For import substrates such as U snRNPs and for exported RNAs such as tRNAs, transport mediators remain to be identified, and it will be of interest to see how they compare with the ones already described. We need to find out if GTP hydrolysis by Ran is the sole source of energy for nuclear import and if Ran is also directly required for RNA export. There is in vivo evidence that suggests a role in export for Ran, its GDP-GTP exchange factor, and its GTPase activating protein [reviewed in (26); see also (77)]. However, the lack of a biochemical system in which to study export makes it impossible to say whether this effect is direct or, for example, is an indirect consequence of blockage of the reimport of an export mediator.

The above questions are important, but all appear to be approachable in a straightforward way. It is more difficult to imagine how we will learn the details of the mechanism of translocation through the pore. The NPC is a large and still rather mysterious structure. We know that 25-nm particles can cross the NPC, but it is not clear if the pore must open just as wide to

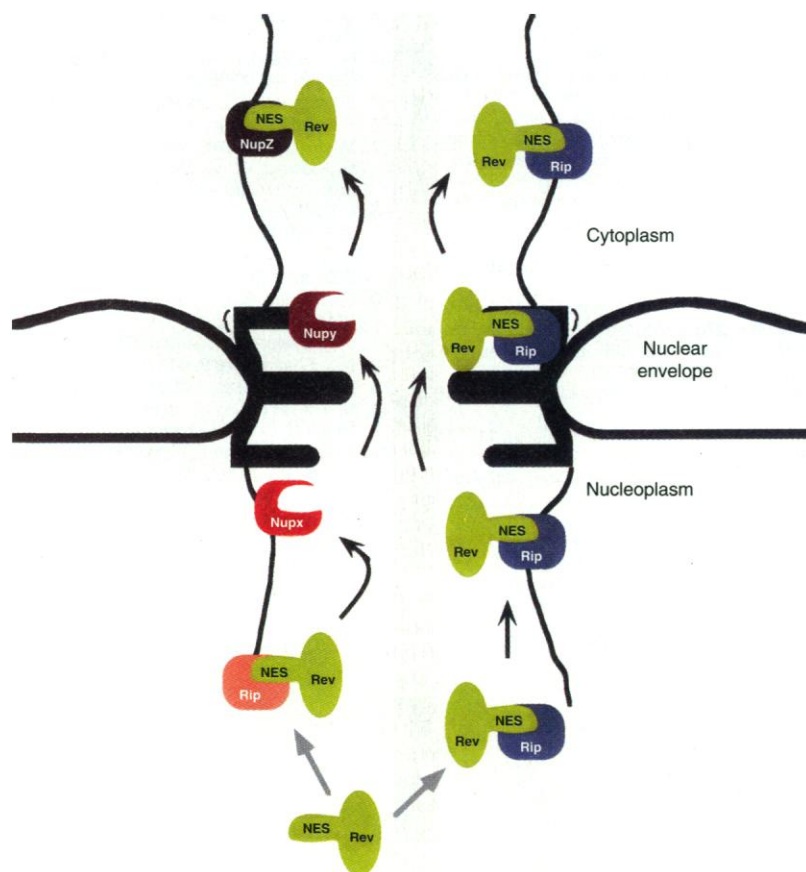


Fig. 2. Two alternative models for Rev-mediated export. The Rev NES interacts directly or indirectly with FG (4) dipeptide-containing repeat domains found in Rip and in some characterized nucleoporins. These interactions could occur sequentially during docking of the Rev-RNA complex at the inner face of the NPC and translocation (left side). Alternatively, the Rip-Rev interaction could occur in the nucleoplasm, and the complex of the two could be translocated through the NPC through interactions between Rip and other nucleoporins (right side). Other similar models can be imagined. For simplicity's sake, we do not show the RNA to which Rev would normally be bound. Rev uptake into the nucleus requires an NLS that is distinct from the NES, and it presumably occurs via the normal protein import pathway (Fig. 1). Rev export and import are thus clearly asymmetrical.

actively transport an average-sized nuclear protein with a diameter of roughly 5 to 6 nm. If it does not, how does the NPC know how far to open; and if it does, how does the NPC maintain a seal around the transport substrate to keep the contents of the nucleus and cytoplasm separated? The problem, as at national borders, is not just to let the desirable cargo through but to keep the undesirable material from passing unnoticed.

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