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Nuclear transport mechanisms

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Abstract. The term nuclear transport, refers to the movement of a large variety of macromolecules both into and out of the nucleus. Transport must be extremely selective, yet also very efficient. A single type of channel, the nuclear pore complex, mediates all movement across the nuclear envelope. Selectivity is achieved through the use of families of soluble factors that target substrates for import and export and deliver them to their appropriate intracellular destinations. We now have a fairly detailed understanding of the basic mechanisms of protein import into the nucleus. Many of these same principles can be applied to protein export and perhaps RNA export. This review will summarize the current status of what is known about various transport pathways and highlight the questions that remain to be answered.

Key words. Nucleocytoplasmic transport; Ran; importin β; nuclear transport factor 2 (NTF2); RNA export.

Introduction

All nucleocytoplasmic trafficking occurs through the nuclear pore complex (NPC). Small molecules such as ions and proteins of 60 kDa or less can diffuse through the pore [1]. Nevertheless, even proteins that are smaller than 20–30 kDa, such as histones, cross the NPC actively in a carrier-mediated fashion [2]. Substrates to be transported into or out of the nucleus contain distinct internal signals that target their directional transport. Transport substrates, which can be thought of as cargoes, include proteins, various types of RNAs and complexes of RNA plus proteins (ribonuclear proteins; RNPs). Proteins to be imported into the nucleus contain sequences termed nuclear localization sequences (NLSs) and proteins to be exported from the nucleus contain nuclear export sequences (NESs). These signals are recognized by soluble factors that work with the RanGTPase cycle to coordinate import to or export from the nucleus.

Ran and the RanGTP gradient

Nucleocytoplasmic transport is orchestrated by the small GTPase, Ran. Ran is an abundant, predominantly nuclear, 25-kDa protein [3]. As with most GTPases [4], Ran hydrolyzes GTP very slowly, and consequently its nucleotide-bound state is regulated through interactions with a variety of effector proteins. These include (i) the Ran GTPase-activating protein, RanGAP (Rna1p in *Saccharomyces cerevisiae*), (ii) the RanGAP-activating protein, RanBP1 (Yrb1p in *S. cerevisiae*), and (iii) the guanine nucleotide exchange factor, RanGEF (RCC1 in higher eukaryotes and Prp20p in *S. cerevisiae*). RanGAP enhances the GTPase activity of Ran ~10,000-fold alone and 100,000-fold in the presence of RanBP1 [5]. RanGAP localizes exclusively to the cytoplasm [6–8]. RanBP1, which has a predominantly cytoplasmic steady state localization, shuttles between the cytoplasm and nucleus [9, 10]. RanGEF, which catalyzes the release of GDP from Ran and consequently the regeneration of the GTP bound form of the protein [11], is tethered to DNA and is therefore found exclusively in the nucleus [12]. The strict compartmentalization of RanGAP to the cytoplasm and RanGEF to the nucleus suggests that Ran is primarily in the GTP-bound state in the nucleus and in the GDP-bound state in the cytoplasm. Consequently, Ran must move between the nucleus and the cytoplasm to undergo a complete round of GTP binding and hydrolysis (fig. 1).

Another important group of Ran-binding proteins is the importin-β family of proteins, which function as nuclear

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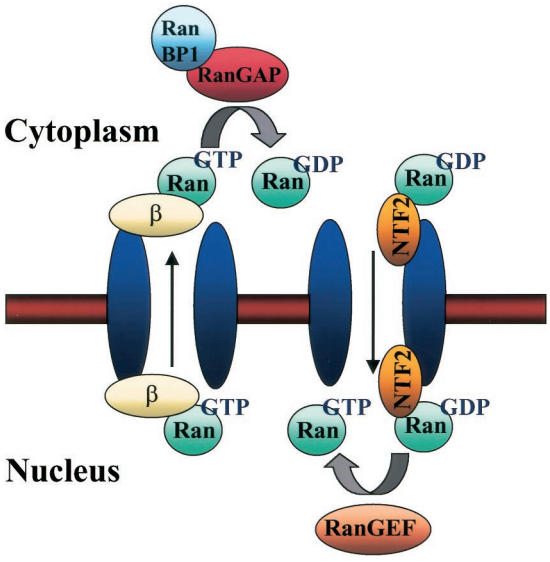

Figure 1. The Ran cycle. RanGDP is imported into the nucleus by NTF2. Once in the nucleus, GDP is exchanged for GTP by the Ran exchange factor, RanGEF. RanGTP then interacts with members of the importin-β family of proteins and is exported to the cytoplasm complexed with importin-β proteins. In the cytoplasm, the GTPase activity of Ran is stimulated by the Ran GTPase-activating protein, RanGAP complexed with RanBP1, resulting in the production of RanGDP.

transport receptors. Importin-β proteins, also known as karyopherins, are characterized by a conserved RanGTP binding domain at the N-terminus [1]. These proteins use Ran to promote their association with or dissociation from their transport substrates (reviewed in [13]) The current model for Ran's role in nucleocytoplasmic transport is based on the asymmetric distribution of Ran, with RanGTP in the nucleus and RanGDP in the cytoplasm [1]. Cargo to be imported into the nucleus associates with an importin-β receptor in the cytosol where RanGTP is scarce. Once inside the nucleus this complex dissociates because RanGTP binds to the importin-β receptor, resulting in a conformational change that causes release of the cargo [14]. Within the nucleus, cargo to be exported binds to its importin-β receptor as part of a trimeric complex containing RanGTP. This complex is subsequently dissociated in the cytoplasm due to the hydrolysis of GTP stimulated by RanGAP. Therefore, the asymmetric distribution of Ran acts as a cellular marker to distinguish between the nuclear and cytoplasmic sides of the nuclear pore. Consistent with this idea, translocation of transport complexes is independent of GTP hydrolysis by Ran [15]. The energetic contribution of RanGTP hydrolysis to nucleocytoplasmic transport against a concentration gradient appears to be through the recycling of receptors to the cytoplasm.

The unidirectional movement of RanGTP out of the nucleus in association with transport receptors would deplete the nucleus of Ran. Therefore, there must be a mechanism for regenerating nuclear pools of RanGTP. Recent evidence indicates that nuclear transport factor 2 (NTF2) mediates RanGDP import into the nucleus [16, 17]. NTF2 interacts with both RanGDP and nuclear pore proteins simultaneously [18] to import RanGDP into the nucleus. Once the NTF2/RanGDP complex reaches the nucleus, it encounters RanGEF, which exchanges the GDP on Ran for GTP. As NTF2 has no detectable affinity for RanGTP [18, 19], RanGTP is then released into the nucleus.

There are four additional Ran-interacting factors whose functions are not yet clear. The first two are p15/NXT1, an NTF2-related RanGTP-binding protein that localizes to the NPC and is implicated in nuclear export [20, 21], and MOG1, a multicopy suppressor of *S. cerevisiae* Ran (*GSP1*) temperature-sensitive mutants [22]. The other two Ran-interacting factors are yeast YRB2, which is implicated in protein export [23], and human RanBP3, a nuclear protein [24, 25]. How each of these proteins impacts Ran function and fits into the RanGTP gradient model of nuclear transport remains to be elucidated.

Protein transport: recognition and targeting

Proteins to be transported into or out of the nucleus contain internal signals, NLSs and NESs, which are recognized by soluble receptors. In the classical import pathway (fig. 2), an NLS composed of a stretch of basic amino acids is recognized by an importin-α adaptor protein, which mediates the interaction between the transport substrate and an importin-β receptor of ~95 kDa (β95). These importin-α adaptor proteins interact with importin β95 through a conserved amino-terminal IBB (importin β binding) domain [26]. The IBB domain of importin α itself has an NLS-like sequence, which modulates the affinity of importin α for the NLS-containing cargo by acting as an auto-inhibitory domain [27]. Binding of importin α to importin β displaces this auto-inhibitory domain and allows the formation of the cargo/importin-α/importin-β95 complex [28]. Importin β95 subsequently targets the complex to the nuclear pore by binding to nuclear pore proteins, collectively termed nucleoporins [29].

The identification of a family of importin-β proteins that do not require an importin-α adaptor protein led to the recognition of distinct nuclear transport pathways (reviewed in [30]). Table 1 lists the various importin β homologs in *S. cerevisiae* and humans and their known substrates. The plethora of nuclear targeting receptors suggests that extracellular signals or the cell cycle may regulate specific aspects of nucleocytoplasmic transport. In such models, different importins with different signal recognition properties would be specifically activated, and the repertoire of active importins would determine

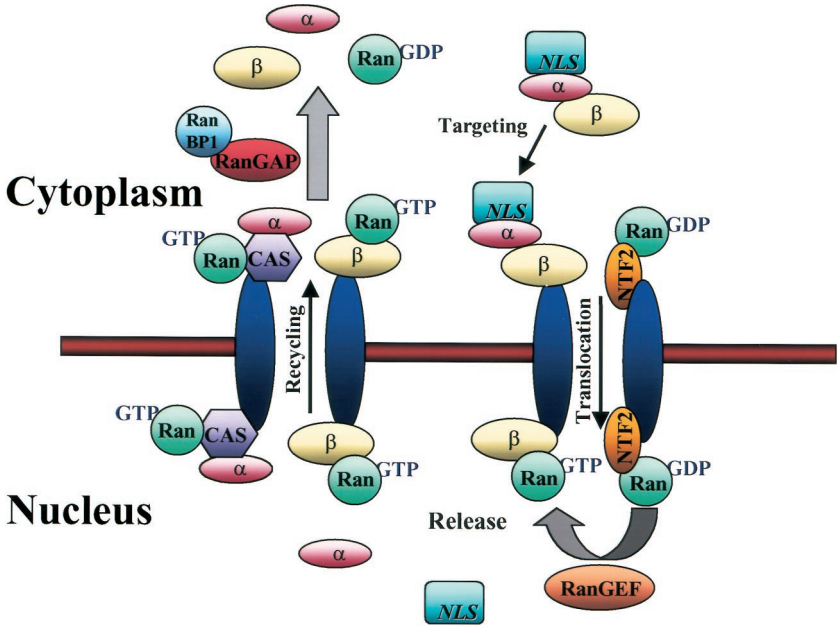

Figure 2. Model of nucleocytoplasmic trafficking of NLS-containing proteins. Proteins bearing a classical NLS are recognized by importin α. The cargo/importin-α complex then interacts with importin-β. Importin-β targets the import complex to the nuclear pore for translocation into the nucleus. In the nucleus, RanGTP interacts with importin-β and disassociates the import complex. Importin α is now free to bind its export receptor, CAS, which interacts with RanGTP to form a trimeric complex that is exported to the cytoplasm thus recycling importin α to undergo another round of import. The importin-β/RanGTP complex also exits the nucleus, recycling importin-β for another round of import. In the cytoplasm, RanGTP interacts with RanGAP/RanBP1 to produce RanGDP, which is then reimported into the nucleus by NTF2.

which proteins were targeted for import into or export from the nucleus in response to these signals. In addition, whereas only one importin-α protein is present in *S. cerevisiae,* higher eukaryotes possess multiple importin-α-like proteins (reviewed in [30]) which are differentially expressed in tissues [31, 32]. Thus, in higher eukaryotes it is possible that different importin-α adaptors could also contribute to the regulation of nuclear transport.

Protein transport: translocation

Once the cargo is targeted to the nuclear pore by its importin-β receptor, the complex must then traverse 200–300 Å through the pore and deliver the cargo to the appropriate compartment. Translocation through the pore is the least well understood step of nucleocytoplasmic transport. Importin-β proteins interact with nucleoporins that contain the repeated motifs FXFG and GLFG (reviewed in [33]). These repeat-containing nucleoporins are located throughout the NPC [34]. Two general models for translocation have been proposed. In one model, movement through the pore occurs by random nondirected diffusion; upon reaching some point, an irreversible mechanism would commit the complex to release cargo into the proper compartment [34, 35]. The second model involves a series of directed associations and dissociations of importin β with specific nucleoporins [36], possibly involving a gradient of affinities between various nucleoporins and importin β as the complex travels through the pore [37]. The recent identification and localization of all the nucleoporins that comprise a yeast NPC [34], as well as the solution of the cocrystal structure of importin β95 with the FXFG region of the nucleoporin Nsp1p, are important steps towards understanding the transport mechanism [36]. These advances should make it possible to distinguish between the two models of translocation and determine how nucleoporins cooperate with receptors to generate directional movement through the NPC.

Protein transport: release

As previously described, the nucleotide-bound state of Ran plays a critical role in dissociating both import and export complexes, and thus regulates the release of the cargo into the appropriate compartment. In the classical import pathway, when the cargo/importin-α/importin-β complex reaches the nuclear face of the NPC, importin β is proposed to bind RanGTP, resulting in disassembly of the complex and release of both importin α and the cargo into the nucleus [38, 39] (fig. 2). Conversely, during export, receptors interact with their cargo only in the pres-

Table 1. Importin-β homologues in *S. cerevisiae* and human.

Importin-β homologue (alternative names)	Example of known substrate	References
S. cerevisiae		
Importin β95 (Rsl1p, Kap95)	Import of classical NLS cargo in conjunction with importin-α	76
Crm1p (Xpo1p, Kap124p, Exportin 1)	Classical leucine rich NES cargo export	77
Cse1p (Kap109p)	Importin-α export	41, 78
Pse1p (Kap121p)	Pho4p import, ribosomal protein import	79, 80
Kap123p (Yrb4p)	Ribosomal protein import	80
Transportin (Kap104p)	Nab2p, Hrp1p import (hnRNP proteins)	81
Kap120p	None known	
Nmd5p (Kap119p)	TFIIs (transcription elongation factor), Hog1p import	82, 83
Sxm1p (Kap108p)	Lha1p (La) import, ribosomal protein import	84, 85
Msn5p (Kap142p)	Pho4p, Far1p export	79, 86
Kap114p (HRC1004p)	TATA binding protein import	87
Mtr10p (Kap111p)	Npl3p import	88
Pdr6p (Kap122p)	TFIIA import	89
Los1p	tRNA export	49, 90
Human		
Importin-β (p97, PTAC97, karyopherin β1)	Import of classical NLS cargo in conjunction with importin-α	29, 91
Transportin 1 (Importin β2, karyopherin β2)	Import of hnRNP proteins	92
Importin 5 (RanBP5, karyopherin β3)	Import of ribosomal proteins	93
Importin 7 (RanBP7)	Import of ribosomal proteins and histone H1	93, 94
Transportin SR	Import of proteins with SR domains	95
CRM1	Classical NES-cargo export	52, 96
CAS	Export of importin-α proteins	97
Exportin-t	Export of tRNA	47

ence of RanGTP [1]. The trimeric complex then traverses the NPC to the cytoplasm, where RanGAP in the presence of RanBP1 stimulates the GTPase activity of Ran, producing RanGDP for which the receptor has no affinity [1]. This causes the disassembly of the export complex and release of the export cargo into the cytoplasm.

Protein transport: recycling

Nucleocytoplasmic transport is a continuous process, and therefore the soluble factors must constantly be recycled back to their site of action to support multiple rounds of transport. The mechanism of this recycling has been demonstrated for several nucleocytoplasmic transport factors. For example, after importin α disassociates from importin β95 in the nucleus, α forms a trimeric complex with both RanGTP and CAS (Cse1p in *S. cerevisiae*), another member of the importin-β family of transporters. This trimeric complex is then exported to the cytoplasm, enabling importin α to mediate another round of import [40, 41] (fig. 2). Importin β95 is recycled by binding to RanGTP in the nucleus. The importin-β95/RanGTP complex traverses the NPC back to the cytoplasm [42], where the complex encounters the RanGAP and dissociates as described for export complexes (fig. 2). This is proposed to be the general mechanism for recycling all importin-β receptors back to the cytoplasm. However, it is not known whether the recycling of β importins is coupled to the export of a cargo, or whether it is used strictly to recycle the importins.

NTF2 imports RanGDP into the nucleus in a manner that is probably similar to importin-β import. However, it is not known how NTF2 is recycled back to the cytoplasm. This small homodimeric 28-kDa protein is well below the predicted diffusion size of 60 kDa for passage through the NPC. Therefore, it may either simply diffuse across the pore into the cytoplasm for another round of RanGDP import, or there may be additional unidentified factors that mediate the export of NTF2 from the cytoplasm for another round of RanGDP import.

RNA export

In addition to the trafficking of proteins, multiple classes of RNAs, including messenger RNAs (mRNAs), transfer RNAs (tRNAs) and U snRNAs (small nuclear RNAs involved in RNA splicing), must be transported to their sites of action in the cytoplasm. Classical competition experiments demonstrated that the pathways taken by each class of RNA were likely to be distinct [43]. There is now an abundance of information about the factors that are essential for RNA export (reviewed in [44, 45]) but as for protein import, the exact mechanism of translocation remains elusive. RNAs require a great deal of processing and quality control before they reach their mature forms and are allowed to exit the nucleus. Thus, it has been ex-

perimentally challenging to separate activities that are required for RNA processing functions (e.g. splicing) that must be completed prior to export, from factors that represent true mediators of export.

Export of tRNA from the nucleus follows the classical mechanism of nuclear export of macromolecules. The tRNA substrate is directly recognized by a receptor of the importin family named exportin-t (Los1p in *S. cerevisiae*) [46, 47]. A trimeric export complex is formed, consisting of the tRNA substrate complexed with exportin-t and RanGTP. This complex is then exported from the nucleus and disassembled in the cytoplasm where RanGTP is hydrolyzed to RanGDP. Exportin-t appears to interact preferentially with mature processed, modified and appropriately aminoacylated tRNAs [47–49]. Thus, the specific affinity of the receptor for mature tRNAs provides a gatekeeper function to assure that only correctly-processed tRNAs are exported to the cytoplasm.

U snRNAs are synthesized in the nucleus and a subset are then exported to the cytoplasm where they associate with protein components of mature small ribonucleoproteins (snRNPs). These mature snRNP complexes are then reimported to the nucleus where they function in mRNA splicing [50]. The mono-methylated G cap of the newly synthesized snRNP is recognized by the cap binding complex (CBC) [43, 51, 52], and exported from the nucleus by the importin-β family member CRM1. There is currently no evidence that the CBC binds directly to CRM1, so it seems likely that an adaptor protein will be involved. There is precedent for such an export adaptor for U snRNAs since mature cytosolic snRNPs are imported into the nucleus by an adaptor, snurportin, complexed with importin-β [53, 54].

Ribosomal RNAs (rRNAs) represent a special case, because rRNAs are exported as very large fully assembled large and small ribosomal subunits after their assembly in the nucleus. Export of ribosomes has been shown by many methods to depend on Ran, but there is still controversy as to whether Ran plays a direct role in ribosomal export or whether its activity is required to import hypothetical components that directly mediate ribosome export [55, 56]. Interestingly, several specific nucleoporins have been implicated in ribosomal export, but the question of whether an importin family receptor is required remains unresolved [55, 56].

mRNA export

Much of the effort towards understanding the mechanism of RNA export has focused on the export of mature mRNAs (reviewed in [44, 57]). These studies clearly showed that mRNAs are not exported to the cytoplasm as naked nucleic acids, but as ribonucleoprotein complexes in which several different proteins associate with a single RNA molecule. The popular consensus is that the export machinery recognizes signals within these proteins, rather than the RNA itself. An example of this mechanism is the export of unspliced human immunodeficiency virus (HIV) transcripts from the nucleus. The unspliced HIV RNA contains a sequence known as the Rev response element (RRE) which binds specifically to the viral Rev protein [58]. The Rev protein contains a classical leucine-rich NES sequence that is recognized by CRM1 [59]. Because unspliced mRNAs are not normally allowed to exit the nucleus, HIV virus appears to have enhanced and exploited an existing mRNA export pathway [60].

There are numerous candidates for mRNA export factors. Many fall into the general class of hnRNP proteins (heterogeneous ribonucleoproteins) that interact with polyadenylated RNA in vivo [61]. In addition to interacting with poly(A)$^+$ RNA, many of these hnRNP proteins shuttle between the cytoplasm and the nucleus, and are thus positioned to recycle after escorting RNAs through the NPC [62]. In addition, the mammalian TAP/p15 protein complex and its functional yeast counterpart, Mex67p/Mtr2p [20], are promising candidates for direct mediators of RNA export. The TAP protein was originally identified as a factor required for the export of simian type D viral RNAs that contain a CTE (constitutive transport element) [63]. TAP is also required to export endogenous mRNAs [64]. In yeast, the loss of Mex67 function causes an immediate defect in the export of poly(A)$^+$ RNA [65]. Furthermore, Tap (Mex67) shuttles between the cytoplasm and the nucleus and interacts with both RNAs and nucleoporins [65, 66]. Thus, it could potentially target bound RNAs directly to the nuclear pore for export.

Other candidate RNA exporters that bind RNA include hnRNPA1 [67], Npl3p [68], Gle1p [69] and Gle2p/*rae1* [70, 71]. Other RNA-binding proteins with possible roles include Hrp1p [72], which is required for poly(A) tail cleavage, and Nab2p [73], another yeast hnRNP protein. An important goal for future studies on the mechanism of mRNA export is to understand whether all these factors cooperate in one export pathway or whether there are numerous potentially overlapping pathways.

Unresolved questions

Despite the many advances in our understanding of nucleocytoplasmic transport, important unanswered questions remain that are critical to understanding the mechanisms of macromolecular movement into and out of the nucleus.

One key question is how cargo/receptor complexes actually translocate through the NPC. Although a number of interactions between transport factors and nucleoporins have been documented, we don't know how these interac-

tions lead to directional movement through the pore. To answer this question we will need to address another issue, the overall structure of the NPC. Several recent studies have advanced our knowledge of the composition and spatial organization of components of the NPC [34, 74]. For example, do all transport factors interact with the same nucleoporins, or are there distinct interactions required for different pathways? The use of fluorescence resonance energy transfer (FRET) technology to examine in vivo protein-protein interactions between transporters and nucleoporins [75] may help to address these questions.

The second key question is the role of the importin-β family of transport receptors. Various members of this family have been implicated in either import or export, but each factor itself needs to shuttle into and out of the nucleus to fulfil its role in nuclear trafficking. It remains possible that each transporter can carry cargo on both legs of the journey. So rather than being either importins (involved solely in cargo import) or exportins (involved solely in cargo export) each may serve a dual role in both import and export, as this seems a more efficient way to accomplish trafficking in both directions.

The third, and long-debated, question is whether energy is required to move cargo into or out of the nucleus. Current models suggest that the only energy requirement is to regenerate RanGTP from RanGDP to mediate the recycling of transport factors to the cytoplasm [15]. This seems rather counterintuitive since the large distance that substrates must travel through the NPC suggested models in which transport was driven by motor proteins within the pore. However, at least in yeast, there are no motor proteins in the nuclear pore complexes [34], supporting the idea that transport through the pore occurs strictly by some form of facilitated diffusion.

A fourth question relates to the function of importin-β family members, where there is controversy as to whether any member of the importin-β receptor family plays a role in mRNA export. This question is also linked to whether Ran plays a direct role in mRNA export. So far, wherever a member of the importin family has been shown to mediate export, this export requires the formation of a trimeric complex with RanGTP. If Ran is truly not required for mRNA export, one would conclude that export of mRNA is mediated by a mechanism that is distinct from the importin family members. It is currently thought that the RanGTP gradient plays an indirect role in mRNA export, through the recycling of presumed RNA export factors.

Final comments

Many questions about the specific mechanisms by which macromolecules move into and out of the nucleus have been answered. However, we realize that there are many pathways for targeting substrates to the nuclear pore for translocation, raising many new and interesting questions for researchers to investigate in the years to come.

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