Traffic 2005; **6:** 187–198
Blackwell Munksgaard

doi: 10.1111/j.1600-0854.2005.00270.x

Review

Mechanisms of Receptor-Mediated Nuclear Import and Nuclear Export

Lucy F. Pemberton[1,2] and Bryce M. Paschal[1,3,*]

[1]Center for Cell Signaling, University of Virginia, Charlottesville, VA 22908, USA
[2]Department of Microbiology, University of Virginia, Charlottesville, VA 22908, USA
[3]Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA 22908, USA
**Corresponding author: Bryce M. Paschal, paschal@virginia.edu*

Nuclear transport of proteins and RNA occurs through the nuclear pore complex and is mediated by a superfamily of transport receptors known collectively as karyopherins. Karyopherins bind to their cargoes by recognition of specific nuclear localization signals or nuclear export signals. Transport through the nuclear pore complex is facilitated by transient interactions between the karyopherins and the nuclear pore complex. The interactions of karyopherins with their cargoes are regulated by the Ras-related GTPase Ran. Ran is assisted in this process by proteins that regulate its GTPase cycle and subcellular localization. In this review, we describe several of the major transport pathways that are conserved in higher and lower eukaryotes, with particular emphasis on the role of Ran. We highlight the latest advances in the structure and function of transport receptors and discuss recent examples of steroid hormone receptor import and regulation by signal transduction pathways. Understanding the molecular basis of nuclear transport may provide insight into human diseases by revealing how nucleocytoplasmic trafficking regulates protein activity.

Key words: exportin, importin, karyopherin, nuclear export signal, nuclear localization signal, nucleoporin, RanGTP, steroid hormone receptor

Received 15 December 2004, revised and accepted for publication 5 January 2004

The transport of proteins and RNAs into and out of the nucleus occurs through the nuclear pore complex (NPC), a large macromolecular structure embedded in the double membrane of the nuclear envelope (1–3). The import and export of most proteins, ribosomal subunits and some RNAs is mediated by a large, evolutionarily conserved family of transport factors, the karyopherin-β family (referred to here as karyopherins). Most karyopherins mediate either nuclear import (also called importins) or nuclear export (also called exportins), and all karyopherins interact directly with their cargoes, although some also use adapter proteins. The best-characterized adapter protein is the evolutionarily conserved protein, karyopherin-α (also called importin-α) (4). Karyopherins that mediate import bind to their cargoes in the cytoplasm via recognition of the nuclear localization signal (NLS) (Figure 1). The karyopherin : cargo complex translocates through the NPC via interactions with NPC proteins (referred to as nucleoporins). Once in the nucleus, the karyopherin encounters RanGTP, and the ensuing karyopherin : RanGTP complex leads to dissociation of the cargo from the karyopherin, whereby the karyopherin is recycled back to the cytoplasm. Conversely, karyopherins that mediate export bind cargo in the nucleus via recognition of a nuclear export signal (NES). Karyopherin binding to export cargo occurs co-operatively with RanGTP, resulting in a karyopherin : cargo : RanGTP ternary complex (Figure 1). Hence, Ran regulates both the assembly and the disassembly of karyopherin : cargo complexes. In this review, we discuss key findings that have advanced our understanding of karyopherin : cargo interactions, including how Ran regulators affect the transport cycle. We also review nuclear transport of steroid hormone receptors, transcription factors whose compartmentalization has important implications for understanding diseases including cancer.

Cargoes and Signals

Each eukaryotic cell must accomplish the rapid and vectorial transport of thousands of proteins and RNAs into and out of the nucleus. Most nucleocytoplasmic transport pathways are mediated by members of the karyopherin family. While human cells contain at least 20 members, in yeast this family comprises 14 members (1) (Table 1). The large number of cargoes relative to the number of karyopherins raises the question of how karyopherin : cargo recognition occurs. Studies have shown that proteins that undergo nuclear import or export generally contain an NLS or NES, respectively (2,3). Characterization of the NLSs from SV40 Large T antigen and nucleoplasmin first elucidated the basic or classical NLS, which consists of one or two clusters of basic amino acids separated by a linker (5). The import of many nuclear proteins is thought to be mediated by the basic NLS. It is now appreciated, however, that import signals unrelated to the basic NLS exist; indeed, the characterization of most NLSs is still in its infancy. Regulation of NLS activity can occur by several

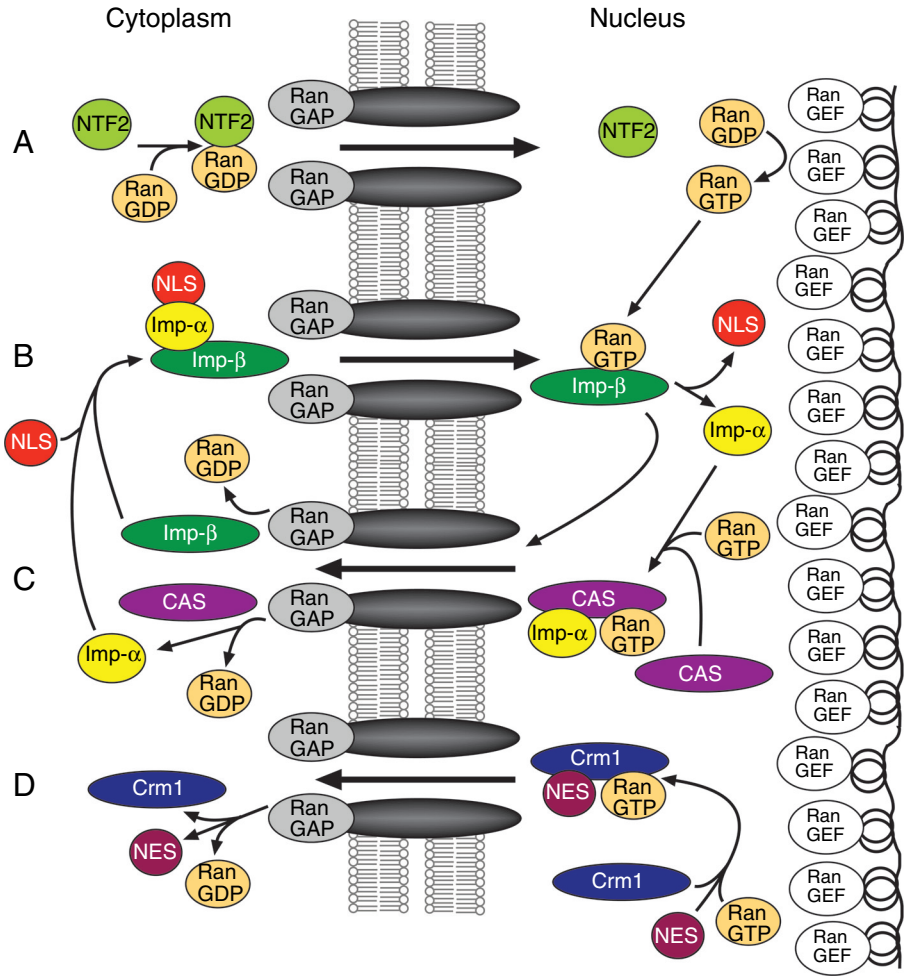

Figure 1: Overview of some major nuclear transport pathways in eukaryotic cells. A) Nuclear import of RanGDP mediated by NTF2. B) Nuclear import of nuclear localization sequence (NLS) cargo mediated by the karyopherin-α : importin-β1 heterodimer (abbreviated Imp-α and Imp-β). Nuclear import of NLS cargo mediated by direct binding to importin-β1 and other karyopherin-β family members is not shown. C) Nuclear export pathways that mediate recycling of importin-β1 and karyopherin-α; the latter requires CAS as an export receptor. D) Nuclear export of nuclear export sequence (NES) cargo mediated by Crm1. RanGAP is anchored to the cytoplasmic side of the NPC and RanGEF is shown bound to chromatin. For simplicity, the model depicts only the minimal components necessary to form the pretranslocation transport complexes, and post-translocation intermediates and accessory factors are not shown. See text for details.

mechanisms including modification or signal masking (6). A recent study has shown that phosphorylation of an amino acid proximal to the basic NLS decreases its binding to karyopherin-α (7). Regulation of nuclear transport by phosphorylation is covered in detail in another article in this volume (8).

Karyopherin-α and the Basic NLS

The basic NLS binds adapter protein karyopherin-α. The karyopherin-α forms a heterodimer with importin-β and mediates import of NLS cargoes (4). A crystal structure of karyopherin-α with an SV40 Large T NLS peptide provided the first insight into karyopherin : NLS interactions (9,10). Karyopherin-α forms a super helix consisting of 10 armadillo (ARM) repeats (9,10). Armadillo repeats were so named because they were first discovered in the Drosophila ARM protein (11). The ARM repeat consists of approximately 40 amino acids arranged into three helices. A series of ARM repeats generate a super helical structure that has a shallow, concave groove, creating a binding pocket for the NLS (9,10). Karyopherin-α has been crystallized with several different basic NLSs, and in general, the NLS is recognized through interactions based on both charge and hydrophobicity (9,10,12–14). There are extensive contacts between several of the ARM repeats and the NLS, which is surprising given the small size of the basic NLS (9,10,12–14).

NLSs Recognized by Karyopherin-β

Most karyopherin-β proteins bind directly to cargoes and therefore do not rely on an adapter (2,3). The NLSs recognized by these karyopherin-β have often proved harder to define. In some cases, the NLS contains several basic amino acids as has been determined for core histones (15–17), ribosomal proteins (18) and arginine–glycine-rich NLSs observed in some RNA-binding proteins (19,20). In other cases, the NLS domain is relatively large, such as the M9 NLS, which consists of 38 amino acids and which is glycine rich and deficient in basic amino acids (21). Examples where even larger domains are necessary for NLS activity have been identified, raising the possibility that the three-dimensional structure of the protein is critical (22).

Table 1: Karyopherin-β family members

Human	Cargo	Yeast	Cargo	Essential gene
Import				
Importin-β1	Many cargoes, cargoes with basic NLSs via karyopherin α, UsnRNPs via snurportin	Kap95	Many cargoes including those with basic NLS via karyopherin α	Yes
Karyopherin-β2	hnRNPA1, histones, ribosomal proteins	Kap104	Nab2, Hrp1	ts
Transportin SR1	SR proteins	Mtr10/Kap111	Npl3, Hrb1	ts
Transportin SR2	HuR	.....		
Importin 4	Histones, ribosomal proteins	Kap123	Ribosomal proteins, histones	No
Importin 5	Histones, ribosomal proteins	Kap121	Ribosomal proteins, histones, Pho4, others	Yes
Importin 9	Histones, ribosomal proteins	Kap114	TBP, histones, Nap1p	No
Importin 7	HIV RTC, Glucocorticoid receptor, ribosomal proteins	Nmd5/Kap119	TFIIS, Hog 1, others	No
		Sxml/Kap108	Lhp1, ribosomal proteins	No
Importin 8	SRP19	.....		
Importin 11	UbcM2, rpL12	.....		No
.....		Kap122	TFIIA	
Export				
Crm1	Leucine rich NES cargoes	Crml	Leucine rich NES cargoes	Yes
Exportin-t	tRNA	Los1	tRNA	No
CAS	Karyopherin α	Cse1	Karyopherin α	Yes
Exportin 4	eIF-5 A	.....		
Exportin 5	microRNA precursors	.....		
Exportin 6	Profilin, actin	.....		
Exportin 7	p50Rho-GAP, 14-3-38	.....		
Import/Export				
Importin 13	Rbm8, Ubc9, Pax6 (import) eIF-1 A (export)	.....		
.....		Msn5	Pho4, others including phosphorylated proteins (import) Replication protein A complex (export)	No
Uncharacterized				
RanBP6	undefined	.....		
RanBP17	undefined	.....		
.....		Kap120	undefined	No

NES, nuclear export signal; NLS, nuclear localization signal. Members of the karyopherin-β family from human and yeast are shown, and orthologues as well as examples of their characterized cargoes are grouped. ''Essential gene'' indicates the phenotype of the yeast gene deletion. Dotted lines indicate orthologues have not been identified. Although Nmd5/Kap119 and Sxm1/Kap108 are shown as orthologues of Importin 7, these proteins show a similar level of sequence relatedness to Importin 8. References are contained within the text.

Ultimately, defining the NLS in these more complicated examples will require analysis of the atomic structures of karyopherin : cargo complexes.

NESs and Exportins

Export karyopherins also recognize specific signals, referred to as NESs (2,3). The NESs of several cargoes have been defined, and the most well characterized is the hydrophobic NES, which is a loosely conserved motif containing three to four hydrophobic residues (e.g. Rev NES sequence is LPPLERLTL) (23). The hydrophobic NES is utilized in all eukaryotes, and is recognized by the karyopherin Crm1 (24,25). At least 75 proteins containing this NES have been identified to date (26). These include many transcription factors and cell-cycle regulators, as well as the viral HIV Rev protein and the protein kinase A inhibitor where the hydrophobic NES was first described (23,27). Like importin-β1, Crm1 can also mediate the export of several cargoes via adapter proteins (28–30).

Cargoes that lack the hydrophobic NES have also been analysed. Some of the most well characterized are the cargoes of the yeast karyopherin, Msn5p (1). This karyopherin is unusual in that it can mediate both nuclear import and export (Table 1) (31,32). Interestingly, most Msn5p export cargoes identified thus far have been shown to be positively regulated by phosphorylation, suggesting the phosphosite is either part of the NES, or phosphorylation indirectly affects NES recognition by the receptor (1,6).

RNA Export

Several other exportins have been characterized, including CAS which exports karyopherin-α from the nucleus, thereby performing a critical recycling function (33). In addition to protein cargoes, several types of RNA constitute a diverse group of export cargoes (34). At least two exportins, exportin-t and exportin 5, have been shown to bind RNA directly (35–37). Exportin-t mediates the export of tRNAs from the nucleus through recognition of an export signal that is part of the RNA structure (35,36). In higher eukaryotes, exportin 5 exports microRNA precursors from the nucleus and recognizes an RNA hairpin structure with a 3′ overhang (37,38). Export of mRNA does not appear to be mediated by any member of the karyopherin family, but is instead mediated by the members of the structurally unrelated TAP/NXF family (34). The specific signals governing mRNA export have yet to be precisely defined; however, some of the factors involved in the export of mRNA also function in mRNA transcription, processing and splicing, thus ensuring that these processes are tightly coupled (34).

The Karyopherin Family

Intrinsic to the function of karyopherins in nuclear import and export is the ability of these proteins to bind their cargoes directly (or indirectly), interact with nucleoporins and interact with RanGTP (1–3). Characterization of these proteins in both yeast and vertebrate systems have indicated that karyopherin function is restricted to either import or export, with the exception of two family members (one in yeast and one in human) that function in both import and export (Table 1) (1–3). Thus far, there are more import receptors that export receptors. In yeast, for example, there are 10 import karyopherins, three export karyopherins and one import/export karyopherin (1) (Table 1). There are many more cargoes than karyopherins, suggesting that each karyopherin has multiple cargoes. Recent structural studies have begun to unravel the mystery of how a single karyopherin can recognize NLSs with limited sequence identity.

Karyopherins are Composed of HEAT Repeats

Karyopherins have comparable molecular weights (95–145 kDa), acidic isoelectric points and a similar domain structure (1–3). This includes an N-terminal Ran-binding domain, a central nucleoporin-binding domain and a C-terminal cargo-binding domain (39,40). However, as described below, several exceptions to these general rules have already been noted. So far, structural data are available for four members of the karyopherin family (39–42). Importin-β1 has been the most extensively studied and structures of this karyopherin with at least three cargoes, as well as with Ran, and with a nucleoporin have been solved (43–47). In addition, structures of karyopherin-β2 with Ran, a fragment of the exportin Crm1, and the exportin Cse1p complexed with RanGTP and its cargo karyopherin-α have all been solved (41,42,48). These atomic structures suggest that each karyopherin is typically composed of 20 HEAT repeats, an approximately 40-amino acid repeats composed of two antiparallel helices connected by a short turn (41–43,48). The HEAT repeat is named after the motif Huntington, elongation factor 3, 'A' subunit of protein phosphatase 2A and TOR1, proteins in which the motif was first discovered (49). The HEAT repeats form a super helix, providing extensive interaction surfaces in the form of N-terminal and C-terminal arches (39,40).

Karyopherins and their Import Cargoes

An unforeseen feature of karyopherins revealed by structural studies is the presence of more than one cargo-binding site (39,40). Co-crystal structures of importin-β1 with a fragment of karyopherin-α, a fragment of the transcription factor SREBP-2, and with the parathyroid hormone-related cargo protein (PTHrP) show that distinct contacts are made between the karyopherin and each of its cargoes (43–45). This suggests that each karyopherin may have multiple binding sites and explains how a limited number of karyopherins can import diverse cargoes with no apparent sequence similarity. In addition, these structures show that the karyopherin is capable of adopting different conformations depending on the cargo (43–45). A study using small-angle X-ray scattering has allowed visualization of the overall shape of several members of the karyopherin family (50). This analysis further suggested that there was considerable conformational variability between individual karyopherins bound to different substrates and between different karyopherins (50). Thus, the super helical shape of the karyopherins may help impart flexibility that enables recognition of a variety of cargoes.

Karyopherins and Ran

Crystallographic studies have also provided insight into how karyopherins interact with RanGTP (47,48). It has been known for some time that karyopherins involved in import and export have different affinities for RanGTP (2,3). Import karyopherins have a relatively high affinity for RanGTP, and RanGTP binding leads to the dissociation of their import cargoes, whereas export karyopherins which form a trimeric complex of RanGTP and cargo have a relatively low affinity for Ran in the absence of cargo (2,3). Atomic structures for two import receptors and Ran have been solved, karyopherin-β2 and RanGTP, and an N-terminal fragment of importin-β1 and RanGTP (47,48). These proteins share many similarities in their Ran binding, most notably that Ran contacts the concave surface of the N-terminal arch (47,48). The Ran-binding site appears to have two separate domains, a more N-terminal domain and a more C-terminal domain (39). Interestingly, in the C-terminal binding site,

Ran contacts an acidic loop found between two HEAT repeats in the centre of the protein (39). Characterization of the acidic loop in karyopherin-β2 led to the prediction that this loop would also contact the cargo and may explain how RanGTP binding results in cargo dissociation (39). Mutation of this loop uncouples cargo release from RanGTP binding (51). RanGTP contact with the acidic loop may therefore regulate the association of the cargo with karyopherin-β2.

Exportins

The recently reported structure of the exportin Cse1p (CAS in mammals) in a complex with RanGTP and its cargo karyopherin-α has highlighted the difference between import and export complexes (42). Cse1p forms a super helix with N-terminal and C-terminal arches that clamp around RanGTP. Cse1p simultaneously makes extensive contacts with karyopherin-α, and in so doing locks karyopherin-α into a conformation where it cannot interact with cargo. Unexpectedly, karyopherin-α also makes direct contact with RanGTP. The interaction between Cse1p and RanGTP was different from that observed between importin-β1 and karyopherin-β2 and may explain why exportins bind RanGTP with high affinity only in the presence of cargo. Cse1p has two Ran-binding sites, and weak binding between Cse1p and RanGTP was mediated by the N-terminal site. In the presence of cargo, however, Cse1p wraps around the RanGTP so that both binding sites are engaged. The authors suggest this complex is 'spring loaded' for dissociation in the cytoplasm, where RanGTP is hydrolysed to RanGDP, a finding that emphasizes the extraordinary flexibility of the karyopherin structure (42).

A fragment of Crm1-containing amino acids 707–1027 has also been crystallized (41). By aligning the Crm1 sequence with the HEAT repeats in karyopherin-β2 and importin-β1, the authors deduced the locations of the HEAT repeats not represented in the Crm1 structure. This alignment indicated that there was significant structural relatedness between Crm1 and karyopherin-β2. Using a combination of sequence alignment and modelling, EM reconstruction and *in vitro* assays with mutant proteins, it was predicted that like karyopherin-β2, Crm1 contains a flexible loop in the centre of the protein (41). The authors proposed a model whereby the loop masks the cargo-binding site and hinders the stable association of Ran with Crm1. However, when either Ran or cargo binds, the loop changes conformation and stable binding of the ternary complex is promoted. This would predict a common mechanism for the assembly and disassembly of cargo complexes, each involving conformational changes in the Ran-binding loop. The structure of the full-length Crm1 as well as the additional karyopherins will be required to verify this model. It is interesting to note that Cse1p lacks a loop in this position, suggesting this mechanism may not be universal, although it does contain an extension within HEAT repeat 19 that contacts cargo and RanGTP (42).

Karyopherins and Nucleoporins

All karyopherins bind transiently to nucleoporins as they move through the NPC; these interactions underlie current models of transport (52). Proteomic characterization of the mammalian and yeast NPC has shown that it comprises approximately 30 proteins (53,54). Within the NPC is a central channel through which transport occurs. There are several classes of nucleoporins including transmembrane proteins that anchor the NPC in the nuclear membrane, FG-containing nucleoporins and a newly emerging class of nucleoporins that contain a structural motif referred to as the WD repeats or seven-blade propeller motif (52,54–56). FG nucleoporins are thought to constitute about half of NPC mass, and while most are distributed symmetrically, a few FG nucleoporins are restricted to the cytoplasmic or nuclear side of the NPC (53). FG nucleoporins provide NPC-binding sites for the karyopherins (52). Despite the critical role played by FG nucleoporins in nuclear transport, it has been shown that at least half the FG-repeat mass of the NPC in yeast can be deleted with little effect on transport (57). In addition, with one exception, each FG-repeat domain could be deleted, with no effect on viability (57).

The crystal structure of an N-terminal fragment of importin-β1 bound to a fragment of the yeast FG-repeat nucleoporin has been solved (46). This shows that the interactions are primarily hydrophobic and mediated by the phenylalanines of the nucleoporin. Two hydrophobic pockets are formed in the karyopherin by the side chains of residues between HEAT repeats 5 and 6 and, 6 and 7, into which the phenylalanines of FG repeats are inserted (46). More recent studies have also shown that importin-β1 contains another nucleoporin-binding region in the C-terminus (58).

Several studies have indicated that different karyopherins have a range of binding affinities for specific nucleoporins (52). Thus, it is possible that the transport of individual karyopherins through the NPC could be regulated by preferential use of different nucleoporin-binding sites. In an intriguing study, it has been shown that molecular rearrangements of the NPC may take place during the cell cycle (59). The authors showed that affinity of a particular karyopherin for a specific nucleoporin was altered during mitosis. When the affinity was very high, the passage of the karyopherin through the NPC was impeded, allowing the downregulation of specific import pathways (59).

It has also been suggested that the asymmetrically located nucleoporins are crucial for vectorial transport and cargo release. Import karyopherins often display high-affinity binding to the nucleoplasmic face of the NPC, whereas exportins seem to bind the cytoplasmic face of the NPC with high affinity (60–62). In yeast, recent studies have shown that all asymmetric nucleoporins can be deleted with only modest effects on transport, and indeed, the

asymmetry of the NPC can be reversed by swapping the FG-repeat domains between nuclear and cytoplasmic nucleoporins (57,63). Although these data suggest that the asymmetric distribution of nucleoporins is not required for transport, there is still obviously much to learn about the contribution of these nucleoporins and non-repeat-containing nucleoporins to the transport process.

Ran Regulators

Ran plays a direct role in nuclear transport by controlling the assembly state of transport complexes (3). Ran activity is tightly regulated through interactions with evolutionarily conserved proteins that help specify its subcellular localization, and its interconversion between GTP- and GDP-bound states (5). The predominant nuclear distribution of Ran observed by fluorescence microscopy belies the high rate of Ran efflux from the nucleus, which exceeds 10^5 molecules/second per nucleus (64,65). This occurs because RanGTP is constantly exported from the nucleus by exiting karyopherins. Ran import into the nucleus is mediated by NTF2, a homodimeric protein originally purified as a factor that stimulates NLS-dependent import in permeabilized cell assays (66–69). NTF2 interacts with the GDP-bound form of Ran, which is the predominate form of Ran in the cytoplasm. The rapid translocation of NTF2 : RanGDP from the cytoplasm into the nucleus occurs because, like karyopherins, NTF2 interacts directly (and with low affinity) with FG-repeat-containing nucleoporins (Figure 1) (70). Ran transport mediated by NTF2 is rendered unidirectional by conversion of RanGDP to RanGTP on the nucleoplasmic side of the NPC (Figure 1). NTF2 binds to the Switch II region of RanGDP, the conformation of which is different in RanGTP (71). Thus, after delivering RanGDP to the nucleoplasm, NTF2 returns to the cytoplasm 'empty-handed' to mediate another round of Ran import.

Perhaps, the least understood Ran-binding protein is Mog1, a protein that upon overexpression suppresses certain mutant alleles of Ran in *Saccharomyces cerevisiae*. Defective import of NLS cargo caused by Mog1 deletion or mutation can be suppressed by overexpression of NTF2 (72). These observations suggest that Mog1 and NTF2 fulfil related functions, but unlike NTF2, Mog1 binds preferentially to RanGTP (73,74). Thus, while it is clear that NTF2 is the Ran import receptor, genetic interactions between NTF2, Ran and Mog1 suggest that additional aspects of Ran import remain to be elucidated.

The conversion of RanGDP to RanGTP involves nucleotide exchange and occurs in the nucleus when RanGDP encounters the guanine nucleotide exchange factor RanGEF (Figure 1). Historically known as RCC1, RanGEF is a chromatin-associated protein that is present at approximately one copy per nucleosome where it binds directly to core histones H2A and H2B (75–77). RanGEF stimulates nucleotide exchange on Ran by up to five orders of magnitude (76). Biochemical and crystallographic analysis revealed that RanGEF inserts a loop (termed a β-wedge) into Ran to promote nucleotide dissociation (78). RanGEF binding also stabilizes the nucleotide-free form of Ran long enough for subsequent nucleotide binding to occur. Nucleotide exchange mediated by RanGEF does not favour GTP over GDP *in vitro*, but GTP loading onto Ran is favoured *in vivo* because of the high cellular GTP : GDP ratio.

The early impression that RanGEF is stably bound to chromatin, which was based largely on biochemical analysis, has given way to a much more dynamic view of RanGEF in the nucleus. Photobleaching analysis of GFP fusions demonstrated that RanGEF is highly mobile both in interphase and in mitosis (79,80). This raised the interesting question of whether nucleotide exchange of Ran occurs while RanGEF is bound to chromatin or while it is mobile in the nucleoplasm. The facts that core histones can modestly enhance RanGEF activity, and that Ran itself can interact with histones, have been taken as indications that nucleotide exchange occurs while RanGEF is in a chromatin-bound state (77). Further support for this view came with the finding that a mutant form of Ran (T24N) that resists nucleotide exchange can effectively immobilize RanGEF on chromatin (79). Taken together, the data suggest that during interphase, RanGEF mediates nucleotide exchange while it is in a chromatin-bound state, and that GTP loading onto Ran is necessary to release both proteins from chromatin. RanGEF-mediated nucleotide exchange on chromatin is believed to be important during mitosis because the high local concentration of RanGTP contributes to microtubule assembly near the surface of the chromosome (81). The roles of Ran in mitosis were recently reviewed (1,82).

High-affinity interactions between RanGTP and karyopherins exiting the nucleus occur both in the context of transporting cargo to the cytoplasm (exportins) and for the purpose recycling (import karyopherins) (Figure 1). Both types of reactions result in the translocation of RanGTP : karyopherin complexes to the cytoplasmic side of the NPC. It is on the cytoplasmic side of the NPC that RanGTP : karyopherin complexes are acted upon by RanGAP, a protein that potently stimulates the otherwise weak GTPase activity of Ran by a factor of 10^5 *in vitro* (83) (Figure 1). RanGTP is not fully inaccessible to RanGAP in the context of the RanGTP : karyopherin complex. This effect is relieved by the RanGTP-binding protein RanBP1 (84). Depending on the karyopherin, RanBP1 either generates a disassembly intermediate (RanBP1 : RanGTP : karyopherin), whereby RanGTP is rendered accessible to RanGAP, or promotes actual RanGTP release from the karyopherin (5). In either case, RanGTP is hydrolysed to RanGDP.

Localization of RanGEF activity in the nucleus and RanGAP activity in the cytoplasm provides a conceptual basis for how a steep gradient of RanGTP can be generated between these compartments (85). Supporting evidence for the RanGTP gradient in interphase nuclei was obtained

using fluorescence resonance energy transfer (FRET) sensors that can register the presence of RanGTP. Measurements from the FRET-based analysis indicated a RanGTP concentration difference of more than 100-fold between the nucleus and the cytoplasm (81), which is similar to estimates derived from computational modelling of nuclear transport of Ran (64,65).

Given the central role of RanGTP in regulating both nuclear import and export, and the fact that cytoplasmic RanGTP would disrupt nuclear transport by premature disassembly of import complexes, it comes as no surprise that cells have highly effective targeting mechanisms to ensure proper localization of the Ran regulators. Nucleotide exchange on Ran is restricted to the nucleus by two different import mechanisms for RanGEF (86,87). GTP hydrolysis on Ran is restricted to the cytoplasm because RanGAP is too large to cross the NPC by diffusion. In addition, modification of RanGAP by a small ubiquitin-like modifier results in targeting of RanGAP to the cytoplasmic face of the NPC (88,89). The RanGAP cofactor RanBP1 is small enough to enter the nucleus by diffusion, but RanBP1 is effectively excluded from the nucleus by the recognition of its hydrophobic NES by Crm1. Another RanGAP cofactor is Nup358, a nucleoporin located on the cytoplasmic face of the NPC (90,91). Nup358 contains four Ran-binding domains that are thought to help disassemble RanGTP : receptor complexes on the cytoplasmic side of the NPC.

The karyopherins together with Ran and its regulators discussed above represent the core machinery for nuclear transport; however, nuclear transport efficiency is enhanced by accessory factors. Npap60 promotes nuclear import at least in part through bridging interactions that increase the affinity of karyopherin-α for importin-β1 (92). After import into the nucleus, the quaternary complex that contains Npap60 : importin-β1 : karyopherin-α : NLS cargo is acted upon by RanGTP and CAS, the exportin that mediates karyopherin-α export. Concomitant with NLS cargo release is the formation of two export complexes, Npap60 : importin-β1 : RanGTP and CAS : karyopherin-α : RanGTP. An unusual feature of the Npap60 mechanism is that the protein adopts different binding modes for importin-β1 during the transport cycle, which led the authors to propose that Npap60 functions as a tri-stable switch (92). A functional counterpart to Npap60 that operates in nuclear export is RanBP3, an accessory factor with the same domain organization as Npap60 (93). RanBP3 enhances Crm1-dependent export by forming a RanBP3 : Crm1 complex that has a higher affinity for RanGTP and NES cargo than Crm1 alone (93).

Nuclear Transport of Steroid Hormone Receptors

The regulation of transcription factors and ultimately gene expression by nuclear transport is now appreciated as an important paradigm in biology. The 48-member nuclear receptor superfamily of ligand-regulated transcription factors controls a diverse array of physiological processes including growth and development. Within the nuclear receptor superfamily are the steroid hormone receptors, which translocate into the nucleus in response to binding cognate hormone (Figure 2). In the nucleus, steroid hormone receptors bind to hormone response element DNA and recruit the factors necessary for assembling preinitiation complexes (94). Although it has been known for some time that nuclear receptors such as the glucocorticoid receptor (GR) undergo ligand-dependent import, new information on the molecular basis of nuclear receptor import is emerging. Nuclear receptors also undergo nuclear export, and the combined import–export cycle, or nucleocytoplasmic shuttling, has been viewed as a potential mechanism for terminating transcription, and for integrating transcription with signalling events in the cytoplasm (95,96). Nuclear export could also regulate the participation of nuclear receptors in non-genomic functions in the cytoplasm. Interest in nuclear receptor transport derives from the critical roles of these factors in normal physiology, but also in the context of human diseases. Understanding the molecular basis of androgen receptor (AR) and oestrogen receptor (ER) transport, which each regulate multiple genes that control proliferation, could reveal targets that are relevant to cancers of the prostate and breast, respectively.

As with other transport pathways, the starting point for understanding nuclear receptor transport is the identification of transport signals and transport receptors. Here, we focus primarily on GR, because it is the most thoroughly studied with regard to nuclear transport. Like all nuclear receptors, GR has a modular structure with an N-terminal transactivation domain, a DNA-binding domain (DBD), a short hinge region and a ligand-binding domain (LBD) that also has transactivation function. Glucocorticoid receptor contains a basic NLS that overlaps the DBD-hinge region (termed NLS-1), and a second NLS activity that maps within the LBD (termed NLS-2 97). NLS-1 in GR is the basic bipartite type found in nucleoplasmin, and bipartite NLSs are found in the same region of AR, ER and the progesterone receptor (PR) (98–101). NLS-2 activity resides in the approximately 200 amino acid LBD of GR, and import activity has been detected in the LBDs of AR, ER and PR (97,100,102,103). The physiological reason for the presence of two import signals in steroid hormone receptors is unclear, but careful mutation and deletion studies have revealed some important differences between the signals (102). NLS-1 in GR is a more potent NLS and appears to be responsible for relatively rapid ($t_{1/2}$ approximately 5–15 min) nuclear import. In addition, NLS-1-dependent import is induced by agonist or antagonist binding, and at relatively high GR expression levels, NLS-1 can also mediate import in the absence of ligand. By contrast, nuclear import mediated by NLS-2 is relatively slow ($t_{1/2}$ approximately 45 min) and is agonist specific. Attempts to dissect NLS-2 activity in nuclear receptors have failed to yield a linear NLS, indicating that the import signal probably resides within the three-dimensional structure of the LBD.

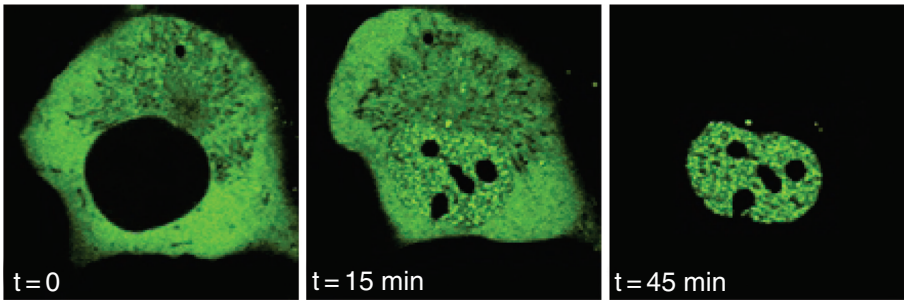

Figure 2: Nuclear import of the androgen receptor (AR) is induced by androgen. Cos7 cells expressing AR fused to green fluorescent protein (GFP-AR) were treated with synthetic androgen (R1881, 1 nM) and monitored by real-time confocal microscopy. GFP-AR is excluded from the nucleus prior to androgen addition (t = 0 min) and is readily visible in the nucleus soon after androgen addition (t = 15 min).

Analysis of the nuclear import receptors that recognize NLS-1 and NLS-2 in GR and the role of ligand binding in these interactions have recently yielded unanticipated results (102,104). Using *in vitro* transport assays, it was shown that GR import can be mediated by the karyopherin-α : importin-β1 heterodimer or by the importin-7, and either import receptor can stably bind to NLS-1 (Table 1). NLS-2 can be recognized by importin-7 or importin-8; however, the exact contribution of import receptor interactions with NLS-2 in the context of full-length GR has not yet been elucidated. The surprise came with the finding that karyopherin-α, importin-7 and importin-8 interactions with GR are ligand independent (104). Thus, the popular model that ligand binding induces dissociation of a chaperone such as Hsp90 that otherwise masks the NLS in GR appears to be incorrect. Ligand binding may instead regulate a critical chaperone-dependent step that occurs after NLS recognition by the import machinery (104–106). Chaperones have also been shown to facilitate nuclear receptor interactions with the cytoskeleton, suggesting that there may be subcellular locales for cytoplasmic retention and/or transformation (96).

Analysis of the signals and receptors that mediate steroid receptor export is still an active area of investigation (96). Nuclear export activity has been mapped to the DBD in multiple nuclear receptors, but there is also evidence for an NES in the LBD of AR (107,108). Notably, sequence analysis has suggested that only one of the 48 nuclear receptor family members (NGIF-B) has a hydrophobic NES recognized by Crm1 (109). Nonetheless, application of leptomycin B, which is a specific inhibitor of Crm1, has in some cases implicated this karyopherin in nuclear receptor export. Export in the heterokaryon assay, for example, is inhibited by leptomycin B in some laboratories and uninhibited in others (110,111). It deserves mention that the observation that GR export does not require Ran seems to argue against Crm1-dependent export, since RanGTP is a stoichiometric component of Crm1 export complexes (24,112,113).

Based on the available data, it may be inferred that nuclear receptors probably undergo either Crm1-dependent or Crm1-independent export, possibly depending on the cellular conditions. Given that nuclear receptors as a group appear to lack a hydrophobic NES, it is conceivable that other proteins such as 14-3-3 family members and p160 coactivators that bind nuclear receptors contain the export signal and thereby function as adapters for Crm1-dependent export (114,115). Adding to the list of possible mechanisms are experiments showing that ligand binding and nuclear receptor heterodimerization can determine whether nuclear export is Crm1 dependent or Crm1 independent (116). One Crm1-independent pathway for GR export is regulated by calreticulin, a chaperone found both in the endoplasmic reticulum and in the cytosol (117). The mechanism responsible for calreticulin-dependent export of GR has not been fully characterized, though it is known to involve the DBD because point mutations within the DNA recognition helix reduce both CRT binding and GR export (107). The general role of calreticulin in GR export has been challenged with the finding that polyethylene glycol used to fuse cells in the heterokaryon assay may release calreticulin from the endoplasmic reticulum (118). This observation does not, however, explain why GR export is deficient in fibroblasts derived from calreticulin-knockout mice (117).

While there has been a major emphasis on understanding how ligand binding controls the nuclear transport of nuclear receptors, there is a substantial interest in defining how signal transduction pathways contribute to nuclear receptor localization. Nuclear import of GR can be triggered by shear stress activation of MAP and PI-3 kinases (119). MAP kinases have also been implicated in the regulation of ER, PR and GR export (96). In the case of PR, epidermal growth factor signalling through the MAP kinase pathway results in PR phosphorylation, nuclear export and degradation in the cytoplasm (120). It is not known how phosphorylation facilitates nuclear export of nuclear receptors, but the possibilities include phosphoregulation of the export signal or the proteins that mediate nuclear export.

Nuclear Transport and Disease

Four different nucleoporins that make up the NPC have been identified as chromosomal translocations that generate

oncogenic fusion proteins (121). To date, however, there are no known disease-causing mutations in the soluble components of the nuclear transport machinery, which includes karyopherins, Ran and its regulators. The absence of mutations and chromosomal translocations underscores the essential nature of soluble transport components even at the single cell level. Nonetheless, it is plausible that alterations in nuclear trafficking of specific cargoes could influence proliferation, and thereby contribute to human diseases. AR, for example, is a key regulator of prostate cell proliferation in both normal and cancer cells. Nuclear import and transactivation of AR are usually regulated by androgen binding (Figure 2) (122). In a disease progression model of prostate cancer, xenografts can switch from androgen-dependent growth to androgen-independent growth in castrated mice. Strikingly, during disease progression in this model, AR acquires the ability to undergo androgen-independent nuclear import and androgen-independent transactivation (123). AR is not mutated during progression, indicating there is a gain-of-function in critical aspects of the AR import and transactivation pathways. The androgen-independent mechanism that controls AR localization is currently unknown, but it could involve MAP kinase pathways that are known to be elevated in many prostate cancers (124). Continued analysis of nuclear transport pathways and regulation of this process by signalling pathways is expected to provide new insights into how compartmentalization contributes to cell regulation in both normal and pathophysiological states.

References

1. Mosammaparast N, Pemberton LF. Karyopherins: from nuclear-transport mediators to nuclear-function regulators. Trends Cell Biol 2004;14:547–556.
2. Fried H, Kutay U. Nucleocytoplasmic transport: taking an inventory. Cell Mol Life Sci 2003;60:1659–1688.
3. Weis K. Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. Cell 2003;112:441–451.
4. Goldfarb DS, Corbett AH, Mason DA, Harreman MT, Adam SA. Importin alpha: a multipurpose nuclear-transport receptor. Trends Cell Biol 2004;14:505–514.
5. Gorlich D, Kutay U. Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol 1999;15:607–660.
6. Kaffman A, O'Shea EK. Regulation of nuclear localization: a key to a door. Annu Rev Cell Dev Biol 1999;15:291–339.
7. Harreman MT, Kline TM, Milford HG, Harben MB, Hodel AE, Corbett AH. Regulation of nuclear import by phosphorylation adjacent to nuclear localization signals. J Biol Chem 2004;279:20613–20621.
8. Poon IKH, Jans DA. Regulation of nuclear transport: central role in development and transformation. Traffic 2005;6:173–186.
9. Conti E, Uy M, Leighton L, Blobel G, Kuriyan J. Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin alpha. Cell 1998;94:193–204.
10. Kobe B. Autoinhibition by an internal nuclear localization signal revealed by the crystal structure of mammalian importin alpha. Nat Struct Biol 1999;6:388–397.
11. Peifer M, Berg S, Reynolds AB. A repeating amino acid motif shared by proteins with diverse cellular roles. Cell 1994;76:789–791.
12. Conti E, Kuriyan J. Crystallographic analysis of the specific yet versatile recognition of distinct nuclear localization signals by karyopherin alpha. Structure Fold Des 2000;8:329–338.
13. Fontes MR, Teh T, Kobe B. Structural basis of recognition of monopartite and bipartite nuclear localization sequences by mammalian importin-alpha. J Mol Biol 2000;297:1183–1194.
14. Fontes MR, Teh T, Jans D, Brinkworth RI, Kobe B. Structural basis for the specificity of bipartite nuclear localization sequence binding by importin-alpha. J Biol Chem 2003;278:27981–27987.
15. Mosammaparast N, Jackson KR, Guo Y, Brame CJ, Shabanowitz J, Hunt DF, Pemberton LF. Nuclear import of histone H2A and H2B is mediated by a network of karyopherins. J Cell Biol 2001;153:251–262.
16. Mosammaparast N, Ewart CS, Pemberton LF. A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. EMBO J 2002;21:6527–6538.
17. Muhlhausser P, Muller EC, Otto A, Kutay U. Multiple pathways contribute to nuclear import of core histones. EMBO Rep 2001;2:690–696.
18. Jakel S, Gorlich D. Importin beta, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. EMBO J 1998;17:4491–4502.
19. Senger B, Simos G, Bischoff FR, Podtelejnikov A, Mann M, Hurt E. Mtr10p functions as a nuclear import receptor for the mRNA-binding protein Npl3p. EMBO J 1998;17:2196–2207.
20. Leslie DM, Zhang W, Timney BL, Chait BT, Rout MP, Wozniak RW, Aitchison JD. Characterization of karyopherin cargoes reveals unique mechanisms of Kap121p-mediated nuclear import. Mol Cell Biol 2004;24:8487–8503.
21. Pollard VW, Michael WM, Nakielny S, Siomi MC, Wang F, Dreyfuss G. A novel receptor-mediated nuclear protein import pathway. Cell 1996;86:985–994.
22. Rosenblum JS, Pemberton LF, Bonifaci N, Blobel G. Nuclear import and the evolution of a multifunctional RNA-binding protein. J Cell Biol 1998;143:887–899.
23. Fischer U, Huber J, Boelens WC, Mattaj IW, Luhrmann R. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. Cell 1995;82:475–483.
24. Fornerod M, Ohno M, Yoshida M, Mattaj IW. CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 1997;90:1051–1060.
25. Stade K, Ford CS, Guthrie C, Weis K. Exportin 1 (Crm1) is an essential nuclear export factor. Cell 1997;90:1041–1050.
26. la Cour T, Gupta R, Rapacki K, Skriver K, Poulsen FM, Brunak S. NESbase, version 1.0: a database of nuclear export signals. Nucleic Acids Res 2003;31:393–396.
27. Wen W, Meinkoth JL, Tsien RY, Taylor SS. Identification of a signal for rapid export of proteins from the nucleus. Cell 1995;82:463–473.
28. Paraskeva E, Izaurralde E, Bischoff FR, Huber J, Kutay U, Hartmann E, Luhrmann R, Gorlich D. CRM1-mediated recycling of snurportin 1 to the cytoplasm. J Cell Biol 1999;145:255–264.
29. Ohno M, Segref A, Bachi A, Wilm M, Mattaj IW. PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. Cell 2000;101:187–198.
30. Johnson AW, Lund E, Dahlberg J. Nuclear export of ribosomal subunits. Trends Biochem Sci 2002;27:580–585.
31. Kaffman A, Rank NM, O'Neill EM, Huang LS, O'Shea EK. The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. Nature 1998;396:482–486.
32. Yoshida K, Blobel G. The karyopherin Kap142p/Msn5p mediates nuclear import and nuclear export of different cargo proteins. J Cell Biol 2001;152:729–740.

33. Kutay U, Bischoff FR, Kostka S, Kraft R, Görlich D. Export of importin α from the nucleus is mediated by a specific nuclear transport factor. Cell 1997;90:1061–1071.
34. Lei EP, Silver PA. Protein and RNA export from the nucleus. Dev Cell 2002;2:261–272.
35. Kutay U, Lipowsky G, Izaurralde E, Bischoff FR, Schwarzmaier P, Hartmann E, Gorlich D. Identification of a tRNA-specific nuclear export receptor. Mol Cell 1998;1:359–369.
36. Arts GJ, Kuersten S, Romby P, Ehresmann B, Mattaj IW. The role of exportin-t in selective nuclear export of mature tRNAs. EMBO J 1998;17:7430–7441.
37. Kim VN. MicroRNA precursors in motion: exportin-5 mediates their nuclear export. Trends Cell Biol 2004;14:156–159.
38. Zeng Y, Cullen BR. Structural requirements for pre-microRNA binding and nuclear export by exportin 5. Nucleic Acids Res 2004;32: 4776–4785.
39. Chook YM, Blobel G. Karyopherins and nuclear import. Curr Opin Struct Biol 2001;11:703–715.
40. Conti E. Structures of importins. Results Probl Cell Differ 2002;35: 93–113.
41. Petosa C, Schoehn G, Askjaer P, Bauer U, Moulin M, Steuerwald U, Soler-Lopez M, Baudin F, Mattaj IW, Muller CW. Architecture of CRM1/Exportin1 suggests how cooperativity is achieved during formation of a nuclear export complex. Mol Cell 2004;16:761–775.
42. Matsuura Y, Stewart M. Structural basis for the assembly of a nuclear export complex. Nature 2004;432:872–877.
43. Cingolani G, Petosa C, Weis K, Muller CW. Structure of importin-beta bound to the IBB domain of importin-alpha. Nature 1999;399:221–229.
44. Cingolani G, Bednenko J, Gillespie MT, Gerace L. Molecular basis for the recognition of a nonclassical nuclear localization signal by importin beta. Mol Cell 2002;10:1345–1353.
45. Lee SJ, Sekimoto T, Yamashita E, Nagoshi E, Nakagawa A, Imamoto N, Yoshimura M, Sakai H, Chong KT, Tsukihara T, Yoneda Y. The structure of importin-beta bound to SREBP-2: nuclear import of a transcription factor. Science 2003;302:1571–1575.
46. Bayliss R, Littlewood T, Stewart M. Structural basis for the interaction between FxFG nucleoporin repeats and importin-beta in nuclear trafficking. Cell 2000;102:99–108.
47. Vetter IR, Arndt A, Kutay U, Gorlich D, Wittinghofer A. Structural view of the Ran–importin beta interaction at 2.3A resolution. Cell 1999;97:635–646.
48. Chook YM, Blobel G. Structure of the nuclear transport complex karyopherin-beta2-Ran × GppNHp. Nature 1999;399:230–237.
49. Andrade MA, Bork P. HEAT repeats in the Huntington's disease protein. Nat Genet 1995;11:115–116.
50. Fukuhara N, Fernandez E, Ebert J, Conti E, Svergun D. Conformational variability of nucleo-cytoplasmic transport factors. J Biol Chem 2004;279:2176–2181.
51. Chook YM, Jung A, Rosen MK, Blobel G. Uncoupling Kapbeta2 substrate dissociation and ran binding. Biochemistry 2002;41: 6955–6966.
52. Suntharalingam M, Wente SR. Peering through the pore: nuclear pore complex structure, assembly, and function. Dev Cell 2003;4: 775–789.
53. Rout MP, Aitchison JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT. The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 2000;148:635–651.
54. Cronshaw JM, Krutchinsky AN, Zhang W, Chait BT, Matunis MJ. Proteomic analysis of the mammalian nuclear pore complex. J Cell Biol 2002;158:915–927.
55. Weirich CS, Erzberger JP, Berger JM, Weis K. The N-terminal domain of Nup159 forms a beta-propeller that functions in mRNA export by tethering the helicase dbp5 to the nuclear pore. Mol Cell 2004;16:749–760.
56. Devos D, Dokudovskaya S, Alber F, Williams R, Chait BT, Sali A, Rout MP. Components of coated vesicles and nuclear pore complexes share a common molecular architecture. PLoS Biol 2004;2:e380.
57. Strawn LA, Shen T, Shulga N, Goldfarb DS, Wente SR. Minimal nuclear pore complexes define FG repeat domains essential for transport. Nat Cell Biol 2004;6:197–206.
58. Bednenko J, Cingolani G, Gerace L. Importin beta contains a COOH-terminal nucleoporin binding region important for nuclear transport. J Cell Biol 2003;162:391–401.
59. Makhnevych T, Lusk CP, Anderson AM, Aitchison JD, Wozniak RW. Cell cycle regulated transport controlled by alterations in the nuclear pore complex. Cell 2003;115:813–823.
60. Ben-Efraim I, Gerace L. Gradient of increasing affinity of importin beta for nucleoporins along the pathway of nuclear import. J Cell Biol 2001;152:411–417.
61. Pyhtila B, Rexach M. A gradient of affinity for the karyopherin Kap95p along the yeast nuclear pore complex. J Biol Chem 2003;278: 42699–42709.
62. Shah S, Tugendreich S, Forbes D. Major binding sites for the nuclear import receptor are the internal nucleoporin Nup153 and the adjacent nuclear filament protein Tpr. J Cell Biol 1998;141:31–49.
63. Zeitler B, Weis K. The FG-repeat asymmetry of the nuclear pore complex is dispensable for bulk nucleocytoplasmic transport in vivo. J Cell Biol 2004;167:583–590.
64. Smith AE, Slepchenko BM, Schaff JC, Loew LM, Macara IG. Systems analysis of Ran transport. Science 2002;295:488–491.
65. Gorlich D, Seewald MJ, Ribbeck K. Characterization of Ran-driven cargo transport and the RanGTPase system by kinetic measurements and computer simulation. EMBO J 2003;22:1088–1100.
66. Moore MS, Blobel G. Purification of a Ran-interacting protein that is required for protein import into the nucleus. Proc Natl Acad Sci USA 1994;91:10212–10216.
67. Paschal BM, Gerace L. Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. J Cell Biol 1995;129:925–937.
68. Smith A, Brownawell A, Macara IG. Nuclear import of Ran is mediated by the transport factor NTF2. Curr Biol 1998;8:1403–1406.
69. Ribbeck K, Lipowsky G, Kent HM, Stewart M, Gorlich D. NTF2 mediates nuclear import of Ran. EMBO J 1998;17:6587–6598.
70. Bayliss R, Leung SW, Baker RP, Quimby BB, Corbett AH, Stewart M. Structural basis for the interaction between NTF2 and nucleoporin FxFG repeats. EMBO J 2002;21:2843–2853.
71. Stewart M, Kent HM, McCoy AJ. Structural basis for molecular recognition between nuclear transport factor 2 (NTF2) and the GDP-bound form of the Ras-family GTPase Ran. J Mol Biol 1998;277:635–646.
72. Oki M, Nishimoto T. A protein required for nuclear-protein import, Mog1p, directly interacts with GTP-Gsp1p, the *Saccharomyces cerevisiae* Ran homologue. Proc Natl Acad Sci USA 1998;95:15388–15393.
73. Oki M, Nishimoto T. Yrb1p interaction with the gsp1p C terminus blocks Mog1p stimulation of GTP release from Gsp1p. J Biol Chem 2000;275:32894–32900.
74. Steggerda SM, Paschal BM. The mammalian Mog1 protein is a guanine nucleotide release factor for Ran. J Biol Chem 2000;275:23175–23180.
75. Ohtsubo M, Kai R, Furuno N, Sekiguchi T, Sekiguchi M, Hayashida M, Kuma K, Miyata T, Fuukushige S, Murotsu T, Matsubara K, Nishimoto T. Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. Genes Dev 1987;1:585–593.
76. Bischoff FR, Ponstingl H. Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. Nature 1991;354:80–82.
77. Nemergut ME, Mizzen CA, Stukenberg T, Allis CD, Macara IG. Chromatin docking and exchange activity enhancement of RCC1 by histones H2A and H2B. Science 2001;292:1540–1543.

78. Renault L, Kuhlmann J, Henkel A, Wittinghofer A. Structural basis for guanine nucleotide exchange on Ran by the regulator of chromosome condensation (RCC1). Cell 2001;105:245–255.
79. Li HY, Wirtz D, Zheng Y. A mechanism of coupling RCC1 mobility to RanGTP production on the chromatin in vivo. J Cell Biol 2003;160: 635–644.
80. Li HY, Zheng Y. Phosphorylation of RCC1 in mitosis is essential for producing a high RanGTP concentration on chromosomes and for spindle assembly in mammalian cells. Genes Dev 2004;18:512–527.
81. Kalab P, Weis K, Heald R. Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts. Science 2002;295: 2452–2456.
82. Harel A, Forbes DJ. Importin Beta; conducting a much larger cellular symphony. Mol Cell 2004;16:319–330.
83. Bischoff FR, Klebe C, Kretschmer J, Wittinghofer A, Ponstingl H. RanGAP1 induces GTPase activity of nuclear Ras-related Ran. Proc Natl Acad Sci USA 1994;91:2587–2591.
84. Bischoff FR, Krebber H, Smirnova E, Dong W, Ponstingl H. Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. EMBO J 1995;14:705–715.
85. Izaurralde E, Kutay U, von Kobbe C, Mattaj IW, Gorlich D. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. EMBO J 1997;16:6535–6547.
86. Nemergut ME, Macara IG. Nuclear import of the ran exchange factor, RCC1, is mediated by at least two distinct mechanisms. J Cell Biol 2000;149:835–850.
87. Talcott B, Moore MS. The nuclear import of RCC1 requires a specific nuclear localization sequence receptor, karyopherin alpha3/Qip. J Biol Chem 2000;275:10099–10104.
88. Matunis MJ, Coutavas E, Blobel G. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. J Cell Biol 1996;135:1457–1470.
89. Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell 1997;88:97–107.
90. Wu J, Matunis MJ, Kraemer D, Blobel G, Coutavas E. Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. J Biol Chem 1995;270:14209–14213.
91. Yokoyama N, Hayashi N, Seki T, Pante N, Ohba T, Nishii K, Kuma K, Hayashida T, Miyata T, Aebi U et al. A giant nucleopore protein that binds Ran/TC4. Nature 1995;376:184–188.
92. Lindsay ME, Plafker K, Smith AE, Clurman BE, Macara IG. Npap60/Nup50 is a tri-stable switch that stimulates importin-alpha: beta-mediated nuclear protein import. Cell 2002;110:349–360.
93. Lindsay ME, Holaska JM, Welch K, Paschal BM, Macara IG. Ran-binding protein 3 is a cofactor for Crm1-mediated nuclear protein export. J Cell Biol 2001;153:1391–1402.
94. McKenna NJ, O'Malley BW. Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 2002;108:465–474.
95. DeFranco DB. Navigating steroid hormone receptors through the nuclear compartment. Mol Endocrinol 2002;16:1449–1455.
96. Shank LC, Paschal BM. Nuclear transport of steroid hormone receptors. Crit Rev Eukaryot Gene Expr 2005; in press.
97. Picard D, Yamamoto KR. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. EMBO J 1987;6:3333–3340.
98. Zhou ZX, Sar M, Simental JA, Lane MV, Wilson EM. A ligand-dependent bipartite nuclear targeting signal in the human androgen receptor. Requirement for the DNA-binding domain and modulation by NH2-terminal and carboxyl-terminal sequences. J Biol Chem 1994;269:13115–13123.
99. Simental JA, Sar M, Lane MV, French FS, Wilson EM. Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem 1991;266:510–518.
100. Ylikomi T, Bocquel MT, Berry M, Gronemeyer H, Chambon P. Cooperation of proto-signals for nuclear accumulation of estrogen and progesterone receptors. EMBO J 1992;11:3681–3694.
101. Picard D, Kumar V, Chambon P, Yamamoto KR. Signal transduction by steroid hormones: nuclear localization is differentially regulated in estrogen and glucocorticoid receptors. Cell Regul 1990;1:291–299.
102. Savory JG, Hsu B, Laquian IR, Giffin W, Reich T, Hache RJ, Lefebvre YA. Discrimination between NL1- and NL2-mediated nuclear localization of the glucocorticoid receptor. Mol Cell Biol 1999;19:1025–1037.
103. Poukka H, Karvonen U, Yoshikawa N, Tanaka H, Palvimo JJ, Janne OA. The RING finger protein SNURF modulates nuclear trafficking of the androgen receptor. J Cell Sci 2000;113:2991–3001.
104. Freedman ND, Yamamoto KR. Importin 7 and importin {alpha}/importin {beta} are nuclear import receptors for the glucocorticoid receptor. Mol Biol Cell 2004;15:2276–2286.
105. Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 1997;18:306–360.
106. Davies TH, Ning YM, Sanchez ER. A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem 2002;277:4597–4600.
107. Black BE, Holaska JM, Rastinejad F, Paschal BM. DNA binding domains in diverse nuclear receptors function as nuclear export signals. Curr Biol 2001;11:1749–1758.
108. Saporita AJ, Zhang Q, Navai N, Dincer Z, Hahn J, Cai X, Wang Z. Identification and characterization of a ligand-regulated nuclear export signal in androgen receptor. J Biol Chem 2003;278: 41998–42005.
109. Katagiri Y, Takeda K, Yu ZX, Ferrans VJ, Ozato K, Guroff G. Modulation of retinoid signalling through NGF-induced nuclear export of NGFI-B. Nat Cell Biol 2000;2:435–440.
110. Liu J, DeFranco DB. Protracted nuclear export of glucocorticoid receptor limits its turnover and does not require the exportin 1/CRM1-directed nuclear export pathway. Mol Endocrinol 2000;14:40–51.
111. Itoh M, Adachi M, Yasui H, Takekawa M, Tanaka H, Imai K. Nuclear export of glucocorticoid receptor is enhanced by c-Jun N-terminal kinase-mediated phosphorylation. Mol Endocrinol 2002;16:2382–2392.
112. Yang J, Liu J, DeFranco DB. Subnuclear trafficking of glucocorticoid receptors in vitro: chromatin recycling and nuclear export. J Cell Biol 1997;137:523–538.
113. Stade K, Ford CS, Guthrie C, Weis K. Exportin 1 (Crm1p) is an essential nuclear export factor. Cell 1997;90:1041–1050.
114. Kino T, Souvatzoglou E, De Martino MU, Tsopanomihalu M, Wan Y, Chrousos GP. Protein 14-3-3sigma interacts with and favors cytoplasmic subcellular localization of the glucocorticoid receptor, acting as a negative regulator of the glucocorticoid signaling pathway. J Biol Chem 2003;278:25651–25656.
115. Amazit L, Alj Y, Tyagi RK, Chauchereau A, Loosfelt H, Pichon C, Pantel J, Foulon-Guinchard E, Leclerc P, Milgrom E, Guiochon-Mantel A. Subcellular localization and mechanisms of nucleocytoplasmic trafficking of steroid receptor coactivator-1. J Biol Chem 2003;278:32195–32203.
116. Prufer K, Barsony J. Retinoid X receptor dominates the nuclear import and export of the unliganded vitamin D receptor. Mol Endocrinol 2002;16:1738–1751.
117. Holaska JM, Black BE, Love DC, Hanover JA, Leszyk J, Paschal BM. Calreticulin is a receptor for nuclear export. J Cell Biol 2001;152:127–140.
118. Walther RF, Lamprecht C, Ridsdale A, Groulx I, Lee S, Lefebvre YA, Hache RJ. Nuclear export of the glucocorticoid receptor is accelerated by cell fusion-dependent release of calreticulin. J Biol Chem 2003;278:37858–37864.

119. Ji JY, Jing H, Diamond SL. Shear stress causes nuclear localization of endothelial glucocorticoid receptor and expression from the GRE promoter. Circ Res 2003;92:279–285.
120. Qiu M, Olsen A, Faivre E, Horwitz KB, Lange CA. Mitogen-activated protein kinase regulates nuclear association of human progesterone receptors. Mol Endocrinol 2003;17:628–642.
121. Cronshaw JM, Matunis MJ. The nuclear pore complex: disease associations and functional correlations. Trends Endocrinol Metab 2004;15:34–39.
122. Black BE, Paschal BM. Intranuclear organization and function of the androgen receptor. Trends Endocrinol Metab 2004;15: 411–417.
123. Zhang L, Johnson M, Le KH, Sato M, Ilagan R, Iyer M, Gambhir SS, Wu L, Carey M. Interrogating androgen receptor function in recurrent prostate cancer. Cancer Res 2003;63:4552–4560.
124. Gioeli D, Mandell JW, Petroni GR, Frierson HF Jr, Weber MJ. Activation of mitogen-activated protein kinase associated with prostate cancer progression. Cancer Res 1999;59:279–284.