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Distinct Nuclear Import and Export Pathways Mediated by Members of the Karyopherin β Family

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Abstract Transport of proteins into and out of the nucleus occurs through nuclear pore complexes (NPCs) and is mediated by the interaction of transport factors with nucleoporins at the NPC. Nuclear import of proteins containing classical nuclear localization signals (NLSs) is mediated by a heterodimeric protein complex, composed of karyopherin α and β1, that docks via β1 the NLS-protein to the NPC. The GTPase Ran; the RanGDP binding protein, p10; and the RanGTP binding protein, RanBP1 are involved in translocation of the docked NLS-protein into the nucleus. Recently, new distinct nuclear import and export pathways that are mediated by members of the karyopherin β family have been discovered. Karyopherin β2 mediates import of mRNA binding proteins, whereas karyopherin β3 and β4 mediate import of a set of ribosomal proteins. Two other β karyopherin family members, CRM1 and CAS, mediate export of proteins containing leucine-rich nuclear export signals (NES) and reexport of karyopherin α, respectively. This growing family contains new members that constitute potential transport factors for cargoes yet to be identified in the future. The common features of the members of karyopherin β family are the ability to bind RanGTP and the ability to interact directly with nucleoporins at the NPC. The challenge for the future will be to identify the distinct or, perhaps, overlapping cargo(es) for each member of the karyopherin β superfamily and to characterize the molecular mechanisms of translocation of karyopherins together with their cargoes through the NPC. J. Cell. Biochem. 70:231–239, 1998.

Key words: nuclear pore complexes; nuclear localization signals; nuclear export signals

The cytoplasmic and nuclear compartments of the eukaryotic cell are separated by the nuclear envelope. Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC), which is a large and complex structure, approximately 30 times the size of the ribosome. Although ions and small molecules can passively diffuse through the 9-nm NPC channels, macromolecules are actively transported through the 26-nm central channel [for review see Rout and Wente, 1994]. Nucleocytoplasmic transport of macromolecules is signal mediated, energy dependent, and highly selective, being mediated by saturable transport receptors [for review see Görlich and Mattaj, 1996].

Much of the work on nucleocytoplasmic pathways is of recent vintage and, because of its fundamental and practical significance, has attracted the attention of many laboratories. One consequence of this intense effort is the proliferation of redundant nomenclature. We have adopted a nomenclature based on karyopherins.

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Karyopherin β1 Together With Karyopherin α Mediates Nuclear Import of NLS-Proteins

The nuclear import of proteins with classical nuclear localization signals (NLSs) is the best characterized nucleocytoplasmic transport pathway [for reviews see Görlich and Mattaj, 1996; Moroianu, 1997]. The NLS contains one or several clusters of basic amino acids [Dingwall and Laskey, 1991; Garcia-Bustos et al., 1991] and is recognized in the cytoplasm by a heterodimeric protein complex, composed of karyopherin α and β1 (known also as importin α and β, NLS receptor, and p97; yeast Kap60 and Kap95) (Table I) [for review see Moroianu, 1997]. The α subunit of karyopherin binds to the NLS [Adam and Adam, 1994; Imamoto et al., 1995; Moroianu et al., 1995a,b; Rexach and Blobel, 1995; Weis et al., 1995], whereas the β1 subunit mediates docking at the NPC to nucleoporins that contain peptide repeats [Görlich et al., 1995; Moroianu et al., 1995b] (see Fig. 1). There are at least four human karyopherin α, and homo-

TABLE I. Distinct Nuclear Import Pathways Mediated by Members of Karyopherin β Family

Mammalian α karyopherins	Mammalian β karyopherins	Yeast αβ karyopherins	Other names	Import signal	Imported cargoes
α1, α2	β1	Kap60	Importin αβ, NLS	Classic NLS (cluster(s) of basic residues)	NLS-proteins
α3, α4		Kap95	receptor, and p97		
	β2	Kap104	Transportin	M9	hnRNP proteins
	β3	Kap121	RanBP5	ND[a]	Ribosomal proteins
	β4	Kap123	RanBP6		

[a]ND, not determined.

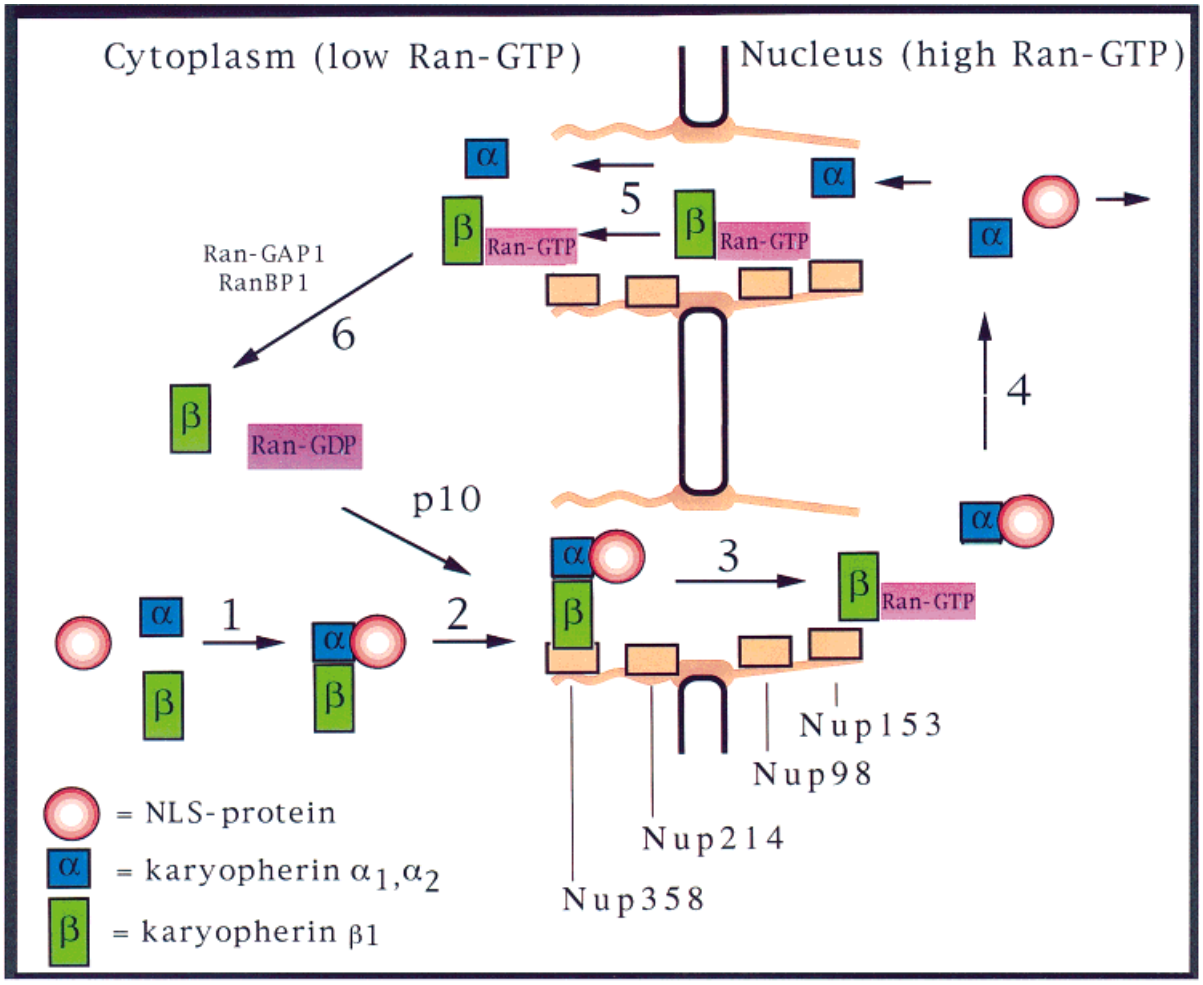

Fig. 1. A model of nuclear import of NLS-proteins mediated by karyopherin αβ1 heterodimer.

logues have been characterized in yeast and *Drosophila melanogaster* [for review see Moroianu, 1997]. All karyopherin α homologues have a common structure. The N- and C-terminal domains are very hydrophilic, whereas the middle part consists of eight copies of a hydrophobic motif present in other proteins, such as armadillo, plakoglobin, β-catenin, adenomatous polyposis coli (APC) protein, and smgGDS [Peifer et al., 1994]. Two of the human α, α1 (corresponding to hSRP/NPI1) and α2 (corresponding to Rch1/NPI3) have been shown to bind to the same β1 and to the NLS of SV40T antigen [Moroianu et al., 1995a,b; Weis et al., 1995], and their binding domains have been mapped. The N-terminal domain of either α1 or

α2 contains the binding site for β1 [Görlich et al., 1996a; Moroianu et al., 1996a; Weis et al., 1996), whereas the C-terminal domain contains binding determinants for different NLSs [Gallay et al., 1996; Moroianu et al., 1996; Fischer et al., 1997]. It is likely that different α subunits have both distinct and overlapping specificities for different NLSs.

During translocation through the NPC the karyopherin heterodimer dissociates: karyopherin α and the NLS-protein enter and accumulate in the nucleoplasm, whereas karyopherin β1 accumulates at the NPC [Görlich et al., 1995; Moroianu et al., 1995b] (see Fig. 1). The GTPase Ran [Drivas et al., 1990; Belhumeur et al., 1993] and GTP hydrolysis by Ran are required for translocation of NLS-proteins through the NPC [Melchior et al., 1993; Moore and Blobel, 1993; Melchior et al., 1995; Schlenstedt et al., 1995a; Palacios et al., 1996; Weis et al., 1996]. Two Ran-interacting proteins, p10/NTF2 that interacts with Ran-GDP and RanBP1 that interacts with Ran-GTP, stimulate nuclear import in vitro [Moore and Blobel, 1994; Paschal and Gerace, 1995; Paschal et al., 1996; Nehrbass and Blobel, 1996; Coutavas et al., 1993; Chi et al., 1996]. In vivo studies have also shown that p10 and RanBP1 are involved in nuclear import [Schlenstedt et al., 1995b; Nehrbass and Blobel, 1996; Corbett and Silver, 1996].

It is not yet clearly established how Ran's GTP cycle is translated into the movement of the NLS-protein/karyopherin αβ1 trimeric complex through the NPC. What is known until now is that nuclear import requires cytoplasmic Ran to be in its GDP form [Görlich et al., 1996b; Nehrbass and Blobel, 1996]. Then, RanGTP generated at the NPC binds to karyopherin β1 and dissociates the karyopherin αβ1 heterodimer [Rexach and Blobel, 1995; Chi et al., 1996; Nehrbass and Blobel, 1996; Görlich et al., 1996b; Moroianu et al., 1996b], causing also release of karyopherin β1 at least from some of the docking sites at the NPC [Rexach and Blobel, 1995]. The asymmetric distribution of the Ran regulatory proteins controls Ran-GTP distribution in the nucleus and thus dictates that subunits of karyopherin αβ1 associate in the cytoplasm and dissociate at the NPC. Thus, RanGAP1, the GTPase-activating protein that causes conversion of RanGTP into RanGDP [Bischoff et al., 1995a; Becker et al., 1995] and RanBP1, which binds to RanGTP and facilitates the GTPase activation by RanGAP1 [Coutavas et al., 1993; Bishoff et al., 1995b; Richards et al., 1995] are localized in the cytoplasm where they deplete RanGTP [Hopper et al., 1990; Richards et al., 1996]. Moreover, a distinct pool of RanGAP is bound to Nup358/RanBP2 at the cytoplasmic filaments of the NPC through a covalent modification with a ubiquitin-related protein [Mahajan et al., 1997; Matunis et al., 1997]. In contrast, RCC1, the guanine nucleotide exchange factor (GEF) for Ran is chromatin bound [Ohtsubo et al., 1989] and generates RanGTP in the nucleus [Bishoff and Ponstingl, 1991; Dasso, 1993]. This localization would suggest a gradient of RanGTP across the NPC, with low RanGTP concentration in the cytoplasm and high RanGTP in the nucleus. When the cytoplasmic RanGTP concentration is raised with a GAP-resistant Ran mutant, nuclear import of NLS-proteins is blocked [Schlenstedt et al., 1995; Carey et al., 1996; Palacios et al., 1996; Corbett et al., 1995], most likely because of a premature dissociation of karyopherin αβ1 heterodimer by Ran-GTP. Also, nuclear import is affected when the RanGTP concentration in the nucleus is decreased, as in the RCC1 temperature-sensitive cell line, tsBN2 [Tachibana et al., 1994].

Once the NLS-protein gets into the nucleus, the two karyopherin subunits recycle back into the cytoplasm on separate pathways. Nuclear export of karyopherin α requires RanGTP, does not require β1, but seems to require the participation of one of the other members of the karyopherin β family that binds RanGTP [Koepp et al., 1996; Kutay et al., 1997a,b; Moroianu et al., 1997] (see the section on nuclear export). An interaction of karyopherin α with the nucleoplasmic nucleoporin, Nup153, is one of the steps in karyopherin α nuclear export [Moroianu et al., 1997]. On the other hand, nuclear export of yeast karyopherin β1 is mediated by a leucine-rich type nuclear export sequence (NES) [Iovine and Wente, 1997]. Complete recycling of karyopherin β1 requires, in addition, the dissociation of the karyopherin β1/RanGTP complex. It has been recently shown for yeast transport factors that the karyopherin β1/RanGTP complex is recycled efficiently in a reaction that involves karyopherin α, RanBP1, and RanGAP [Floer et al., 1997]. First, the karyopherin β1/RanGTP complex is disassembled by karyopherin α through active release as karyopherin α stimulates the dissocia-

tion by at least 3 orders of magnitude [Floer et al., 1997]. The proposed displacement mechanism is supported by data indicating that karyopherin α and RanGTP have partially overlapping binding sites on karyopherin β1 [Moroianu et al., 1996b; Kutay et al., 1997a].

The experimental information accumulated suggests a model for nuclear import of NLS-proteins mediated by karyopherin αβ1, schematically shown in Figure 1. The NLS-protein binds in the cytoplasm to the karyopherin αβ1 heterodimer (step 1), which is stable because of low cytoplasmic levels of RanGTP (see the paragraph above). The resulting trimeric complex docks to peptide repeat-containing nucleoporins at the cytoplasmic fibers of NPC (step 2) and is translocated through the NPC (step 3). Translocation is mediated by the interaction between karyopherin β1 and nucleoporins and requires Ran, GDP-GTP exchange reactions, and GTP hydrolysis by Ran. RanGDP is docked at the NPC through p10, and exchange of GDP to GTP on Ran occurs at the NPC in the presence of karyopherin αβ1 heterodimer [Nehrbass and Blobel, 1996]. Then, RanGTP dissociates the karyopherin αβ1 heterodimer and thus releases the α subunit with the NLS-protein into the nucleus. In the nucleus the karyopherin α/NLS-protein complex dissociates, releasing the NLS-protein for its destination within the nucleus (step 4). After the completion of the import reaction, karyopherin α and karyopherin β1/RanGTP are exported separately into the cytoplasm (step 5) where the β1/RanGTP complex is recycled in a reaction requiring karyopherin α, RanBP1, and RanGAP (step 6).

Interestingly, both influenza virus RNA and HIV-RNA use the cellular classic NLS protein import pathway. Nuclear import of influenza virus RNA is mediated by the viral NP that contains classical basic NLSs and interacts with both karyopherin α1 and α2 [O'Neill and Palese, 1994] and thus requires the karyopherin αβ1/Ran/p10 transport system [O'Neill et al., 1995; Whittaker et al., 1996]. HIV nucleic acid import is mediated in part by the viral matrix protein (MA) that contains a classical NLS [Bukrinsky et al., 1993; von Schwedler et al., 1994] and interacts with karyopherin α2 [Gallay et al., 1996]. It is likely that nuclear entry of nucleic acids via associated viral/cellular NLS-proteins and a classical NLS pathway is common to other viruses.

Karyopherin β2 Mediates Nuclear Import of a Set of mRNA Binding Proteins

An exciting discovery was the nuclear import pathway of hnRNP proteins mediated by a member of karyopherin β family, called transportin [Pollard et al., 1996] or karyopherin β2 [Bonifaci et al., 1997], or in yeast, Kap104 [Aitchison et al., 1996] (Table I). Among the 20 different mammalian hnRNP proteins, some are retained in the nucleus, including hnRNP C1, C2, U, but others, like hnRNP A1, A2, and K, shuttle back and forth between the nucleus and cytoplasm [Pinol-Roma and Dreyfuss, 1992].

Transportin/karyopherin β2 mediates nuclear import of hnRNP A1 [Pollard et al., 1996; Bonifaci et al., 1997] and hnRNP F [Siomi et al., 1997]. It also binds in overlay assays to other hnRNP proteins, such as B, D, and E, suggesting that it might be involved in their nuclear import [Siomi et al., 1997]. A 38 amino acid signal, called M9, is functional in both nuclear import [Siomi and Dreyfuss, 1995] and export of hnRNP A1 [Michael et al., 1995]. It was suggested that hnRNP A1 or other proteins containing an M9 signal play a role in nuclear export of mRNA [Izaurralde et al., 1997a]. The M9 signal is recognized by the C-terminal domain of karyopherin β2 (amino acids 518 to the end), and the heterodimeric complex is docked at the NPC through karyopherin β2 [Pollard et al., 1996]. Like karyopherin β1, karyopherin β2 binds to several nucleoporins containing characteristic peptide repeat motifs and can compete for binding with β1 [Bonifaci et al., 1997]. Translocation of the docked karyopherin β2/hnRNP A1 complex into the nucleus requires the GTPase Ran and GTP hydrolysis by Ran [Bonifaci et al., 1997]. RanGTP causes dissociation of karyopherin β2/hnRNP A1 complexes in vitro, suggesting that this might happen in vivo after nuclear import [Siomi et al., 1997]. The released hnRNP A1 could then function in mRNA nuclear export [Pinol-Roma and Dreyfuss, 1992].

A homologue of transportin, called transportin 2, does not bind in overlay assays to the hnRNP proteins recognized by transportin. This suggests that the nuclear import substrates of transportin 2, if any, are different from those of transportin. The two proteins are highly homologous, and the most notable difference is the presence of an extra sequence near the

COOH end of transportin 2, which probably modifies its interactions [Siomi et al., 1997]. Recently, it was reported that the hnRNP K protein contains a novel shuttling domain, termed KNS, that uses a separate import pathway from either classical NLSs or M9 [Michael et al., 1997]. It remains to be established which member of karyopherin β family is mediating this pathway.

The yeast karyopherin β2, known as Kap104, mediates nuclear import of two mRNA binding proteins, Nab2p and Nab4p [Aitchison et al., 1996]. Depletion of Kap104 results in a rapid shift of Nab2p from the nucleus to the cytoplasm without affecting the nuclear localization of other nuclear proteins, including Nsr1p, Nop1p, Npl3p, and the SV40 large T NLS fused to the green fluorescent protein. Poly(A)$^+$ RNA accumulates in the nucleus after a temperature shift, but in only a subpopulation of cells after 3 hours, suggesting that Kap104p does not directly affect mRNA export [Aitchison et al., 1996]. Interestingly, Npl3p is also an RNA-binding protein that shuttles in and out of the nucleus, and it was suggested to be involved in nuclear export of mRNA [Lee et al., 1997]. Npl3p does not contain either a classical NLS or M9 signal, and its import is not blocked in Kap104 mutant [Aitchinson et al., 1996], suggesting that it might use a distinct import pathway mediated by another member of karyopherin β family.

Karyopherin β3 and β4 Mediate Nuclear Import of a Set of Ribosomal Proteins

A major distinct nuclear import pathway mediated by the yeast member of the karyopherin β family, Kap123p/karyopherin β4, was recently discovered [Rout et al., 1997]. Kap123p binds directly to its import substrates, to a set of ribosomal proteins, to peptide repeat-containing nucleoporins, and to RanGTP. Interestingly, another member of the β family, Pse1p/Kap121/karyopherin β3, can functionally substitute for Kap123p (Table I). Both Kap123 and Kap121 can direct the ribosomal protein NLS reporter L25 NLS-β-galactosidase into the nucleus in vivo [Rout et al., 1997]. Although Kap123p binds many ribosomal proteins, it does not associate with mature ribosomes, most likely because their NLSs are masked. Because not all ribosomal proteins are recognized in an overlay assay by Kap123p, some of them may use other import pathways [Rout et al., 1997]. A mammalian homologue of yeast Kap121/Pse1p, karyopherin β3, was cloned and characterized. Like karyopherin β1 and β2, β3 proteins bind to peptide repeat-containing nucleoporins and to Ran-GTP [Yaseen and Blobel, 1997]. Although it was shown that β3 interacts with two mammalian ribosomal proteins, RL13 and RL23, in an overlay assay, it remains to be established if β3 is involved in their nuclear import [Yaseen and Blobel, 1997].

Members of Karyopherin β Family That Mediate Nuclear Export Pathways

Recently, additional members of karyopherin β family were identified. The human homologue of yeast CRM1, a protein known to be required for maintenance of correct chromosome structure [Adachi and Yanagida, 1989], was found to associate with the repeat-containing nucleoporin Nup214 [Fornerod et al., 1997a]. This human CRM1 is localized both at the NPC and in the nucleoplasm and seems to shuttle between the nucleus and cytoplasm. Significantly, hCRM1 shares an N-terminal domain of homology with karyopherin β1, β2, β3, β4, and with other uncharacterized yeast and vertebrate proteins [Fornerod et al., 1997a], suggesting that hCRM1 and these other proteins might be transport factors. It should be noted that the N-terminal domain of karyopherin β1 contains binding determinants for nucleoporins/NPC [Chi and Adam, 1997; Kutay et al., 1997a; Moroianu et al., 1997], and this might be the case for the other members of the karyopherin β family.

Recently, it was reported that hCRM1 mediates nuclear export of Rev, an HIV protein that contains a leucine-rich nuclear export signal (NES) [Fisher et al., 1995], and of UsnRNAs [Fornerod et al., 1997b] (Table II). Leptomycin B (a cytotoxin) specifically inhibits both nuclear

TABLE II. Distinct Nuclear Export Pathways Mediated by Members of the Karyopherin β Family

Mammalian β karyopherins	Export signal/domain	Exported cargo
CRM1	Leucine-rich NES	Rev protein of HIV UsnRNA
CAS	ND[a]	Karyopherin α2

[a]ND, not determined.

export of Rev and UsnRNA and the formation of the trimeric complex between CRM1, RanGTP, and the NES of Rev [Fornerod et al., 1997b]. The asymmetric distribution of RanGTP in the nucleus and RanGDP in the cytoplasm would promote formation of the trimeric export complex in the nucleus and its disassembly in the cytoplasm.

The yeast CRM1 also shuttles between nucleus and cytoplasm and is involved in export of NES-proteins. A ts mutation in CRM1 blocks the export of an import/export substrate, NES-GFP-NLS. Interestingly, the crm^{ts} mutation also blocks mRNA export [Stade et al., 1997].

Among the new members of the karyopherin β family that have been shown to bind RanGTP are the yeast Cse1 [Xiao et al., 1993], the human homologue of Cse1, CAS [Brinkmann et al., 1995], and the yeast Msn5p [Görlich et al., 1997]. In addition to RanGTP binding, Cse1p and Los1p show an NPC-like cellular localization [Irninger et al., 1995; Simons et al., 1996]. Human CAS is mainly nuclear in HeLa cells and is rapidly exported out of the nucleus in the presence of RanGTP [J. Moroianu and G. Blobel, unpublished data]. Recently, it was reported that CAS is involved in nuclear reexport of karyopherin α [Kutay et al., 1997b] (Table II). CAS binds strongly to karyopherin α only in the presence of RanGTP, forming a karyopherin α/CAS/RanGTP export complex in the nucleus. After nuclear export, karyopherin α is released from the complex in the cytoplasm under the combined action of RanBP1 and RanGAP1 [Kutay et al., 1997b].

Several major types of cargoes, mRNA, tRNA, and ribosomal subunits, are waiting for the identification of their export factors. Because depletion of nuclear RanGTP by injection of RanGAP1 blocks export of NES-containing proteins [Richards et al., 1997], karyopherin α, tRNA, and several mRNAs [Izaurralde et al., 1997b], it is likely that nuclear export of tRNA and mRNA is also mediated by other members of karyopherin β superfamily.

Other Karyopherin β Family Members Are Waiting for the Identification of Their Cargoes

A new nuclear transport factor called RanBP7 (Ran binding protein 7) was copurified with karyopherin β1. Xenopus RanBP7 interacts with karyopherin β1, RanGTP, can bind directly to NPC, and can shuttle between the nucleus and cytoplasm [Görlich et al., 1997]. Another member, called RanBP8 (human), has 61% identity to RanBP7 and also binds RanGTP. RanBP7 and RanBP8 have similarity to yeast *S. cerevisiae* Nmd5p (26%), *S. cerevisiae* D9509.15p and Lph2p, and also to karyopherin β1, β2, β3, β4, CAS, CRM1, and other proteins in the database. The most significant homology between all the members of karyopherin β family is found within the N-terminal 150 amino acids [Görlich et al., 1997]. The potential to bind both RanGTP and the NPC and to shuttle between nucleus and cytoplasm suggests that these new members might be nuclear import/export factors for yet to be identified cargoes.

Members of Karyopherin β Family Can Compete for Docking Sites at the NPC

An interesting discovery was that different members of karyopherin β family can compete with each other for binding to peptide repeat-containing nucleoporins and therefore, also for import into the nucleus. Thus, karyopherin β1 and β2 compete for binding to Nup98 and also for nuclear import of their specific cargoes, NLS-protein and hnRNP A1, respectively [Bonifaci et al., 1997]. Karyopherin β3 competes with β1 for binding to peptide repeat-containing nucleoporins in overlay blot assays [Yaseen and Blobel, 1997]. Binding of RanBP7 and RanBP8 to NPC is competed by karyopherin β1. Also, karyopherin β2 and RanBP7 compete with each other for binding to NPC [Görlich et al., 1997]. Moreover, karyopherin β1 cross-competes with major export pathways, such as export of proteins containing leucine-rich NES, mRNA, and UsnRNA [Kutay et al., 1997a]. Also, both karyopherin β1 and β2 compete with nuclear export of karyopherin α [J. Moroianu and G. Blobel, unpublished data]. All these data would suggest that distinct nucleocytoplasmic pathways mediated by members of the karyopherin β family partially merge at the level of docking to nucleoporins at the NPC and that in the cell the karyopherins may act in dynamic competition.

CONCLUSION

The nucleocytoplasmic transport field is in a period of exciting development. An entire superfamily of transport factors has been discovered during the last few years, and so far three nuclear import pathways and two export pathways have been identified. Karyopherin β1, β2,

and β3/β4 mediate the nuclear import of NLS-proteins, of mRNA binding proteins, and of a set of ribosomal proteins, respectively. CRM1 and CAS mediate nuclear export NES-proteins and karyopherin α, respectively. The task for the future is to identify the cargoes and, thus, the distinct transport pathways mediated by each of the other members of karyopherin β family. Establishing the mechanisms of translocation of karyopherins together with their cargoes through the NPC will require fine analysis of their interaction with the nucleoporins at the NPC, high-resolution structures of different import/export complexes and defining how the RanGTP cycle is translated into movement through the NPC. Finally, an entire field that will emerge is the cellular regulation of all these complex pathways during cell growth, development, viral infections, and in different diseases.

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