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REVIEW

Diversity in Nucleocytoplasmic Transport Pathways

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ABSTRACT. **Significant progress has been made toward our understanding of the basic principle of nucleocytoplasmic transport, and the structure of transport factors, as well as the diversity of nucleocytoplasmic transport pathways. This review outlines the current knowledge of transport, and discusses the problems that remain as to how eukaryotic cells acquire additional levels for the regulation of gene expression from a diversity of nucleocytoplasmic transport pathways.**

Key words: nuclear localization signal/nuclear export signal/nuclear transport factor/importin/exportin/nuclear pore complex/small GTPase Ran

In eukaryotic cells, DNA is sequestered in the nucleus by a double membrane called the nuclear envelope, that separates the central genetic process of DNA replication and RNA synthesis from the cytoplasm, where the genetic message is translated into the protein. A continuous exchange of molecules occurs through the nuclear pore complex (NPC), which is present in the nuclear envelope, in order to coordinate cytoplasmic and nuclear events. This exchange of molecules is important in order for cells to maintain their homeostasis and adapt to their extracellular environment. The nucleocytoplasmic transport provides an additional level of regulation for gene expression that does not exist in prokaryotic cells.

Nucleocytoplasmic exchange is a very dynamic activity, in which a vast number of molecules enter and exit the nucleus in rapid, accurate, and often regulated manner. Numerous molecules, which enter and exit the nucleus, contain specific amino acids sequences, which are referred to as nuclear localization signal (NLS), or nuclear export signal (NES). Since the identification of the first transport factor, which is referred to as importin α and β, and which mediates nuclear import of classical basic NLS-containing substrates, significant progress has been made toward our understanding of the mechanism of nucleocytoplasmic transport, as well as the diversity of nucleocytoplasmic transport pathways. This review outlines the current knowledge of nucleocytoplasmic transport, and tries to bring out what problems remain in our understanding of the regulation of transport, and how eukaryotic cells acquire additional levels for regulation of gene expression.

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Abbreviations: NLS, nuclear localization signal; NES, nuclear export signal; NPC, nuclear pore complex; GAP, GTPase activating protein; NTF2, nuclear transport factor 2; IBB domain, importin β-binding domain; HEAT, huntingtin-elongation-A subunit-TOR.

The basic concept of transport

The discoveries of the nuclear transport factors, termed importins and exportins, and the involvement of small GTPase Ran in nuclear import led to the development of the basic concept for receptor-mediated active nuclear import and export (for reviews, see Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Nakielny and Dreyfuss, 1999). Importins are the nuclear transport factors that recognize specific import signals present within the import cargoes, and carry the bound cargoes from cytoplasm into the nucleus. Exportins recognize specific export signals present in cargoes and carry the bound cargoes from the nucleus out to the cytoplasm. The small GTPase Ran assures the directional transport of the cargoes by regulating the loading and unloading of cargoes to the importins and exportins (Izzaurralde *et al*., 1997). Binding of RanGTP to importins reduces the affinity for their import cargoes whereas binding to exportins stabilizes the affinity for their export cargoes. Ran shuttles between the nucleus and cytoplasm, and its GTPase cycle is regulated across the nuclear envelope. Since the Ran GTPase activating protein, Ran GAP1, is predominantly localized in the cytoplasm or cytoplasmic face of NPC (Mahajan *et al*., 1997; Matunis *et al*., 1996), and since the

GDP/GTP exchange factor for Ran, RCC1, is localized predominantly in the nucleus (Ohtsubo *et al*., 1989), nuclear Ran is thought to be predominantly in its GTP form, whereas cytoplasmic Ran is predominantly in its GDP form. This steep gradient of Ran-GTP and Ran-GDP across the nuclear envelope allows import complexes to form in the cytoplasm and dissociate in the nucleus, whereas export complexes form in the nucleus and dissociate in the cytoplasm. As a result, import and export cargoes are recognized by importins in the cytoplasm and exportins in the nucleus, respectively, translocate through the NPC, and are then released at their destination, allowing the unidirectional transport for each cargo.

The precise mechanism of NPC translocation process is very poorly understood. There is no evidence that for Ran or any other soluble factors participate directly in this process. Rather, the present evidence indicates that NPC-translocation is driven by direct interaction of transport factors with nucleoporins (see below).

Different transport pathways are mediated by many different transport factors

The first nuclear localization signal (NLS) of SV40 large T-antigen was identified in the mid-1980s (Landford and Butel, 1984; Kalderon *et al*., 1984). For about 10 years after the initial identification of the signal, our knowledge on nuclear localization signal had been almost restricted to single-type (SV40 T-antigen-like NLS) or bipartite-type (nucleoplasmin-like NLS) basic NLS that consists of fairly short sequences rich in basic amino-acid residues (for review see Dingwall and Laskey, 1991). However, at present, our knowledge has broadened remarkably and we now know that there are many transport signals that show considerable variation in complexity, from different peptide sequence motifs to a large protein domain, and even a structure formed by protein assembly or post translational modifications (see Table I).

Our present knowledge of transport diversity has evolved from the initial identification of importin α and β, import mediator of classical NLS-containing proteins (Görlich *et al*., 1994, 1995; Chi *et al*., 1995; Enenkel *et al*., 1995; Imamoto *et al*., 1995a,b; Moroianu *et al*., 1995; Radu *et al*., 1995; Weis *et al*., 1995). In this transport pathway, importin α recognizes the NLS, and functions as an adaptor molecule that loads cargoes to importin β. Subsequent identification of importin β-related proteins, such as transportin (mammals) and Kap104 (yeast), which function as transport factors for different import signals, led to the notion that an extended family of importin β that mediates different transport pathways exists (Pollard *et al*., 1996; Aitchison *et al*., 1996). Finally, the identification of exportins, for leucine-rich NES, exportin 1/CRM1 (Fornerod *et al*., 1997; Fukuda *et al*., 1997; Stade *et al*., 1997), or importin α, exportin 2/CAS (Kutay *et al*., 1997a), established that importin β-related proteins can be all classified into either nuclear import or export factors.

There are numerous transport factors that recognize many signals and mediate different transport pathways (reviewed in Nakielny and Dreyfuss, 1999). The genome of *S. cerevisiae* encodes 14 importin β-related proteins, and one importin α, which can be recognized by sequence homology. Nine of these are importins, and four are exportins, and one remains uncharacterized. In mammals, a similar number of importin β-related proteins and seven importin α proteins are characterized and published at present, but this number can be expected to grow. Multicellular organisms contain larger number of transport factors and adaptors than unicellular organism. For example, genomic database searches in *Caenorhabditis elegans* show the presence of more than 20 importin β-related proteins and 3 importin α proteins .

The more complex organism could be equipped with larger number of transport pathways in order for different transport pathways to function in different cell types of different tissues as well as under different cellular conditions.

Transport diversity in importin β mediated transport

The complexity of nuclear transport pathways does not evolve simply from the number of transport factors. As shown in Table 1 and Fig. 1, the most well characterized transport factor, importin β, mediates nuclear import of various proteins that contain different signals in many different ways. Importin β was initially identified as a nuclear import mediator for classical NLS containing proteins in conjunction with importin α. By employing different members of the importin α family, importin β is able to mediate the nuclear import, not only of classical NLS-containing proteins, but also of nuclear proteins such as Stat1 whose NLSs are different from the basic-type NLS. Stat1 is recognized by different portion of specific importin α family member (Sekimoto *et al.*, 1997).

Importin β employs different adaptor molecules other than importin α, such as snurportin (Huber *et al*., 1998), RIP α (Jullien *et al.*, 1999) or importin 7 (Jäkel *et al*., 1999), to mediate nuclear import of UsnRNP, Replication protein A, and histone H1, respectively. Importin α , RIP α and snurportin share a similar Arg-rich motif at their N-terminus, which is required for importin β-binding (called IBB domain, Görlich *et al*., 1996; Weis *et al*., 1996). Importin 7 is a member of the importin β superfamily that forms a trimeric complex with histone H1 and importin β to mediate the nuclear import of histone H1.

Importin β also mediates the nuclear import of certain cargoes without the need for adaptor proteins. These cargoes include ribosomal proteins (Jäkel and Görlich, 1998), HIV Rev and Tat (Truant and Cullen, 1999), the Rex protein of human T-cell leukemia virus type 1 (Palmeri and

Table I. EXAMPLES OF SEQUENCES AND STRUCTURES REQUIRED FOR NUCLEAR TRASNPORT

<Import>		
Cargo		**Transport factor**
	Peptide Sequence Motifs	
Sv40 large T-antigen (wide range of protein)	**PKKKRKV (monopartite classical NLS)**	**Importin α/Importin β**
nucleoplasmin (wide range of protein)	**KRPAAIKKAGQAKKKK (bioartite classical NLS)**	**Importin α/Importin β**
HIV-1 Rev	**RQARRNRRNRRRRWR**	**Importin β**
hnRNPA1	**NQSSNFGPMKGGNFGGRSSGPY GGGGQYEAKPRNQGGY**	**Transportin**
	Large Protein Domain	
SREBP2	**basic helix-loop-helix zip**	**Importin β**
SR proteins [a]*	**SR domains**	**Transportin**
β-catenin	**ARM repeats**	**not required**
ribosomal proteins	**Arg-rich domain (BIB-domain)**	**Importin β (also RanBP5 and importin7)**
	Protein Assembly	
UsnRNP	**m3cap and Sm core protein**	**snurportin/Importin β**
Stat 1	**dimerization**	**Importin α (NPI-1)/Importin β**
	Modification	
NF-AT [b]*	**phosphorylation**	**Importin α/Importin β** (?)
Pho 4 [c]*	**dephosphorylation**	**Pse 1**
<Export>		
Cargo		**Transport fator**
	Peptide Sequence Motifs	
HIV-1 Rev	**LQL PPLERL TLD**	**Exportin 2(CAS)**
Protein kinase inhibitor	**LALKLAGLDIN**	
Map kinase kinase	**LGKKLEELELE**	
	Large Protein Domain or Structure	
Importin α	**about 140 amino acids**	**Exportin 2(CAS)**
tRNA [d]*	**Tφ C arms are important**	**Exportin-t**
	Protein Assembly	
U snRNA	**CBC and m7G cap**	**PHAX/Exportin 1(CRM1)**
	Modification	
Pho4p [e]*	**phosphorylation**	**Msn5p**
Far 1p [f]*	**phosphorylaiton**	**Msn5p**
eIF-5A [g]*	**hypusine modification**	**Exportin 4**

*References which are not cited in text are given below.
a) Kataoka *et al*. (1999) J. Cell Biol. 145: 1145–1152.
b) Znu & Mckeon (1999) Nature 398: 256–260.
c) Kaffman *et al.* (1998) Genes Dev. 12: 2673–2683.
d) Kutay *et al.* (1998) Mol. Cell 1: 359-369. Arts *et al*. (1998) EMBO J., 17: 7430–7441.
e) Kaffman *et al*. (1998) Nature 396: 482–485.
f) Pines (1999) Nat. Cell Biol. 1: E73–E79.
g) Lipowsky *et al.* (2000) EMBO J. 19: 4362–4371.

Malim, 1999), and a transcription factor SREBP-2 (Nagoshi *et al*., 1999). The BIB (beta-like importin-binding) domain of ribosomal proteins, and helix-loop-helix zip motif of SREBP-2 have been identified as novel NLSs that interact directly with importin β. It is noteworthy that these importin β binding sequences share no obvious sequence similarity with one another and the IBB domain. The IBB domain of importin α, the BIB domain of ribosomal proteins, and importin 7 bind to different regions of importin β (Fig. 2). Therefore, besides employing different adaptor molecules, importin β sorts out various cargoes by means of interaction with its own different regions. All these reported cargoes bind to importin β in the absence of RanGTP, and are released upon RanGTP binding to importin β, following the basic concept of importin β import pathway.

What is the benefit of having such diversity in the importin β transport pathway? Expression of importin β is not tissue specific, but the expression of importin α in mam-

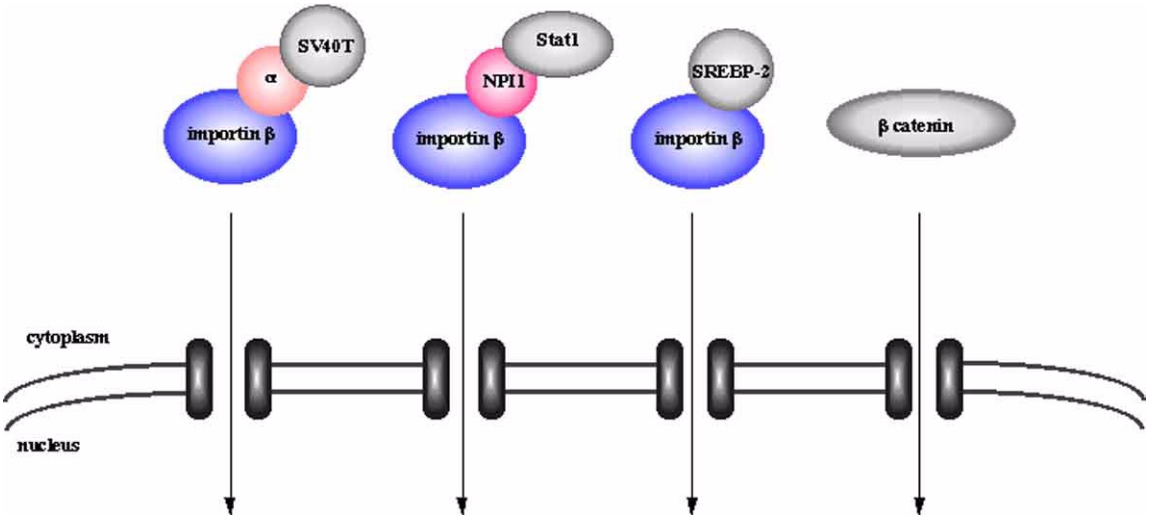

Fig. 1. Importin β mediates nuclear import of various proteins in many different ways. For example, by employing different members of importin α family, it mediates nuclear import not only of classical basic NLS-containing proteins, but also a regulated import of Stat1 that occurs in response to extracellular signal stimulation of interferon γ. Importin β also mediates a nuclear import of transcription factor SREBP-2, by recognizing its helix-loop-helixzip domain directly. There are a number of importin β-related proteins that mediate different nuclear import and export pathways. On the other hand, there exists a transport pathway which is not mediated by soluble transport factor family members, as revealed by nuclear import of β-catenin.

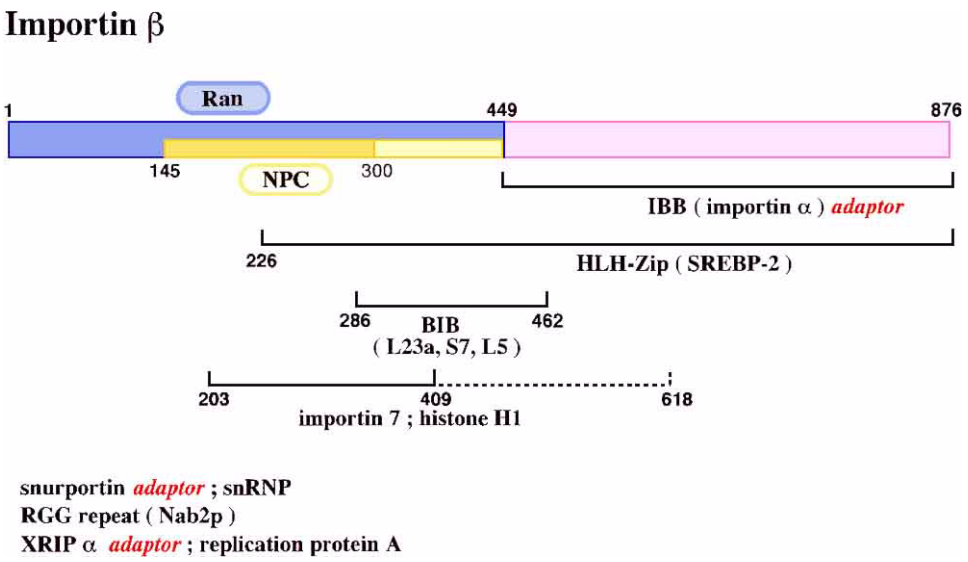

Fig. 2. Functional regions of importin β. Functional regions of importin β required for binding to RanGTP, NPC and various cargoes are indicated.

mals shows strict tissue specificity (reviewed in Imamoto *et al.*, 1998; Yoneda, 2000). Therefore, employing the specific importin α as an adaptor molecule may allow importin β to function in a tissue specific manner. Importin β-based transport that employs an adaptor molecule shows somewhat lower sensitivity to the loss of RCC1 *in vivo*, compared to the transport that does not use an adaptor molecule. It is possible that a pathway with or without importin α may be regulated differently upon alternation of RanGTP concentration or activity of its regulatory factors in cell.

Recently, nuclear export of spliceosomal U snRNPs was found to be mediated by CRM1 (exportin 1), in conjunction with a protein named PHAX (phosphorylated adaptor for RNA export) (Ohno *et al*., 2000). This is a unique example of CRM1-mediated export using an adaptor-like molecule. PHAX is required for U snRNA export but not for CRM1-mediated export in general. Interestingly, phosphorylation of PHAX is essential for export complex assembly while its dephosphorylation causes export complex disassembly. PHAX is phosphorylated in the nucleus and dephosphory-

lated in the cytoplasm when exported with RNA. This novel example shows that by employing a specific adaptor molecule, CRM1 gains an additional regulatory mechanism for mediation of its nuclear export of particular cargo.

Structural features of importin β

Importin β is the most well characterized transport factor, and all other importin β-related proteins share common biochemical features with importin β which are essential to function as transport factors. These are their ability to bind to NPC components and RanGTP. Importin β-related proteins share the homologous N-terminal amino acid sequences, which are involved in RanGTP binding (Görlich *et al*., 1997). All transport factors possess ability to shuttle between the nucleus and the cytoplasm. As in the case of importin β, interaction with NPC components plays a key role during the NPC translocation-step of transport, as well as their recycling that proceeds back through the NPC (Kose *et al*., 1999). Whether these importin β-related proteins function as importin or exportin depends largely on their affinity with RanGTP. In general, affinity of importins to RanGTP is high, while those of exportins are low. Therefore, importin/RanGTP complexes are sufficiently stable by themselves, while exportins form stable complexes with RanGTP only in the presence of their export cargoes. (reviewed in Mattaj and Englmeier, 1998; Görlich and Kutay, 1999).

The crystal structures of importin β complexed with the IBB domain, and N-terminal fragment of importin β complexed with RanGTP or FxFG repeats of nucleoporin, and its uncomplexed form have been determined (Cingolani *et al*., 1999; Vetter *et al*., 1999; Bayliss *et al*., 2000; Lee *et al*., 2000). Some of these structures are shown in Fig. 3. Full-length importin β consists of 19 copies of tandemly arrayed HEAT sequence repeats. Each HEAT sequence contains approximately 40 residues and is constructed from two α helices connected by a short turn. The HEAT repeats are joined by a short linker and are arranged in a right-handed superhelical molecule. The IBB domain and RanGTP interacts with inner, concave surface of HEAT repeats, whereas FxFG repeats interact with outer, convex side of HEAT repeats.

The full length importin β, when complexed with the IBB domain, shows a snail-like appearance in which the N- and C-terminal HEAT repeats are located in close proximity to one another, while the phosphatase 2A PR65/A subunit, which forms a left-handed superhelix, shows a much more open conformation (Groves *et al*., 1999). One other full length importin β-related protein, karyopherin β2/transportin has been crystallized in the form of a complex with RanGTP (Chook and Blobel, 1999). Transportin is also an all-helical protein consisting of HEAT repeats organized in a right-handed superhelix. The RanGTP-bound form of transportin shows a twisted S-like structure. These reported structures of the HEAT repeat helical proteins suggest that they are capable of undergoing rather dramatic conformational changes. The structure of IBB/importin β complex and RanGTP/importin β complex explains how binding of importin α and RanGTP to importin β can be mutually ex-

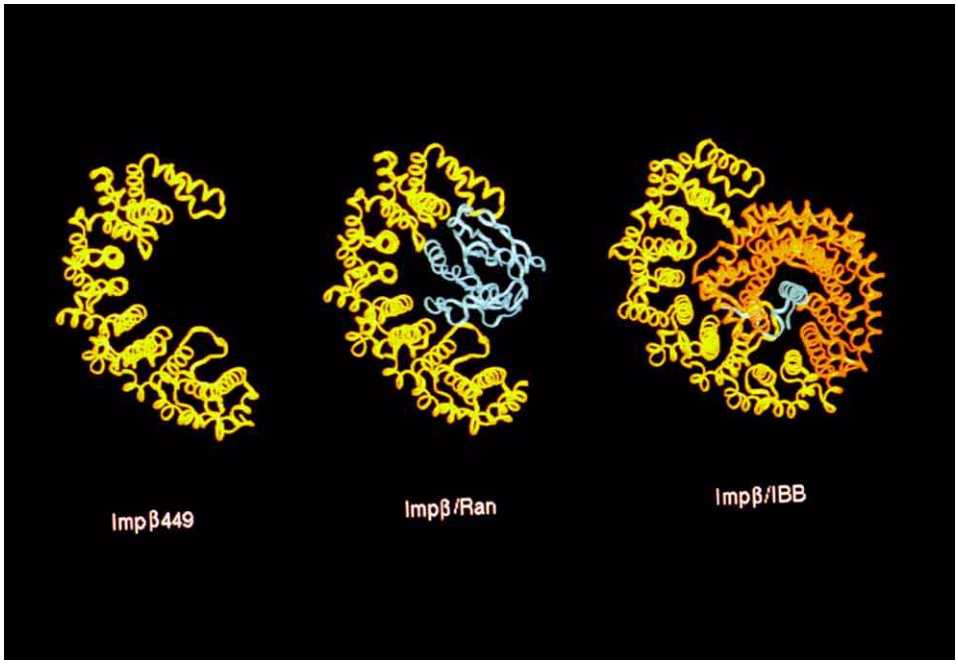

Fig. 3. Crystal structures of importin β. Structure of full length importin β complexed with IBB (impβ/IBB), and N-terminal fragment of importin β complexed with RanGTP (impβ /Ran) and its uncomplexed form (impβ449) are shown. Ran in impβ /Ran, or IBB in impβ/IBB are shown in blue. Amino acids 1-449 of importin β are shown in yellow.

clusive. Moreover, a dynamic conformational change of importin β, due to the flexible nature of HEAT repeat helical proteins, could further contribute to trigger the substrate release upon binding of RanGTP to importin β.

The superhelical structure of importin β , and its binding mode with RanGTP and FxFG repeat of nucleoporin, allows us to speculate that importin β can mediate nuclear import of different cargoes and adaptors in a Ran-dependent manner, if they are loaded onto the concave surface of importin β.

Precise structural comparison of the uncomplexed form of importin β, and the IBB or RanGTP complexed form of importin β, has revealed the flexible nature of importin β in more detail (Lee *et al*., 2000). The characteristics of internal motion can be visualized from the architecture of the hypothetically constructed superhelix shown in Fig. 4. The snail-like constructions of the superhelices of uncomplexed form, and the RanGTP- or IBB-complexed form show differences in both diameter and pitch, indicating that this protein has the ability not only to bend, but also to twist, these structural changes being similar to the elastic motion of a mechanical spring.

An analysis of the inter-helix angle revealed the distribution of local and largest conformational changes occurring at heat repeats 4 to 7 (Fig. 5). This is particularly interesting because this portion of importin β corresponds to its NPC binding domain, which is sufficient and necessary for its NPC translocation. Moreover, the crystal structure of importin β complexed with FxFG nucleoporin repeats revealed that primary binding sites of FxFG repeats resides in the hydrophobic pocket generated by side chains of the HEAT repeats 5 and 6 (Bayliss *et al*., 2000), a portion which shows the largest conformational changes. Although the mechanism of how protein translocation occurs through the nuclear pores largely remains a mystery, the local conformational changes and flexible motion of importin β could be important essentials for its movement through nuclear pores.

Translocation efficiency at nuclear pore complex.es

A central mediator of nucleocytoplasmic traffic is the nuclear pore complex (NPC), a large supramolecular structure of protein complex consisting of about 50 to 100 different polypeptides with an estimated molecular mass of 125 megadaltons (Mda) in vertebrates. Yeast NPC consists of a slightly lower number of polypeptide (~30) with a smaller molecular mass (~66 Mda), but its structure is very similar to vertebrate NPC. Detailed information on structure, components, and location of components within the NPC structure have been recently described (for review see Stoffler *et al.*, 1999; Allen *et al.*, 2000; Ryan and Wente, 2000).

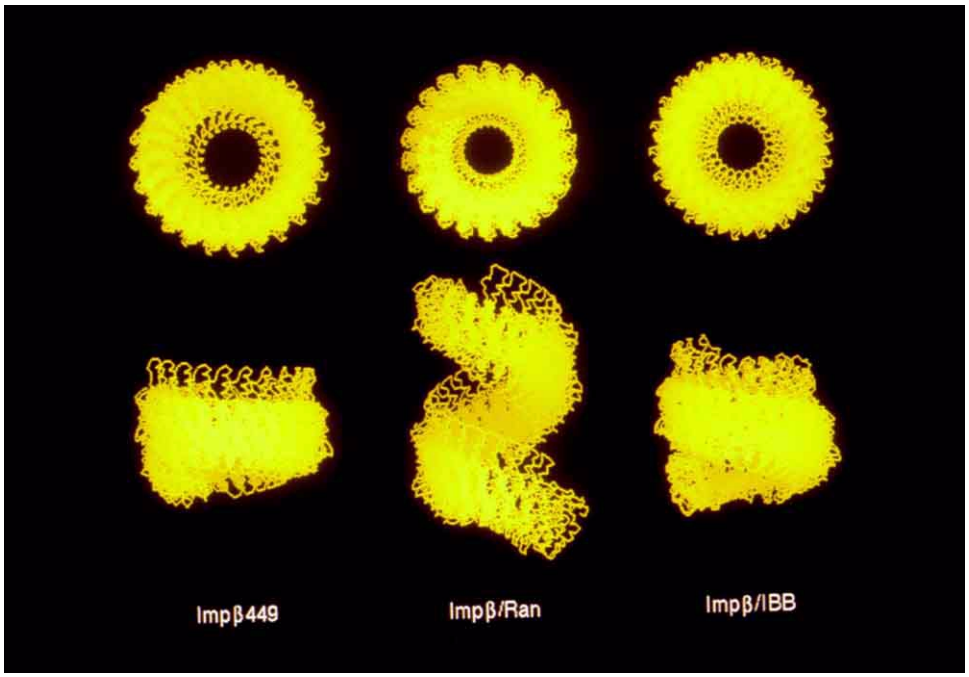

Fig. 4. Flexible nature of importin β. HEAT repeat helices from A3 to B9 (see Fig. 5, right panel), stacked side by side, are superimposed. Each superhelix is comprised of 24 molecules (impβ449), 23 molecules (impβ/IBB) and 23 molecules (impβ/Ran). impβ449, impβ/IBB, and impβ/Ran are the superhelices of uncomplexed form, IBB complexed form, and RanGTP complexed form, respectively. Estimated diameter and pitch of impβ449, impβ/IBB, and impβ/Ran are 106 Å and 31 Å, 102 Å and 42 Å, and 96 Å and 94 Å, respectively. The diameter of impβ449 is larger than those of impβ/Ran and impβ/IBB, representing a more open conformation of helix stacking. The pitch of impβ/Ran is about three times larger than that of the uncomplexed form of impβ449, demonstrating the presence of a "twisted motion".

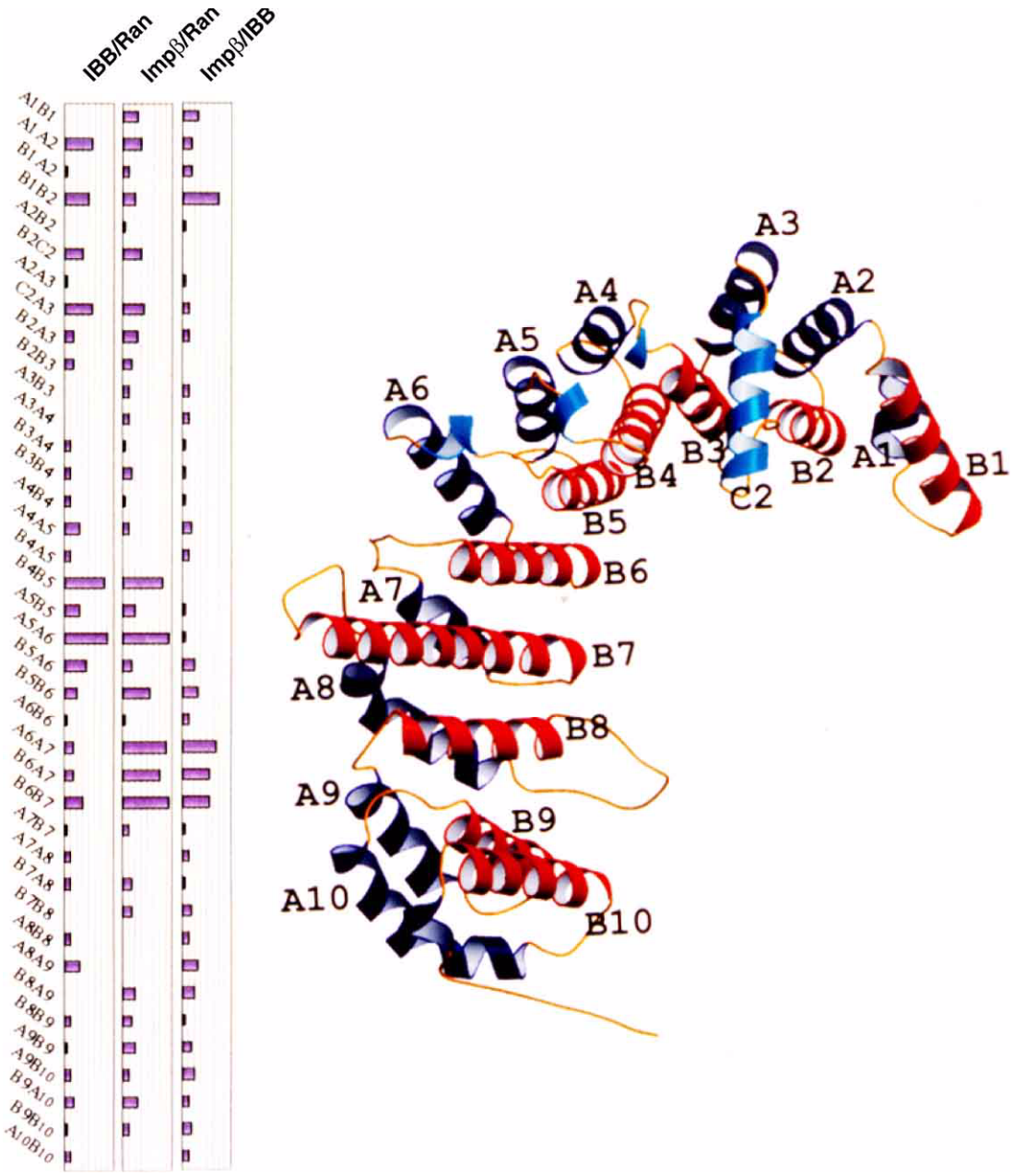

Fig. 5. Distribution of local conformational changes.
Right panel: HEAT repeat helices in uncomplexed form of 1-449 N-terminal amino acid fragments of importin β. The convex side of helices of A1-A10 and the concave side of the helices of B1-B10 and 310 helices are shown in red, blue and light blue, respectively. C2 helix is shown in green.
Left panel: The distributions of interhelix angle differences between impβ449&impβ/IBB (rightmost), impβ449&impβ/Ran (middle), impβ/Ran&impβ/IBB (leftmost) are represented by bar graphs. One increment for thin lines corresponds to 1 degree.

Any requirement of energy source or soluble factors has never been detected for the translocation reaction through the NPC (Kose *et al*., 1997; Ribbeck *et al*, 1998; Schwoebel *et al*., 1998; Englmeier *et al*., 1999, Yokoya *et al.*, 1999). Such evidence has led to the one model that nuclear transport factors move through the NPC by repeated association-dissociation reactions with NPC proteins, a process that has been called "facilitated diffusion".

More than one million macromolecules are estimated to pass through the nuclear envelope every minute in dividing cells. About 3,000 to 5,000 NPCs are present per nuclei, which means each NPC needs to translocate at least 5 molecules per second. Electron microscopic studies have shown that molecules must travel for 200 nm from the cytoplasmic

face to the nucleoplasmic face of NPC for the translocation (reviewed in Ohno *et al.*, 1998). If, for instance, molecule translocate through the NPC one by one, each molecule needs to translocate through the NPC in the order of 10^{-1}s. This is the rate of kinensin-driven motor protein movement. Actin driven motor protein movement is much faster, as it takes about 10^{-2}s to travel for 200 nm distance. If NPC binds to a larger number of transport factors at once (for example, 10 to 100 molecules), a much increased retention time (1s to 10s) is still sufficient to achieve passage through the nuclear envelope in a cell on the order of a million molecules per minute. If each NPC possesses a large capacity to bind transport factors, efficient transport is possible with much slower movement compared to the movement of those driven by motor proteins. Important issues that need to be considered for the efficiency of transport would then be the affinity of a transport factor with the NPC, the capacity of NPC to bind a transport factor, and its translocation rate (retention time).

How the diversity of transport pathways accommodates at the NPC level?

The presence of distinct transport factors between the cytoplasm and the nucleus raises questions as to how the diversity of transport complexes accommodates at the level of nuclear pore machinery. Importin β-like nuclear transport factors, as well as proteins which are capable of translocating through the NPC on its own, have all been reported to interact with FxFG or GLFG repeat motifs of nucleoporins. These molecules include β-catenin (Fagotto *et al*., 1998; Yokoya *et al*., 1999), p10/NTF2 (carrier of RanGDP) (Paschal and Gerace, 1995, Ribbeck *et al.*, 1998), and Mex67p/Mtr2p, implicated as a mediator of mRNA export (Straber *et al*., 2000). Point mutations introduced in the primary FxFG binding sites of importin β (Bayliss *et al*., 2000), and in the FxFG interacting sites of p10/NTF2 (Bayliss *et al.*, 1999) had significant effect on their import activity, providing convincing evidence that these interactions are important for NPC translocation.

Kutay *et al*. (1997b) showed that all transport pathways examined (NLS, M9, leucine-rich NES, mRNAs, UsnRNAs) can be competitively blocked by importin β lacking Ran-binding activity, and suggested that most import and export pathways share the same or overlapping NPC-binding sites for the translocation. More recently, Lane *et al*. (2000) indicated that p10/NTF2 and importin β compete for identical and/or overlapping binding sites at the NPC. They showed point-mutated p10/NTF2, that shows stronger affinity for NPC, could alter the import rate of importin β-based import pathway. They predict a dynamic equilibrium between multiple nuclear transport pathways mediated by transport factors sharing the same NPC docking sites, could be shifted by a modification of one transport factor.

However, there are several lines of evidence that the binding of all transport factors to NPC are not exactly equivalent, but differs in sites, mode, and affinity. For example, Shah and Forbes (1998) biochemically showed that different importin β-related proteins bind at different regions of the NUP153. Nakielny *et al*. (1999) have shown that different importin β-related proteins can interact with several regions of NUP153, and that these interactions are regulated differently by RanGTP. Hu *et al*. (1996) showed that 100-fold molar excess of p10/NTF2 did not prevent the binding of importin β to three subunits of p62 complex. Ten-fold molar excess importin β inhibits the in vitro transport of β-catenin, but 20-fold molar excess of β-catenin did not. This suggests that binding sites of these two factors may overlap, but that their affinity in context of NPC may be different (Yokoya *et al*., 1999).

Iborra *et al.* (2000), using immunogold labeling and electron spectroscopic imaging, found that some pores were associated only with poly(A)$^+$ RNA and others only with p10/NTF2, suggesting the presence of distinct populations of NPC. Uv *et al*. (2000) found that a drosophila homologue of mammalian nucleoporin Nup88, named *members only* (mbo), is selectively required for the nuclear import of the Rel family transcription factors Dorsal and Dif, providing in vivo evidence that individual nucleoporins are required for distinct nuclear protein import pathways. Moreover, the zygotic expression and phenotypes of mbo mutant during organogenesis are tissue specific, suggesting that the nuclear import capacities of different cells may be regulated at the NPC level.

As evidence accumulates regarding how the diversity of transport pathways accommodates at the NPC level, it is important to understand in terms of the regulation of transport at that level. The results from many different experiments have yet to lead to one solid conclusion. Care must be taken when viewing the results of the competition studies since the addition of excess pore-binding molecules may not only saturate the binding sites of transport factors, but may also alter the structure or function of NPC, and thus indirectly prevent transport. Biochemical studies provide direct molecular evidence, but it may not necessarily represent the interaction of molecules in the context of assembled NPC structure. In vivo evidence is important, but require further molecular information to be convincing. However, recent advances in the field provides an optimistic view that these results will come together in the near future.

Fundamental questions to the inquiry

The presence of so many distinct transport factors and pathways obliges us to raise a naive but fundamental question: What is the benefit to having such a complexity of nuclear transport pathways leading to regulatory changes of gene expression *in vivo*? This, of course, needs to be addressed from several different aspects. What particular cargoes are carried by the respective transport factors? In what

tissue, cell type, or under what different cellular conditions do different transport pathways function? What affects the efficiency of transport? Are they regulated differently or specifically under particular cellular conditions? Tremendous genomic information from several organisms, as well as new tools of imaging and measurement are being made available that should also help us to answer these questions, by providing new perspectives on biological phenomena in which the exchange of molecular information between nucleus and cytoplasm play an important role.

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