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Gradient of Increasing Affinity of Importin β for Nucleoporins along the Pathway of Nuclear Import

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Abstract. Nuclear import and export signals on macromolecules mediate directional, receptor-driven transport through the nuclear pore complex (NPC) by a process that is suggested to involve the sequential binding of transport complexes to different nucleoporins. The directionality of transport appears to be partly determined by the nucleocytoplasmic compartmentalization of components of the Ran GTPase system. We have analyzed whether the asymmetric localization of discrete nucleoporins can also contribute to transport directionality. To this end, we have used quantitative solid phase binding analysis to determine the affinity of an importin β cargo complex for Nup358, the Nup62 complex, and Nup153, which are in the cytoplasmic, central, and nucleoplasmic regions of the NPC, respectively. These nucleoporins are proposed to provide progressively more distal binding sites for importin β during import. Our results indicate that the importin β transport complex binds to nucleoporins with progressively increasing affinity as the complex moves from Nup358 to the Nup62 complex and to Nup153. Antibody inhibition studies support the possibility that importin β moves from Nup358 to Nup153 via the Nup62 complex during import. These results indicate that nucleoporins themselves, as well as the nucleocytoplasmic compartmentalization of the Ran system, are likely to play an important role in conferring directionality to nuclear protein import.

Key words: nuclear import • nuclear pore complex • importin β • Nup62 complex • Nup153

Introduction

Nucleocytoplasmic transport occurs through nuclear pore complexes (NPCs),[1] ~125-mD proteinaceous assemblies that span the nuclear envelope (for reviews see Ohno et al., 1998; Gorlich and Kutay, 1999; Stoffler et al., 1999; Ryan and Wente, 2000). NPCs are composed of ~50–100 different proteins called nucleoporins. The major framework of the NPC consists of eight spokes, which are flanked by nuclear and cytoplasmic rings that surround a central channel structure. Extending outward from the ring–spoke assembly are ~35–50-nm-long cytoplasmic fibrils and 50–100-nm-long nuclear fibrils that are joined in a basket-like structure. Ions, metabolites, and small proteins (<20–40 kD) move through the NPC by passive diffusion, but most larger molecules are transported by signal- and energy-dependent mechanisms. Most signal-dependent nuclear transport is mediated by nucleoplasmic shuttling receptors of the importin/karyopherin β family (for reviews see Ohno et al., 1998; Gorlich and Kutay, 1999). Nuclear localization signals (NLSs) or nuclear export signals in protein or RNA cargo are recognized directly by transport receptors or indirectly via adaptor proteins that bind to the receptors. After a receptor–cargo complex is formed, it is translocated through the NPC by a multistep process that apparently involves the sequential binding of the transport receptor to nucleoporins in different NPC regions. Cargo is then released from the receptor and the latter is recycled. The small GTPase Ran, which interacts with importin β–type receptors, plays a key role in driving both nuclear import and export (see below).

The classical NLS is characterized by a basic amino acid–rich sequence, which is present in a simple or bipartite motif. This NLS is recognized by the adaptor importin α, which binds to the import receptor importin β through its importin β binding (IBB) domain. Importin β–related receptors have been shown to bind directly to several nucleoporins that contain FG repeat motifs, and these bind-

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[1] *Abbreviations used in this paper:* FG, phenylalanine-glycine; GST, glutathione *S*-transferase; IBB, importin β binding; NLS, nuclear localization sequence; NPC, nuclear pore complex; RanBP, Ran binding protein; RBD, Ran binding domain.

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ing interactions could be involved in cargo transport and receptor recycling.

Nuclear transport mediated by importin β–related receptors is strongly directional (Keminer et al., 1999): import receptors transfer NLS-containing cargo from the cytoplasm to the nucleus, and export receptors transfer nuclear export signal–containing cargo from the nucleus to the cytoplasm. Understanding how this directionality is determined is a key question. Mounting evidence indicates that the Ran system makes a major contribution to transport directionality (for reviews see Ohno et al., 1998; Gorlich and Kutay, 1999). The RanGTPase activating protein (RanGAP1) and the RanGTP binding protein, RanBP1, which stimulate the hydrolysis of GTP by Ran, are localized to the cytoplasm, whereas the Ran guanine nucleotide exchange factor RCC1, which regenerates RanGTP, is concentrated in the nucleus. The compartmentalization of Ran regulators is predicted to result in a substantially higher concentration of RanGTP in the nucleus than in the cytoplasm. In vitro studies have shown that RanGTP promotes the dissociation of cargo from import receptors and the binding of cargo to export receptors. Thus, RanGTP in the nucleus can promote the assembly of export complexes and the disassembly of import complexes. Moreover, the dissociation of RanGTP from export complexes coupled with RanGTP hydrolysis in the cytosol can terminate the export reaction (Bischoff and Gorlich, 1997; Floer et al., 1997; Kehlenbach et al., 1999).

An additional mechanism for directionality could be built into the NPC itself, because several FG repeat nucleoporins implicated in the binding of transport receptors have distinctive localizations in the three-dimensional structure of the NPC. For example, in vertebrates Nup214/CAN and Nup358/RanBP2 are localized to the cytoplasmic fibrils, Nup153 and Nup98 are localized to the nuclear fibrils, and the Nup62 complex, consisting of Nup62, Nup58, Nup54, and Nup45, is restricted to the central channel region of the NPC (for review see Stoffler et al., 1999).

To address the possibility that the intrinsic design of the NPC could promote transport directionality, we measured the affinity of importin β import complexes for Nup358, Nup62 complex proteins, and Nup153, all of which have been shown previously to interact with importin β. Based on their localizations in the NPC, these nucleoporins are predicted to represent early, intermediate, and late binding sites, respectively, for import complexes. Strikingly, we found that the affinity of importin β for its nucleoporin binding partner increases progressively from Nup358 to the Nup62 complex to Nup153. Antibody inhibition studies support the possibility that importin β interacts sequentially with these three proteins during import. These observations suggest that the net movement of importin β transport complexes through the NPC is intrinsically directional, and that this is due in part to the presence of specific nucleoporins in distinctive localizations.

Materials and Methods

Expression and Purification of Recombinant Proteins

All proteins were expressed in *Escherichia coli* BL21 (strain DE30) as described previously: glutathione *S*-transferase (GST)-tagged Nup62, Nup58, and Nup54 (Hu et al., 1996), Nup358 fragments (358-1 and 358-4) (Yaseen and Blobel, 1999), 6× his-S–tagged importin β (Chi and Adam, 1997), 6× his–tagged importin α (Hu et al., 1996), 6× his–tagged IBB domain (Weis et al., 1996), nuclear transport factor 2 (Paschal and Gerace, 1995), Ran and RanQ69L (Melchior et al., 1995), and RanBP1 (Kehlenbach et al., 1999). An expression clone for the 6× his–tagged COOH-terminal segment of human Nup153 comprising amino acids 609–1475 was constructed by subcloning an XbaI–BglI fragment into the blunted HindIII site of pET28a (Novagen). An expression clone for full length 6× his–tagged human Nup98 was constructed by subcloning a HindIII–XhoI fragment into the same restriction sites of pET28b. Cells transformed with C-153 or Nup98 were grown at 37°C to an OD_{600} of 0.6. Protein was induced with 1 mM IPTG for 3 h and was purified on Talon beads (CLONTECH Laboratories, Inc.).

Nuclear Import Assays

The nuclear import assay was carried out in NRK cells supplemented with HeLa cytosol. For the antibody inhibition assay, NRK cells were trypsinized and washed with transport buffer (TB: 20 mM Hepes, pH 7.4, 110 mM KOAc, 2 mM MgOAc, 2 mM DTT, 1 μg/ml of pepstatin, leupeptin, and aprotinin). The cells were then permeabilized with digitonin (Adam et al., 1992) and subsequently incubated with 10 μg/ml RanQ69L and 10 μg/ml RanBP1 for 15 min at 30°C to deplete them of endogenous importin β (Kehlenbach, R., personal communication). The cells were then preincubated on ice with 0.5 mg/ml anti-Nup62 Fab fragment or with an equivalent volume of TB, and nuclear import was carried out and quantified by flow cytometry as described (Melchior, 1998).

To analyze the binding of his-S–importin β to nucleoporins in cells preincubated with the anti-Nup62 Fab fragment, cells were incubated with 73.5 nM his-S–importin β, 330 nM his–importin α, 500 nM Ran, 670 nM nuclear transport factor 2, 25 μg/ml FITC-NLS-BSA and an energy regenerating system at 30°C for 30 min. Next, the cells were washed with TB, and a lysate prepared with NP-40 buffer containing 0.3 M NaCl (Kehlenbach et al., 1999) was subsequently incubated with S protein–agarose. The bound proteins were analyzed by SDS-PAGE followed by immunoblotting with affinity-purified anti-Nup153 and anti-Nup358 antibodies. To analyze the effect of anti-Nup62 Fab fragment on the interaction between recombinant Nup62 and importin β, GST-Nup62 absorbed to glutathione beads was preincubated with the anti-Nup62 Fab fragment. Beads were then incubated with importin β and analyzed by SDS-PAGE.

Preparation and Affinity Purification of Antibodies

For antibody production, Nup62 was expressed as an untagged protein in *E. coli* and purified as described (Paschal and Gerace, 1995), with an additional step involving chromatography on a DEAE column with a 0–0.3 M NaCl gradient. Nup62 and a keyhole lympet hemocyanin conjugate of amino acids 921–930 of Nup153 were used to immunize rabbits. The resulting antibodies were affinity purified on a resin coupled to Nup62 or to the Nup153 peptide. The Fab fragment of the anti-Nup62 antibodies was prepared using papain-agarose beads (Pierce Chemical Co.).

Microtiter Plate Binding Assay

Solid phase binding assays were carried out on microtiter plates (Maxisorp; Nunc) coated with 25 ng of nucleoporin. Assays were conducted as described (Delphin et al., 1997) except the bound his-S–importin β was detected using anti–S tag antibodies (CLONTECH Laboratories, Inc.) and horseradish peroxidase–conjugated secondary antibodies (Pierce Chemical Co.). Colorimetric detection was carried out using 3,3′, 5,5′-tetramethylbenzidine (Calbiochem). Values were corrected for background binding of importin β to GST alone. For 6× his–tagged proteins, wells adsorbed with BSA served as the control. To study the effect of RanGTP on the binding of importin β to nucleoporins, recombinant Ran was loaded with GTP and used for binding assays (Delphin et al., 1997).

Results and Discussion

Characterization of the Binding of Importin β and an Importin β–IBB Complex to Nucleoporins

To investigate whether the affinity of the importin β transport complex for nucleoporins changes as the complex moves through the NPC, we carried out quantitative solid

Importin β Importin β + IBB

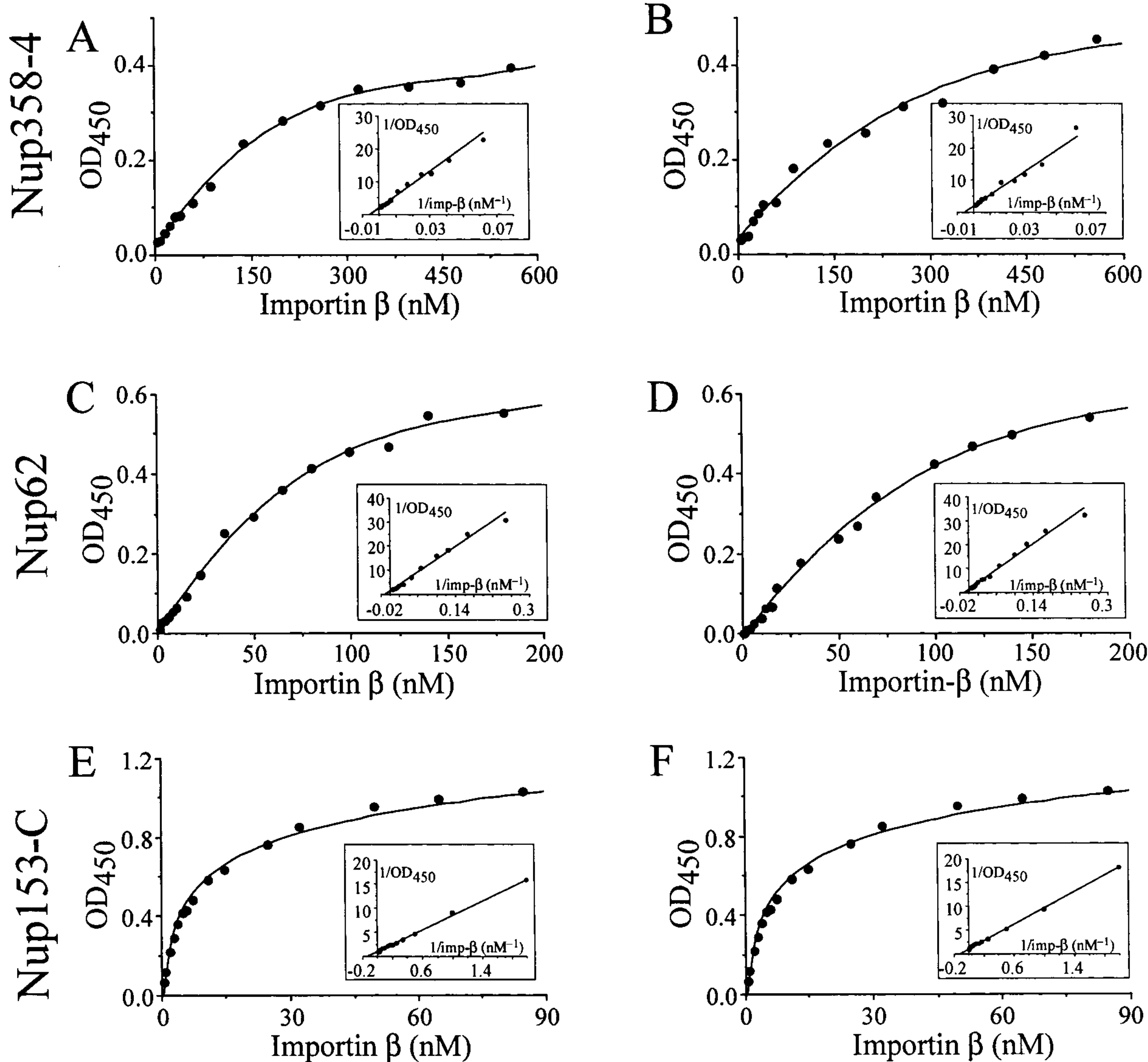


Figure 1. Characterization of the binding of importin β to nucleoporins. Binding was analyzed in the absence (A, C, and E) or presence (B, D, and F) of the IBB domain. Increasing concentrations of importin β were incubated with Nup358-4 (A and B), Nup62 (C and D), and Nup153 (E and F) and the bound importin β was measured (see Materials and Methods). The results are from duplicates of a single typical experiment. The standard deviation was $<5\%$ of the mean. A Lineweaver-Burke plot, presented as an inset in each binding isotherm, provides the apparent binding constant (K_d apparent; see Table I). Curves E and F were fitted for a logarithmic function and the rest for a polynomial function using Cricket Graph software. The correlation coefficients for the curve fits were always >0.99.

phase binding analysis with several FG repeat nucleoporins that are relatively abundant components of the NPC (Snow et al., 1987) and that have been shown previously to interact with importin β in qualitative assays. We analyzed Nup358 (Yaseen and Blobel, 1999), which is in the cytoplasmic fibrils, the Nup62, Nup58, and Nup54 subunits of the Nup62 complex (Hu et al., 1996), which are near the central channel of the NPC, and Nup153 (Shah et al., 1998), which is in the nucleoplasmic fibrils. Based on their localization, these proteins are predicted to be involved in early, intermediate, and late steps of transit through the NPC, respectively. In these binding studies, we analyzed full length Nup62, Nup58, and Nup54. Since it currently is not possible to obtain recombinant full length Nup358 and Nup153, we examined two FG repeat–containing fragments of Nup358 (Nup358-1, amino acids 996–1963; Nup358-4, amino acids 2500–3224) and the COOH-terminal region of Nup153 (Nup153-C, amino acids 609–1475)

Table I. Dissociation Constants for Nucleoporins and Importin β in the Presence or Absence of the IBB Domain

Recombinant nucleoporin	K_d apparent*	
	Importin β	Importin β + IBB domain
	nM	*nM*
Nup 358-1 (996–1963)‡	216 ± 16.6 (3)	210 ± 15 (3)
Nup 358-4 (2500–3224)‡	225 ± 14.4 (4)	220 ± 16 (3)
Nup 62‡	100 ± 8 (6)	105 ± 6 (6)
Nup 58‡	101 ± 2 (5)	106.7 ± 8 (5)
Nup 54‡	111.5 ± 6.5 (4)	108.5 ± 8.5 (4)
Nup 153-C (609–1475)§	9 ± 2.5 (6)	9.4 ± 3 (5)

*Data represent the mean ± SD (number of repeats in parentheses) for the apparent dissociation constant K_d (see legend to Fig. 1).
‡GST-tagged recombinant nucleoporins.
§His-tagged recombinant nucleoporins.

that contains the only detectable binding site for importin β (Shah et al., 1998).

The binding experiments were conducted both with importin β alone and with importin β bound to the IBB domain of importin α (Fig. 1 and Table I). The IBB domain behaves as an authentic import cargo for importin β and closely resembles certain NLSs that bind to importin β in an importin α–independent fashion (for review see Gorlich and Kutay, 1999). The binding isotherms for each of the six proteins tested showed saturable binding of both importin β and the importin β–IBB domain complex, as evidenced by linear double reciprocal plots (Fig. 1; data not shown). The apparent affinity of importin β for the nucleoporins tested is similar in the presence and absence of the IBB domain. This argues that the region of importin β involved in nucleoporin binding is not conformationally altered by cargo binding. Interestingly, the affinity of importin β was lowest for each of the Nup358 fragments (K_d = 210–225 nM), increased ~2-fold for each of the Nup62 complex proteins (K_d = 100–105 nM), and increased another ~10-fold for Nup153-C (K_d = 9 nM) (Table I). Thus, there is a progressively increasing affinity of importin β for nucleoporins that occur progressively closer to the nucleoplasmic periphery of the NPC.

We also analyzed binding of importin β to Nup98, an FG repeat nucleoporin that does not appear to be required for importin β–mediated import, since import still occurs in nuclei assembled from *Xenopus* egg extracts depleted of Nup98 (Powers et al., 1995). No specific binding was seen by the microtiter plate binding assay with the concentration range of importin β analyzed for the other nucleoporins (data not shown), as the low level association of importin β with recombinant Nup98 that we observed was nonsaturable and was not inhibited by RanGTP (see below). It should be noted that in a previous study, we did not detect a difference in the affinity of importin β for Nup358 purified from rat liver compared with recombinant Nup62 (Delphin et al., 1997). This difference from our current results may be explained by our finding that

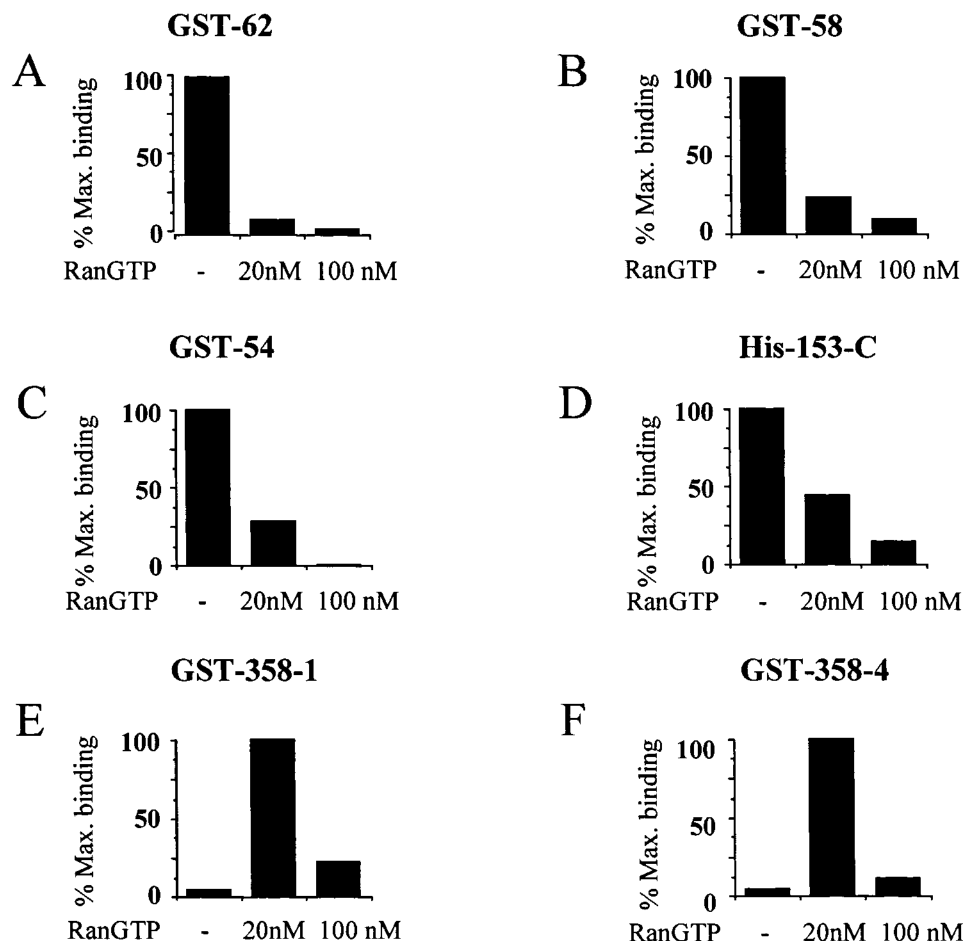
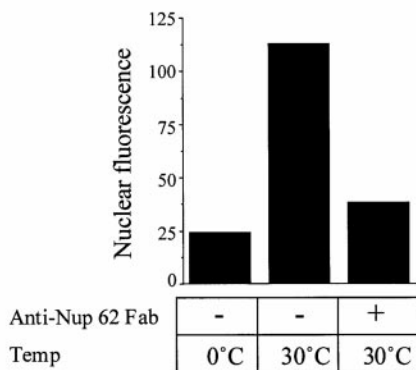
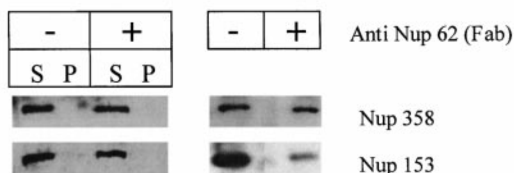


Figure 2. The binding of importin β to nucleoporins in the solid phase assay is sensitive to RanGTP. The binding of 10 nM importin β to nucleoporins adsorbed to microtiter wells was analyzed in the absence of RanGTP, or in the presence of 20 or 100 nM RanGTP, as indicated. The bound importin β was quantified as described in the legend to Fig. 1.

A Nuclear import



B Nup solubilization Nup association with Imp β



C Importin β binding to Nup62

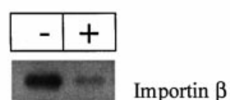


Figure 3. Role of the Nup62 complex in nuclear import. Shown are the effects of anti-Nup62 Fab fragment on (A) NLS-mediated import in NRK cells; (B) the solubilization of Nup358 and Nup153 (left) and the association of importin β with these Nups (right); and (C) the binding of importin β to GST-Nup62.

the 6× his–tagged importin β used in the previous analysis was much less active in nuclear import than the 6× his-S–tagged importin β used in the present study (our unpublished observations), and by the O-linked *N*-acetylglucosamine (Snow et al., 1987) present on the rat liver Nup358 but absent from recombinant Nup358 fragments.

Previous studies have shown that RanGTP strongly diminishes the binding of importin β to most nucleoporins as well as to intact NPCs, probably because importin β has an altered conformation when present in a complex with RanGTP (for reviews see Ohno et al., 1998; Gorlich and Kutay, 1999). This suggests a role for RanGTP in regulating importin β–Nup interactions during import. In our assay, we found that the binding of importin β to the Nup62, Nup58, Nup54, and Nup153-C was strongly diminished by the addition of RanGTP at a 2:1 molar ratio to importin β (Fig. 2, A–D), conditions under which most of the importin β is expected to be complexed to Ran, based on its high binding affinity (for reviews see Ohno et al., 1998; Gorlich and Kutay, 1999). The binding of importin β to these nucleoporins was further diminished with a 10:1 Ran/β ratio (Fig. 2, A–D). These data indicate that the importin β binding to this group of nucleoporins is inhibited by RanGTP, as is expected if the binding assays measure interactions relevant to nuclear import.

The binding of importin β to Nup358 can occur by two different mechanisms (Delphin et al., 1997; Yaseen and Blobel, 1999). One involves the binding of importin β by itself to FG repeat regions of Nup358, and is blocked in the RanGTP–importin β complex. The second mechanism, which is higher affinity, involves the binding of the importin β–RanGTP complex to the Ran binding domains (RBDs) of Nup358 via RanGTP. We measured the binding of importin β to the two fragments of Nup358 described above (Fig. 2, E and F). We found that a 2:1 ratio of RanGTP/importin β enhanced the binding of the importin β to the Nup358 fragments, as expected by the presence of one RBD in each fragment. By contrast, a 10:1 ratio diminished the binding, apparently reflecting the competition of free RanGTP with the RanGTP–importin β complex for the RBDs. These results agree with previous findings made with intact Nup358 (Delphin et al., 1997).

Pathway of Nucleoporin Associations in Importin β–mediated Import

A role for the Nup62 complex in nuclear import has been suggested by the observation that transport is inhibited in nuclei assembled from *Xenopus* egg extracts from which the Nup62 complex has been depleted (Finlay et al., 1991). To obtain further evidence for a role of the Nup62 complex in importin β–mediated nuclear import, and to examine whether this protein may be an intermediate in the (direct or indirect) transfer of the import complex from Nup358 to Nup153, we carried out antibody inhibition experiments. We pretreated permeabilized NRK cells with the Fab fragment derived from anti-Nup62 IgG and then analyzed both nuclear import and the levels of importin β associated with Nup358 and Nup153. The anti-Nup62 antibodies reacted only with an ~62-kD band on an immunoblot of NRK cells and showed strong nuclear rim staining in immunofluorescence, as expected (data not shown). As shown in Fig. 3 A, the anti-Nup62 Fab fragment inhibited import on average by 84% compared with a control reaction lacking the antibody, when transport is corrected for the 0°C control. This suggests that an interaction of importin β with Nup62 is essential for NLS-mediated import. Strong inhibition of import was also obtained with intact anti-Nup62 IgG, as well as with anti-Nup58 and anti-Nup54 IgG (data not shown). By contrast anti-Nup62 IgG and Fab do not inhibit in vitro nuclear export (our unpublished observations).

To analyze the effect of the anti-Nup62 Fab fragment on the interaction of importin β with Nup358 and Nup153, NRK cells that had been pretreated with the antibodies were analyzed in an import assay containing his-tagged importin β (see Materials and Methods). The cells were then solubilized and the association of importin β with Nup358 and Nup153 in the absence or presence of the anti-Nup62 Fab fragment was determined by immunoprecipitation and immunoblotting. As shown in Fig. 3 B, most of Nup358 and Nup153 were solubilized under our conditions, whether or not cells were preincubated with the antibody. Although there was no effect of the Fab fragment on the level of Nup358 that coprecipitated with importin β, there was a considerable decrease in the amount of

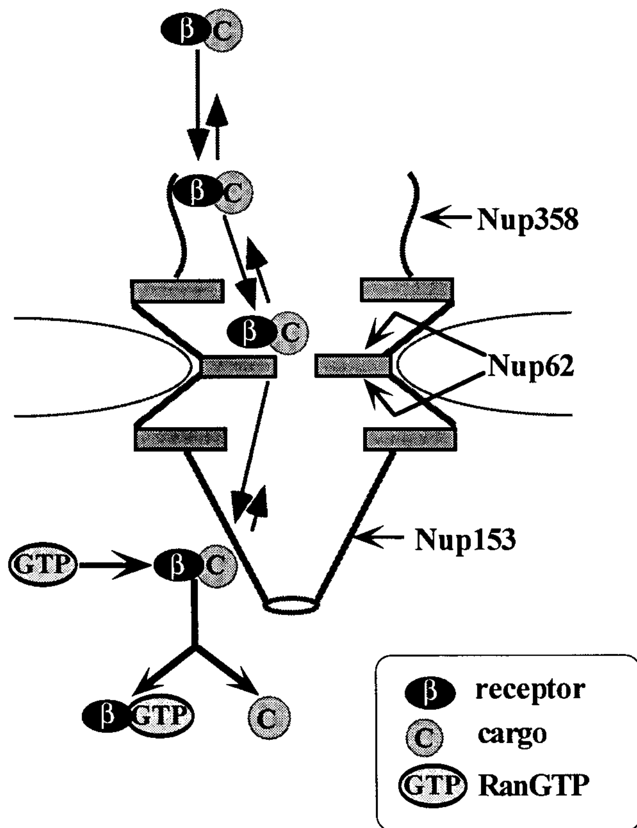


Figure 4. Model for the directional movement of an importin β cargo complex through the NPC. See text for details.

Nup153 that coprecipitated with importin β in the Fab fragment-treated cells as compared with the control (Fig. 3 B). Immunofluorescence microscopy demonstrated that the binding of anti-Nup62 antibodies did not dissociate Nup153 from the NPC (data not shown).

Because the solubilization conditions we used for immunoprecipitation released only a small fraction of the Nup62 complex from the permeabilized cells, we were unable to measure the association of importin β with the Nup62 complex in this experiment. However, using an alternative approach, we found that the anti-Nup62 Fab fragment strongly diminished the amount of importin β bound to column-immobilized recombinant GST-Nup62 in vitro compared with the untreated control (Fig. 3 C). This suggests that the antibody may inhibit import in permeabilized cells by blocking a binding site on Nup62 for importin β, although it cannot be excluded that the antibody acts by sterically inhibiting the binding to another nearby subunit protein of the Nup62 complex. Considered together, our data argue that the Nup62 complex is directly involved in nuclear import, and that the complex is an intermediate NPC binding site for importin β as it traverses the NPC between binding sites at Nup358 and Nup153.

Implications for the Mechanism of Nuclear Protein Import

Several models for the movement of transport complexes through the NPC have been discussed (Rexach and Blobel, 1995; Nachury and Weis, 1999). We believe that the simplest model for importin β–mediated nuclear import that is consistent with the observations presented in this study is an "affinity gradient" mechanism (Fig. 4). Our antibody inhibition and biochemical analyses support the possibility that movement of importin β through the NPC involves its transfer from Nup358 to Nup153 via the Nup62 complex. Although other unidentified nucleoporin intermediates may also be involved, it is possible that Nup358 and Nup153, which are components of the flexible cyto/nucleoplasmic fibrils, might be able to directly interact with the Nup62 complex. Based on the progressive increase in the affinity of importin β for Nup358, Nup62 complex proteins, and Nup153, we suggest that the movement of the importin β cargo complex through the NPC has a strong cytoplasmic-to-nuclear directional bias due in part to increasing affinity of the transport complex for the nucleoporin binding sites that it sequentially encounters. In the simplest situation, transfer between nucleoporin pairs could occur in either a forward or backward direction at each step, but forward movement would be favored by an increase in the affinity of transport complexes for more distal nucleoporins. Release from the terminal nucleoporin binding site could be mediated by RanGTP (Gorlich et al., 1996). It is striking that two different FG repeat regions of Nup358 bound to importin β with nearly identical affinity, as did three different subunits of the Nup62 complex. This suggests that the affinity of import complexes for a specific region of the NPC may be an important parameter in specifying directionality (see below).

Precisely how the transport complex is transferred between two nucleoporins is unclear. In one scenario, this could be a concerted reaction whereby an importin β cargo complex bound to one nucleoporin is induced to release from the first binding site upon interacting with a second nucleoporin. Consistent with this possibility, importin β may have at least two distinct binding sites for nucleoporins (Kutay et al., 1997). We attempted to investigate this model by monitoring the ability of Nup153 to release importin β from Nup62, and by the ability of Nup62 to release importin β from the Nup358 fragments. Unfortunately these experiments were not informative, since under our experimental conditions Nup153, Nup62, Nup58, and the Nup358 fragments interact with each other (our unpublished results).

The on-rate for association of importin β with nucleoporins may be in the range of 10^7–10^8 M^{-1} s^{-1} (Berg and Von Hippel, 1985; Chaillan-Huntington et al., 2000), and so the measured dissociation constants for importin β binding to Nup358, to the Nup62 complex, and to Nup153 would imply off-rates on the order of 2–20/s, 1–10/s, and 0.1–1/s, respectively. Since the rate of nuclear transport is thought to be in the range of 10–100 events/s (for review see Gorlich and Kutay, 1999), it would seem that these interactions, especially the binding to Nup153, would predict an interaction persistence time that is too long to support import by simple on/off binding reactions. However, much more rapid transfer between nucleoporin pairs could occur at each step if a concerted transfer were involved (see above). Moreover, additional factors, such as RanGTP (Rexach and Blobel, 1995; Gorlich et al., 1996), could promote the transfer/release reactions. An analogous mechanism involving a gradient of increasing nucleoporin bind-

ing affinity may function in nuclear export, to promote directional movement of export complexes from the nuclear to the cytoplasmic surface of the NPC. For example, chromosome maintenance region 1-containing nuclear export complexes have a substantially higher affinity for the cytoplasmic fibril protein Nup214/CAN than for more proximal nucleoporin binding sites in the export pathway including the Nup62 complex and Nup153 (Kehlenbach et al., 1999; Kehlenbach, R., and L. Gerace, unpublished). Finally, a similar mechanism may be involved in the recycling of importin β to the cytoplasm after import, since the RanGTP–importin β complex that is thought to be created in the nucleus by dissociation of the import complex has a much higher affinity for the cytoplasmic periphery of the NPC than for any other NPC region (Delphin et al., 1997).

In conclusion, we propose that the increasing affinity of importin β for nucleoporins that are localized progressively closer to the nucleoplasmic surface of the NPC contributes to the directional movement of import complexes through the NPC. This mechanism would clearly enhance the efficiency of directional nuclear transport that is promoted by compartmentalization of different components of the Ran system.

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