The nonpermissive temperature, importin-β encodes a guanine nucleotide exchange factor of Ran. At the same time, the nuclear export of importin-β indicates that its export is not mediated by a leucine-domain of this molecule. Insensitivity to leptomycin B for the export of importin-β was not inhibited by co-injection with a GTPase-deficient Ran mutant (G19V). The cell line tsBN2 contains a temperature-sensitive point mutation in the RCC1 gene, which encodes a guanine nucleotide exchange factor of Ran. At the nonpermissive temperature, importin-β was exported from the nucleus of these cells, even when RanGAP1, a GTPase-activating protein for Ran, was co-injected. These results not only provide support for the view that Ran-dependent GTP hydrolysis is not required for the nuclear export of importin-β but also indicate that nuclear RanGTP is not essential for its export. As a result, we propose that importin-β can be recycled from the nucleus alone in a Ran-independent manner.

It is well known that the nucleocytoplasmic transport of macromolecules occurs through NPCs and that the selective nuclear import and export of proteins occur via a signal- and temperature-dependent pathway (1, 2). Conventional nuclear localization signal (NLS)-containing proteins target the nuclear pore complex (PTAC), in the cytoplasm (3–5). This complex is composed of a karyophilic protein and two cytosolic components. One of these (PTAC58/importexin-α/kyrophin-α/human SRP1/Srp1/Kap60p) functions as an NLS receptor (2). The other component (PTAC97/importin-β/kyrophin-β/p97/Kap95p) mediates the targeting of the NLS substrate bound to the NLS receptor to the nuclear pores by binding both the NLS receptor and components of the NPC (2). The NPC-binding domain of importin-β is able to migrate from the cytoplasm to the nucleus through the NPC, suggesting that importin-β is translocated through the NPC by binding directly to nucleoporins (6, 7). After targeting of the trimeric complex to the NPC, the translocation step through the NPC occurs in an energy-dependent manner and requires additional soluble factors, namely a small GTPase Ran and its interacting protein, p10/NTF2 (2). The direct binding of RanGTP to importin-β causes the dissociation of the importin-αβ heterodimer (6, 8, 9). This event is generally thought to occur on the nucleoplasmic side of the NPC, resulting in the release of NLS substrate into the nucleus (6).

After the translocation of the NLS substrate into the nucleus, its carrier molecules, importin-α and -β must return to the cytoplasm in order to transport the next NLS substrate into the nucleus. CAS has recently been identified as a nuclear export mediator of importin-α (10). CAS is also related to importin-β and binds simultaneously to both importin-α and RanGTP. In addition, it has been reported that Kap95p, which is an Saccharomyces cerevisiae homologue of importin-β, requires a leucine-rich NES in the molecule for its nuclear export in mammalian and yeast cells (11). Recently, an export receptor (CRM1/exportin 1/XPO1) for a leucine-rich NES has been identified (12–15). CRM1 is related to importin-β and binds directly to the leucine-rich NES and RanGTP, and this trimeric complex is then translocated to the cytoplasm through the NPC (12). However, it is not yet known whether or not the nuclear export of mammalian importin-β requires a leucine-rich NES in mammalian cells.

Ran is a key component in the nucleocytoplasmic transport of proteins (2, 16). Ran is an abundant, small GTPase of the Ras superfamily, which is predominantly localized in the nucleus (17, 18). Like other GTPases, Ran is thought to function as a molecular switch by cycling between a GDP- and a GTP-bound state. This cycle is catalyzed mainly by two molecules, RCC1 and RanGAP1 (19). RCC1 was first identified as a causative gene for the temperature sensitivity of tsBN2 cells derived from hamster BHK21 cells (20). RCC1 is located on the chromatin (21) and functions as a guanine nucleotide exchanging factor of Ran, which enhances the rate of guanine nucleotide exchange on Ran by about 100,000-fold (17, 22). In addition, RanGAP1 is located in the cytoplasm and on the cytoplasmic fibers extending from the NPC (23, 24) and functions as a GTPase-activating protein for Ran, which enhances the rate of GTP hydrolysis on Ran by about 100,000-fold (22, 25). The asymmetric distribution of these two factors across the nuclear envelope suggests that nuclear Ran may be predominantly the GTP-bound form and that cytoplasmic Ran is the GDP-bound form. Furthermore, it has been proposed that the asymmetric
distribution of RanGTP across the nuclear envelope assures the directional movement of proteins between the cytoplasm and the nucleus (6, 26).

In this study, we show that mouse importin-β is exported from the nucleus to the cytoplasm through the NPC in living mammalian cells and that this nuclear export of importin-β is temperature-dependent. Moreover, we demonstrate that the nuclear export of mammalian importin-β depends on the NPC-binding domain of this molecule and is not mediated by CRM1. Experiments using a GTPase-deficient Ran mutant show that the nuclear export of importin-β does not require Ran-dependent GTP hydrolysis. Further, microinjection experiments using tsBN2 cells cultured at the nonpermissive temperature show that importin-β can be transported from the nucleus in a nuclear RanGTP-independent manner. The requirement of Ran on nucleocytoplasmic shuttling of importin-β is discussed.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—MDCK, BHK21, and tsBN2 cells** were incubated in Dulbecco’s modified Eagle’s minimum essential medium supplemented with 5% fetal bovine serum at 37 °C (MDKB, BHK21) or 33.5 °C (tsBN2). Cells were grown on coverslips for 48 h at 37 °C (BHKB1) or 39.5 °C (tsBN2) prior to use in microinjection experiments. **Cell Fusion by the Hemagglutinating Virus of Japan (HVJ, Sendai Virus)—**BHKB1 or tsBN2 cells were fused by HVJ as described previously (27). Homokaryons were incubated for 3 h at 37 °C (BHKB1) or 39.5 °C (tsBN2) prior to microinjection. In experiments using leptomycin B, BHKB1 cells were fused by HVJ after preincubation with leptomycin B (2 μg/ml in Dulbecco’s modified Eagle’s minimum essential medium supplemented with 5% fetal bovine serum) for 3 h at 37 °C.

**Purification of Recombinant Proteins—**Expression and purification of recombinant mouse importin-β, the amino acid (aa) 448–876 mutant, and importin-α were performed as described previously (7, 28, 29). To construct the expression vector of the aa 145–449 mutant, the region of aa 145–449 of mouse importin-β was amplified with PCR using the synthetic oligonucleotides 5′-GCGGCTTC-3′ and 5′-GAAAGGATCCAGTACGATGTTCCCG-3′ and 5′-GGATCCATGGCCTCGGAAGACATTGCCAAG-3′, and this PCR product was inserted into BamHI and KpnI sites of the pRSETa vector (Invitrogen Corp.). The green fluorescence protein (GFP)-fragment amplified by PCR was then inserted into a BamHI site. Recombinant GFP (145–449) protein was expressed by 0.5 mM isopropyl-1-thio-galactopyranoside for 18 h at 20 °C in E. coli strain BL21(DE3). An expression vector of GST-GFP containing the amino acid sequence of SV40 large T-antigen (6, 26) was kindly provided by Dr. S. Morita. Recombinant GFP-(145–449) protein was expressed by 0.5 mM isopropyl-1-thio-galactopyranoside for 48 h at 37 °C (BHKB1) or 24 h at 39.5 °C (tsBN2) from the pGEX-2T vector (Amersham Pharmacia Biotech). The recombinant proteins, which were trapped in glutathione-Sepharose, were eluted with 600 mM NaCl. Peak fractions containing RanGAP1 were dialyzed against 20 mM Hepes (pH 7.3), 110 mM potassium acetate, and 2 mM DTT.

**Expression and purification of the recombinant fusion protein—**RanGAP1. GST and thrombin were separated from the recombinant protein on a MonoQ column at a flow rate of 0.5 ml/min with a linear gradient from 0 to 1.0 m NaCl in 20 mM Hepes (pH 7.3) and 2 mM DTT. Recombinant protein was eluted with 600 mM NaCl. Peak fractions containing RanGAP1 were pooled and dialyzed against 20 mM Hepes (pH 7.3), 110 mM potassium acetate and 2 mM DTT.

**Electrophoresis and Western blot analysis—**Aliquots of each recombinant protein were frozen in liquid nitrogen and stored at –80 °C. Ran(G19V) (31) and GST-NES-GFP proteins (32) were prepared as described previously.

**Conjugation of Texas Red with Bovine Serum Albumin (TR-BSA)—**Bovine serum albumin (BSA) was dissolved at 5 mg/ml in 0.1 M NaHCO3, 0.5 mg of Texas Red (TR) was added per 5 mg of BSA, and the solution was incubated for 2 h at room temperature. Free fluorophore was removed by gel filtration on PD10 (Amersham Pharmacia Bio- tech) equilibrated with 10 mM Hepes (pH 7.3), 110 mM potassium acetate. Peak fractions containing TR-BSA were collected and dialyzed against 10 mM Hepes (pH 7.3), 110 mM potassium acetate.

**Microinjection—**Microinjection experiments were performed essentially as described previously (33). After microinjection and incubation, cells were fixed with 3.7% formaldehyde in PBS, 0.7 mM KCl, 8.1 mM Na2HPO4, 1 mM KH2PO4, pH 7.2 for 30 min at room temperature. The injected fluorescence-labeled proteins were detected by Axiohot 2 microscope (Carl Zeiss, Inc.).

**Ran Overlay Assay—**RanGTP overlay assay was performed as described previously (7). Recombinant importin-β, its aa 145–449 mutant, and RanBP1 were separated by 12.5% SDS-polyacrylamide gel electro- phoresis and transferred to nitrocellulose and then incubated with transport buffer (20 mM Hepes (pH 7.3), 110 mM potassium acetate, 5 mM magnesium acetate, 0.25% Tween 20, 0.5% BSA, and 5 mM DT and then preincubated for 30 min at room temperature in binding buffer (20 mM Hepes (pH 7.3), 100 mM potassium acetate, 5 mM magnesium acetate, 0.05% Tween 20, 0.5% BSA, and 5 mM DT) in the presence of 100 μM GTP. GTP was then washed with binding buffer and then overlaid with 1.25 μg/ml of BSA (specific activity 1550 epm/pan/ml) in binding buffer for 30 min at room temperature.

**Preparation of an NLS-containing Transport Substrate (T-APC)—**Allophycocyanin (Calbiochem) was chemically conjugated to synthetic peptide containing the amino acid sequence of SV40 large T-antigen NLS (CYSGGPKKKRVEDP), as described previously (28).

**Cell-free Import Assay—**Digitonin-permeabilized MDCK cells were prepared as described previously (25, 34). Digitonin-permeabilized cells were incubated with transport buffer (200 mM Hepes (pH 7.3), 110 mM potassium acetate, 2 mM magnesium acetate, 5 mM sodium acetate, 1 mM glycothjeridaminetraacetic acid, 2 mM DTT, and 1 μg/ml each of aprotinin, leupeptin, and pepstatin) containing test proteins, in 10 μl, under the conditions indicated in the figure legends. After incubation, cells were fixed with 3.7% formaldehyde in transport buffer.

**Antibodies—**Rabbit anti-mouse importin-β and anti-mouse importin-α antibodies were prepared as described previously (28, 29).

**Indirect Immunofluorescence—**Cells were washed twice in PBS and fixed with 3.7% formaldehyde in PBS for 30 min at room temperature. After permeabilization with 0.5% Triton X-100 in PBS for 5 min at room temperature, cells were incubated with 10 μg/ml of affinity-purified antibodies for 1.5 h at room temperature. Rabbit antibodies were detected with fluorescein isothiocyanate-conjugated goat antibodies to rabbit IgG (TAGO). The samples were examined using an Axiohot 2 microscope (Carl Zeiss Inc.).

**RESULTS**

**Nuclear Export of Importin-β in Homokaryons of Mammalian Cells**—It is proposed that importin-β is recycled back to the cytoplasm after termination of translocation of NLS protein-carrier complexes via the binding of RanGTP to importin-β at the nucleoplasmic side of the NPC. However, the precise mechanism involved in the recycling of importin-β remains obscure. Therefore, we attempted to better understand the
mechanism of how importin-β is exported from the nucleus to the cytoplasm after dissociating from the cargo in the nucleus. To accomplish this, we constructed homokaryons by using the HVJ, injected recombinant GFP-fused mouse importin-β proteins into a nucleus of multinucleated cells, and then examined the intracellular localization of GFP-importin-β. As shown in Fig. 1C, GFP-importin-β was exported from the nucleus to the cytoplasm and then reimported into all of the nuclei in the homokaryons within 30 min at 37 °C.

We next examined whether the export of importin-β is temperature-dependent. After the BHK21 cells were fused by HVJ, the homokaryons were preincubated for 10 min on ice and the recombinant GFP-importin-β proteins were then injected into a nucleus. After incubation for 30 min on ice, the injected GFP-importin-β proteins were largely detected only in the injected nucleus (Fig. 2A). This finding indicates that importin-β is exported from the nucleus in a temperature-dependent manner. We further examined whether wheat germ agglutinin (WGA) inhibits the nuclear export of importin-β. As shown in Fig. 2C, the nuclear export of importin-β was inhibited by the co-injection of WGA (2 mg/ml in a needle), whereas the passive diffusion of GFP (about 27 kDa) from the nucleus was not (Fig. 2B). These results indicate that the nuclear export of importin-β observed herein does not result from the passive diffusion of the degradation products of GFP-importin-β fusion proteins.

Nuclear Export of Importin-β Is Not Inhibited by Leptomycin B—Recently, an export receptor (CRM1) for leucine-rich NES was identified (12–15). Meanwhile, it was reported that Kap95p, a yeast homologue of importin-β, contains a region (amino acid residues 55–65) that is similar to the leucine-rich
NES motifs and that this region of Kap95p is sufficient to mediate the active nuclear export of Kap95p (11). More recently, an antibiotic, leptomycin B was demonstrated to specifically inhibit the leucine-rich NES-mediated nuclear export by binding directly to CRM1 (35–37). Therefore, we investigated whether nuclear export of mouse importin-β is inhibited by leptomycin B. BHK21 cells were preincubated with leptomycin B at 2 ng/ml in a culture medium for 3 h at 37 °C and then fused by HVJ. As reported previously, nuclear export of GST-NES-GFP was inhibited in these homokaryons, which had been preincubated with leptomycin B (Fig. 3A). However, the nuclear export of the importin-β was completely unaffected by leptomycin B. These results strongly suggest that mouse importin-β is not carried by CRM1 and does not possess a leucine-rich NES such as those in Rev and PKI (protein kinase A inhibitor) (discussed below).

We next attempted to determine the region required for the nuclear export of importin-β. Since it was previously found that importin-β contains the NPC-binding domain (amino-terminal region) as well as the importin-α binding domain (carboxyl-terminal region) (7), we constructed two deletion mutants containing the NPC-binding region (aa 145–449) and the importin-α binding region (aa 448–876). As expected, the aa 145–449 mutant failed to bind the importin-α (data not shown) and Ran (Fig. 1B, left), but migrated into the nucleus in vivo and in vitro (summarized in Fig. 1A), consistent with our previous results (7). Further, in the digitonin-permeabilized cells, NPC-
targeting of NLS-containing proteins was competitively inhibited by the addition of the aa 145–449 mutant, indicating that the aa 145–449 mutant contains a domain that interacts with the NPC (Fig. 1B, right).

It was found that, similar to the case of wild type (WT) importin-β, the aa 145–449 mutant was exported from the nucleus and reimported into the nuclei (Fig. 1C). Moreover, it was also shown that the nuclear export of the aa 145–449 mutant is temperature-dependent, WGA-sensitive, and unaffected by leptomycin B treatment, similar to WT importin-β (Figs. 1C, 2A, 2C, and 3B). In contrast, the NPC binding-deficient mutant, aa 448–876, was not exported from the nucleus at all (Fig. 1C). These results indicate that the NPC-binding domain of importin-β is involved in not only nuclear import but also the nuclear export of importin-β.

**Importin-β Can Be Exported in the Absence of RanGTP as Well as Its GTP Hydrolysis**—Richards et al. (38) showed that a leucine-rich NES-mediated nuclear export requires RanGTP but not the GTP hydrolysis of Ran. In order to determine if the hydrolysis of RanGTP is required for the nuclear export of importin-β, we investigated the effect of a Ran mutant, Ran(G19V), which is known to be a GTPase-deficient mutant (39). Consistent with the previous results on the leucine-rich NES-containing substrates was strongly inhibited. In addition, the nuclear export of importin-β occurred in tsBN2 cells incubated for 24 h at 39.5 °C followed by treatment of 35.5 μM cycloheximide for 3 h at 39.5 °C to prevent the resynthesis of RCC1 (data not shown). Moreover, it was also found that the nuclear export of the Ran binding-deficient mutant, aa 145–449, was not inhibited under the same assay conditions (Fig. 6).

These results indicate that mouse importin-β can be exported from the nucleus through the NPC without the support of RanGTP as well as its GTP hydrolysis in living mammalian cells, while the possibility that importin-β bound to RanGTP is also exported without its GTP hydrolysis cannot be excluded. The actual role of Ran in the export of importin-β is discussed below.

**DISCUSSION**

**Nuclear Export of Importin-β Is Temperature-dependent and WGA-sensitive**—The NLS substrate targets to the NPC through nuclear pore-targeting complex formation with the importin-αβ heterodimer and is then translocated through the NPC into the nucleus in an energy-dependent manner. After translocation of the NLS substrate-carrier complex into the nucleus, carrier molecules are required to return to the cytoplasm.

As expected, microinjection experiments showed that importin-β was exported from the nucleus to the cytoplasm in living mammalian cells (Fig. 1C). Nuclear export of importin-β did not occur in chilled cells (Fig. 2A), which is consistent with a previous report (11). The nuclear export of importin-β was inhibited by WGA (Fig. 2C), while the passive diffusion of small molecules was not affected (Fig. 2B). These results exclude the possibility that the degradation products of GFP fusion proteins passively diffuse out of the injected nucleus. Moreover, although the precise mechanism of the inhibitory effects of WGA remains unclear, it is generally assumed that some gly-
we found that the aa 145–449 mutant of mouse importin-like motif with GLFG repeat regions. In contrast, in this study, recycling of Kap95p is mediated by the interaction of an NES-manner after injection into the nuclei of cultured mammalian cells. Moreover, they found that the NES-like motif-containing subdomain moved to the cytoplasm in a temperature-dependent manner after injection into the nuclei. These data strongly suggest that the nuclear export of importin-β is promoted by direct association with components of the NPC.

In this study, it was found that the aa 145–449 mutant containing the region for NPC binding involved in the nuclear import of importin-β also possesses nuclear export activity (Fig. 1C). In addition, numerous other studies have shown that several importin-β-related proteins are likely to associate with components of the NPC, suggesting that the binding of importin-β family members to components of the NPC is critical in order for the member to function as a nuclear transport factor. These data strongly suggest that the nuclear export of importin-β is promoted by direct association with components of the NPC.

Recently, it was reported that the nuclear export of proteins containing a leucine-rich NES is mediated by CRM1 (12–15). Leptomycin B is a specific inhibitor of CRM1 (35–37). However, the nuclear export of importin-β was insensitive to leptomycin B treatment (Fig. 3B), suggesting that importin-β is not carried back to the cytoplasm by CRM1. Iovine and Wente (11) previously showed that Kap95p, which is a S. cerevisiae homologue of importin-β, contains a region similar to a leucine-rich NES in the amino terminus (amino acid residues 55LEGRILAALTL65) and that the NES-like motif-mutated Kap95p abolished the binding activity to glycine-leucine-phenylalanine-glycine (GLFG) repeat regions of nucleoporins Nup116p and Nup100p. Moreover, they found that the NES-like motif-containing substrate moved to the cytoplasm in a temperature-dependent manner after injection into the nuclei of cultured mammalian cells. From these findings, they proposed a model in which the recycling of Kap95p is mediated by the interaction of an NES-like motif with GLFG repeat regions. In contrast, in this study, we found that the aa 145–449 mutant of mouse importin-β, which lacks the region (55VARVAAGLQT61) corresponding to the NES-like motif of yeast Kap95p, is fully capable of migrating from the nucleus to the cytoplasm through the NPC (Fig. 1C). Although we cannot clearly explain this contradiction, the differences may result from the source of importin-β family molecules used in these experiments.

A putative vertebrate homologue of yeast Nup116p is human/rat/Xenopus Nup98, which localizes at the nucleoplasmic side of the NPC (43, 44). At present, Nup98 is the only identified vertebrate GLFG repeat-containing nucleoporin. It has been reported that importin-β is able to bind to Nup98 in overlay assays (45). Powers et al. showed that nuclear injection of anti-Xenopus Nup98 antibodies did not inhibit nuclear protein import but did inhibit the nuclear export of RNAs, including small nuclear RNAs, 5 S RNA, large ribosomal RNAs, and mRNA (46), although it has not been determined whether the nuclear export of importin-β is inhibited by the nuclear injection of anti-Xenopus Nup98 antibodies. Further studies are required to elucidate the function of GLFG repeat-containing nucleoporins in the nuclear export of importin-β.

The Roles of Ran in Nuclear Export of Importin-β—In this study, we found that the nuclear export of importin-β is not inhibited by nuclear injection of Ran(G19V)GTP (Fig. 4), which is consistent with a previous report concerning NES-mediated nuclear export (38). Moreover, it appeared that the recycling of importin-β, but not importin-α, occurred in tsBN2 cells cultured at the nonpermissive temperature (Fig. 5), in which endogenous mutated RCC1 is inactive and the level of nuclear RanGTP would be expected to be quite low (18). The export of importin-β was not inhibited by nuclear co-injection of RanGAP1, which lowers the level of free RanGTP and, in turn, elevates that of RanGDP, into the tsBN2 cells at nonpermissive temperature (Fig. 6). In addition, we showed that the aa 145–449 mutant, which lacks the Ran-binding domain, is capable of migrating from nucleus to cytoplasm through the NPC (Figs. 1C, 3B, and 6). These results suggest that nuclear RanGTP is not essential for the nuclear export of importin-β. In contrast, Izaurralde et al. (26) demonstrated that the nuclear export of human importin-β was significantly, but not completely, inhibited by the injection of Rna1p, a Saccharomyces pombe homologue of RanGAP1, into Xenopus laevis oocyte nuclei. However, their data showed that a considerable amount of importin-β was exported into the cytoplasm, even when Rna1p was injected into Xenopus oocyte nuclei at higher concentration than that which nearly completely inhibited the nuclear export of importin-α. These findings suggest that at least part of importin-β can be exported via a pathway that is unaffected by Rna1p. Alternatively, since we did not examine the export kinetics of importin-β, we cannot exclude the possibility that the decrease of nuclear RanGTP may affect export efficiency and that the export rate of importin-β may be decreased by nuclear injection of RanGAP1 in mammalian cells as in Xenopus oocytes.

It should be noted that the nuclear reimport of WT importin-β was considerably inhibited when Ran(G19V)GTP was co-injected in a nucleus of multinucleated cells, whereas the aa 145–449 mutant lacking the Ran-binding domain was not inhibited by nuclear co-injection of RanGAP1, which lowers the level of free RanGTP and, in turn, elevates that of RanGDP, into the tsBN2 cells at nonpermissive temperature (Fig. 4). From these findings, we speculate that the WT importin-β-Ran(G19V)GTP complex formed in the nucleus is exported to the cytoplasm, and the disassembly of the importin-β-RanGTP complex through GTP hydrolysis of Ran is involved in the reimport of WT importin-β. Moreover, these results suggest that WT importin-β, which is complexed with RanGTP in the nucleus, can be exported from the nucleus without GTP hydrolysis of Ran.

From these and other findings, we propose that importin-β is recycled from the nucleus in two distinct ways: 1) in the form of a complex with RanGTP and 2) alone in a Ran-independent manner. In either case, the export does not require the GTP hydrolysis of Ran. Since importin-β binds RanGTP with high
affinity and nuclear Ran would be expected to be the predominant GTP-bound form, it is plausible that nuclear RanGTP binds importin-β to dissociate the nuclear pore-targeting complex after its translocation through the NPC and that the importin-β-RanGTP complex recycles back to the cytoplasm without hydrolysis of its GTP. However, in our previous study, it was demonstrated that importin-β is translocated alone into the nucleus through the NPC in a Ran-independent manner when it does not carry the importin-α-NLS substrate complex (7). Furthermore, we found that the NPC-binding domain of importin-β, which had been shown to be involved in nuclear import of importin-β, was also required for the nuclear export. Therefore, we speculate that importin-β traverses the NPC into and out of the nucleus in two ways; “Ran-dependent” and “Ran-independent.” As a result, we assume that importin-β, which is imported independently from the NLS substrates, “Ran-independent.” As a result, we assume that importin-β binds importin-β in a Ran-independent manner after its translocation through the NPC and that the importin-β-RanGTP will be expected to be the predominant RanGTP-bound form, it is plausible that nuclear RanGTP binds importin-β to dissociate the nuclear pore-targeting complex after its translocation through the NPC and that the importin-β-RanGTP complex recycles back to the cytoplasm without hydrolysis of its GTP. However, in our previous study, it was demonstrated that importin-β is translocated alone into the nucleus through the NPC in a Ran-independent manner when it does not carry the importin-α-NLS substrate complex (7). Furthermore, we found that the NPC-binding domain of importin-β, which had been shown to be involved in nuclear import of importin-β, was also required for the nuclear export. Therefore, we speculate that importin-β traverses the NPC into and out of the nucleus in two ways; “Ran-dependent” and “Ran-independent.” As a result, we assume that importin-β, which is imported independently from the NLS substrates, “Ran-independent.” As a result, we assume that importin-β binds importin-β in a Ran-independent manner after its translocation through the NPC and that the importin-β-RanGTP will be expected to be the predominant RanGTP-bound form, it is plausible that nuclear RanGTP binds importin-β to dissociate the nuclear pore-targeting complex after its translocation through the NPC and that the importin-β-RanGTP complex recycl...
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