

Nucleocytoplasmic Protein Transport and Recycling of Ran

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ABSTRACT. The active transport of proteins into and out of the nucleus is mediated by specific signals, the nuclear localization signal (NLS) and nuclear export signal (NES), respectively. The best characterized NLS is that of the SV40 large T antigen, which contains a cluster of basic amino acids. The NESs were first identified in the protein kinase inhibitor (PKI) and HIV Rev protein, which are rich in leucine residues. The SV40 T-NLS containing transport substrates are carried into the nucleus by an importin α/β heterodimer. Importin α recognizes the NLS and acts as an adapter between the NLS and importin β , whereas importin β interacts with importin α bound to the NLS, and acts as a carrier of the NLS/importin α/β trimer. It is generally thought that importin α and β are part of a large protein family. The leucine rich NES-containing proteins are exported from the nucleus by one of the importin β family molecules, CRM1/exportin 1. A Ras-like small GTPase Ran plays a crucial role in both import/export pathways and determines the directionality of nuclear transport. It has recently been demonstrated in living cells that Ran actually shuttles between the nucleus and the cytoplasm and that the recycling of Ran is essential for the nuclear transport. Furthermore, it has been shown that nuclear transport factor 2 (NTF2) mediates the nuclear import of RanGDP. This review largely focuses on the issue concerning the functional divergence of importin α family molecules and the role of Ran in nucleocytoplasmic protein transport.

Key words: importin/exportin/Ran/nuclear protein transport/nuclear localization signal (NLS)/nuclear export signal (NES)

In eukaryotic cells, the compartment for DNA replication and RNA synthesis, the nucleus, is sequestered from the cytoplasm, where protein synthesis occurs, by a double membrane, the nuclear envelope. Proteins are synthesized from RNA templates on ribosomes in the cytoplasm, whereas the expression and duplication of genetic information is regulated in the nucleus in response to information from the cytoplasm. Therefore, molecules are continuously and bi-directionally transported between these two compartments in order for a cell to communicate. Intracellular communication between the nucleus and the cytoplasm occurs through the nuclear pore complexes (NPCs) which are present in the nuclear envelope.

Ions, small metabolites and proteins which are smaller than 40–60 kDa can passively diffuse through aqueous channels (~10 nm in diameter) which exist

around the perimeter of the NPC. Most macromolecules which are larger than 40–60 kDa, and which are too large to diffuse, are transported across the NPC by an active process. The vertebrate NPC is a huge proteinaceous structure composed of ~100 different species of proteins and is estimated to have the total mass of ~125 MDa. The core structure of the NPC is highly symmetrical, consisting of a spoke-ring structure surrounding the so-called central transporter, which is believed to mediate the active transport of macromolecules. Short fiber-like structures extend from the cytoplasmic side of the NPC into the cytoplasm, while basket-like structures extend from the nucleoplasmic side of the NPC (6, 8, 33, 42). Although a great deal of similarity exists between the fundamental structure of the NPCs from various species, the NPCs of the yeast *Saccharomyces cerevisiae* appear to be smaller than those of vertebrates (47).

Numerous experiments have indicated that active nuclear import and export of proteins are mediated by

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Table I. TRANSPORT SIGNALS

| Signal | Sequence |
|-----------------------------------|--|
| Nuclear Localization Signal (NLS) | |
| Basic monopartite type | |
| SV40 large T-antigen | PKKKRKV |
| Adenovirus E1A | KRPRP |
| p53 | PQPKKKP |
| Basic bipartite type | |
| Nucleoplasmin | KRPAAIKKAGQAKKKK |
| CBP80 (cap-binding protein 80) | RRRHSDENDGGQPHKRRK |
| Other | |
| hnRNP A1 (M9 sequence) | NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY |
| Nuclear Export Signal (NES) | |
| Protein kinase inhibitor | LALKLAGLDIN |
| HIV-1 Rev | LQLPPLERLTLD |
| MAP kinase kinase | LGKKLEEELE |

short amino acid stretches termed nuclear localization signals (NLSs) and nuclear export signals (NESs), respectively (Table I) (11, 31). The classical NLSs, which are most intensively characterized, contain a cluster of basic amino acids, and, as a result, the signals are also called basic type NLSs. Several different types of NLSs such as M9 of the heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) have now been identified that show no apparent homology to each other. Meanwhile, the NESs which have been identified to date are rich in hydrophobic amino acids such as leucine or isoleucine, and the leucine residues are critical for their activity. Therefore, such signals are called leucine-rich NESs.

The NLS-mediated nuclear import and NES-mediated nuclear export require specific soluble factors. The import of substrates containing the classical NLS such as that of SV40 large T-antigen is initiated by the formation of NLS-dependent complex, termed the nuclear pore-targeting complex, in the cytoplasm to target the NPC (15, 51). A small GTPase Ran and its interacting protein NTF2 (nuclear transport factor 2) are involved in the subsequent translocation of the complex through the NPC. This targeting complex is composed of two essential components, importin α (karyopherin α) and importin β (karyopherin β). Importin β transports the classical NLS-substrate bound to importin α into the nucleus via its interaction with the NPC (11, 31, 51). The M9 import sequence is directly recognized and carried into the nucleus by transportin, one of the importin β -like molecules (35). Leucine-rich NES containing substrates form a ternary complex with CRM1/exportin 1, one of the importin β homologues, in conjunction with the GTP-bound form of Ran (RanGTP) in the nucleus and are exported as a tri-

meric complex to the cytoplasm (44).

Thus, it has been demonstrated that importin β constitutes a large, interspecies protein family. The complete yeast genome reveals that at least 13 open reading frames appear to encode proteins which belong to the importin β family. Although it has yet to be demonstrated whether all of these 13 proteins are actually involved in nuclear import/export, all the proteins which have already been investigated have turned out to function as a nuclear import/export receptor for proteins or RNAs. The details for these importin β family molecules are reviewed elsewhere (45).

In this review, we will focus on another series of soluble transport factors, importin α and Ran. In particular, we will pay attention to the biological significance of the issues that the mammalian importin α constitutes a large family, and that Ran shuttles between the nucleus and the cytoplasm.

I. Functional divergence of importin α molecules

Importin α recognizes the basic-type NLS and binds to importin β via its N-terminal sequences which are rich in basic amino acids, and referred to as the IBB (importin β binding) domain (10). That is, importin α functions as an adapter molecule between the NLS and importin β . One of the most remarkable features of the structure of this protein is the tandemly repeated modules known as armadillo (arm) motifs which are located in the central domain (9, 16, 34). The crystal structure of the 50 kDa central portion of the yeast importin α (full size 60 kDa) shows that this molecule contains a tandem array of ten armadillo repeats, organized in a right-handed superhelix of helices (3). Moreover, it has been found that the NLS binds to two

sites within a helical surface groove, which is consistent with the proposal that the arm repeats contain the binding site for the basic type NLS (38).

Yeast importin α , named Srp1, was first identified as a suppressor of temperature-sensitive RNA polymerase I mutation and found to be a product of an essential gene (48). The complete yeast genome reveals that *S. cerevisiae* has a single gene for importin α . In contrast, importin α of higher eukaryotes constitutes a multigene family which has a considerable degree of sequence homology. In mammals, it has been demonstrated that the importin α family molecules can be classified into three distinct subgroups, subfamilies of so-called Rch1, NPI-1, and Qip1 (28, 29). These subfamilies show an $\sim 50\%$ amino acid identity with one another, and individuals within the same subfamily

show more than an 80% amino acid identity (43).

Although it was demonstrated that Rch1 and NPI-1 are able to transport the SV40 T-NLS substrates into the nucleus with nearly equal efficiency in a permeabilized cell-free transport assay, it is also apparent that these three structurally distinct importin α molecules have functional divergence. Qip1 is the most efficient in mediating the nuclear import of the substrate containing the NLS of DNA helicase Q1, but transports the substrates containing the monopartite NLS of SV40 T-antigen and the bipartite NLS of CBP80 (cap-binding protein 80) in a very inefficient manner (28). Moreover, it has been found that efficient and specific NLS recognition by Qip1 requires both the core basic NLS sequence of the helicase and its upstream sequence, despite the fact that only the core NLS sequence is re-

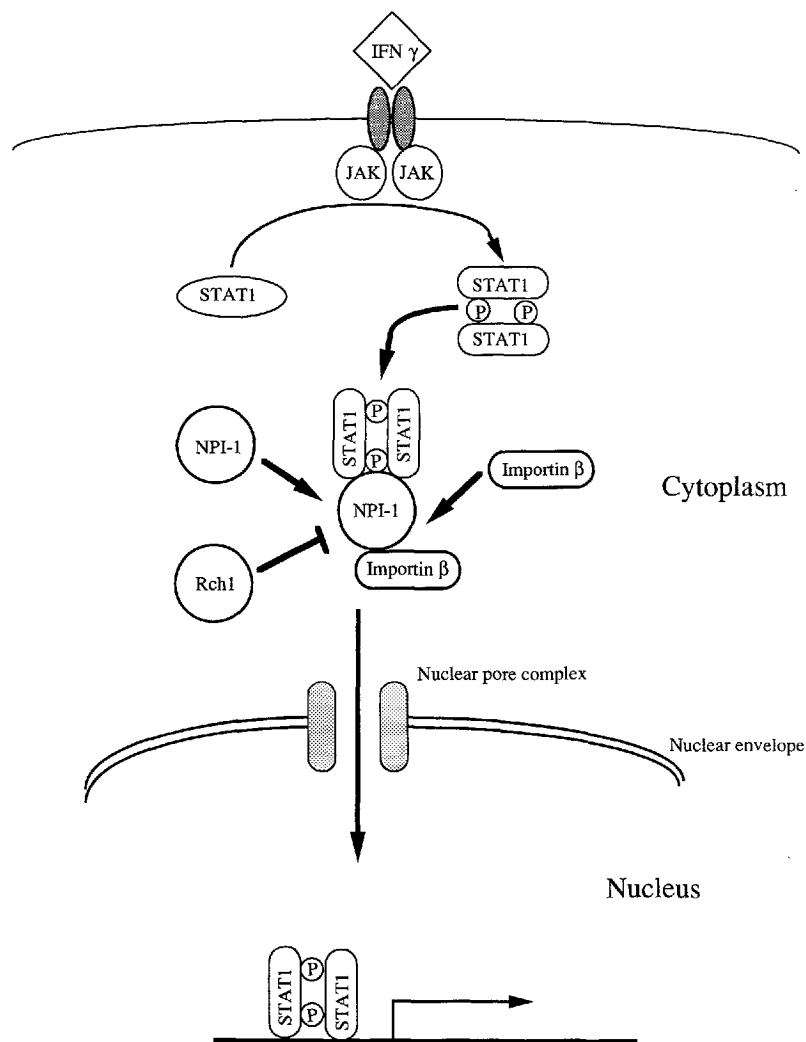


Fig. 1. Scheme for STAT1 import. In response to interferon- γ stimulation, STAT1 is tyrosine-phosphorylated by Jaks and forms a dimer. The STAT1 dimer is recognized by NPI-1, but not Rch1. Importin β binds to NPI-1 to target nuclear pores.

quired and sufficient for the recognition by Rch1 and NPI-1. It was concluded that the upstream region, rather than the basic cluster of the NLS, plays a crucial role in the efficient recognition of helicase Q1-NLS by Qip1, although the issue of how Qip1 specifically recognizes the upstream region near the NLS remains unknown (28). That is, Qip1 may be referred to as a cargo-specific NLS receptor.

Meanwhile, it has become evident that the NPI-1 family is functionally distinct from the Rch1 family. The interferon- γ -dependent nuclear accumulation of STAT1 (signal transducers and activators of transcription 1) is mediated by the NPI-1 family, but not the Rch1 family, in conjunction with importin β (38, 39). Interestingly, the STAT1-binding domain of NPI-1 is located in the C-terminal non-armadillo region, which is clearly distinct from the SV40 T-NLS-binding region located in the arm-motifs (38). The amino acid sequences of the C-terminal non-armadillo region of these family members have a low degree of similarity, and it is likely that the non-conserved C-terminal region provides the functional divergence of each family member. That is, the NPI-1 family, but not the Rch1 family, mediates the conditional nuclear import as well as the constitutive nuclear import, although it is not yet determined whether the Qip1 family is involved in the conditional nuclear import.

Recently, it was demonstrated by using the subfamily-specific antibodies that these three subclasses of importin α are differentially expressed in adult mouse tissues (19), which is consistent with the previous studies, based on Northern blotting of mRNAs from various tissues (20, 29, 30, 43). In particular, in brain, the expression of the Rch1 family is not detected, whereas the NPI-1 family is significantly expressed in Purkinje cells and pyramidal cells of the hippocampus and cerebral cortex. In addition, the expression is located not only in the soma but also in the dendrites (19). Note that these cells receive input signals at their neurites and transmit output signals to other regions along their axons. Considering the fact that NPI-1 plays an important role in the nuclear import of signaling molecules such as STAT1, it is possible that the NPI-1 family molecules are critically involved in the signal transduction in neurons.

II. Identification of adapter molecules other than importin α for importin β

It has been shown that functional redundancy of the nuclear import by importin β is further established by other types of adapter molecules, one of which is snurportin 1 (14). This 45 kDa protein was shown to be specifically involved in the 5'-trimethylguanosine cap structure-mediated nuclear import of U snRNPs. This pro-

tein has an IBB domain at its N-terminus but does not contain any structure similar to the arm-motif of importin α . More recently, another example, XRIP α , was identified as a protein which interacts with replication protein A (RPA) by means of yeast two-hybrid screening (18). It was demonstrated that XRIP α is required for the nuclear import of RPA but not for nucleoplasmin, which contains a bipartite type of classical NLS. Although XRIP α does not have a typical IBB domain, it interacts directly with recombinant importin β and reconstitutes RPA import in the presence of recombinant importin β . Therefore, it is possible that XRIP α functions as an adapter to link RPA to importin β . Thus, although it has been shown that adapter molecules for importin β have structural and functional diversity, the common feature among these adapters is that they are dissociated from importin β by RanGTP.

III. The directionality of nuclear transport of proteins and Ran

A small GTPase Ran is essential for the active nuclear import and export of molecules through the NPC (11, 31, 51). Ran is predominantly, but not exclusively, located in the nucleus (41). Judging from the findings described below, it can be concluded that a steep gradient might exist for RanGTP across the nuclear envelope, with a high level in the nucleus and a low level in the cytoplasm (17). Like other small GTPases, the Ran GTPase cycle is regulated by various interacting proteins. RCC1 functions as the GDP/GTP exchange factor of Ran (RanGEF), which charges Ran with GTP, and is localized in the nucleus (37). RanGAP1 (Ran GTPase-activating protein 1) enhances the GTPase activity, thus accelerating the conversion of RanGTP to RanGDP (1, 4). RanBP1 and RanBP2 further stimulate the activity of RanGAP1 through their interaction with RanGTP (5, 27). RanBP1 is exclusively cytoplasmic and RanBP2 is located on the cytoplasmic fibrils of the NPC (17, 50). RanGAP1, modified with the ubiquitin-related protein SUMO-1 (small ubiquitin-related modifier 1), is associated with RanBP2, and a fraction of RanGAP1 is also cytoplasmic (25). That is, the conversion of the GDP-bound form of Ran to the GTP-bound form occurs exclusively in the nucleus, whereas the opposite reaction occurs in the cytoplasm.

Biochemical studies have shown that import carriers, such as importin β and transportin interact with their cargoes in the absence of RanGTP and that RanGTP dissociates the cargo/import carrier complex (12). This dissociation reaction would be expected to occur in the nucleus where the concentration of RanGTP is estimated to be high. The import carrier molecules form a stable complex with RanGTP and the complex is disassembled by RanGAP1 and RanBP1

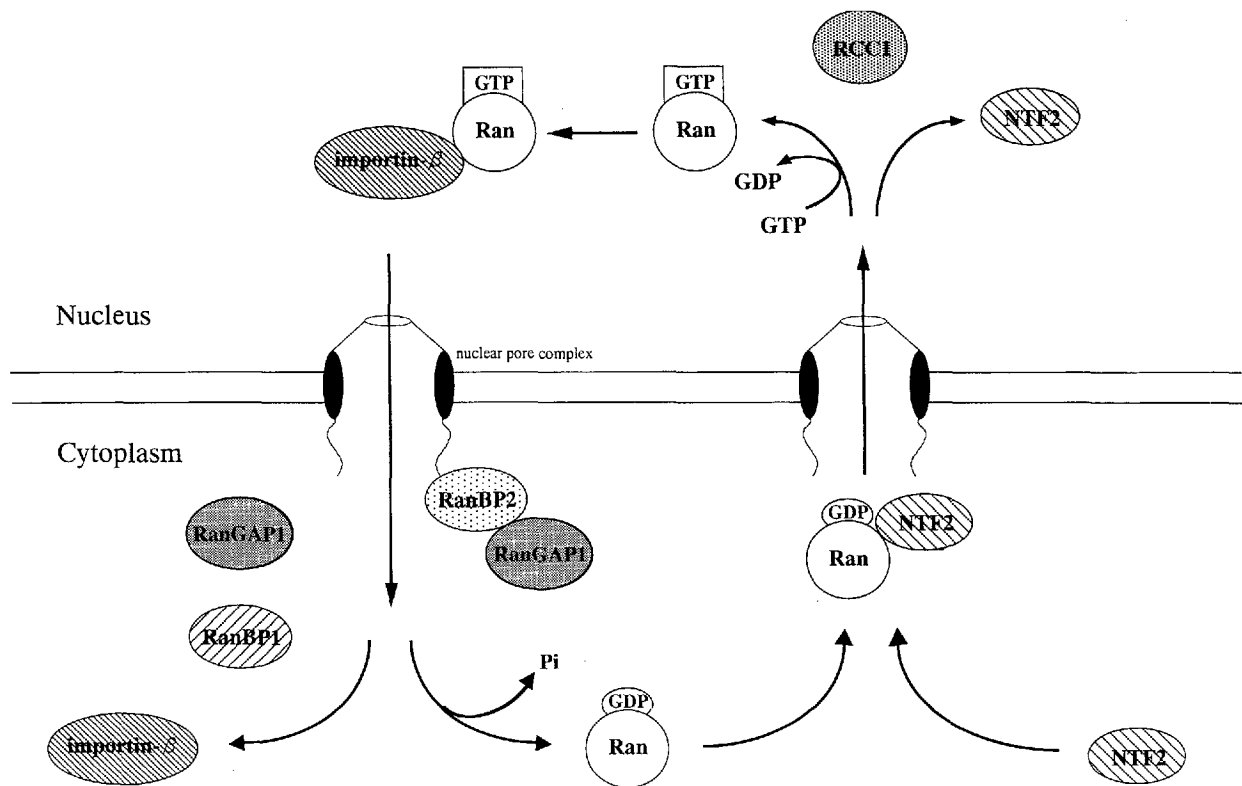


Fig. 2. Recycling of Ran. RanGTP is exported from the nucleus as a complex along with importin β family molecules. After translocation through the NPC, RanGTP is converted to RanGDP with the aid of RanGAP1 and RanBP1/RanBP2 in the cytoplasm and dissociates from the importin β family molecules. NTF2 carries RanGDP into the nucleus. In the nucleus, the NTF2/Ran complex dissociates and the nucleotide exchange occurs by RCC1 to generate RanGTP.

simultaneously with the conversion of RanGTP to RanGDP (2). A similar reaction appears to be induced by the SUMO-1-modified RanGAP1 complexed with RanBP2 at the cytoplasmic fibrils of the NPC. In contrast, the binding of export carriers such as CRM1 and exportin-t (export carrier for tRNA) to their cargoes is significantly enhanced by the simultaneous binding of RanGTP (7, 24, 40). The GTP hydrolysis of Ran stimulated by RanGAP1 and RanBP1 (or RanBP2) results in the dissociation of the export cargo/export carrier/RanGTP complex and release of the cargo. This reaction would be expected to occur in the cytoplasm or at the cytoplasmic face of the NPC.

Based on these findings, the following scheme is proposed. The classical NLS is recognized by importin α and the importin β binds to importin α bound to the NLS-substrate to form a heterotrimeric complex in the cytoplasm. The complex docks at the NPC via interactions of importin β with the NPC proteins (nucleoporins), and is then translocated through the NPC, and RanGTP binds to importin β in the nucleus thus triggering the dissociation of the complex (termination of import). The RanGTP/importin β complex is trans-

ported from the nucleus to the cytoplasm for the next cycle of the import. Conversely, the leucine-rich NES is recognized by CRM1 in the presence of RanGTP, giving rise to the NES/CRM1/RanGTP trimer in the nucleus. The trimer is translocated through the NPC to the cytoplasm without GTP hydrolysis. After the translocation, RanGTP is converted to RanGDP with the aid of RanGAP1 and RanBP1/RanBP2 in the cytoplasm and the trimer dissociates (26, 32). Thus, Ran plays a critical role in the directionality of nucleocytoplasmic transport, and ensures that the transport factors can carry their cargoes unidirectionally.

IV. Recycling of Ran and nuclear protein transport

As described above, it has been proposed that RanGTP is exported from the nucleus as complexes with importin β family molecules such as importin β , transportin, CRM1 and CAS (the export factor for importin α). Recently, direct *in vivo* evidence for this proposal has been obtained. A monoclonal antibody against human Ran, designated ARAN1, which recognizes an epitope in the C-terminal acidic portion of

Ran, binds to Ran when complexed with importin β , transportin and CAS, but not RanGDP or RanGTP alone. When ARAN1 was injected into the nucleus, it was exported to the cytoplasm, indicating that the Ran/importin β family protein complex is exported as a complex to the cytoplasm in living cells. Moreover, it was found that endogenous Ran accumulates in the cytoplasm and that the nuclear import of SV40 T-NLS substrates is prevented in ARAN1-injected cells, thus providing *in vivo* evidence that Ran is actually recycled from the cytoplasm to the nucleus and that this recycling is critical for nuclear protein transport (13).

It is interesting to note that NTF2, the precise function of which had remained unclear, has been shown to mediate the nuclear import of Ran as a carrier (36). It has also been demonstrated that NTF2 binds cytoplasmic RanGDP to dock on the cytoplasmic face of the NPC, and the NTF2/RanGDP complex is then trans-

located through the NPC, although the precise mechanism of the translocation step remains obscure. In addition, biochemical analysis shows that NTF2 inhibits the dissociation of GDP, but not GTP, from Ran by RCC1, the only known mammalian guanine nucleotide exchange factor for Ran (46). These results indicate that NTF2 functions as a GDP dissociation inhibitor for Ran (RanGDI). Thus, NTF2 appears to be crucial in maintaining the RanGTP concentration gradient across the nuclear envelope. Considering these two recent studies, it has been proposed that NTF2 carries RanGDP into the nucleus with maintaining Ran in the GDP-bound form through its GDI activity. Thus, although Ran is sufficiently small to diffuse passively, the transport of Ran through the NPC is mediated by importin β family molecules for export and by NTF2 for import.

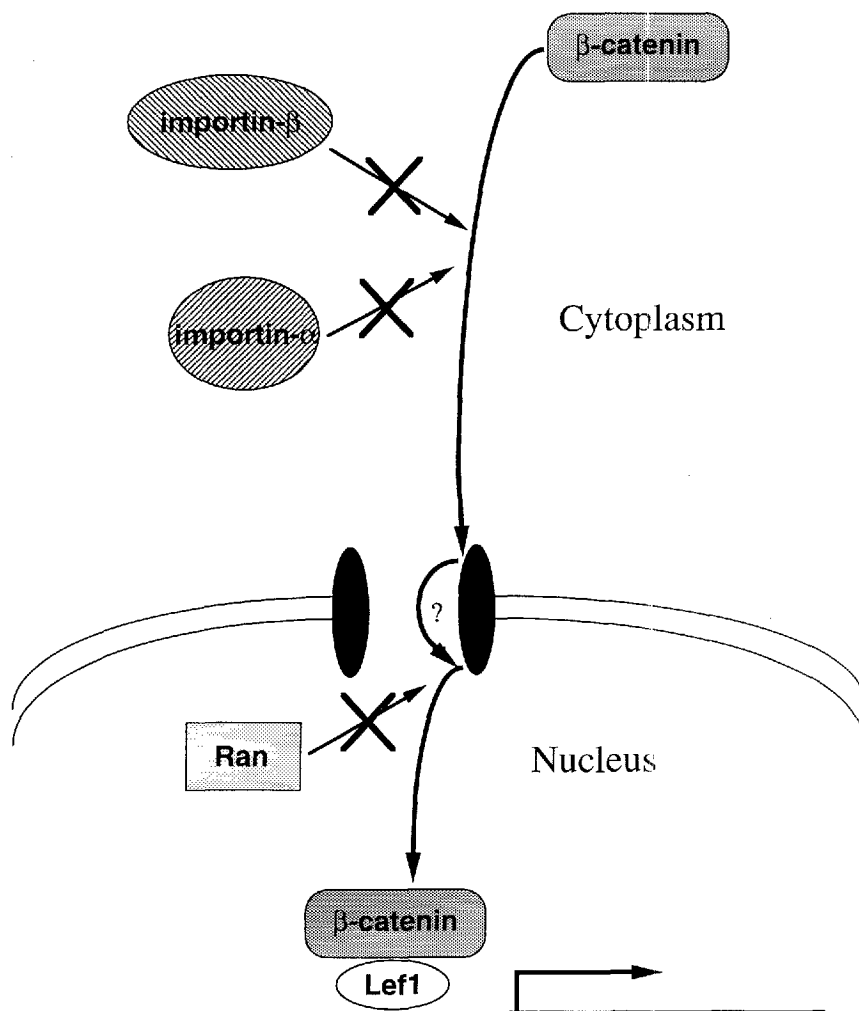


Fig. 3. Nuclear import of β -catenin. β -Catenin alone migrates into the nucleus without the aid of importin-like transport factors in a Ran-independent manner.

V. Ran-independent transport

A large number of studies have indicated the significance of Ran in relation to the nucleocytoplasmic transport of molecules. However, it has been reported that molecules exist which can be transported through the NPC in a Ran-independent manner. The first obvious example was shown by a study of the behavior of importin β (21). The use of deletion mutants of importin β showed that the nuclear import of importin β requires neither Ran- nor importin α -binding but only the NPC-binding domain. Furthermore, a dominant-negative mutant Ran, defective in GTP-hydrolysis, failed to inhibit the nuclear import of importin β . These results indicate that Ran is required only when importin β carries an importin α /NLS substrate complex into the nucleus. Similar observations have been reported for other importin β -related molecules, suggesting that Ran-independent import could be a common feature for importin β -related proteins (23). Moreover, it has been demonstrated that importin β alone can be exported from the nucleus in a manner dependent on the NPC-binding domain of this molecule in a nuclear RanGTP-independent manner (22). Therefore, it is likely that importin β is recycled from the nucleus in two distinct ways: 1) as a complex with RanGTP, and 2) alone in a Ran-independent manner.

Another example of Ran-independent transport became evident from a study of the nuclear accumulation of β -catenin, which is important for the Wingless/Wnt signaling. The Wingless/Wnt signal triggers an increase in the cytoplasmic pool of β -catenin, and free cytoplasmic β -catenin accumulates in the nucleus, where it activates the transcription of Wingless-responsive genes, along with DNA-binding proteins called the LEF/TCF (lymphocyte enhancer factor/T-cell factor) family proteins. It had previously been thought that β -catenin migrates into the nucleus in the form of a complex with the LEF/TCF family of molecules, since LEF/TCF family proteins have typical basic-type NLS but β -catenin does not. However, it was recently reported that β -catenin possesses the ability to constitutively translocate through the NPC in a manner very similar to that of importin β in a Ran-independent manner without the aid of any transport factors (49). This import requires specific molecular interactions at the NPC. These findings will provide a new insight into the yet unsolved question regarding the molecular mechanism by which proteins move through the NPC with directionality.

VI. Conclusion

Significant progress has been made in our understanding of the molecular mechanism of the nucleo-

cytoplasmic transport of proteins through the NPC. At present, intensive studies are focussed on two types of key molecules, importin β -like carrier molecules and Ran. A number of importin β -related proteins have been identified as import and export factors. These factors have two common properties, namely, their ability to bind to NPC components (nucleoporins) as well as to RanGTP. The binding of RanGTP to the import factors causes the dissociation of import cargoes from the import factors, whereas binding to the export factors results in the stabilization of the export cargo/export factor complex and the dissociation occurs with GTP hydrolysis. Thus, Ran acts as a determinant for both the initiation and termination of transport. Although it has been demonstrated that Ran ensures the directionality of the transport through the NPC, the issue of precisely how the transport complexes move across the NPC remains obscure (32). Meanwhile, whereas the importin β family molecules have been intensively characterized, the issue concerning the biological significance of the molecular divergence of the importin α family and other adapter molecules remains unanswered. More efforts toward understanding the translocation steps through the NPC as well as the issue of how many transport pathways exist in cells are urgently needed (51).

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References

1. BISCHOFF, F.R., KLEBE, C., KRETSCHMER, J., WITTINGHOFFER, A., and PONSTINGL, H. 1994. RanGAP1 induces GTPase activity of nuclear Ras-related Ran. *Proc. Natl. Acad. Sci. USA*, **91**: 2587–2591.
2. BISCHOFF, F.R. and GÖRLICH, D. 1997. RanBP1 is crucial for the release of RanGTP from importin β -related nuclear transport factors. *FEBS Lett.*, **419**: 249–254.
3. CONTI, E., UY, M., LEIGHTON, L., BLOBEL, G., and KURIYAN, J. 1998. Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin α . *Cell*, **94**: 193–204.
4. CORBETT, A.H., KOEPP, D.M., SCHLENSTEDT, G., LEE, M.S., HOPPER, A.K., and SILVER, P.A. 1995. Rna1p, a Ran/TC4 GTPase activating protein, is required for nuclear import. *J. Cell Biol.*, **130**: 1017–1026.
5. COUTAVAS, E., REN, M., OPPENHEIM, J.D., D'EUSTACHIO, P., and RUSH, M.G. 1993. Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature*, **366**: 585–587.
6. DAVIS, L.I. 1995. The nuclear pore complex. *Annu. Rev. Biochem.*, **64**: 865–896.
7. FORNEROD, M., OHNO, M., YOSHIDA, M., and MATTAJ, I.W. 1997. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell*, **90**: 1051–1060.
8. GOLDBERG, M.W. and ALLEN, T.D. 1995. Structural and func-

- tional organization of the nuclear envelope. *Curr. Opin. Cell Biol.*, **7**: 301–309.
9. GÖRLICH, D., PREHN, S., LASKEY, R.A., and HARTMANN, E. 1994. Isolation of a protein that is essential for the first step of nuclear protein import. *Cell*, **79**: 767–778.
 10. GÖRLICH, D., HENKLEIN, P., LASKEY, R.A., and HARTMANN, E. 1996. A 41 amino acid motif in importin- α confers binding to importin- β and hence transit into the nucleus. *EMBO J.*, **15**: 1810–1817.
 11. GÖRLICH, D. and MATTAJ, I.W. 1996. Nucleocytoplasmic transport. *Science*, **271**: 1513–1518.
 12. GÖRLICH, D., PANTÉ, N., KUTAY, U., AEBI, U., and BISCHOFF, F.R. 1996. Identification of different roles for RanGDP and RanGTP in nuclear protein import. *EMBO J.*, **15**: 5584–5594.
 13. HIEDA, M., TACHIBANA, T., YOKOYA, F., KOSE, S., IMAMOTO, N., and YONEDA, Y. 1999. A monoclonal antibody to the COOH-terminal acidic portion of Ran inhibits both the recycling of Ran and nuclear protein import in living cells. *J. Cell Biol.*, **144**: 645–655.
 14. HUBER, J., CRONSHAGEN, U., KADOKURA, M., MARSHALLSAY, C., WADA, T., SEKINE, M., and LÜHRMANN, R. 1998. Snurportin 1, an m³G-cap-specific nuclear import receptor with a novel domain structure. *EMBO J.*, **14**: 4114–4126.
 15. IMAMOTO, N., TACHIBANA, T., MATSUBAE, M., and YONEDA, Y. 1995. A karyophilic protein forms a stable complex with cytoplasmic components prior to nuclear pore binding. *J. Biol. Chem.*, **270**: 8559–8565.
 16. IMAMOTO, N., SHIMAMOTO, T., TAKAO, T., TACHIBANA, T., KOSE, S., MATSUBAE, M., SEKIMOTO, T., SHIMONISHI, Y., and YONEDA, Y. 1995. *In vivo* evidence for involvement of a 58 kDa component of nuclear pore-targeting complex in nuclear protein import. *EMBO J.*, **14**: 3617–3626.
 17. IZAURRALDE, E., KUTAY, U., VON KOBEE, C., MATTAJ, I.W., and GÖRLICH, D. 1997. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J.*, **16**: 6535–6547.
 18. JULLIEN, D., GÖRLICH, D., LAEMMLI, U.K., and ADACHI, Y. 1999. Nuclear import of RPA in *Xenopus* egg extracts requires a novel protein XRIP α but not importin α . *EMBO J.*, **18**: 4348–4358.
 19. KAMEI, Y., YUBA, S., NAKAYAMA, T., and YONEDA, Y. 1999. Three distinct classes of the α -subunit of the nuclear pore-targeting complex (importin- α) are differentially expressed in adult mouse tissues. *J. Histochem. Cytochem.*, **47**: 363–372.
 20. KÖHLER, M., ANSIEAU, S., PREHN, S., LEUTZ, A., HALLER, H., and HARTMANN, E. 1997. Cloning of two novel human importin- α subunits and analysis of the expression pattern of the importin- α protein family. *FEBS Lett.*, **417**: 104–108.
 21. KOSE, S., IMAMOTO, N., TACHIBANA, T., SHIMAMOTO, T., and YONEDA, Y. 1997. Ran-unassisted nuclear migration of a 97-kDa component of nuclear pore-targeting complex. *J. Cell Biol.*, **139**: 841–849.
 22. KOSE, S., IMAMOTO, N., TACHIBANA, T., YOSHIDA, M., and YONEDA, Y. 1999. β -Subunit of nuclear pore-targeting complex (importin- β) can be exported from the nucleus in a Ran-independent manner. *J. Biol. Chem.*, **274**: 3946–3952.
 23. KUTAY, U., BISCHOFF, F.R., KOSTKA, S., KRAFT, R., and GÖRLICH, D. 1997. Export of importin α from the nucleus is mediated by a specific nuclear transport factor. *Cell*, **90**: 1061–1071.
 24. KUTAY, U., LIPOWSKY, G., IZAURRALDE, E., BISCHOFF, F.R., SCHWARZMAIER, P., HARTMANN, E., and GÖRLICH, D. 1998. Identification of a tRNA-specific nuclear export receptor. *Mol. Cell*, **1**: 359–369.
 25. MAHAJAN, R., DELPHIN, C., GUAN, T., GERACE, L., and MELCHIOR, F. 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell*, **88**: 97–107.
 26. MATTAJ, I.W. and ENGLMEIER, L. 1998. Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.*, **67**: 265–306.
 27. MELCHIOR, F. and GERACE, L. 1998. Two-way trafficking with Ran. *Trends Cell Biol.*, **8**: 175–179.
 28. MIYAMOTO, Y., IMAMOTO, N., SEKIMOTO, T., TACHIBANA, T., SEKI, T., TADA, S., ENOMOTO, T., and YONEDA, Y. 1997. Differential modes of nuclear localization signal (NLS) recognition by three distinct classes of NLS receptors. *J. Biol. Chem.*, **272**: 26375–26381.
 29. NACHURY, M., RYDER, U.W., LAMOND, A.I., and WEIS, K. 1998. Cloning and characterization of hSRP1 γ , a tissue-specific nuclear transport factor. *Proc. Natl. Acad. Sci. USA*, **95**: 582–587.
 30. NADLER, S.G., TRITSCHLER, D., HAFFAR, O.K., BLAKE, J., BRUCE, A.G., and CLEAVELAND, J.S. 1997. Differential expression and sequence-specific interaction of karyopherin α with nuclear localization sequences. *J. Biol. Chem.*, **272**: 4310–4315.
 31. NIGG, E.A. 1997. Nucleocytoplasmic transport: signals, mechanisms and regulation. *Nature*, **386**: 779–787.
 32. OHNO, M., FORNEROD, M., and MATTAJ, I.W. 1998. Nucleocytoplasmic transport: the last 200 nanometers. *Cell*, **92**: 327–336.
 33. PANTÉ, N. and AEBI, U. 1995. Exploring nuclear pore complex structure and function in molecular detail. *J. Cell Sci.*, **Supplement 19**: 1–11.
 34. PEIFER, M., BERG, S., and REYNOLDS, A.B. 1994. A repeating amino acid motif shared by proteins with diverse cellular roles. *Cell*, **76**: 789–791.
 35. POLLARD, V.W., MICHAEL, W.M., NAKIELNY, S., SIOMI, M.C., WANG, F., and DREYFUSS, G. 1996. A novel receptor-mediated nuclear protein import pathway. *Cell*, **86**: 985–994.
 36. RIBBECK, K., LIPOWSKY, G., KENT, H.M., STEWART, M., and GÖRLICH, D. 1998. NTF2 mediates nuclear import of Ran. *EMBO J.*, **17**: 6587–6598.
 37. SEKI, T., HAYASHI, N., and NISHIMOTO, T. 1996. RCC1 in the Ran pathway. *J. Biochem.*, **120**: 207–214.
 38. SEKIMOTO, T., IMAMOTO, N., NAKAJIMA, K., HIRANO, T., and YONEDA, Y. 1997. Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. *EMBO J.*, **16**: 7067–7077.
 39. SEKIMOTO, T. and YONEDA, Y. 1998. Nuclear import and export of proteins: the molecular basis for intracellular signaling. *Cytokine Growth Factor Rev.*, **9**: 205–211.
 40. STADE, K., FORD, C.S., GUTHRIE, C., and WEIS, K. 1997. Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell*, **90**: 1041–1050.
 41. TACHIBANA, T., IMAMOTO, N., SEINO, H., NISHIMOTO, T., and YONEDA, Y. 1994. Loss of RCC1 leads to suppression of nuclear protein import in living cells. *J. Biol. Chem.*, **269**: 24542–24545.
 42. TALCOTT, B. and MOORE, M.S. 1999. Getting across the nuclear pore complex. *Trends Cell Biol.*, **9**: 312–318.
 43. TSUI, L., TAKUMI, T., IMAMOTO, N., and YONEDA, Y. 1997. Identification of novel homologues of mouse importin α , the α subunit of the nuclear pore-targeting complex, and their tissue-specific expression. *FEBS Lett.*, **416**: 30–34.
 44. ULLMAN, K.S., POWERS, M.A., and FORBES, D.J. 1997. Nu-

- clear export receptors: from importin to exportin. *Cell*, **90**: 967–970.
45. WOZNIAK, R.W., ROUT, M.P., and AITCHISON, J.D. 1998. Karyopherins and kissing cousins. *Trends Cell Biol.*, **8**: 184–188.
46. YAMADA, M., TACHIBANA, T., IMAMOTO, N., and YONEDA, Y. 1998. Nuclear transport factor p10/NTF2 functions as a Ran-GDP dissociation inhibitor (Ran-GDI). *Curr. Biol.*, **8**: 1339–1342.
47. YANG, Q., ROUT, M.P., and AKEY, C.W. 1998. Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. *Mol. Cell*, **1**: 223–234.
48. YANO, R., OAKES, M., YAMAGISHI, M., DODD, J.A., and NOMURA, M. 1992. Cloning and characterization of *SRP1*, a suppressor of temperature-sensitive RNA polymerase I mutations, in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **12**: 5640–5651.
49. YOKOYA, F., IMAMOTO, N., TACHIBANA, T., and YONEDA, Y. 1999. β -Catenin can be transported into the nucleus in a Ran-unassisted manner. *Mol. Biol. Cell*, **10**: 1119–1131.
50. YOKOYAMA, N., HAYASHI, N., SEKI, T., PANTÉ, N., OHBA, T., NISHII, K., KUMA, K., HAYASHIDA, T., MIYATA, T., AEBI, U., FUKUI, M., and NISHIMOTO, T. 1995. A giant nucleopore protein that binds Ran/TC4. *Nature*, **376**: 184–188.
51. YONEDA, Y. 1997. How proteins are transported from cytoplasm to the nucleus. *J. Biochem.*, **121**: 811–817.

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