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How Proteins Are Transported from Cytoplasm to the Nucleus[1]

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Nuclear proteins are transported actively through nuclear pores by a selective, mediated process. The process is mediated by a nuclear localization signal (NLS), and can be divided into at least two steps, (a) targeting to the pores and (b) translocation through the pores. The first step involves the formation of a stable complex containing a nuclear protein, termed nuclear pore-targeting complex (PTAC), in the cytoplasm. The second step, translocation, requires at least two soluble factors, a small GTPase Ran and its interacting protein p10/nuclear transport factor 2 (NTF2), along with nuclear pore complex components. These findings have been generally obtained by using the NLS of SV40 large T-antigen, and data concerning the detailed mechanism are now accumulating. Transport pathways other than for the SV40 T-antigen, for example, extracellular signal-dependent nuclear protein import pathway, have also recently been studied. Considering all these observations, one should be able to attain an understanding of the mechanism of intracellular information transduction between cytoplasm and the nucleus.

Key words: nuclear localization signal, nuclear pore complex, nuclear pore-targeting complex, nucleocytoplasmic transport, semi-intact cell.

One of the most remarkable features of eukaryotic cells is the variety of organelles that they contain, among are the nucleus and mitochondria, both of which are surrounded by lipid bilayers. Although each organelle has its own specific and unique role and functions independently, in a whole cell, all organelles function in harmony with each other, thereby maintaining homeostasis. Proteins are accurately synthesized on ribosomes in the cytoplasm based on information contained by mRNAs. However, proteins are not functional until they are transported accurately to their destination after their synthesis. Cells, therefore, have a precise system for protein sorting and transport.

The nucleus, in which the genomic DNA is sequestered, contains a double membrane, the nuclear envelope. Nuclear (karyophilic) proteins are synthesized on free ribosomes in the cytoplasm and transported efficiently and precisely to the nucleus through the nuclear pore complexes (NPCs) present in the nuclear envelope (*1, 2*). The NPC is a huge proteinaceous structure having a molecular mass of about 125 MDa and is currently thought to consist of 100-200 different proteins (*3, 4*). The pore complex contains an aqueous diffusion channel, approximately 10 nm in diameter, which allows non-selective passive diffusion of molecules smaller than 20-40 kDa, such as, for example, ions and metabolites. Karyophilic proteins can be actively transported through the nuclear pores by a selective, mediated process, even though they are larger than 40-60 kDa (*2-4*).

Recent electron microscopic observations indicate that the NPC consists of octagonal spoke-ring structures, and that a short fiber-like structure extends into the cytoplasm from the cytoplasmic ring, while an unusual basket-like structure extends from the nucleoplasmic ring (*2-4*). Many nuclear pore proteins (nucleoporins) are characterized by *O*-linked *N*-acetylglucosamine modifications and the presence of multiple FXFG or GLFG peptide repeats (*3*).

Various molecules, such as proteins and RNAs, are transported through the nuclear pores in both directions in order to communicate between the cytoplasm and the nucleus. Because the nucleocytoplasmic transport machinery is critical for cells, in order to maintain life, considerable efforts have been made in recent years in terms of developing an understanding of the mechanism of nucleocytoplasmic transport. Numerous experiments indicate that selective, active nuclear protein transport is mediated by a "signal" contained in the karyophilic proteins, which is referred to as a nuclear localization signal (NLS) (Table I) (*5, 6*). It is also noteworthy that the nuclear export signal (NES) required for the export of proteins from the nucleus has been recently identified (Table I) (*7-9*), but the detailed mechanism and machinery necessary for export remains unknown (*7, 8, 10*).

Signals for protein import into the nucleus

The discovery of the NLSs present in karyophilic proteins has allowed an understanding of the molecular mechanism of nuclear protein import into the nucleus. The first example to be identified at the amino-acid sequence level is the NLS of simian virus 40 (SV40) large T-antigen (*5*). Since this initial finding, a number of other NLSs have been identified by deletion or point mutagenesis experiments involving karyophilic protein genes (*5*). Unlike the signal sequences for the targeting of proteins into rough endoplasmic reticulum (rough ER) or mitochondria, the NLS is not deleted after or during translocation and remains a part of the mature molecule. Another interesting

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TABLE I. **Nuclear localization signals and nuclear export signals.**

Protein	Amino acid sequence
Nuclear localization signals	
1) Single basic type	
SV40 large T-antigen	PKKKRKV
c-Jun	RKRKL
2) Bipartite basic type	
Nucleoplasmin	KRPAAIKKAGQAKKKK
CBP80	RRRHSDENDGGQPHKRRK
3) Unclassified	
hnRNP A1	NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY
Nuclear export signals	
PKI	LALKLAGLDI
Rev	LQLPPLERLTL
MAPKK	ALQKKLEELELDE
TFIIIA	QPDASKADPLPVLENLTLK

feature of the NLS is that synthetic peptides which contain the NLS are capable of functioning as transport signals when chemically conjugated with a nonnuclear protein, such as bovine serum albumin (BSA) or immunoglobulin G (IgG) (*11, 12*).

The NLS of SV40 large T-antigen contains a basic amino acid-stretch, and nearly all the NLSs identified thereafter have been found to contain some basic amino acids. It is therefore thought that a characteristic feature of NLS's includes a basic amino acid cluster in the primary amino acid sequence of the karyophilic proteins, even though there might be no obvious consensus sequences (*5*). It was later proposed that the "classical" NLSs can be divided into two groups: (1) single basic type (for example, SV40 large T-antigen) and (2) bipartite basic type (such as nucleoplasmin), as shown in Table I (*6*).

Recently, the signal within the heterogeneous nuclear ribonucleoprotein (hnRNP) A1 that mediates its nuclear import has been determined (Table I) (*13*). A 38 amino acid region, referred to as M9, has been shown to be necessary and sufficient to localize the protein to the nucleus, but does not contain the stretches of basic amino acids that are typical for the classical NLS (*13*). Interestingly, this sequence has also been shown to act as an NES (*14*). These findings suggest that the M9-mediated import pathway is different from that mediated by the classical NLS.

Nuclear pore-targeting complex

Previous saturation kinetic experiments indicated that nuclear protein import is a receptor-mediated process. It has been shown that the selective nuclear import process can be divided into at least two steps: (1) the NLS-dependent and ATP-independent binding of nuclear proteins to the cytoplasmic surface of the nuclear pores and (2) their ATP-dependent translocation through the nuclear pores (Fig. 1) (*8, 15, 16*). Recently, a very convenient cell-free transport assay system was developed by using digitonin-permeabilized semi-intact cells, and has facilitated the identification of factors involved in nuclear protein import (*17*).

By using the cell-free transport assay, we were able to identify a stable protein complex of approximately 500 kDa containing a transport substrate (T-BSA: BSA conjugated with T-antigen NLS peptides), which has nuclear rim binding activity, even in the absence of any other soluble proteins (*18*). We therefore refer to this biologically active complex as the nuclear pore-targeting complex (PTAC)

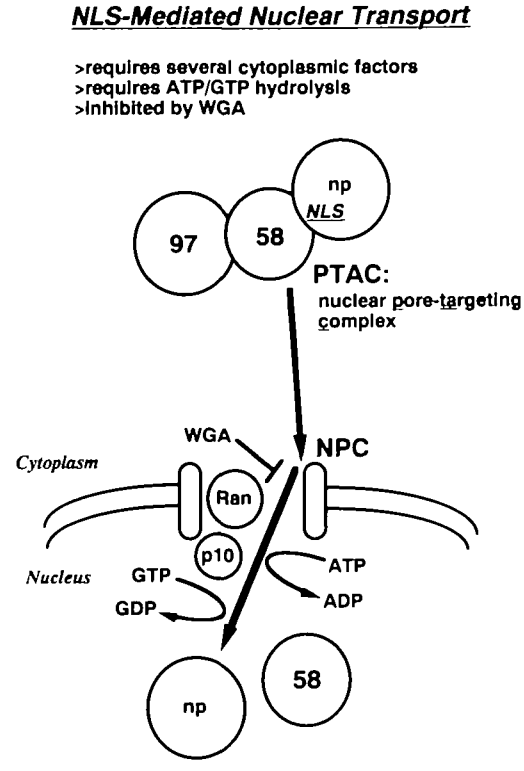

Fig. 1. **The process and soluble factors required for nuclear protein transport.** An NLS-containing karyophile forms a stable complex, termed the nuclear pore-targeting complex (PTAC), to target the nuclear pores. After binding to the cytoplasmic face of the nuclear pore complex (NPC), the complex translocates through the pores in cooperation with a small GTPase Ran and its interacting protein, p10/NTF2, in the presence of ATP/GTP. During or after the translocation step, the PTAC dissociates into the individual components. Wheat germ agglutinin (WGA) is well known to inhibit the active nuclear protein transport by binding to *O*-linked *N*-acetylglucosamine-containing nucleoporins.

(Fig. 1) (*16, 18*). Furthermore, the purification and identification of two essential components of the PTAC, PTAC58 and PTAC97, was achieved using mouse Ehrlich ascites tumor cells as the source (*19, 20*). From these findings, it was found that a karyophilic protein actually forms a complex with cytoplasmic proteins during the process of active nuclear protein import. We therefore propose that the first step of transport can be divided into two further

steps: (1) formation of the pore-targeting complex in the cytoplasm and (2) subsequent binding of the complex to the nuclear pores (*16, 19*).

At about the same time, using the cell-free *in vitro* transport assay, two soluble factors required for the translocation step (the second step) of transport were identified (*8, 15, 16, 21*). The first is the small guanosine triphosphatase (GTPase) Ran, and the second its interacting protein p10/nuclear transport factor 2 (NTF2). In the presence of these four soluble factors, the transport substrates efficiently migrate into the nucleus in the semi-intact cells (Fig. 1).

PTAC58

PTAC58 showed a 93% amino acid identity with human Rch1 (hSRP1α), 61% with *Xenopus* importin-α, and 52% with *Drosophila* pendulin (OHO31) (*19*). Human Rch1 was identified in yeast two hybrid screen with the recombination-activating protein called RAG-1. Importin-α was originally isolated from *Xenopus laevis* as an essential factor involved in the nuclear binding-step of the transport (*22*). *Drosophila* pendulin was identified as the tumor suppressor gene. They were shown to be homologues of yeast *Saccharomyces cerevisiae SRP1*, which was originally isolated as a suppressor of mutants defective in RNA polymerase I (*23*). These proteins consist of eight tandem repeats known as the armadillo structure (arm motif), which is rich in hydrophobic amino acids and was first identified in the *Drosophila* segment polarity gene armadillo (*24*). Although these homologues have been referred to by a variety of names, in this review, they will be referred to as "PTAC58" (Table II).

PTAC58 was demonstrated to directly bind to the NLS in a solution binding assay. Moreover, we found that cytoplasmic injection of anti-PTAC58 antibodies caused aggregation of T-BSA with antibodies in the cytoplasm (*19*). The *in vitro* and *in vivo* results strongly suggest that PTAC58 functions in nuclear protein import in living cells *via* association with the NLS in the cytoplasm prior to nuclear pore-targeting (*16, 19*). Detailed information relative to the exact binding site is controversial at this time. One report suggests that the C-terminal acidic residues recognize the NLS, indicating that its interaction might be electrostatic (*25*). In contrast, other studies suggest that the NLS binds directly to the arm repeats, although the number of repeats involved in its interaction are not known (*8*). Thus, further experiments are required in order to determine the exact region which is necessary and sufficient for NLS-binding.

In view of the fact that NLSs appear to have no obvious consensus sequences, it is very important to know how many isoforms of PTAC58 exist and how many NLSs are recognized by one isoform. *S. cerevisiae* is known to have one PTAC58-homologue, Srp1p, which is an essential gene product (*23*). However, a family of PTAC58 is identified in higher eukaryotes. For example, in humans, Rch1 and NPI-1 (hSRP1), which have about 50% amino acid-identity with each other, are expressed, even in the same cell (*26–28*). Moreover, *Drosophila* pendulin (OHO31) is not essential, and has been identified as the tumor suppressor gene for the malignant transformation of hematopoietic cells, which suggests the possible existence of functionally different family members which are unrelated to hematopoietic cell transformation in *Drosophila* (*29*). Moreover, since it was found that both Rch1 and NPI-1 can support almost equally the nuclear import of T-BSA in permeabilized cells, it would be interesting to understand how the NLS is recognized by the two distinct molecules in the cytoplasm, and whether they function equally as an NLS-receptor in a single cell.

TABLE II. **Nomenclature for soluble transport factors.**

Higher eukaryotes	Yeast
1) PTAC58 family	
PTAC58 (m-importin)	Srp1p
Importin-α	Kap60p
Karyopherin-α	
NLS-receptor	
hSRP1/NPI-1	
hSRP1α	
Rch1	
Pendulin/OHO31	
2) PTAC97 family	
PTAC97	Kap95p
Importin-β	Kap104p
Karyopherin-β	Kap123p
p97	Pse1p
Transportin	
3) Small GTPase Ran/TC4	
Ran	Gsp1p
TC4	
4) Ran interacting factor	
p10	
NTF2	
pp15	

PTAC97

PTAC97 is the mouse homologue of the β-subunit of importin or karyopherin, and p97. I here call it PTAC97 (Table II). Using recombinant proteins, we provided *in vitro* evidence that the first-step in nuclear protein import occurs through the formation of a complex of PTAC97 with PTAC58, bound to a karyophile (*20*). The PTAC58 binds directly to PTAC97 in a molar ratio of 1:1 (*20*). It was recently shown that PTAC97 binds to the N-terminal 41 amino acid motif of PTAC58 called the importin-β binding domain (IBB domain) (*30*). Furthermore, the fusion protein of the IBB domain to a heterologous protein is transported into the nucleus, depending on PTAC97, which indicates that PTAC97 confers the interaction with the NPC. That is, PTAC97 functions to dock the PTAC to the NPC by binding to PTAC58.

Ligand overlay assays showed that PTAC97 binds specifically to the GLFG or FXFG repeats of a subgroup of nucleoporins (*31, 32*). Moreover, it has been found that PTAC97 binds directly to Ran-GTP, but not Ran-GDP (*32, 33*). PTAC97 was found to bind to PTAC58 *via* its internal region containing two acidic loops in the *in vitro* binding assay (*34*). Interestingly, the binding site of PTAC97 for Ran-GTP overlaps with that for PTAC58, although further downstream regions are required for Ran-binding (*35*). Furthermore, it has been shown that Ran-GTP binds to PTAC97 with higher affinity than does PTAC58. From these findings, it is proposed that Ran-GTP may competitively dissociate PTAC58 from PTAC97 to form a PTAC-97/Ran-GTP heterodimer for the translocation process of the nuclear import (*35*).

Although, in the case of PTAC58, many homologous

proteins have been identified in various species, how many homologues are there for PTAC97? Earlier studies have suggested that more than one active nuclear import pathway may exist. Recently, a novel receptor-mediated nuclear import pathway was actually demonstrated at the molecular level by using a novel transport signal in the hnRNP A1, termed M9. The M9-mediated nuclear import was found to proceed *via* a specific interacting protein, called transportin (*36*). Transportin is distantly related to PTAC97. Almost simultaneously, three additional proteins, which are structurally homologous to Kap95p (yeast homologue to PTAC97), Kap104p, Kap123p, and Pse1p, were identified from the complete yeast genome database (*37*). One of these, referred to as Kap104p, has been shown to function in the nuclear import of a specific class of proteins, specifically, mRNA binding proteins, such as Nab2p and Nab4p (*37*). Thus, Kap104p is involved in a nuclear import pathway independent of the classical NLS-import pathway which requires both PTAC58 and PTAC-97. Interestingly, these PTAC97-homologous proteins interact directly with its substrates and nucleoporins to carry the substrates into the nucleus without the necessity of an NLS-binding adapter protein.

Ran and p10/NTF2

The translocation step of nuclear protein import, after the docking of the PTAC to the NPC, requires a small G protein, Ran, and p10/NTF2 in addition to the PTAC components. It has become evident that Ran plays a crucial role in nuclear protein import, and many investigators have studied it. However, the role of Ran is too complex to be clearly elucidated. Further, how p10/NTF2 acts on the translocation of the PTAC through the nuclear pore remains unclear.

Ran is located predominantly in the nucleus, but small amounts also exist in the cytoplasm. Like other GTPases, Ran acts through the cycling between GDP- and GTP-bound form. One of the most interesting features of Ran is that it interacts with a variety of proteins in a cell. At present, it is known that RCC1, which is the sole GDP-GTP exchange factor, is located only in the nucleus, and that RanGAP, which is the sole Ran GTPase activating protein, is located only in the cytoplasm. In addition to RCC1 and RanGAP, Ran is known to interact with many other molecules, such as RanBP1 and RanBP2 (*38, 39*). These molecules have been demonstrated to participate in nuclear protein import (*39, 40*).

In previous studies, using a temperature-sensitive RCC1 mutant cell line, tsBN2, we showed that the loss of RCC1 function leads to the suppression of nuclear protein import efficiency in living cells (*41*). In the same study, it was suggested that, for tsBN2 cells cultured at non-permissive temperatures, an inhibitory factor may accumulate in the cytoplasm, which was suspected to be GDP-bound form of Ran. Later, another group provided support for these observations by showing that the thermolability of nuclear protein import in tsBN2 cells is suppressed by microinjection of Ran-GTP into the cytoplasm (*42*). The *S. cerevisiae* gene, *RNA1*, encodes a protein homologous to the mammalian RanGAP. The *RNA1* gene was initially identified for mutants defective in mRNA processing. By using these mutant cells, it was demonstrated that Rna1p (RanGAP) is required for protein import across the nuclear membrane (*40*).

It is very helpful in terms of the mechanism of the nuclear protein import to understand that Ran-GTP interacts with PTAC97 *via* a sequence similar to the cytoplasmic Ran-GTP binding protein, RanBP1 (*35*). Incubation of the PTAC58/PTAC97 heterodimer with Ran-GTP leads to the dissociation of PTAC58 from PTAC97 and, in turn, to an association of Ran with PTAC97. Incubation of the PTAC containing NLS-substrate with an FXFG repeat region of nucleoporins leads to the dissociation of the NLS-substrate from the PTAC58/PTAC97 heterodimer (*32, 35*). From these findings, one model can be proposed which explains the translocation mechanism through the nuclear pore, namely that the NLS-substrate/PTAC58/PTAC97 complex migrates through the nuclear pores *via* repeated cycles of docking, release and diffusion of the complex (*32*). Alternatively, the PTAC containing NLS-substrate may move from one site on the pore complex to the next, without dissociation, with Ran providing the energy for the movement (*8, 43*). In this model, it is suggested that once the pore-targeting complex docks to the NPC, it is carried into the nucleus as a single entity without intermediate disassembly. Considering the demonstration that the Ran binding sites of the NPC involved in the translocation would be expected to induce nucleotide exchange, it appears likely that cytoplasmic Ran-GDP binds to the NPC, and the nucleotide exchange and GTP hydrolysis on NPC-bound Ran occur to generate the movement of the targeting complex through the nuclear pore (*8*).

Although more complicated to understand, it was recently suggested that a GTPase, distinct from Ran, may be involved in the nuclear protein transport (*44*). By using a mutant form of Ran, which binds specifically to xanthosine triphosphate (XTP), it was found that at least one additional GTPase may play a role in the transport. Although the GTPase was not completely identified, it is certain that the existence of other GTPase than Ran must be considered for an understanding of the exact mechanism of the translocation step.

The role of p10/NTF2 on the nuclear protein import is much less clear. p10/NTF2 binds to Ran-GDP and PTAC-97, but not to Ran-GTP. It also binds to several peptide repeat-containing nucleoporins. Furthermore, it has been shown that p10/NTF2 coordinates the formation of pentameric complex containing nucleoporin, Ran-GDP, PTAC-58/PTAC97 heterodimer and p10/NTF2 (*45*). When GTP is added to the pentameric complex, PTAC58 dissociates from the complex through *in situ* conversion of Ran-GDP to Ran-GTP. From these findings and the data described above, it is proposed that p10/NTF2 may coordinate the cycles of docking, release and diffusion of the PTAC by binding Ran-GDP with PTAC docked to nucleoporins (*45*).

We recently found that, when injected into the cytoplasm of living cells, p10/NTF2 strongly inhibits the nuclear import of co-injected NLS-substrates in a dose-dependent manner (*46*). These effects of p10/NTF2 on nuclear protein import were reproduced in the *in vitro* assay, showing that an imbalance in the concentration of p10/NTF2 relative to other import factors causes the suppression of nuclear protein import. From these findings, we speculate that p10/NTF2 not only plays an essential role on nuclear protein transport, but also regulates the nuclear transport efficiency by controlling the expression of p10/NTF2 in

cells. Alternatively, the expression of p10/NTF2 in cells may be strictly controlled so as to support constant and efficient nuclear transport (*46*).

Regulation of nuclear protein import

The intracellular behavior of some karyophilic proteins is regulated by cell cycle or extracellular signal. Nuclear accumulation of viral Jun (v-Jun) but not of cellular Jun (c-Jun) is known to be cell cycle dependent. This dependency is controlled by phosphorylation of a serine residue near its NLS (*47*). Similarly, the nuclear import of lamin-B2, inner nuclear membrane-lining protein, is inhibited by phosphorylation of serine residues adjacent to the NLS. In contrast, a transcription factor, NFκB, cannot be transported into the nucleus until its cytoplasmic anchoring protein, IκB, is phosphorylated and dissociates from NFκB after extracellular stimuli. The molecular mechanism of the nuclear import of these proteins will be discussed below.

As described above, information concerning the fundamental machinery for nuclear protein import is now increasing exponentially at the molecular level. However, little is known relative to how the import machinery is regulated, for example, by cell cycle or environmental factors. It was previously demonstrated that proliferating cells have higher import capacity than serum-starved quiescent cells (*8*). In addition, data suggesting a close relationship between protein phosphorylation and nuclear protein import are gradually accumulating (*48, 49*). Studies on the role of protein phosphorylation, analyzed in semi-intact cells, showed that phosphoproteins, probably on the NPC, are involved in the binding of NLS-substrate to the NPC and its subsequent translocation, suggesting the possibility that nuclear import can be regulated by phosphorylation-dephosphorylation reactions (*48*). We recently found that synthetic NLS-peptides stimulate the phosphorylation of several cellular proteins both *in vivo* and *in vitro* (*49*). Although the kinase which is specifically stimulated by NLS-peptides has not yet been identified, it is possible that the nuclear import efficiency may be controlled by the amount of NLS-containing proteins to be transported into the nucleus *via* this putative "NLS-dependent kinase" (*49*).

Extracellular signal-dependent conditional nuclear import *versus* constitutive nuclear import

Thus far, extensive studies have been mainly concentrated on developing an understanding of the molecular mechanism of nuclear import of the SV40 T-NLS containing substrate, and as a result, many significant findings have been obtained, as described above. The SV40 T-antigen is a good candidate for karyophilic proteins which are transported constitutively and immediately after synthesis on free ribosomes in the cytoplasm. However, how proteins, which preexist in the cytoplasm at steady state or at some phase during cell cycle, migrate into the nucleus, in response to extracellular signal or in a cell-cycle dependent manner, remains unknown. Do these proteins enter the nucleus by the same transport machinery as do SV40 T-antigen-like karyophiles?

To answer this question, we used a transcription factor, a Stat (signal transducers and activators of transcription) protein, as a model substrate (*50*). In response to interferon-γ, Stat1 is phosphorylated by the Jak family of tyrosine kinases, which results in the dimer formation, and translocation into the nucleus to directly activate target genes. In order to investigate the mechanism of interferon-γ-dependent nuclear import of Stat1, we expressed HA (an epitope of hemagglutinin of influenza virus)-tagged human Stat1, purified it to homogeneity, and microinjected it into the cytoplasm of human cells. Injected HA-tagged Stat1 was actively transported into the nucleus in an interferon-γ-dependent manner, indicating that an *in vivo* assay system for examining the Stat1 transport had been established. By using this *in vivo* system, we were able to demonstrate that the interferon-γ-dependent nuclear import also requires GTP-hydrolysis of Ran (*50*). Consistent with our data, it was found that the dexamethasone-dependent nuclear accumulation of glucocorticoid receptor-green fluorescent protein fusion was dramatically inhibited by the mutant Ran proteins, which do not cycle between the GDP- and GTP-bound form (*51*). These results suggest that it is very likely that Ran is involved in the extracellular signal-dependent nuclear import of at least some proteins, although whether its role in transport is identical to that of constitutive nuclear import of proteins, such as SV40 T-antigen, remains unclear.

Furthermore, it will be also necessary to investigate whether other essential factors, PTAC58, PTAC97, and p10/NTF2, which have been already shown to be required for the nuclear import of SV40 T-NLS substrate, are also required for the extracellular signal-dependent nuclear protein transport. In addition, the issue of whether the Ran-requiring step of these proteins compete with each other or not also remains to be elucidated. Such investigations could lead to a complete understanding of the number of independent pathways that are required for nuclear protein import in cells.

"Nuclear networking"

In eukaryotic cells, each organelle has its own specific role and appears to function separately. On the other hand, in order for a cell to function, these organelles need to communicate with each other. Interorganelle communication, especially between the nucleus and other organelles is important for the maintenance of homeostasis. This suggests that information network centered around the nucleus, which I call "nuclear networking," exists in cells, although how the network is constructed around the nucleus remains unclear.

The interaction between the nucleus and mitochondria has been investigated. A considerable number of nuclear genes encode proteins which are required for the function of mitochondria, including mitochondrial gene expression, the replication and transmission of mitochondrial DNA, and mitochondrial biogenesis. Otherwise, it has been recently suggested that mitochondria can regulate the expression of nuclear genes (*52, 53*). For example, the expression of the yeast *CIT2* nuclear gene encoding peroxisomal citrate synthase, was found to be influenced by the mitochondrial state, and some of the factors which regulate this expression were identified (*53*). Likewise, ER-nuclear signal transduction has been studied in yeast (*54*). The accumulation of malfolded proteins in the ER appears to transfer signals to the nucleus. Factors which are activated for ER-nuclear signal transduction have been actually identifi-

ed (*54*). Furthermore, it was recently found that NFκB participates in a novel ER-nuclear signal transduction pathway distinct from the unfolded-protein-dependent pathway (*55*).

However, the transport mechanism and machinery required for these organelle-nuclear communication remains unknown. It would be interesting to know whether the organelle-nuclear signal transduction pathway is distinct from the constitutive nuclear import pathway which carries the SV40 T-NLS substrate and which is described extensively above.

Future problems remaining

Many problems remain unsolved in the study of the nucleocytoplasmic transport. Although, as described above, four soluble factors required for the nuclear protein import in the *in vitro* system have been identified and appear to be able to reconstitute the import, are no other factors involved in the *in vivo* nuclear protein import? Other proteins, which has not yet been identified, may regulate the efficiency of the transport. Additional accessory factors also may play a critical role, for example, at the recognition step of the NLS by the PTAC58 family of molecules. It is important to understand how each of the PTAC58 family of molecules properly recognize an NLS, although they appear to co-exist in the same cell. Does a karyophilic protein arrive at the NPC by diffusion after PTAC formation in the cytoplasm? At the present time, there is no positive evidence that cytoskeletons (railways) are required for the nuclear protein transport. However, the issue of whether the cytoskeletal meshwork is involved in the transport or not needs to be examined, probably by new experimental approaches, since the possibility exists that its involvement may not be observed in the *in vitro* systems that are now used widely. Are the NPCs heterogeneous? Do both the import and export of proteins occur simultaneously through the same NPC? Or, does each import and export pathway have their exclusive NPC? Although the information concerning the components of the NPC and their own function is now increasing, a complete understanding of NPC function will require further, additional studies. Moreover, it can be anticipated that the mechanism of the export of proteins from the nucleus will be more completely elucidated. If these studies progress, we will be in a position to completely understand the exchange mechanism of intracellular information between the nucleus and the cytoplasm.

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