Review

Importin α: functions as a nuclear transport factor and beyond

By Masahiro OKA[*1] and Yoshihiro YONEDA[*1,†]

(Communicated by Shizuo AKIRA, M.J.A.)

Abstract: Nucleocytoplasmic transport is an essential process in eukaryotes. The molecular mechanisms underlying nuclear transport that involve the nuclear transport receptor, small GTPase Ran, and the nuclear pore complex are highly conserved from yeast to humans. On the other hand, it has become clear that the nuclear transport system diverged during evolution to achieve various physiological functions in multicellular eukaryotes. In this review, we first summarize the molecular mechanisms of nuclear transport and how these were elucidated. Then, we focus on the diverse functions of importin α, which acts not merely an import factor but also as a multi-functional protein contributing to a variety of cellular functions in higher eukaryotes.

Keywords: nuclear transport, nuclear localization signal, small GTPase Ran, nuclear pore-targeting complex, importin α, importin β

Introduction

One of the characteristic features of eukaryotic cells is that they have functional compartments called organelles such as the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, and others, which are surrounded by lipid bilayers (Fig. 1). In the nucleus, DNA is transcribed into a variety of RNAs, whereas proteins are translated from coding mRNAs on ribosomes in the cytoplasm. Thus, mature mRNAs must be accurately transported from the nucleus to the cytoplasm. For cells to function normally, after translation in the cytoplasm, proteins should be selectively and efficiently transported to their destination compartments where they play their roles. Then, proteins are degraded by the ubiquitin-proteasome system and/or autophagy after they have completed their function, although the timing of degradation depends on the features of individual proteins or cellular conditions.

The nucleus has a double membrane called the nuclear envelope. In order to move between the nucleus and the cytoplasm, proteins and RNAs must be transported efficiently through nuclear pore complexes (NPCs) that penetrate the nuclear envelope. The NPC is a large, multimeric structure that acts as a permeability barrier. Karyophilic proteins such as transcription factors, replication factors, DNA repair factors, and cell cycle regulators that function in the nucleus need to be efficiently and correctly transported into the nucleus through the NPC after their synthesis in the cytoplasm. Therefore, it is important to understand how karyophilic proteins are transported from the cytoplasm into the nucleus in order to understand their cellular physiological functions.

Approaches for understanding the molecular machineries and mechanisms for nucleocytoplasmic transport of proteins have been performed from two fundamental aspects. One is to understand the characteristic features of cargo molecules (karyophilic proteins themselves), and the other is to discover and elucidate the transport machineries and determine their characteristics. In this review, we first focus on the characteristics of karyophilic proteins that are transported in cells as cargo. Then, we will address how these cargoes are transported from the

[*1] National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka, Japan.

[†] Correspondence should be addressed: Y. Yoneda, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan (e-mail: y-yoneda@nibiohn.go.jp).

Abbreviations: NPC: nuclear pore complex; SV40: simian virus 40; NLS: nuclear localization signal; cNLS: classical nuclear localization signal; NTR: nuclear transport receptor; Nup: nucleoporin; FG repeat: phenylalanine-glycine repeat; PTAC: nuclear pore-targeting complex; KPNA: karyopherin α; KPNB: karyopherin β; IBB: importin β-binding; Arm: armadillo.

doi: 10.2183/pjab.94.018
©2018 The Japan Academy

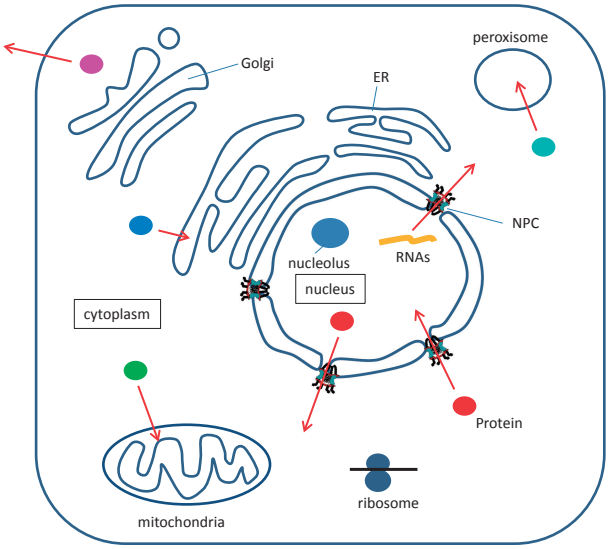


Fig. 1. Organelles surrounded by lipid bilayers and intracellular trafficking in eukaryotic cells. The transcribed RNAs are exported from the nucleus to the cytoplasm. After translation, proteins have to be targeted to their destination. The mechanism of protein delivery varies depending on the destination organelle. Proteins and RNAs are efficiently transported through the NPC, bi-directionally. NPC; nuclear pore complex, ER; endoplasmic reticulum.

cytoplasm to the nucleus and especially focus on one of the transport factors, importin α. Finally, we will discuss the diverse physiological functions of importin α.

Nuclear localization signal

It was very elegantly demonstrated that karyophilic proteins require a signal to reach the nucleus as their destination using nucleoplasmin, a nuclear protein from *Xenopus laevis*, which functions as a chaperone for nucleosome formation.[1] Nucleoplasmin (molecular mass ~30 kDa/monomer) forms a pentamer and can be biochemically cleaved into two domains, core and tail.[1] A variety of biochemically prepared nucleoplasmin fragments containing tail domains attached to a core domain were microinjected into the cytoplasm of *Xenopus* oocytes, and their subcellular localization was determined. As a result, it was found that the fragments containing more than one tail domain could enter the nucleus, whereas just the core domain without a tail domain could not, which meant that the tail domain has a specific signal for nuclear localization.

Next, using simian virus 40 (SV40), it was first demonstrated that there exists a short stretch of amino acids that direct nuclear transport in karyophilic proteins. The genome sequence of SV40 encodes several proteins including the large T antigen, which is involved in the replication of viral DNA and functions in host cell nuclei. One of the virus mutants had a point mutation that resulted in the suppression of nuclear localization of the large T antigen.[2),3)] Using this virus mutant gene, it was found that the seven amino acids, Pro-Lys-Lys-Lys-Arg-Lys-Val, act as a signal for nuclear transport, and this sequence was named the nuclear localization signal (NLS).[3)] After that, NLSs were identified one by one in various karyophilic proteins.[4)]

Because most NLSs that were initially identified usually consisted of basic amino acids such as lysine and arginine, these were called basic-type or classical NLSs (cNLSs). cNLSs are divided into two types, a monopartite type such as the NLS of the SV40 large T antigen (PKKKRKV) consisting of one cluster of basic amino acids, and a bipartite type such as that of nucleoplasmin (KRPAATKKAGQAKKKK) composed of two clusters of basic amino acids separated by linker amino acids.[5)] On the other hand, there are many karyophilic proteins whose NLSs have not yet been identified experimentally.

Breakthroughs to identify factors required for nuclear protein import

Next, researchers sought to understand nuclear transport machineries and mechanisms, and two major breakthroughs were made. One was the use of biochemical protein conjugates with synthetic peptides containing a cNLS. When a peptide consisting of the cNLS was conjugated to a non-nuclear protein, such as bovine serum albumin, and the conjugate was injected into the cytoplasm, the conjugate rapidly migrated into the nucleus of *Xenopus* oocytes or mammalian cells, meaning that the conjugate was an artificial protein but its behavior was identical to that of a native karyophilic protein.[6)–8)] Therefore, conjugates can be used as a convenient probe to identify the molecular machineries for nuclear protein import. This was one of the breakthroughs in this field, because researchers have been able to obtain soluble karyophilic proteins easily without complicated purification steps with native karyophilic proteins, which usually have low solubility and are not easy to purify.

Using such conjugates, extensive efforts have been made to establish an *in vitro* system to reproduce the nuclear import of proteins in living cells. Finally, a reproducible system was established using digitonin-permeabilized semi-intact cells (Fig. 2).[9)] Briefly, cultured cells were treated with

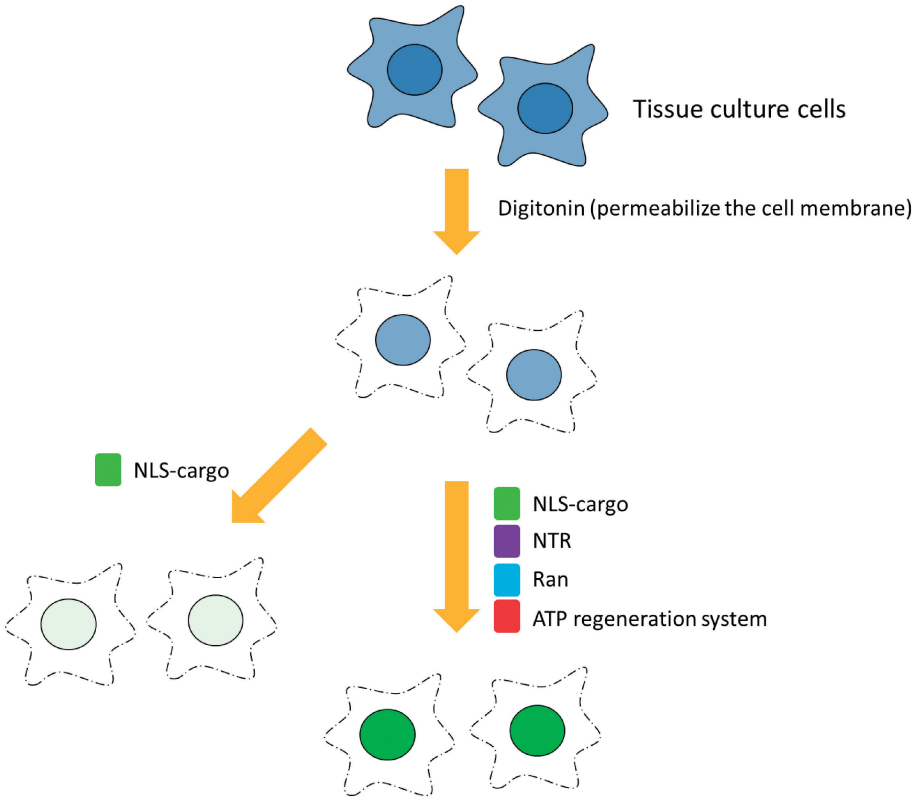


Fig. 2. *In vitro* nuclear transport system. The detergent digitonin permeabilizes the plasma membrane while leaving the nuclear membrane intact, causing the extraction of cytoplasmic materials. Addition of the nuclear transport receptors, Ran, and an ATP regeneration system is required for efficient protein import. NTR; nuclear transport receptor.

an appropriate concentration of digitonin, which specifically permeabilizes the cholesterol-rich plasma membrane, but not the cholesterol-poor nuclear envelope. This resulted in the selective permeabilization of the plasma membrane to remove cytoplasmic soluble factors, whereas the nuclear envelope and nuclear contents were intact. After permeabilization, transport substrates such as the NLS-peptide conjugates, cytosolic extracts, and an ATP-regenerating system are added to the semi-intact cells. Then, the nuclear localization of the transport substrates can be observed only in the presence of soluble factors, which means that the karyophilic protein can enter the nucleus in a factor-dependent manner. This was another breakthrough in nuclear transport research.

The nuclear transport machinery

Using the *in vitro* transport system and other methods, it has been elucidated that eukaryotic cells have dedicated machinery for the nucleocytoplasmic transport of macromolecules. This machinery consists of NPCs, nuclear transport receptors (NTRs), and the small GTPase Ran system.

Nuclear pore complex

The NPC is a large, proteinaceous structure that allows the transport of functional molecules between the cytoplasm and nucleus. Although the size of the NPC varies from ∼66 MDa in yeast to ∼125 MDa in vertebrates, its overall structure is conserved across species.[10)–15)] NPCs are composed of multiple copies of approximately 30 different proteins called nucleoporins (Nups) that can be grouped according to their sequence motifs, structural folds, location, or primary function. One characteristic sequence motif of Nups is the tandem phenylalanine-glycine repeats (FG repeats), which are found in approximately one-third of Nups.[10)] It has been suggested that the central region of the NPC consists of a meshwork of FG repeats that confer the molecular sieve function of the NPC.[16),17)] That is, FG-containing Nups form a barrier that inhibits the passive diffusion of macromolecules through the NPC, whereas NTR-bound cargoes or small molecules can pass through.

The NPC shows eight-fold rotational symmetry around its central axis.[18),19)] There are three major

octagonal rings: the cytoplasmic ring, the central spoke ring, and the nuclear ring. In contrast to the NPC central region, eight of the filament-like structures called cytoplasmic filaments extend from the cytoplasmic ring into the cytoplasm, whereas the basket-like structures called the nuclear basket extend from the nuclear ring into the nucleoplasm. Peripheral regions such as cytoplasmic filaments and the nuclear basket consist of asymmetrically arranged nucleoporins. Cytoplasmic filaments are predominantly composed of Nup88, Nup214 (CAN), and Nup358 (RanBP2), whereas the main constituents of the nuclear basket are Tpr, Nup153, and Npap60 (Nup50). The directionality of transport through NPCs is precisely regulated. Most Ran-binding nucleoporins such as Nup358, Nup153, and Npap60 that localize in peripheral regions of the NPC may play important roles in providing directionality. However, the exact mechanism for directional translocation through the NPC remains to be determined.

Nucleocytoplasmic transport receptors

The *in vitro* transport assay using digitonin-permeabilized semi-intact cells clearly indicated that the NLS-substrate does not solely enter the nucleus, but the addition of cytosolic extract can reproduce its nuclear import in the presence of an energy source, meaning that nuclear protein import requires additional factors. After biochemical purification of the cytosolic extract, we isolated a stable complex called the nuclear pore-targeting complex (PTAC) containing the NLS-substrate to target the nuclear pore and found that the complex contains two essential components, a 58-kDa protein called PTAC58 and a 97-kDa protein called PTAC97.[20),21)] Several groups independently and almost simultaneously identified these two types of NTRs, and named the molecules importin α or karyopherin α for PTAC58 and importin β or karyopherin β for PTAC97.[22)–24)] In general, the terms importin α and importin β are now widely used.

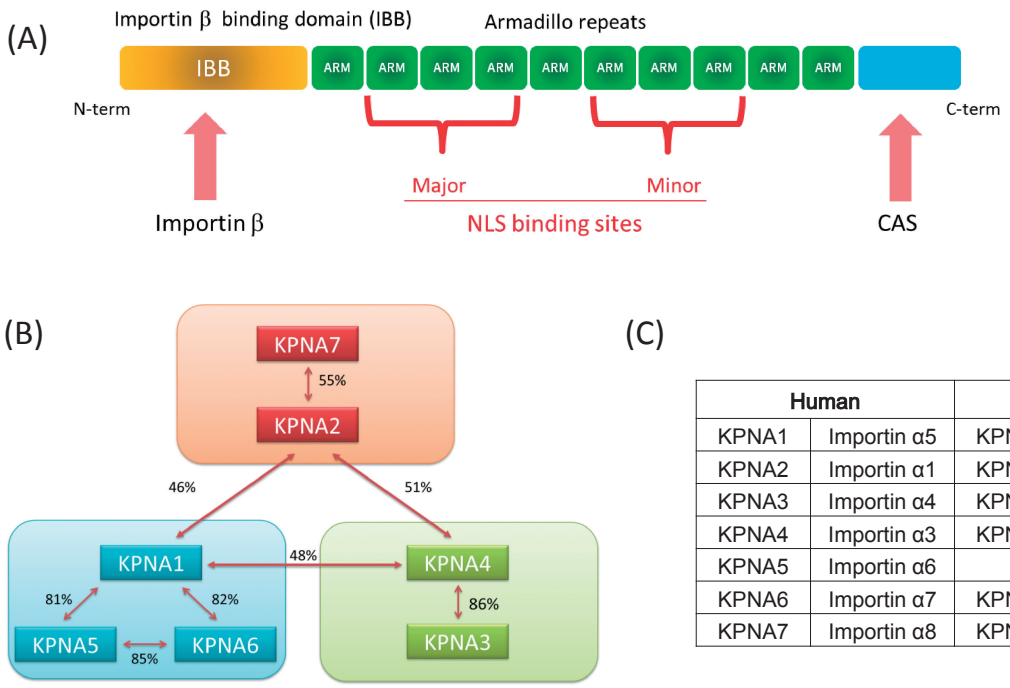
Importin α. Importin α, also known as karyopherin α (KPNA), was first identified as an adaptor protein linking cNLS-containing proteins with importin β, also called karyopherin β (KPNB). This protein has three key structural domains: an N-terminal importin β-binding (IBB) domain, armadillo (Arm) repeats that function as internal cargo cNLS-binding sites, and a C-terminal region that binds to the nuclear export factor of importin α, CAS/CSE1L (Fig. 3A).[25)–27)] Although there is a single importin α gene in budding yeast, mouse and human genomes encode 6 and 7 subtypes, respectively. Importin α subtypes are classified into three subfamilies based on their sequence similarity. In human importin α, for example, there exists an α1 subfamily (importin α5 (KPNA1), α6 (KPNA5), and α7 (KPNA6)), α2 subfamily (importin α1 (KPNA2) and α8 (KPNA7)), and α3 subfamily (importin α3 (KPNA4) and α4 (KPNA3)) (Fig. 3B).[28),29)]

The central portion of importin α consists of 10 repetitive motifs of a relatively hydrophobic sequence of approximately 42–43 amino acids (Arm repeats). A cNLS-containing cargo binds to two sites within the Arm repeats that are referred to as major (Arm repeats 2–4) and minor (Arm repeats 6–8) NLS binding sites. Typical monopartite cNLSs, such as the SV40 large T antigen NLS, bind to the major binding site, whereas bipartite cNLSs, such as the nucleoplasmin NLS, bind to both the major and minor binding sites.[30)]

Importin β. Importin β, which is now called importin β1, was first identified as a carrier molecule for importing cNLS cargoes together with importin α.[21),23),31),32)] Then, it was demonstrated that importin β1 functions as an NTR and transports a variety of NLS cargoes by binding to importin α carrying cNLS cargoes or through direct binding to the cargo molecules. Importin β also constitutes a large family. Importin β family molecules (14 members in budding yeast and 20 members in humans) participate in the nucleocytoplasmic transport of proteins and RNAs.[33)] Namely, the importin β family includes nuclear import receptors (called importins), export receptors (called exportins), and bidirectional receptors. Each member of the importin β family transports specific cargoes and mediates a variety of nucleocytoplasmic transport pathways.[34),35)] Among the importin β family members, only importin β1 utilizes adaptor molecules to bind to cargoes. Importin β family proteins, independently of cargo loading,[36),37)] can overcome the NPC permeability barrier composed of FG-nucleoporins through binding to the FG repeats, possibly due to the HEAT repeats composed of multiple flexible helices connected by a short linker.[38)]

Ran GTPase

Another key molecule is the small GTPase Ran, which was originally identified as one of the factors that encode a Ras-like sequence.[39)] Ran is a very abundant ~25-kDa protein that is located predominantly in the nucleus. Like other small



(C)

Human		Mouse	
KPNA1	Importin α5	KPNA1	Importin α1
KPNA2	Importin α1	KPNA2	Importin α2
KPNA3	Importin α4	KPNA3	Importin α3
KPNA4	Importin α3	KPNA4	Importin α4
KPNA5	Importin α6		
KPNA6	Importin α7	KPNA6	Importin α6
KPNA7	Importin α8	KPNA7	Importin α8

Fig. 3. The subtypes of importin α. (A) The structure of importin α. (B) Three distinct subfamilies of human importin α. The percentage indicates the protein homology between indicated subtypes. Note that even though the similarity between KPNA2 and KPNA7 is markedly low, they are currently grouped in the same clade.[72),127)] (C) The subtypes of human and mouse importin α.

GTPases, the function of Ran is regulated by binding to either GTP or GDP. The conversion of the GDP-bound form of Ran (RanGDP) to the GTP-bound form (RanGTP) is mediated by RCC1, a guanine-nucleotide exchange factor for Ran (RanGEF).[40)] RCC1 is a chromatin factor that is located in the nucleus, which means that Ran in the nucleus is mainly in the GTP-bound form. Importin β family molecules have a Ran-binding domain, and the binding of RanGTP to importin β family molecules induces a conformational change.[41)]

On the other hand, although Ran has its own weak hydrolytic activity, the hydrolysis of RanGTP to RanGDP is strongly accelerated by the GTPase-activating protein RanGAP1, in conjunction with Ran binding protein 1 (RanBP1) and/or Ran binding protein 2 (RanBP2, also called Nup358).[42),43)] These Ran-binding proteins are located in the cytoplasm, which means that RanGTP is rapidly converted to RanGDP in the cytoplasm. Therefore, it is believed that there is a steep gradient of RanGTP/GDP between the nucleus and cytoplasm, which is important for the directionality of nuclear transport.[44)] Furthermore, even during the mitotic phase, this gradient of RanGTP/GDP is maintained,[45),46)] which is important for regulating spindle assembly.

Molecular mechanism of classical nuclear protein import

From a variety of *in vivo* and *in vitro* data, a reliable model for the molecular mechanism of classical nuclear protein import has been proposed (Fig. 4). In the cytoplasm, the cNLS-containing cargo protein is initially recognized by an adaptor molecule, importin α, and then importin β1 binds to importin α to form a ternary complex called a nuclear pore-targeting complex. This complex is targeted to the NPCs and translocates through the nuclear pore via importin β1 activity. After translocation into the nucleus, abundant nuclear RanGTP binds to importin β1 to trigger the dissociation of the complex, resulting in the release of the cargo proteins from importin α into the nucleus where they function. In addition, other molecules that bind to importin α, namely, nucleoporin Npap60 or RBBP4 (Retinoblastoma binding protein 4, also called RbAp48) are possibly involved in the disassembly process of the importin α/importin β1/NLS-cargo ternary complex in the nucleus. Npap60 is known to promote the release of cNLS-cargo from importin α,[47)–49)] whereas RBBP4 could bind to the IBB domain of importin α to stimulate the dissociation

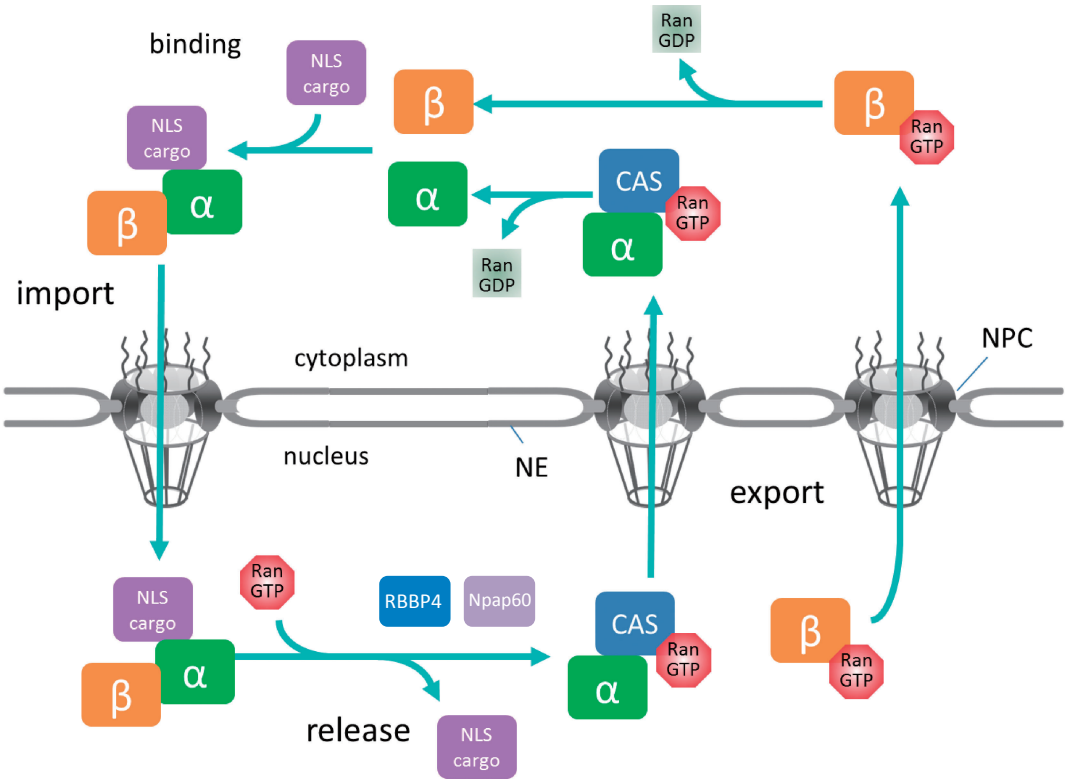


Fig. 4. The mechanism of importin α/β-mediated nuclear import of proteins. The NLS-containing cargo is bound by importin α, and then importin $\beta1$ binds to importin α to form a nuclear pore-targeting complex. After passing through the NPC, the binding of RanGTP to importin $\beta1$ triggers the dissociation of the complex inside the nucleus. CAS functions to export importin α in conjunction with RanGTP. Both importin α and importin $\beta1$ are recycled back to the cytoplasm and reused for the next round of nuclear import.

of importin $\beta1$ from importin α.[50] Then, importin α is exported from the nucleus as a ternary complex with RanGTP and a specific importin β family molecule, CAS/CSE1L, while importin $\beta1$ is also recycled back to the cytoplasm in conjunction with RanGTP.[25),27]

After translocation through the NPCs, the RanGTP/importin $\beta1$ export complex and RanGTP/CAS/importin α export complex are dissociated through the conversion of RanGTP in these complexes to RanGDP by cytoplasmic RanGAP1 with the aid of RanBP1 and/or RanBP2. After this, both importin α and importin $\beta1$ are reused for the next round of transport and RanGDP is imported into the nucleus by nuclear transport factor 2, NTF2 (also called p10), which specifically binds to RanGDP.[51),52] NTF2 is an abundant protein that carries RanGDP into the nucleus and functions as a RanGDP dissociation inhibitor (RanGDI) to keep Ran in the GDP-bound form during the transport process.[53]

Lessons from studies of importin α

Since the discovery of NTRs such as importin α and importin $\beta1$, the functions and pathways related to each NTR have been studied extensively. In fact, the expression of NTRs has been shown to be spatiotemporally regulated and the differential expression to be linked to various biological phenomena. In addition, it has been demonstrated that regulation of the nucleocytoplasmic transport pathways affects cellular physiological states. The biological significance of the importin β family has been discussed in detail elsewhere.[34),54] Hereafter, in this review, we will focus on the primary function and unexpected functions of importin α family members.

The primary function of importin α. To determine the function of importin α at the organism level, its knockdown or knockout has been performed in various species. In budding yeast *Saccharomyces cerevisiae*, there is only a single importin α family member called Srp1, which was originally identified

as a suppressor of RNA Polymerase I mutations.[55] Analysis of Srp1 temperature-sensitive mutants revealed the pleiotropic functions of Srp1, including roles in nuclear division, maintenance of nucleolar structure, and RNA transcription,[56] possibly reflecting defects in general nuclear transport.

The physiological significance of importin α 'subtypes' has been demonstrated in a variety of organisms. Although budding yeast, *S. cerevisiae*, contains a single importin α gene, fission yeast, *Schizosaccharomyces pombe*, possesses two importin α genes.[57),58] Furthermore, genetic analysis of the mutants of these two importin α molecules, cut15 and imp1, revealed that they show synthetic lethality, although they cannot rescue gene-specific defects in each other,[58] demonstrating that these two importin α subtypes possess their own unique physiological roles as well as overlapping roles.

Analysis in the fruit fly *Drosophila melanogaster*, which contains three importin α molecules (importin α1, importin α2, and importin α3; 42–46% homology with each other), showed that importin α1 or importin α2 mutant flies showed defects in gametogenesis, whereas importin α3 mutants die at the first or second instar larval stage.[59),60] In addition, the defects in importin α2-mutated female flies could not be rescued by the other family members.[61] In the nematode *Caenorhabditis elegans*, which expresses three importin α molecules (IMA-1, IMA-2, and IMA-3; 23–35% homology with each other), knocking down IMA-3 caused the arrest of germ cell development,[62] whereas depleting IMA-2 resulted in embryonic lethality with severe chromosome segregation defects and an abnormal nuclear envelope.[63),64] These subtype-specific defects mean that the importin α subtypes play their own physiologically important roles in different species.[65]

The physiological significance of importin α subtypes in mammals. As mentioned above, mouse and human genomes encode 6 and 7 importin α subtypes, respectively. The nomenclature of importin α subtypes differs between human and mouse homologs, which sometimes leads to confusion (Fig. 3C). To avoid confusion, we primarily use the terms KPNA1 to KPNA7 below to refer to human and mouse importin subtypes, because the same term is used for human and mouse homologues.

These importin α subtypes show a cargo-specific affinity to carry broad subtype-specific molecules into the nucleus,[29),66] and are expressed in a tissue-, developmental stage-, or cell-type-specific manner,[67)–79] suggesting that importin α subtypes play some critical roles in cell-type specific function, cell specification, and/or cell differentiation processes. Indeed, by modulating the expression pattern of importin α subtypes *in vitro*, the expression changes of these importin α subtypes have demonstrated to be physiologically significant. For example, the expression patterns of importin α subtypes were shown to change during cell differentiation processes, and, more importantly, its modulation clearly affects cell differentiation processes such as the differentiation of embryonic stem cells into neural cells (KPNA1[73]), myoblasts into myotubes (KPNA2[77]), or maturation of oligodendrocyte progenitors (KPNA1,[80] KPNA4[78]). However, it remains to be determined whether the changes in the nuclear transport of specific subsets of cargoes in fact alter differentiation processes. Moreover, in order to understand the differentiation processes precisely from the viewpoint of importin α subtype expression pattern, it should be considered that importin α is a multi-functional protein, as discussed in the next section.

The physiological function of importin α subtypes in mammals has been revealed *in vivo* by the generation and analysis of importin α knockout mice. KPNA1-null mice develop normally,[81),82] but show hypoplasia in female reproductive organs such as the ovary and uterus with severely reduced serum progesterone levels and progesterone receptor mRNA levels.[81] Analysis of KPNA1 knockout mice further revealed that KPNA1 is important for muscle regeneration.[83] That is, KPNA1 knockout caused muscle satellite cells to prematurely activate and undergo apoptosis, which led to the exhaustion of muscle satellite cells. On the other hand, knocking out KPNA7 results in reduced reproductivity and fetal lethality in females.[84] Of note, it was reported that mutations in KPNA7 are associated with a human neurodevelopmental disease.[85] Although KPNA6 knockout mice were viable, KPNA6-null oocytes showed a complete arrest at the two-cell embryo stage after fertilization,[86] demonstrating that it has an important role in the very early phases of development. Intriguingly, a comparison of KPNA1, KPNA4, and KPNA6 knockout mice revealed that KPNA6 knockout mice are highly resistant to infection with influenza viruses,[87] highlighting the importance of importin α as a determinant of pathogenicity. Thus, the analysis of knockout mice has opened up the analysis of the subtype-specific physiological significance of importin α *in vivo*.

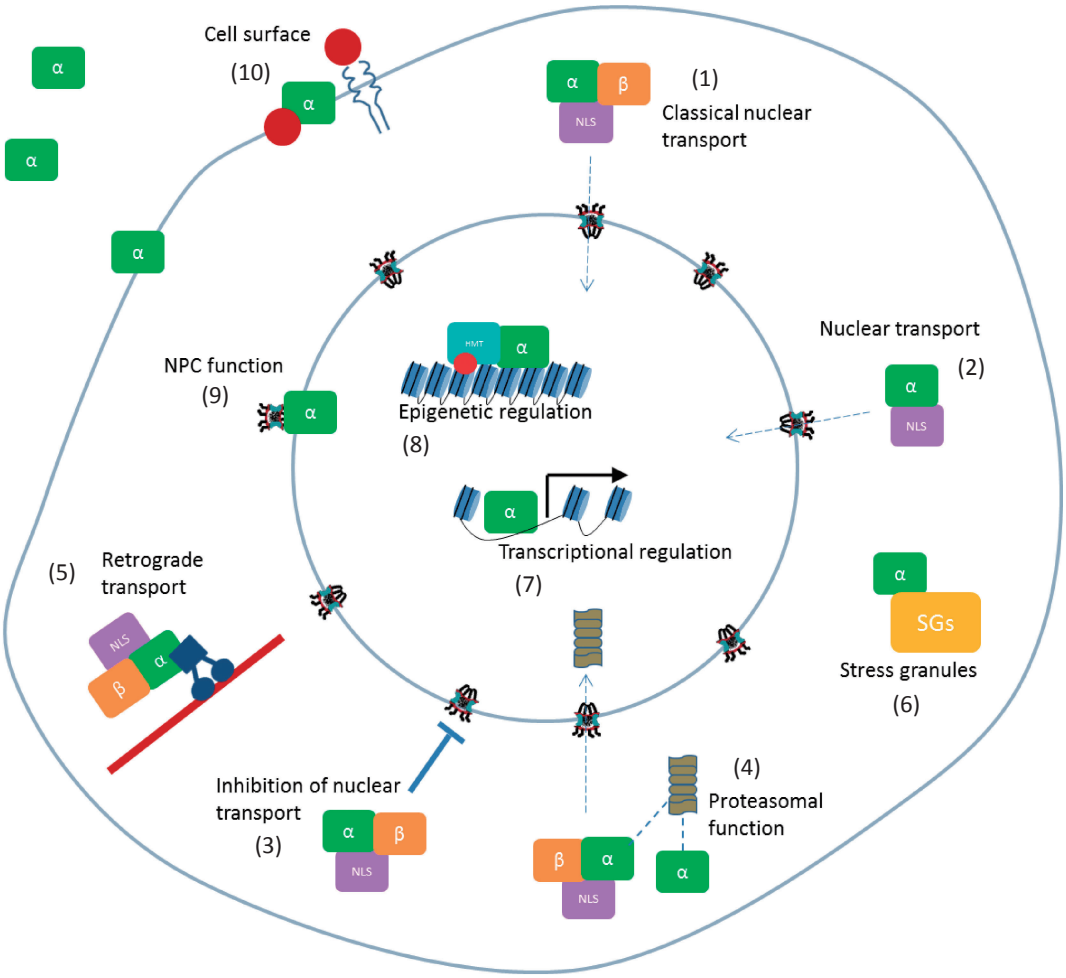


Fig. 5. Importin α is a multi-functional protein. Importin α possesses multiple functions depending on its subcellular localization. In the cytoplasm: (1) classical nuclear transport, (2) nuclear transport without importin β1, (3) negative regulation of nuclear import, (4) proteasomal function, (5) retrograde axonal transport, and (6) stress granule formation. In the nucleus: (7) transcriptional regulation, (8) epigenetic regulation, and (9) NPC function. At the cell surface: (10) cell-surface association with growth factors.

The unexpected functions of importin α

As described above, importin α was primarily identified and is well established as a nuclear transport factor or an NLS receptor. However, detailed analysis of importin α has revealed that this protein is involved in many unexpected cellular processes and localized to various cellular compartments (Fig. 5).

Cytoplasmic functions

Functions other than as an adaptor for importin β1. It has been demonstrated that importin α is not merely an adaptor molecule supporting the nuclear import process mediated by importin β1. That is, it was found that importin α by itself,[88] without importin β1, could transport cargo proteins, such as calcium/calmodulin-dependent protein kinase type IV (CaMKIV) or Vpr from human immunodeficiency virus type 1 (HIV-1).[89),90] In addition, depending on the cell condition, importin α was found to function as a negative regulator of nuclear import. For example, although Snail, a transcriptional repressor, can be imported into the nucleus by importin β1 alone,[91] it was shown that the direct binding of importin α to Snail competes with importin β1-Snail binding to negatively regulate the formation of the transport complex, and a subsequent nuclear import process.[92] This cytoplasmic retention of Snail by importin α eventually triggers its rapid degradation, which could severely affect the epithelial mesenchymal transition. Such effects by importin α were also observed for telomere

repeat factor 1 (TRF1)[93] and cdc7.[94] It has also been shown that, in undifferentiated ES cells, high expression of KPNA2 inhibited the nuclear translocation of the POU-domain transcription factors Brn2 or Oct6 through the binding of its atypical C-terminal region to these cargoes.[95] Thus, KPNA2 supposedly retains these differentiation factors in the cytoplasm to maintain the undifferentiated state through suppressing their transcriptional activities.

A connection to proteasome function

A study of Srp1, a budding yeast importin α, demonstrated that importin α is potentially a multifunctional protein. While an *srp1-31* mutant was defective in nuclear transport, another mutant, *srp1-49*, showed normal classical nuclear transport activity. Instead, the *srp1-49* mutant showed defects in protein degradation by the ubiquitin-proteasome system, which was possibly due to defects in nuclear localization of the proteasome.[96),97] Furthermore, these two mutants showed intragenic complementation. These results suggested that Srp1 carries out differential functions *in vivo*.[98]

Spindle formation

During open mitosis, when there is no intact nucleus due to the breakdown of the nuclear envelope, importin α/importin β1 binds to NLS-containing proteins, not to transport them, but to regulate their function. In particular, importin α binds to spindle assembly factors such as TPX2, NuMA, and XCTK2 to inhibit their function.[99)–101] The RanGTP concentration gradient formed surrounding mitotic chromosomes is important to regulate the function of importin α in this process. Namely, importin α can bind to spindle assembly factors to form a stable ternary complex with importin β1 to inhibit their function in the cytoplasm; however, around mitotic chromosomes, where the RanGTP concentration is high due to the presence of RCC1,[45),46] the binding of RanGTP to importin β1 triggers the dissociation of importin β1 and importin α from spindle assembly factors, leading to local activation of the spindle assembly factors.

It has also been shown that importin α is involved in regulating spindle scaling during *Xenopus* development, by inhibiting the microtubule-destabilizing activity of the kinesin-13, kif2a.[102] Although the microtubule-destabilizing activity of kif2a was inhibited by soluble importin α during early phases of development, kif2a becomes more active later in development (stage 8), when importin α is more sequestered to the plasma membrane.

Among the importin α subtypes, KPNA7 is the newest member and is specifically expressed in ovaries and mature oocytes,[72),84),103] where it functions as an NLS receptor.[104),105] Knockdown and knockout studies showed that KPNA7 was required for early embryogenesis.[72),84] Of note, mouse KPNA7 is localized in the nucleus or spindles in oocytes, depending on the maturation stage. Therefore, it is possibly involved in regulating spindle formation or gene expression during early embryogenesis; however, the exact function of KPNA7 remains to be established.

Retrograde axonal transport in neurons

Unexpectedly, it has been demonstrated that KPNA1 also functions in the retrograde transport of molecules for axonal injury signaling.[106] Sciatic nerve injury in KPNA1-null mice resulted in a significant increase in apoptotic neurons compared with the number in wild-type mice. Further study suggested that the retrograde transport, but not nuclear transport, of STAT3 transcription factors along axons is impaired in KPNA1 knockout mice, implying a novel role of KPNA1 as a cargo-binding adaptor to dynein.

Stress granules

Importin α molecules (KPNA1, KPNA2, and KPNA3) also exist in cytoplasmic stress granules (SGs) that were induced by arsenite or heat shock.[107),108] Furthermore, knocking down importin α (KPNA2) delayed SG formation upon exposure to arsenite, implying its novel regulatory role in the process of SG assembly.[107]

Nuclear functions

It has been observed that importin α often accumulates in the nucleus in some cancer cells[109] or in various stress conditions,[110)–112] suggesting that importin α plays roles other than nuclear transport within the nucleus. Indeed, it has been demonstrated that nuclear importin α binds to chromatin to regulate the expression of genes such as *STK35*.[113]

A recent study showed that a mutation of *dim-3* (defective in methylation-3), which encodes NUP-6 (importin α in *Neurospora*), causes a substantial loss of heterochromatin marks, such as H3K9me3 or DNA methylation.[114] Of note, no obvious defects in nuclear transport of proteins, including factors required for heterochromatin formation, are observed

in *dim-3* mutant cells. Furthermore, heterochromatin targeting, but not nuclear transport, of at least two components of a protein complex that catalyzes H3K9 methylation, DIM-5 and DIM-7, is severely affected in *dim-3* mutant cells. These data suggested that importin α has a unique role in targeting the chromatin modifier to its final destination after its nuclear transport process.

How does importin α work in the nucleus? It is known that the DNA-binding region of karyophilic proteins frequently overlaps with their NLS.[115] Thus, importin α could be involved in regulating the function of DNA-binding proteins through continuous intranuclear interaction after their import into the nucleus. Alternatively, the binding of importin α may modulate the targeting of chromatin modification proteins within the nucleus. Tripartite motif-containing 28 (TRIM28) is known as a component of a repressor complex containing heterochromatin protein 1 (HP1). Of note, the NLS of TRIM28 is located in a region that overlaps with its HP1 binding site (called the HP1 box). Furthermore, HP1 and importin α indeed compete for binding to TRIM28, suggesting that importin α may play a role in delivering TRIM28 to heterochromatin regions enriched with HP1 after nuclear transport.[116]

At the nuclear pore complex

A recent study showed that importin α plays an important role in the NPC. Importin α associates with the NPC by binding to the C-terminus of Nup153[117] and plays a critical role in the import of both cNLS-proteins and importin β-binding domain-containing-artificial cargoes.[118] Therefore, importin α functions as an important component of the NPC to achieve efficient directional nuclear import.

Moreover, importin α, together with importin β1, occupies the NPCs. In particular, the binding of importin α to importin β1 causes the high affinity binding of importin β1 to FG Nups to warrant the barrier function of the NPC.[119] Collectively, importin α complexed with importin β1 at the NPC is critically involved in two essential functions of the NPC: active nuclear transport and the permeability barrier.

Nuclear envelope and lamin assembly, and nuclear scaling

Importin α is also important for nuclear envelope assembly[120] and lamin polymerization[121] as shown by *in vitro* nuclear assembly reactions using *Xenopus* egg extracts. Furthermore, it was also found that *Xenopus* importin α2 functions as a critical factor to determine the nuclear size homeostasis in *Xenopus*,[122] possibly related to its role in the nuclear transport of lamin B3.

At the cell surface

Cell-surface importin α. Unexpectedly, we found that a fraction of importin α is localized on the cell surface of several cancer cells.[123] We further found that cell surface importin α can bind to the NLS, which means that importin α on the cell surface is functional. Of note, some growth factors that are secreted into the culture medium, are known to possess a functional NLS and be actively transported into the nucleus,[124] raising the intriguing possibility that importin α could associate with growth factors on the cell surface to help the function of these factors. Indeed, we found that KPNA2 interacts with FGF1, FGF2, and IGF-BP5 and that adding KPNA2 to the culture media stimulates the downstream signaling pathway of FGF1. It is also known that importin α is detected in the serum of healthy human controls, and at higher levels in those of patients with non-small cell lung carcinoma.[125] These findings provide an interesting scenario in which extracellular importin α helps FGF1 bind to the cell surface FGF receptor to accelerate signaling, and further plays a role in the nuclear transport of FGF1 after its internalization. In addition, future research should examine how functionally active importin α is transported to the cell surface from inside the cells.

Conclusion and perspectives

Approximately 35 years ago, we had little or no information on the mechanisms of nuclear protein transport. First, the NLS sequences were identified in various karyophilic proteins. Then, through the use of synthetic peptides containing the NLSs and the development of an *in vitro* transport system, transport machineries were identified and the mechanism was elucidated so that a concrete model has been proposed. Thereafter, the relationship between the physiological phenomena and the nuclear transport machineries was examined extensively, resulting in the re-confirmation of the importance of the nuclear protein transport system and machineries in cell functions. Furthermore, it has been determined that nuclear transport factors, especially importin α, are multifunctional proteins and have diverse functions other than nuclear protein transport.

In the future, it should be more extensively studied how nuclear protein transport is involved

in various physiological phenomena, such as aging. We have some interesting data showing that the downregulation of nuclear protein transport efficiency affects cell senescence.[50),126)] Thus, understanding the relationship between aging and nuclear protein transport may help promote health and longevity. Furthermore, it will be expected that medical drugs for various diseases, such as infectious diseases, are developed based on the knowledge concerning nuclear transport machineries. For example, compounds inhibiting the nuclear transport of viral proteins may be good, novel candidates to suppress viral infection. Thus, a complete understanding of nuclear protein transport will greatly contribute to medical innovation.

Acknowledgements

We thank Dr. Yoichi Miyamoto and all other members of the Laboratory of Nuclear Transport Dynamics (NIBIOHN). This work was supported in part by JSPS KAKENHI Grant Numbers 25116008, 16H04789, 16K14676, and 17H03679.

References

1) Dingwall, C., Sharnick, S.V. and Laskey, R.A. (1982) A polypeptide domain that specifies migration of nucleoplasmin into the nucleus. Cell **30**, 449–458.
2) Lanford, R.E. and Butel, J.S. (1984) Construction and characterization of an SV40 mutant defective in nuclear transport of T antigen. Cell **37**, 801–813.
3) Kalderon, D., Roberts, B.L., Richardson, W.D. and Smith, A.E. (1984) A short amino acid sequence able to specify nuclear location. Cell **39**, 499–509.
4) Dingwall, C., Robbins, J., Dilworth, S.M., Roberts, B. and Richardson, W.D. (1988) The nucleoplasmin nuclear location sequence is larger and more complex than that of SV-40 large T antigen. J. Cell Biol. **107**, 841–849.
5) Robbins, J., Dilworth, S.M., Laskey, R.A. and Dingwall, C. (1991) Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell **64**, 615–623.
6) Goldfarb, D.S., Gariepy, J., Schoolnik, G. and Kornberg, R.D. (1986) Synthetic peptides as nuclear localization signals. Nature **322**, 641–644.
7) Lanford, R.E., Kanda, P. and Kennedy, R.C. (1986) Induction of nuclear transport with a synthetic peptide homologous to the SV40 T antigen transport signal. Cell **46**, 575–582.
8) Yoneda, Y., Arioka, T., Imamoto-Sonobe, N., Sugawa, H., Shimonishi, Y. and Uchida, T. (1987) Synthetic peptides containing a region of SV 40 large T-antigen involved in nuclear localization direct the transport of proteins into the nucleus. Exp. Cell Res. **170**, 439–452.
9) Adam, S.A., Marr, R.S. and Gerace, L. (1990) Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. J. Cell Biol. **111**, 807–816.
10) Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y. and Chait, B.T. (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. J. Cell Biol. **148**, 635–651.
11) Cronshaw, J.M., Krutchinsky, A.N., Zhang, W., Chait, B.T. and Matunis, M.J. (2002) Proteomic analysis of the mammalian nuclear pore complex. J. Cell Biol. **158**, 915–927.
12) DeGrasse, J.A., DuBois, K.N., Devos, D., Siegel, T.N., Sali, A., Field, M.C. *et al.* (2009) Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor. Mol. Cell. Proteomics **8**, 2119–2130.
13) Tamura, K., Fukao, Y., Iwamoto, M., Haraguchi, T. and Hara-Nishimura, I. (2010) Identification and characterization of nuclear pore complex components in Arabidopsis thaliana. Plant Cell **22**, 4084–4097.
14) Mans, B.J., Anantharaman, V., Aravind, L. and Koonin, E.V. (2004) Comparative genomics, evolution and origins of the nuclear envelope and nuclear pore complex. Cell Cycle **3**, 1612–1637.
15) Asakawa, H., Yang, H.J., Yamamoto, T.G., Ohtsuki, C., Chikashige, Y., Sakata-Sogawa, K. *et al.* (2014) Characterization of nuclear pore complex components in fission yeast *Schizosaccharomyces pombe*. Nucleus **5**, 149–162.
16) Frey, S., Richter, R.P. and Gorlich, D. (2006) FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. Science **314**, 815–817.
17) Frey, S. and Gorlich, D. (2007) A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. Cell **130**, 512–523.
18) Unwin, P.N. and Milligan, R.A. (1982) A large particle associated with the perimeter of the nuclear pore complex. J. Cell Biol. **93**, 63–75.
19) Hinshaw, J.E., Carragher, B.O. and Milligan, R.A. (1992) Architecture and design of the nuclear pore complex. Cell **69**, 1133–1141.
20) Imamoto, N., Shimamoto, T., Takao, T., Tachibana, T., Kose, S., Matsubae, M. *et al.* (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.
21) Imamoto, N., Shimamoto, T., Kose, S., Takao, T., Tachibana, T., Matsubae, M. *et al.* (1995) The nuclear pore-targeting complex binds to nuclear pores after association with a karyophile. FEBS Lett. **368**, 415–419.
22) Gorlich, 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.
23) Radu, A., Blobel, G. and Moore, M.S. (1995) Identification of a protein complex that is required for nuclear protein import and mediates docking of import substrate to distinct nucleoporins. Proc. Natl. Acad. Sci. U.S.A. **92**, 1769–1773.
24) Gorlich, D., Vogel, F., Mills, A.D., Hartmann, E. and Laskey, R.A. (1995) Distinct functions for the two importin subunits in nuclear protein import. Nature **377**, 246–248.
25) Kutay, U., Bischoff, F.R., Kostka, S., Kraft, R. and Gorlich, D. (1997) Export of importin α from the nucleus is mediated by a specific nuclear transport factor. Cell **90**, 1061–1071.
26) Herold, A., Truant, R., Wiegand, H. and Cullen, B.R. (1998) Determination of the functional domain organization of the importin α nuclear import factor. J. Cell Biol. **143**, 309–318.
27) Matsuura, Y. and Stewart, M. (2004) Structural basis for the assembly of a nuclear export complex. Nature **432**, 872–877.
28) Miyamoto, Y., Yamada, K. and Yoneda, Y. (2016) Importin α: a key molecule in nuclear transport and non-transport functions. J. Biochem. **160**, 69–75.
29) Pumroy, R.A. and Cingolani, G. (2015) Diversification of importin-α isoforms in cellular trafficking and disease states. Biochem. J. **466**, 13–28.
30) Stewart, M. (2007) Molecular mechanism of the nuclear protein import cycle. Nat. Rev. Mol. Cell Biol. **8**, 195–208.
31) Gorlich, D., Kostka, S., Kraft, R., Dingwall, C., Laskey, R.A., Hartmann, E. *et al.* (1995) Two different subunits of importin cooperate to recognize nuclear localization signals and bind them to the nuclear envelope. Curr. Biol. **5**, 383–392.
32) Adam, E.J. and Adam, S.A. (1994) Identification of cytosolic factors required for nuclear location sequence-mediated binding to the nuclear envelope. J. Cell Biol. **125**, 547–555.
33) Ström, A.C. and Weis, K. (2001) Importin-β-like nuclear transport receptors. Genome Biol. **2**, REVIEWS3008.
34) Chook, Y.M. and Suel, K.E. (2011) Nuclear import by karyopherin-βs: recognition and inhibition. Biochim. Biophys. Acta **1813**, 1593–1606.
35) Kimura, M., Morinaka, Y., Imai, K., Kose, S., Horton, P. and Imamoto, N. (2017) Extensive cargo identification reveals distinct biological roles of the 12 importin pathways. eLife **6**, e21184.
36) Nakielny, S. and Dreyfuss, G. (1998) Import and export of the nuclear protein import receptor transportin by a mechanism independent of GTP hydrolysis. Curr. Biol. **8**, 89–95.
37) Kose, S., Imamoto, N., Tachibana, T., Shimamoto, T. and Yoneda, Y. (1997) Ran-unassisted nuclear migration of a 97-kD component of nuclear pore-targeting complex. J. Cell Biol. **139**, 841–849.
38) Yoshimura, S.H. and Hirano, T. (2016) HEAT repeats — versatile arrays of amphiphilic helices working in crowded environments? J. Cell Sci. **129**, 3963–3970.
39) Drivas, G.T., Shih, A., Coutavas, E., Rush, M.G. and D'Eustachio, P. (1990) Characterization of four novel *ras*-like genes expressed in a human teratocarcinoma cell line. Mol. Cell. Biol. **10**, 1793–1798.
40) Bischoff, F.R. and Ponstingl, H. (1991) Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. Nature **354**, 80–82.
41) Bayliss, R., Littlewood, T. and Stewart, M. (2000) Structural basis for the interaction between FxFG nucleoporin repeats and importin-beta in nuclear trafficking. Cell **102**, 99–108.
42) Bischoff, F.R., Klebe, C., Kretschmer, J., Wittinghofer, A. and Ponstingl, H. (1994) RanGAP1 induces GTPase activity of nuclear Ras-related Ran. Proc. Natl. Acad. Sci. U.S.A. **91**, 2587–2591.
43) Bischoff, F.R., Krebber, H., Smirnova, E., Dong, W. and Ponstingl, H. (1995) Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. EMBO J. **14**, 705–715.
44) Gorlich, D., Pante, 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.
45) Carazo-Salas, R.E., Guarguaglini, G., Gruss, O.J., Segref, A., Karsenti, E. and Mattaj, I.W. (1999) Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic spindle formation. Nature **400**, 178–181.
46) Kalab, P., Pu, R.T. and Dasso, M. (1999) The ran GTPase regulates mitotic spindle assembly. Curr. Biol. **9**, 481–484.
47) Matsuura, Y. and Stewart, M. (2005) Nup50/Npap60 function in nuclear protein import complex disassembly and importin recycling. EMBO J. **24**, 3681–3689.
48) Lindsay, M.E., Plafker, K., Smith, A.E., Clurman, B.E. and Macara, I.G. (2002) Npap60/Nup50 is a tri-stable switch that stimulates importin-α:β-mediated nuclear protein import. Cell **110**, 349–360.
49) Ogawa, Y., Miyamoto, Y., Asally, M., Oka, M., Yasuda, Y. and Yoneda, Y. (2010) Two isoforms of Npap60 (Nup50) differentially regulate nuclear protein import. Mol. Biol. Cell **21**, 630–638.
50) Tsujii, A., Miyamoto, Y., Moriyama, T., Tsuchiya, Y., Obuse, C., Mizuguchi, K. *et al.* (2015) Retinoblastoma-binding protein 4-regulated classical nuclear transport is involved in cellular senescence. J. Biol. Chem. **290**, 29375–29388.
51) Smith, A., Brownawell, A. and Macara, I.G. (1998) Nuclear import of Ran is mediated by the transport factor NTF2. Curr. Biol. **8**, 1403–1406.
52) Ribbeck, K., Lipowsky, G., Kent, H.M., Stewart, M. and Gorlich, D. (1998) NTF2 mediates nuclear import of Ran. EMBO J. **17**, 6587–6598.
53) 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.

54) Kimura, M. and Imamoto, N. (2014) Biological significance of the importin-β family-dependent nucleocytoplasmic transport pathways. Traffic **15**, 727–748.
55) Yano, R., Oakes, M., Yamaghishi, 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.
56) Azuma, Y., Tabb, M.M., Vu, L. and Nomura, M. (1995) Isolation of a yeast protein kinase that is activated by the protein encoded by SRP1 (Srp1p) and phosphorylates Srp1p complexed with nuclear localization signal peptides. Proc. Natl. Acad. Sci. U.S.A. **92**, 5159–5163.
57) Matsusaka, T., Imamoto, N., Yoneda, Y. and Yanagida, M. (1998) Mutations in fission yeast Cut15, an importin α homolog, lead to mitotic progression without chromosome condensation. Curr. Biol. **8**, 1031–1034.
58) Umeda, M., Izaddoost, S., Cushman, I., Moore, M.S. and Sazer, S. (2005) The fission yeast *Schizosaccharomyces pombe* has two importin-α proteins, Imp1p and Cut15p, which have common and unique functions in nucleocytoplasmic transport and cell cycle progression. Genetics **171**, 7–21.
59) Goldfarb, D.S., Corbett, A.H., Mason, D.A., Harreman, M.T. and Adam, S.A. (2004) Importin α: a multipurpose nuclear-transport receptor. Trends Cell Biol. **14**, 505–514.
60) Ratan, R., Mason, D.A., Sinnot, B., Goldfarb, D.S. and Fleming, R.J. (2008) Drosophila importin α1 performs paralog-specific functions essential for gametogenesis. Genetics **178**, 839–850.
61) Mason, D.A., Fleming, R.J. and Goldfarb, D.S. (2002) *Drosophila melanogaster* importin α1 and α3 can replace importin α2 during spermatogenesis but not oogenesis. Genetics **161**, 157–170.
62) Geles, K.G. and Adam, S.A. (2001) Germline and developmental roles of the nuclear transport factor importin α3 in *C. elegans*. Development **128**, 1817–1830.
63) Askjaer, P., Galy, V., Hannak, E. and Mattaj, I.W. (2002) Ran GTPase cycle and importins α and β are essential for spindle formation and nuclear envelope assembly in living *Caenorhabditis elegans* embryos. Mol. Biol. Cell **13**, 4355–4370.
64) Geles, K.G., Johnson, J.J., Jong, S. and Adam, S.A. (2002) A role for *Caenorhabditis elegans* importin IMA-2 in germ line and embryonic mitosis. Mol. Biol. Cell **13**, 3138–3147.
65) Miyamoto, Y., Boag, P.R., Hime, G.R. and Loveland, K.L. (2012) Regulated nucleocytoplasmic transport during gametogenesis. Biochim. Biophys. Acta **1819**, 616–630.
66) Yoneda, Y. (2000) Nucleocytoplasmic protein traffic and its significance to cell function. Genes Cells **5**, 777–787.
67) 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.
68) Tsuji, 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.
69) 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.
70) Prieve, M.G., Guttridge, K.L., Munguia, J.E. and Waterman, M.L. (1996) The nuclear localization signal of lymphoid enhancer factor-1 is recognized by two differentially expressed Srp1-nuclear localization sequence receptor proteins. J. Biol. Chem. **271**, 7654–7658.
71) Nachury, M.V., Ryder, U.W., Lamond, A.I. and Weis, K. (1998) Cloning and characterization of hSRP1γ, a tissue-specific nuclear transport factor. Proc. Natl. Acad. Sci. U.S.A. **95**, 582–587.
72) Tejomurtula, J., Lee, K.B., Tripurani, S.K., Smith, G.W. and Yao, J. (2009) Role of importin alpha8, a new member of the importin alpha family of nuclear transport proteins, in early embryonic development in cattle. Biol. Reprod. **81**, 333–342.
73) Yasuhara, N., Shibazaki, N., Tanaka, S., Nagai, M., Kamikawa, Y., Oe, S. *et al.* (2007) Triggering neural differentiation of ES cells by subtype switching of importin-α. Nat. Cell Biol. **9**, 72–79.
74) Hogarth, C.A., Calanni, S., Jans, D.A. and Loveland, K.L. (2006) Importin α mRNAs have distinct expression profiles during spermatogenesis. Dev. Dyn. **235**, 253–262.
75) Köhler, M., Fiebeler, A., Hartwig, M., Thiel, S., Prehn, S., Kettritz, R. *et al.* (2002) Differential expression of classical nuclear transport factors during cellular proliferation and differentiation. Cell. Physiol. Biochem. **12**, 335–344.
76) Suzuki, T., Ishigami, Y., Okada, N., Kaneko, A., Fukutomi, R. and Isemura, M. (2008) Differentiation-associated alteration in gene expression of importins and exportins in human leukemia HL-60 cells. Biomed. Res. **29**, 141–145.
77) Hall, M.N., Griffin, C.A., Simionescu, A., Corbett, A.H. and Pavlath, G.K. (2011) Distinct roles for classical nuclear import receptors in the growth of multinucleated muscle cells. Dev. Biol. **357**, 248–258.
78) Laitman, B.M., Mariani, J.N., Zhang, C., Sawai, S. and John, G.R. (2017) Karyopherin Alpha Proteins Regulate Oligodendrocyte Differentiation. PLoS One **12**, e0170477.
79) Hosokawa, K., Nishi, M., Sakamoto, H., Tanaka, Y. and Kawata, M. (2008) Regional distribution of importin subtype mRNA expression in the nervous system: study of early postnatal and adult mouse. Neuroscience **157**, 864–877.
80) Laitman, B.M., Asp, L., Mariani, J.N., Zhang, J., Liu, J., Sawai, S. *et al.* (2016) The transcriptional

activator Krüppel-like factor-6 is required for CNS myelination. PLoS Biol. **14**, e1002467.
81) Moriyama, T., Nagai, M., Oka, M., Ikawa, M., Okabe, M. and Yoneda, Y. (2011) Targeted disruption of one of the importin α family members leads to female functional incompetence in delivery. FEBS J. **278**, 1561–1572.
82) Shmidt, T., Hampich, F., Ridders, M., Schultrich, S., Hans, V.H., Tenner, K. *et al.* (2007) Normal brain development in importin-α5 deficient-mice. Nat. Cell Biol. **9**, 1337–1338, author reply 1339.
83) Choo, H.J., Cutler, A., Rother, F., Bader, M. and Pavlath, G.K. (2016) Karyopherin alpha 1 regulates satellite cell proliferation and survival by modulating nuclear Import. Stem Cells **34**, 2784–2797.
84) Hu, J., Wang, F., Yuan, Y., Zhu, X., Wang, Y., Zhang, Y. *et al.* (2010) Novel importin-α family member Kpna7 is required for normal fertility and fecundity in the mouse. J. Biol. Chem. **285**, 33113–33122.
85) Paciorkowski, A.R., Weisenberg, J., Kelley, J.B., Spencer, A., Tuttle, E., Ghoneim, D. *et al.* (2014) Autosomal recessive mutations in nuclear transport factor KPNA7 are associated with infantile spasms and cerebellar malformation. Eur. J. Hum. Genet. **22**, 587–593.
86) Rother, F., Shmidt, T., Popova, E., Krivokharchenko, A., Hügel, S., Vilianovich, L. *et al.* (2011) Importin α7 is essential for zygotic genome activation and early mouse development. PLoS One **6**, e18310.
87) Gabriel, G., Klingel, K., Otte, A., Thiele, S., Hudjetz, B., Arman-Kalcek, G. *et al.* (2011) Differential use of importin-alpha isoforms governs cell tropism and host adaptation of influenza virus. Nat. Commun. **2**, 156.
88) Miyamoto, Y., Hieda, M., Harreman, M.T., Fukumoto, M., Saiwaki, T., Hodel, A.E. *et al.* (2002) Importin α can migrate into the nucleus in an importin β- and Ran-independent manner. EMBO J. **21**, 5833–5842.
89) Kotera, I., Sekimoto, T., Miyamoto, Y., Saiwaki, T., Nagoshi, E., Sakagami, H. *et al.* (2005) Importin α transports CaMKIV to the nucleus without utilizing importin β. EMBO J. **24**, 942–951.
90) Kamata, M., Nitahara-Kasahara, Y., Miyamoto, Y., Yoneda, Y. and Aida, Y. (2005) Importin-α promotes passage through the nuclear pore complex of human immunodeficiency virus type 1 Vpr. J. Virol. **79**, 3557–3564.
91) Yamasaki, H., Sekimoto, T., Ohkubo, T., Douchi, T., Nagata, Y., Ozawa, M. *et al.* (2005) Zinc finger domain of Snail functions as a nuclear localization signal for importin beta-mediated nuclear import pathway. Genes Cells **10**, 455–464.
92) Sekimoto, T., Miyamoto, Y., Arai, S. and Yoneda, Y. (2011) Importin α protein acts as a negative regulator for Snail protein nuclear import. J. Biol. Chem. **286**, 15126–15131.
93) Forwood, J.K. and Jans, D.A. (2002) Nuclear import pathway of the telomere elongation suppressor TRF1: inhibition by importin α. Biochemistry **41**, 9333–9340.
94) Kim, B.J. and Lee, H. (2006) Importin-β mediates Cdc7 nuclear import by binding to the kinase insert II domain, which can be antagonized by importin-α. J. Biol. Chem. **281**, 12041–12049.
95) Yasuhara, N., Yamagishi, R., Arai, Y., Mehmood, R., Kimoto, C., Fujita, T. *et al.* (2013) Importin alpha subtypes determine differential transcription factor localization in embryonic stem cells maintenance. Dev. Cell **26**, 123–135.
96) Wendler, P., Lehmann, A., Janek, K., Baumgart, S. and Enenkel, C. (2004) The bipartite nuclear localization sequence of Rpn2 is required for nuclear import of proteasomal base complexes via karyopherin $\alpha\beta$ and proteasome functions. J. Biol. Chem. **279**, 37751–37762.
97) Lehmann, A., Janek, K., Braun, B., Kloetzel, P.M. and Enenkel, C. (2002) 20 S proteasomes are imported as precursor complexes into the nucleus of yeast. J. Mol. Biol. **317**, 401–413.
98) Tabb, M.M., Tongaonkar, P., Vu, L. and Nomura, M. (2000) Evidence for separable functions of Srp1p, the yeast homolog of importin α (Karyopherin α): role for Srp1p and Sts1p in protein degradation. Mol. Cell. Biol. **20**, 6062–6073.
99) Gruss, O.J., Carazo-Salas, R.E., Schatz, C.A., Guarguaglini, G., Kast, J., Wilm, M. *et al.* (2001) Ran induces spindle assembly by reversing the inhibitory effect of importin α on TPX2 activity. Cell **104**, 83–93.
100) Nachury, M.V., Maresca, T.J., Salmon, W.C., Waterman-Storer, C.M., Heald, R. and Weis, K. (2001) Importin β is a mitotic target of the small GTPase Ran in spindle assembly. Cell **104**, 95–106.
101) Ems-McClung, S.C., Zheng, Y. and Walczak, C.E. (2004) Importin α/β and Ran-GTP regulate XCTK2 microtubule binding through a bipartite nuclear localization signal. Mol. Biol. Cell **15**, 46–57.
102) Wilbur, J.D. and Heald, R. (2013) Mitotic spindle scaling during *Xenopus* development by kif2a and importin α. eLife **2**, e00290.
103) Wang, X., Park, K.E., Koser, S., Liu, S., Magnani, L. and Cabot, R.A. (2012) KPNA7, an oocyte- and embryo-specific karyopherin alpha subtype, is required for porcine embryo development. Reprod. Fertil. Dev. **24**, 382–391.
104) Kimoto, C., Moriyama, T., Tsujii, A., Igarashi, Y., Robuse, C., Miyamoto, Y. *et al.* (2015) Functional characterization of importin α8 as a classical nuclear localization signal receptor. Biochim. Biophys. Acta **1853**, 2676–2683.
105) Vuorinen, E.M., Rajala, N.K., Rauhala, H.E., Nurminen, A.T., Hytonen, V.P. and Kallioniemi, A. (2017) Search for KPNA7 cargo proteins in human cells reveals MVP and ZNF414 as novel regulators of cancer cell growth. Biochim. Biophys. Acta **1863**, 211–219.
106) Ben-Yaakov, K., Dagan, S.Y., Segal-Ruder, Y., Shalem, O., Vuppalanchi, D., Willis, D.E. *et al.*

(2012) Axonal transcription factors signal retrogradely in lesioned peripheral nerve. EMBO J. **31**, 1350–1363.

107) Fujimura, K., Suzuki, T., Yasuda, Y., Murata, M., Katahira, J. and Yoneda, Y. (2010) Identification of importin α1 as a novel constituent of RNA stress granules. Biochim. Biophys. Acta **1803**, 865–871.

108) Mahboubi, H., Seganathy, E., Kong, D. and Stochaj, U. (2013) Identification of novel stress granule components that are involved in nuclear transport. PLoS One **8**, e68356.

109) Christiansen, A. and Dyrskjot, L. (2013) The functional role of the novel biomarker karyopherin α 2 (KPNA2) in cancer. Cancer Lett. **331**, 18–23.

110) Miyamoto, Y., Saiwaki, T., Yamashita, J., Yasuda, Y., Kotera, I., Shibata, S. *et al.* (2004) Cellular stresses induce the nuclear accumulation of importin α and cause a conventional nuclear import block. J. Cell Biol. **165**, 617–623.

111) Furuta, M., Kose, S., Koike, M., Shimi, T., Hiraoka, Y., Yoneda, Y. *et al.* (2004) Heat-shock induced nuclear retention and recycling inhibition of importin α. Genes Cells **9**, 429–441.

112) Kodiha, M., Chu, A., Matusiewicz, N. and Stochaj, U. (2004) Multiple mechanisms promote the inhibition of classical nuclear import upon exposure to severe oxidative stress. Cell Death Differ. **11**, 862–874.

113) Yasuda, Y., Miyamoto, Y., Yamashiro, T., Asally, M., Masui, A., Wong, C. *et al.* (2012) Nuclear retention of importin α coordinates cell fate through changes in gene expression. EMBO J. **31**, 83–94.

114) Klocko, A.D., Rountree, M.R., Grisafi, P.L., Hays, S.M., Adhvaryu, K.K. and Selker, E.U. (2015) Neurospora importin α is required for normal heterochromatic formation and DNA methylation. PLoS Genet. **11**, e1005083.

115) Cokol, M., Nair, R. and Rost, B. (2000) Finding nuclear localization signals. EMBO Rep. **1**, 411–415.

116) Moriyama, T., Sangel, P., Yamaguchi, H., Obuse, C., Miyamoto, Y., Oka, M. *et al.* (2015) Identification and characterization of a nuclear localization signal of TRIM28 that overlaps with the HP1 box. Biochem. Biophys. Res. Commun. **462**, 201–207.

117) Moroianu, J., Blobel, G. and Radu, A. (1997) RanGTP-mediated nuclear export of karyopherin α involves its interaction with the nucleoporin Nup153. Proc. Natl. Acad. Sci. U.S.A. **94**, 9699–9704.

118) Ogawa, Y., Miyamoto, Y., Oka, M. and Yoneda, Y. (2012) The interaction between importin-α and Nup153 promotes importin-α/β-mediated nuclear import. Traffic **13**, 934–946.

119) Kapinos, L.E., Huang, B., Rencurel, C. and Lim, R.Y.H. (2017) Karyopherins regulate nuclear pore complex barrier and transport function. J. Cell Biol. **216**, 3609–3624.

120) Hachet, V., Kocher, T., Wilm, M. and Mattaj, I.W. (2004) Importin α associates with membranes and participates in nuclear envelope assembly *in vitro*. EMBO J. **23**, 1526–1535.

121) Adam, S.A., Sengupta, K. and Goldman, R.D. (2008) Regulation of nuclear lamin polymerization by importin α. J. Biol. Chem. **283**, 8462–8468.

122) Levy, D.L. and Heald, R. (2010) Nuclear size is regulated by importin α and Ntf2 in *Xenopus*. Cell **143**, 288–298.

123) Yamada, K., Miyamoto, Y., Tsujii, A., Moriyama, T., Ikuno, Y., Shiromizu, T. *et al.* (2016) Cell surface localization of importin α1/KPNA2 affects cancer cell proliferation by regulating FGF1 signalling. Sci. Rep. **6**, 21410.

124) Olsnes, S., Klingenberg, O. and Wiedlocha, A. (2003) Transport of exogenous growth factors and cytokines to the cytosol and to the nucleus. Physiol. Rev. **83**, 163–182.

125) Wang, C.I., Wang, C.L., Wang, C.W., Chen, C.D., Wu, C.C., Liang, Y. *et al.* (2011) Importin subunit alpha-2 is identified as a potential biomarker for non-small cell lung cancer by integration of the cancer cell secretome and tissue transcriptome. Int. J. Cancer **128**, 2364–2372.

126) Nagai, M. and Yoneda, Y. (2013) Downregulation of the small GTPase ras-related nuclear protein accelerates cellular ageing. Biochim. Biophys. Acta **1830**, 2813–2819.

127) Kelley, J.B., Talley, A.M., Spencer, A., Gioeli, D. and Paschal, B.M. (2010) Karyopherin α7 (KPNA7), a divergent member of the importin α family of nuclear import receptors. BMC Cell Biol. **11**, 63.

(Received Feb. 23, 2018; accepted Apr. 19, 2018)

Profile

Yoshihiro Yoneda was born in Nara in 1955 and graduated from Osaka University Medical School in 1981. Then, he entered the Graduate School of Medicine at Osaka University and started cell biological research under the supervision of the late Professor Yoshio Okada, who discovered the cell–cell fusion phenomena and was a member of the Japan Academy. Yoshihiro Yoneda received his Ph.D. degree in 1985 and worked as a postdoctoral fellow at the Institute for Molecular and Cellular Biology, Osaka University. He became Assistant Professor in 1986, Associate Professor in 1991 and Professor in 1992 at the same Institute. He moved to Osaka University Medical School as Professor in 1993. He was elected as Dean of Medical School at Osaka University in 2011 and served a 2-year term. He became Director General of the National Institute of Biomedical Innovation in 2013. Since the two institutes were reorganized, he became Director General of the National Institutes of Biomedical Innovation, Health and Nutrition in 2015. He has been elucidating the molecular mechanism of nucleocytoplasmic transport and its significance on cell functions as one of the world-wide pioneers in the field. For his achievement, he received the Medical Award of The Japan Medical Association in 2009, Medical Award of Takeda Science Foundation in 2013, and Medal with Purple Ribbon in 2015. He is currently a member of the Science Council of Japan.