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Review

Transportin-1 and Transportin-2: Protein nuclear import and beyond

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ABSTRACT

Nearly 20 years after its identification as a new β-karyopherin mediating the nuclear import of the RNA-binding protein hnRNP A1, Transportin-1 is still commonly overlooked in comparison with its best known cousin, Importin-β. Transportin-1 is nonetheless a considerable player in nucleo-cytoplasmic transport. Over the past few years, significant progress has been made in the characterization of the nuclear localization signals (NLSs) that Transportin-1 recognizes, thereby providing the molecular basis of its diversified repertoire of cargoes. The recent discovery that mutations in the Transportin-dependent NLS of FUS cause mislocalization of this protein and result in amyotrophic lateral sclerosis illustrates the importance of Transportin-dependent import for human health. Besides, new functions of Transportin-1 are emerging in processes other than nuclear import. Here, we summarize what is known about Transportin-1 and the related β-karyopherin Transportin-2.
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1. Basis of protein nucleo-cytoplasmic transport

Active nucleo-cytoplasmic transport of proteins is mostly carried out by β-karyopherins, a family of factors functionally divided into importins and exportins. Importins bind to the nuclear localization signal (NLS) of their cargoes in the cytoplasm, either directly or through an adaptor. The importin/cargo complexes cross the nuclear pore complex (NPC) through the interactions of the importin with nucleoporins. In the nucleus, importins are bound by Ran-GTP, which releases the cargo. Importins are then recycled to the cytoplasm in association with Ran-GTP. On the cytoplasmic face of the NPC, Ran hydrolyzes its bound GTP into GDP and dissociates, freeing the importin for a new import cycle (see for example [1] for review). Exportins work in a similar way but in reverse. In the nucleus, exportins cooperatively bind Ran-GTP and a cargo featuring a fitting nuclear export signal (NES). Once the trimeric complexes reach the cytoplasm, they are dissociated and free exportins return to the nucleus to complete the cycle (see for example [2] for review). Thus, while Ran-GTP binding causes importins to release their cargoes, it is required for exportins to bind theirs. Neither importins nor exportins have significant binding affinity for the GDP-bound form of Ran. Therefore, the directionality of the transfers is maintained by mechanisms that ensure that nuclear Ran is bound to GTP and cytoplasmic Ran to GDP. The nuclear part of this task is carried out by the chromatin-associated guanine exchange factor RCC1, which promotes the exchange of GDP for GTP on nuclear Ran. Three factors located on the cytosolic face of the NPC (RanBP1, RanBP2, and RanGAP) collaborate to ensure the hydrolysis of Ran-bound GTP by Ran as soon as it goes out of the nucleus (see for example [3–5] for review).

2. Transportin-1 and -2: two similar β-karyopherins

The β-karyopherin family comprises 14 members in *Saccharomyces cerevisiae* and about 20 in mammals [6]. β-Karyopherins have relatively high molecular weights (95–145 kDa) and acidic

Abbreviations: hnRNP, heterogeneous ribonucleoprotein; NES, nuclear export signal; NLS, nuclear localization signal; NPC, nuclear pore complex; TRN-1, Transportin-1; TRN-2, Transportin-2

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Table 1
Crystal structures available for Transportins. The presence of two PDB entries indicates that two different structures were obtained. *Hs: Homo sapiens*. *Sc: Saccharomyces cerevisiae*.

Importin	Ligand	PDB entry	Refs.
Hs TRN-1	*Hs* Ran-GppNHP	PDB: 1QBK	[20]
Hs TRN-1	*Hs* hnRNP A1 "M9" NLS	PDB: 2H4M	[22]
Hs TRN-1	*Hs* hnRNP M NLS	PDB: 2OT8	[24]
Hs TRN-1	–	PDB: 2Z5J	[27]
	Hs TAP NLS	PDB: 2Z5K/PDB: 2Z5M	
	Hs hnRNP D NLS	PDB: 2Z5N	
	Hs hnRNP DL NLS	PDB: 2Z5O	
Hs TRN-1	–	PDB: 2QMR	[19]
Hs TRN-1	*Hs* FUS NLS	PDB: 4FDD	[29]
Hs TRN-1	*Hs* FUS NLS	PDB: 4FQ3	[28]
Hs TRN-1	*Sc* Nab2p NLS	PDB: 4JLQ	[42]

isoelectric points (4.0–5.5). Their N-terminal region is the most conserved one and binds Ran. Apart from these characteristics, it is the common structural organization of β-karyopherins, rather than their sequence similarity (<20%) that groups them together. All the β-karyopherins whose structures have been determined contain about 20 HEAT repeats spread over their lengths ([4,7]; Table 1 and references therein). These motifs are about 40 a.a. long and consist of two antiparallel α-helixes, A and B, connected by a loop [8].

The founding member of the β-karyopherin family, Importin-β, usually works in tandem with the adaptor Importin-α to import cargoes containing lysine-rich NLSs. The Importin-α/β system is probably responsible for the nuclear import of hundreds of proteins [9] and is generally considered as the general nuclear import machinery. Other importins have been less studied, except maybe for Transportin-1.

Transportin-1 was identified by three independent groups as the import factor for the heterogeneous ribonucleoprotein A1 (hnRNP A1) in mammalian cell lines [10–13]. As hnRNP A1 NLS – the "M9" sequence – also functions as a NES, another group termed the new karyopherin Transportin, suggesting that the new karyopherin might carry hnRNP A1 both ways [10]. The hypothesis was rapidly abandoned upon the observation that Ran-GTP binding dissociates the hnRNP A1/Transportin-1 complex [14,15]. The name Transportin-1 (TRN-1) is retained here because it is the one recommended in the UniProt database. The protein is also referred to as Karyopherin-β2 or Importin-β2.

In the course of isolating full-length Transportin-1 cDNA, a very similar protein was discovered and named Transportin-2 (TRN-2) [14] or Karyopherin-β2B [16]. It was later shown that human Transportin-2 is expressed as two isoforms A and B [17]. Human TRN-2A and TRN-2B sequences share 84% identities and 92% similarities with that of Transportin-1. The most variable sequence between TRN-1 and both isoforms of TRN-2 is the unstructured 62-residue acidic loop that joins helixes A and B of HEAT repeat 8 (45% identities for residues 336–368). TRN-2A HEAT repeat 17 includes a stretch of ten residues that is absent from TRN-2B and TRN-1 [17] (Fig. 1), as well as from the ortholog of Transportins in yeast, Kap104p. Transportin-2 was first suspected to act as an export factor for Transportin-1 cargoes. However, subsequent studies refuted this hypothesis (see [18] and below). A large set of data indicate that the role of Transportin-1 and Transportin-2 in nucleo-cytoplasmic transport is restricted to nuclear import.

The structure of human Transportin-1 has been described (see Table 1 and references therein), but not that of Transportin-2. Transportin-1 consists of twenty HEAT repeats stacked parallel to each other with a slight clockwise twist to form one and a half pitch of a superhelix [19]. The superhelix can also be described as two overlapping arches: a N-terminal one whose inner surface binds Ran-GTP and a C-terminal one whose inner surface is the binding site for most known cargoes. A 62 residue loop connects helixes A and B from the HEAT repeat 8. When Ran-GTP binds Transportin-1, it pushes the loop into the principal cargo-binding site, which causes the release of the cargo [19–22].

The following paragraphs describe the subsets of cargoes that are imported by Transportin-1, -2A and -2B and broach the subject of their role in other cellular processes.

3. Cargoes of mammalian Transportin-1 and -2

3.1. Transportin-1

A few dozens of cargoes with diverse NLS sequences have been experimentally validated for mammalian Transportin-1 (see Table 2). Structural analysis of TRN-1/NLS complexes revealed common patterns among apparently disparate Transportin-1-dependent NLSs and unified many of these sequences – including the M9 sequence of hnRNP A1 – into a new class of modular NLSs termed PY-NLSs (see below). Most of the PY-NLS-containing cargoes that have been experimentally validated are RNA-binding proteins, and about 60% of the human proteins in which a PY-NLS was predicted are classified as involved in RNA transcription or processing [22].

In general, PY-NLS-containing cargoes seem to be specifically imported by Transportins. By contrast, cargoes that are imported by Transportin-1 but do not comprise a PY-NLS frequently use several β-karyopherin-mediated nuclear import pathways. Viral, ribosomal, and histone proteins constitute the bulk of these cargoes (see Table 2 and references therein).

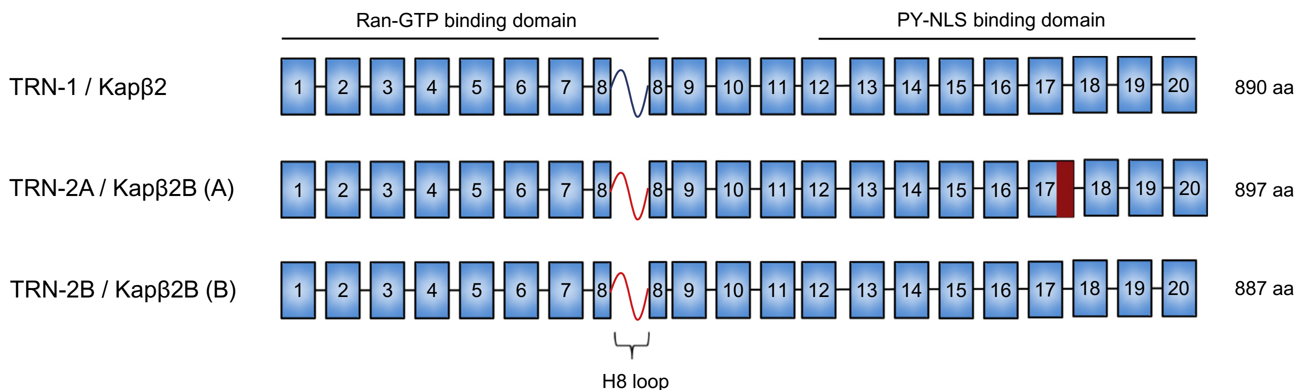

Fig. 1. A comparison between Transportin-1, -2A, and -2B. Each box represents a HEAT repeat. Transportins -2A and -2B are the products of two alternatively spliced isoforms of the *Tnpo2* gene [17]. The least conserved region between Transportin-1 and both isoforms of Transportin-2 is the loop connecting helixes A and B of HEAT repeat 8. Transportin-2A includes 10 supplementary residues in HEAT repeat 17.

Table 2
Mammalian Transportin-1 cargoes documented by experimental evidence. Part 1: cargoes with a PY-NLS. Part 2: potential cargoes without PY-NLS.

Cargoes with PY-NLSs or PY-NLS-like NLSs

Cargo	NLS	Experimental evidence	Other importins potentially used by the cargo	Refs.
CLK3	bPY	P-D/MS, P-D	TRN-SR2	[22,107]
CPSF6	hPY	P-D/MS, P-D	(TRN-2B), TRN-SR2	[18,22,107]
Cyclin T1	≈hPY	P-D/MS, P-D		[18,22]
EWS	hPY	P-D/MS, P-D, mut, M9M		[18,22,38,60,108,109]
FUS	hPY	P-D/MS, P-D, mut, siRNA, M9M, SPR	TRN-2B	[18,22,26,28,29,38]
GLI2, GLI3	≈hPY	P-D		[49]
HCC1	bPY	P-D		[22]
HCMV UL79	hPY	M9M, mut		[110]
HEXIM1	bPY	P-D/MS, P-D	(TRN-2B)	[18,22]
hnRNP A1	hPY	Y2H, P-D, P-D/MS, IPCc, IPCk, M9M, mut, ΦD, BH	TRN-2A, TRN-2B	[10–13,17,18,22,24,111,112]
hnRNP A2/B1	hPY	Y2H, ΦD, BH		[10,111,112]
hnRNP A3	hPY	ΦD		[111]
hnRNP AB	hPY	BH		[112]
hnRNP D	hPY	IPCc, IPCk, P-D, mut, BH		[27,37,112,113]
hnRNP DL/JKTBP	hPY	P-D, BH, IPCc		[27,112,114]
hnRNP F	hPY	P-D/MS, P-D, IPCk, mut		[14,18,22]
hnRNP H1	hPY	P-D/MS, mut, BH	(TRN-2B)	[18,112,115]
hnRNP M	bPY	P-D, M9M, ΦD, mut	(TRN-2B)	[18,22,24,111]
Huntingtin	≈bPY	P-D, mut	β	[31]
HuR	≈hPY	P-D, IPCk, IPCc, (M9M), co-IP	(α1/β), TRN-2A,TRN-2B,	[17,18,24,112,116]
NXF1	hPY	IPCc, IPCk, P-D, mut, M9M	(α/β), β, 4, 11, (TRN-2),	[18,23,27,112,117]
PABPN1	≈bPY	P-D, IPCk,		[46,54,112,118]
PQBP-1	bPY	P-D/MS, P-D		[18,22]
QKI-5	hPY	mut		[119]
RB15B	bPY	P-D		[22]
SAM68	hPY	P-D/MS, PD, IPCk, mut	(TRN-2B)	[18,22,53,112]
SOX14	bPY	P-D		[22]
TAF15	hPY	ΦD, M9M, mut		[38,60,108,111]
WBS16	hPY	P-D		[22]
YBX1	≈bPY	P-D/MS, P-D		[18,22]

Cargoes without PY-NLSs

Cargo	NLS	Experimental evidence	Other importins potentially used by the cargo	Refs.
Ad2 adenovirus core protein V	?	P-D, IPCc, IPCk	α/β	[120]
Ad2 adenovirus core protein VII	82–198	P-D, IPCc, IPCk	α/β, β, 7	[120,121]
ADAR1	dsRBD3	P-D/MS, CoIP, IPCk, IPCc		[122]
CD44 (ICD)	671–697	P-D/MS, CoIP, IPCc	β	[123]
DDX3	?	P-D/MS,		[18,22]
FOS	81–160	P-D/MS, P-D, IPCc, siRNA	(β), β-7,	[124,125]
FOXO4	Around Cys239			[67]
H2A	?	P-D, IPCk	β, 5, (7, 9),	[126,127]
H2B	?	P-D, IPCk	5, 7, 9, (β), α/β	[126,127]
H3	?	P-D, IPCk	5, (β, 7, 9),	[126,127]
H4	?	P-D, IPCk	5, 7, (β, 9),	[126,127]
HIV-1 REV	35–46	IPCk, P-D, M9M, siRNA	9, (5, 7, β),	[32,77,128]
HPV16 E6	121–151	P-D, IPC	α/β, β	[129]
HPV16 L2	1–13	P-D, IPC	α/β, 5	[33]
HPV18 L2	1–12	P-D, (IPC)	α/β, 5	[34]
HSP70	?	P-D/MS	(TRN-2)	[18]
HSP90	?	P-D/MS		[18]
JMJD5	134–151	CoIP	α1/β	[130]
JUN	250–334	P-D, IPC	β, 5, 7, 9	[131]
NPM-ALK	?	P-D/MS	8	[132]
PLK1	396–433	P-D/MS, P-D	(α/β),	[133]
RPL23A	32–74	P-D, IPCk	β, 5, 7, (α/β, 8)	[35,126,134]
RPS7	?	IPCk	α/β, β, 5, 7	[35]
RPL5	?	IPCk	α/β, β, 5, 7	[35]
RPL7	1–54	PD, siRNA	5	[135]
SRP19	?	P-D/MS, P-D, IPCk	(α/β, β, 5, 7), 8	[134]
TAF1A/TAFI48	400–450	P-D	β, 5	[136]
TCP-1-γ	?	P-D/MS, P-D	(TRN-2)	[18,137]
U1 snRNP A	?	(BH), IPC		[112]
U2 snRNP A	?	BH, IPC		[112]

hPY: hydrophobic PY-NLS. bPY: basic PY-NLS. ≈: similarity to but not perfect match with the PY-NLS consensus.
Experimental evidences: BH, evidence of interaction by bead halo assay; P-D, evidence of interaction by pull-down; P-D/MS, evidence of interaction by pull-down/mass spectrometry experiment; Y2H, evidence of interaction by yeast two-hybrid assay; CoIP, evidence of interaction by coimmunoprecipitation; ΦD, evidence of interaction by phage display assay; IPCk, import of the cargo by Transportin-1 in a permeabilized cell assay; IPCc, competition for import between the cargo and a known substrate of Tranportin-1 in a permeabilized cell assay; M9M, diminution of cargo import upon overexpression of the competitive inhibitor M9M; siRNA, diminution of cargo import upon siRNA-mediated knock-down of *Tnpo1*; mut, point mutation in the PY-NLS reduces nuclear import of the cargo.
These symbols are struck through when the experiment contradicts the hypothesis of TRN-1-dependent import.
In the column "Other importins potentially used by the cargo", importin names are between brackets when there is little evidence or contradictory evidence that the importin may import the cargo; importin names are struck through when there is evidence that the importin is not able to import the cargo.

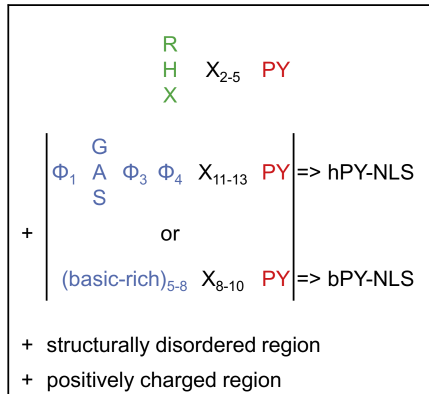

Fig. 2. The three criteria proposed by Lee et al. to define PY-NLSs: consensus sequences, structural disorder, and overall positive charge [22]. Φ_1 is strictly hydrophobic (=I/V/M/L/F/Y/P) while Φ_3 and Φ_4 may also be residues with long aliphatic side chains (R/K). PY residues (in red) constitute epitope 1. The basic residue preceding the PY (in green) constitutes epitope 2. Epitope 3 corresponds to the hydrophobic or basic N-terminal motif (in blue).

3.2. Transportin-2

The first proposed function for Transportin-2B was export the mRNA export factor NXF1 [16]. This hypothesis was abandoned after the contradicting report that the TRN-2/NXF1 interaction is disrupted by Ran-GTP [18]. TRN-2B thus probably imports NXF1 as do many other importins including TRN-1 [23]. TRN-2B actually interacts with many cargoes of TRN-1, among others hnRNP A1, hnRNP H, hnRNP M, FUS, CPSF6 and Sam68 [18].

The extra residues found in Transportin-2A occur within the B helix of HEAT repeat 17 (Fig. 2), which contributes to the interaction of TRN-1 with PY-NLSs [22,24]. Therefore, one might expect TRN-2A to recognize cargoes different from those of Transportin-1 and -2B. In support of this hypothesis, a potential cargo of TRN-2A is the Apobec-1 complementation factor (ACF) involved in nuclear C-to-U RNA editing, whose NLS is devoid of PY-NLS motif and which was reported to interact with TRN-2A but not with TRN-1 [25]. However, hnRNP A1, which contains a PY-NLS, and HuR, which contains a PY-NLS-like sequence, are able to interact with TRN-1, TRN-2A, and TRN-2B [17]. Finally, in HeLa cells, where *Tnpo2* is expressed as Transportin-2A and Transportin-2B [17], simultaneous knockdown of *Tnpo1* and *Tnpo2* is necessary to impair nuclear import of FUS, which suggests that both Transportin-1 and Transportin-2A and/or 2B import FUS [26]. In conclusion, not a single cargo has been demonstrated to be imported exclusively by Transportin-1, -2A or -2B, and some functional redundancy between these three importins is expected (See Fig. 3).

4. PY-NLSs and their interaction with Transportin-1

The concept of PY-NLS was proposed after elucidation of the structure of the complex formed between Transportin-1 and the NLS of hnRNP A1 [22]. This study identified the residues critical

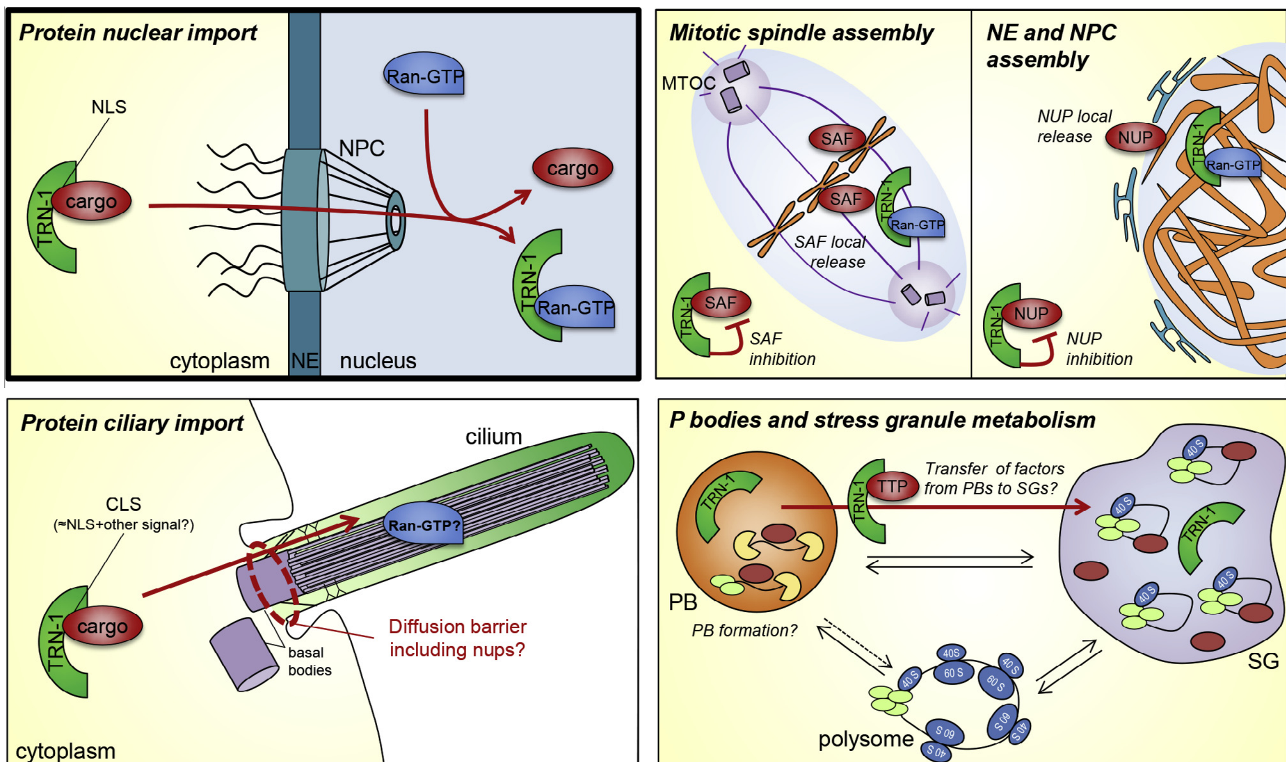

Fig. 3. Models for known and putative functions of Transportin-1 (see references in the text). *Top left panel:* protein nuclear import is the best known function of Transportin-1. In the cytoplasm, cargo proteins that contain suitable NLSs form an import complex with TRN-1, which facilitates movement through NPCs. In the nucleus, Ran-GTP binds to TRN-1, dissociating the complex and releasing the cargo. *NE:* nuclear envelope. *Top right panel:* model for the spatial regulation of mitotic assembly events by Transportin-1. Far from the chromatin, TRN-1 inhibits the assembly of diverse mitotic structures by sequestering key assembly factors. In the vicinity of the chromatin, a "cloud" of Ran-GTP is produced by chromatin-bound RCC1. This allows for Ran-GTP binding to TRN-1, thereby releasing the assembly factor. *SAF:* spindle assembly factors (SAFs). *MTOC:* microtubule organizing center. *NUP:* nucleoporin. *Bottom left panel:* putative model for Transportin-1 function in ciliary transport. In the cytoplasm, TRN-1 forms a ciliary import complex with cargoes that contain suitable CLSs (ciliary localization signals). This facilitates the crossing of a diffusion barrier located at the basis of the cilium (possibly comprising nups) and the ensuing delivery of the cargo into the cilium (possibly through binding of Ran-GTP to TRN-1). *Bottom right panel:* Transportin-1 is present in two classes of RNA granules that contain translationally silent mRNAs: P-bodies (PBs) and stress granules (SGs). TRN-1 may play a role in PB formation and/or in the transport of specific cargoes (e.g. TTP) from PBs to SGs. *Green ovals:* Poly(A)-binding protein and eukaryotic initiation factors. *Red ovals:* RNA-binding proteins. *Blue ovals:* ribosomal subunits. *Yellow pies:* 5′–3′ and 3′–5′ mRNA degradation factors.

for the interaction, which could then be located within the NLSs previously delineated in other cargoes of TRN-1. PY-NLSs consist of a C-terminal (R/K/H-X_{2-5}-P-Y) motif and of an N-terminal motif which can either be a hydrophobic motif fitting the loose Φ-G/A/S-Φ-Φ consensus (where Φ is a hydrophobic amino acid) or a patch enriched in basic residues. The linker separating the N-terminal motif from the C-terminal PY is 8–13 a.a. long. Besides these sequence criteria, canonical PY-NLSs respond to physical rules: they should be included in a basic and structurally disordered region (Fig. 1) [22].

By applying this set of criteria to the human proteome in Swiss-Prot, 13 known Transportin-1 cargoes and 81 new candidate cargoes harboring a PY-NLS were identified. The predictive value of the rules was validated by showing a direct interaction between TRN-1 and five candidate cargoes [22]. Crystal structures of TRN-1 in complex with other NLSs (see Table 1) also confirmed the relevance of the PY-NLS.

Transportin-1 residues involved in the interaction with PY-NLSs are scattered throughout the internal helices of HEAT repeats 8–20 and disposed in the direction antiparallel to that of the NLS. The binding groove is similar for hydrophobic PY-NLS (*hPY-NLSs*) – exemplified by those of hnRNP A1, hnRNP D, hnRNP DL, and FUS – and for basic PY-NLSs (*bPY-NLSs*) – exemplified by that of hnRNP M. The C-terminal PY motifs of these various NLSs are contacted by the same residues in TRN-1. By contrast, the hydrophobic or basic N-terminal parts of PY-NLSs are contacted by partially overlapping sets of residues [22,24,27–29]. The difference in the recognition of bPY-NLSs and hPY-NLSs has implications for the specificity of the karyopherin towards hPY-NLS or bPY-NLSs through evolution (see below).

Mutagenesis and thermodynamical analyses of several Transportin-1/cargo and yeast Kap104p-cargo complexes further documented the multipartite nature of PY-NLSs. They revealed that the distribution of the binding energy along the three epitopes of PY-NLSs – i.e. the hydrophobic or basic N-terminal patch, the PY C-terminal motif, and the basic residue between them – is highly variable. Each epitope can thus accommodate large sequence diversity as long as it is combined with other stronger epitopes. In consequence, the loose rules currently describing the PY-NLSs – which were based on a few known Transportin-dependent NLSs – may still be too restrictive to encompass all PY-NLSs [24,30].

Several cargoes do bind Transportin-1 through NLSs that meet only partially the current PY-NLS consensus criteria. For example, in HuR, the final PY motif is replaced with PG. The length and structure of the linkers do not always correspond exactly to the rules presented in Fig. 2 either. While PY-NLSs have been defined as sequences that lack secondary structures [22], the PY-NLS of FUS contains an α-helix [28,29]. In Huntingtin, the PY motif is separated from the amino-terminal basic motif by a 26 residue-long linker in which lies a β-sheet [31]. In YBX1, Cyclin T1, PABPN1, NXF1, and HuR, the length of the linker between the PY motif and the preceding basic residue is also out of the range permitted in the consensus (see Refs. in Table 2). However, it is likely that relaxing sequence criteria for PY-NLS prediction will increase not only sensitivity but also the occurrence of false positives. Developing accurate and sensitive methods of prediction of PY-NLSs is therefore a formidable challenge which will not be met by simple alignment-based methods. To better define these motifs, it will be necessary to integrate sequence requirements with structural and chemical criteria such as accessibility, disorder, charge, and hydrophobicity.

5. Transportin-1-dependent NLSs that are not PY-NLSs

As mentioned above, PY-NLSs are probably not the only kind of NLS recognized by Transportin-1, as several cargoes of TRN-1 do not seem to contain any PY-NLS-like sequence (see part 2 of Table 2). The NLSs of these cargoes have seldom been characterized in detail. Those that have been delineated are often very basic sequences [32–35] which can be bound by TRN-1 and other importins [32,35]. Biochemical studies suggest that they might, at least in some cases, be recognized by determinants of Transportin-1 distinct from the PY-NLS-binding domain. For example, it was shown that Transportin-1 binding to the M9 PY-NLS of hnRNP A1 and to the non-PY-NLS of rpL23a are both displaced by Ran-GTP but are not mutually exclusive [35]. Structural data and analysis of these NLSs by mutagenesis would be necessary to characterize the mode of recognition of non-PY-NLS-cargoes by Transportin-1 and other karyopherins.

Another interesting issue is whether non-PY-NLS motifs may modulate the interaction of PY-NLS-containing cargoes with TRN-1. The role of accessory domains in the Transportin/cargo interaction is probably underestimated, because all the crystal structures documenting the interaction were obtained with Transportin-1 bound to isolated NLSs (see Table 1), rather than to full-length cargoes. Recently, it was shown that the RNA-binding protein FUS interacts with TRN-1 not only through its PY-NLS motif but also with the adjacent RGG3 domain, when the latter is unmethylated (see also Section 8.1.3). Whether unmethylated RGG domains might generally act as TRN-1-dependent NLSs on their own or as accessory domains extending PY-NLSs remains to be determined.

6. M9M, a potent inhibitor of Transportin-1 and probably of Transportin -2A and -2B

The identification of karyopherin-cargo complexes has usually been performed through the delineation of the NLS involved, the demonstration that the karyopherin interacts with the cargo through the NLS in a Ran-GTP-regulated manner, and the confirmation that the recombinant karyopherin imports the cargo in a digitonin-permeabilized cell assay (see Table 2). Recently developed karyopherin inhibitors were added to the existing toolbox and might facilitate the future identification or validation of karyopherin/cargo complexes [36].

A peptide named M9M was developed by Cansizoglu et al. to inhibit Transportin-1 [24]. Its design is based on the observation that the distribution of binding energy along PY-NLSs is highly variable. In particular, in hnRNP A1 M9, the binding hotspot is the N-terminal hydrophobic motif, whereas in hnRNP M NLS, the binding hotspot is the C-terminal PY motif [24]. Combining hnRNP A1 M9 N-terminal and hnRNP M C-terminal moiety, the M9M peptide binds mammalian TRN-1 with very high affinity (K_D = 107 pM) and therefore competes effectively with wild-type PY-NLSs ($K_D \approx$ 1–50 nM, [21–24,27–29,37]) [24]. In fact, the binding of TRN-1 to M9M even prevents its binding to Ran-GTP (K_D = 2 nM, [21]). In consequence, adding recombinant M9M in pull-down reactions successfully displaces cargoes from TRN-1, and expressing M9M in HeLa cells diminishes the nuclear fraction of TRN-1 cargoes [24,26,38].

Because M9M is essentially a combination of two PY-NLSs and because PY-NLSs seem to be recognized exclusively by TRN-1 and TRN-2, M9M is not expected to target unrelated β-karyopherins. Consistently, it has been demonstrated that M9M expression does not disturb Importin-β-dependent pathways [24,26,39]. On the other hand, the specificity of M9M towards Transportin-1, -2A and -2B has not been thoroughly characterized. As discussed above, a certain redundancy seems to be shared by Transportin-1, -2A and -2B. In particular, all of them were shown to interact with hnRNP A1 NLS [17], which constitutes the N-terminal moiety of M9M. Therefore, it is likely that the M9M spectrum extends to Transportin-2A and/or -2B. In support of this hypothesis, M9M

expression causes a striking redistribution of the FUS cargo to the cytoplasm, while knock-down of individual Transportins does not alter FUS localization [26].

7. Diversification of the Transportin family across evolution

The Transportin subfamily seems to derive from a single ancestral gene [6]. It is still represented by a single member in *S. cerevisiae* (*Kap104* gene) and in *Caenorhabditis elegans* (*imb-2* gene). In bony vertebrates, the ancestral gene underwent duplication, giving rise to the *Tnpo1* and *Tnpo2* genes. Additional events of duplication may have occurred over the course of evolution, notably in the ancestor of ray-finned fishes (such as *Danio rerio*) which have at least two *Tnpo2* genes. In *Drosophila melanogaster*, two Transportin genes (*Trn* and *CG8219*) are also present [6]. Gene losses might have occurred as well; for example, *Tnpo2* seems to have disappeared in *Gallus gallus* [6] and more generally in birds (Twyffels et al., personal observation).

The specificity of Transportin proteins for NLSs has not been studied except for mammalian Transportin-1 and yeast Kap104p. As mentioned previously, mammalian Transportin-1 recognizes hydrophobic PY-NLSs as well as basic PY-NLSs. Interestingly, the acidic interface contacting basic N-terminal motifs seems generally well conserved. By contrast, some of the residues contacting hydrophobic N-terminal motifs are conserved in Transportins from metazoans and plants but not from fungi. This suggests that fungal Transportin may be able to bind bPY-NLSs but not bPY-NLSs [30]. In accordance with this prediction, recombinant yeast Kap104p binds the basic PY-NLS subclass but not the hydrophobic PY-NLS subclass in pull-down assays [30]. Moreover, the four direct cargoes of Kap104p – Nab2p [40–42], Hrp1p [43,44], Tfg2p [45], and Pab2p [46] – all contain basic PY-NLSs. Kap104p is also involved in the import of a trimeric complex consisting of a bPY-NLS-containing adaptor – called Symportin 1 – linked to ribosomal proteins Rpl5p and Rpl11p [47]. These results tend to confirm that yeast Kap104p is not able to import cargoes that contain hydrophobic PY-NLSs.

Recently, we showed that *Drosophila* Transportin is able to import the mammalian hydrophobic PY-NLS-containing hnRNP A1 and that this activity is inhibited by the expression of the M9M peptide [48]. *Drosophila* Transportin was also shown to bind and import an endogenous hPY-NLS-containing cargo, Cubitus interruptus [49]. This also confirms the specificity predictions made on the basis of sequence conservation [24].

All together, these observations indicate that the diversification of the *Tnpo* gene family as well as the broader spectrum of PY-NLS recognized by these β-karyopherins across evolution contributed to the increased diversity of nuclear-imported cargoes in higher eukaryotes.

8. Regulation of Transportin-dependent nuclear import

The regulation of nucleo-cytoplasmic transport can theoretically occur at several levels, from the most specific (the cargo) to the most global (the nuclear pore complex), through an intermediate level (karyopherins) [50,51]. In the following paragraphs, we discuss how these multiple regulation levels can affect Transportin-dependent import pathways.

8.1. Regulation of Transportin-cargo interaction through post-translational modifications of the cargo

The ability of a specific cargo to bind its import or export receptor can be modulated by several mechanisms. First, the NLS or NES of the cargo may be masked by intra- or inter-molecular interactions formed by the cargo. An example of this kind of regulation was recently provided by a study on the import of Ci/Gli transcription factors. These are imported by Transportins through a PY-NLS which can be masked by binding to the Hedgehog pathway component Suppressor-of-fused (Sufu) [49]. Second, the affinity of the NLS/NES for the karyopherin may be modulated by post-translational modifications occurring in or near the NLS/NES. Known examples include phosphorylation, methylation, mono-ubiquitination and poly(ADP-ribosyl)ation. These modifications can either increase or decrease the affinity of the cargo for its carrier (see [50,51] for review).

8.1.1. Phosphorylation within or next to PY-NLSs

Kinases/phosphatases can be regulated by many different cellular signals. Therefore, signal-responsive phosphorylation/dephosphorylation modulating subcellular localization represents a direct link between extracellular stimuli and the cell response in terms of nucleo-cytoplasmic distribution of specific signaling molecules [50].

The localization of several Transportin-1 cargoes can be regulated by phosphorylation. A well-documented example is that of hnRNP A1. Upon osmotic stress, hnRNP A1 is massively redistributed from the nuclear to the cytoplasmic compartment. This cytoplasmic relocalization is dependent on the phosphorylation of serine residues present in the "F-peptide" (amino acids 301–318). While a phosphomimetic hnRNP A1 mutant stays cytoplasmic even in absence of stress, a phosphorylation-deficient mutant remains nuclear upon stress. Furthermore, phosphorylation of the F-peptide down-modulates hnRNP A1 capacity to interact with TRN-1. Because the phosphorylation sites and the M9 motif (amino acids 273–289) are juxtaposed near the C terminus of hnRNP A1, it is likely that phosphorylation of these residues regulates the accessibility of M9 [52].

Another cargo whose Transportin-1-dependent import might also be regulated through phosphorylation is SAM68. In response to EGF signaling, the BRK kinase phosphorylates SAM68 tyrosine residue Y440. While the main pool of SAM68 is nuclear, Y440-phosphorylated Sam68 relocalizes to perinuclear structures [53]. This may result from a decrease in Transportin-1-dependent import of Y440-phosphorylated Sam68 as Y440 is part of the PY motif within Sam68 PY-NLS [22].

It should be anticipated that such phosphorylation-dependent regulation of cargo-Transportin interactions will be uncovered in the future as phosphorylation is a frequently occurring post-translational modification which allows a rapid signal-mediated modulation of protein nuclear import.

8.1.2. Arginine methylation within or next to PY-NLSs

Arginine methylation is another post-translational modification which has been shown to modulate protein nucleo-cytoplasmic distribution.

Several Transportin-1 cargoes are reported to be methylated on arginine residues located within or directly next to their PY-NLS. This is the case of the nuclear poly(A)-binding protein PABP-N1, whose methylated arginines overlap the PY-NLS. Methylation of these residues weakens PABP-N1 association to TRN-1 and favors PABP-N1 binding to RNA. Therefore, this modification appears to modulate the competitive association of PABP-N1 with TRN-1 or RNA. Modification of TRN-1 binding affinity by arginine methylation is consistent with structural data as the modified residues correspond to contact points with TRN-1 [54].

Besides PABPN1, other Transportin-1 cargoes can be arginine-methylated within or next to their NLS. For example, the Transportin-dependent NLSs of the RNA-binding proteins HuR [17] and hnRNP D [27] comprise arginine residues which can be methylated [55,56]. The transcription elongation factor SPT5 is also arginine-

methylated [57] in its predicted PY-NLS [22]. Although the effect of methylation on the nuclear localization of these cargoes has not been investigated yet, it can be expected that methylation of arginine side chains directly interacting with TRN-1 modifies cargo-TRN-1 interactions.

In yeast proteins Hrp1p and Nab2p, the Kap104p-dependent NLS is of the basic PY-NLS subtype. It includes a terminal PY or PL motif and a basic motif that comprises two or three RGG repeats [30]. Methylation of the arginine from these RGG repeats has been implicated in stimulating nuclear export (see [58,59] and references therein). It remains to be determined whether it also affects the recognition of the NLS by Kap104p.

8.1.3. Methylation of the RGG domain of FET proteins

We already mentioned that the RGG3 domain of FUS can contribute to the interaction of FUS with TRN-1 [60]. Interestingly, FUS RGG3 domain undergoes extensive asymmetric dimethylation of arginines, and this modification strongly reduces its affinity for TRN-1. When unmethylated, the RGG3 domain rescues weaker binding of a PY-NLS mutant. Moreover, a PY-NLS mutant that is cytoplasmic in basal conditions recovers a nuclear localization upon inhibition of methyltransferase activity or expression. Therefore, FUS unmethylated RGG3 might be an independent TRN-1-binding motif, sufficient by itself to achieve recognition and import by TRN-1 [60]. Determining the structure of full-length FUS bound to TRN-1 should improve our understanding of the unmethylated RGG domain-TRN-1 interaction, which is likely to occur in other proteins. The best candidates are the two other FET proteins, EWS and TAF15. Both present a domain organization similar to that of FUS, with a PY-NLSs preceded by an extensively methylated RGG domain. Moreover, mutants of EWS and TAF15 in which the PY-NLS is impaired recover a nuclear localization upon inhibition of methyltransferases, as does FUS [60]. In conclusion, these data suggest that all three FET proteins could bind TRN-1 with their unmethylated RGG domain as well as through their PY-NLS.

This is an important biomedical issue because a link between the Transportin-1-dependent import of FET proteins and two neurodegenerative disorders is currently under investigation. Mutations that disable the PY-NLS of FUS have recently been described as a cause of familial Amyotrophic Lateral Sclerosis (fALS) [26]. The neurons and glial cells of fALS patients present abnormal cytoplasmic protein inclusions that contain methylated FUS, but not the other FET proteins or TRN-1. The primary cause of fALS could therefore be the selective cytoplasmic accumulation of FUS PY-NLS mutants; this mislocalization could be aggravated by physiological conditions favoring the methylation of FUS RGG3 domain [60]. By contrast, a set of similar neurodegenerative disorders collectively known as FTLD-FUS is characterized by neuronal inclusions in which unmethylated FUS is deposited along with TRN-1, EWS and TAF15, but not with other RNA-binding cargoes of TRN-1 (see [38,60] and references therein). In FTLD-FUS, hypomethylation of the three FET proteins might contribute to the pathological deposition of the FET proteins through several mechanisms, including altered import [60]. Further studies are needed to understand the physiological role of arginine methylation in the RGG domain of FET proteins and the mechanism behind their potential hypomethylation in FTLD-FUS.

RGG domains are present in several Transportin-1 cargoes beyond FET proteins, but whether Transportin-1 is able to bind them has not been tested yet, and caution should be exercised when drawing parallels. For example, the RGG domain of hnRNP A2 comprises arginines that are asymmetrically dimethylated, like those in FET proteins; however, treatment with a methyltransferase inhibitor shifts the localization of hnRNP A2 from the nucleus to the cytoplasm, contrary to what was shown for PY-NLS mutants of FET proteins. This suggests that RGG methylation increases nuclear import, decreases nuclear export or decreases cytoplasmic retention of hnRNP A2 [61]. RGG methylation can thus have contradictory effects on the nucleo-cytoplasmic shuttling of different Transportin-1 cargoes. Besides, RGG methylation can influence other properties of RGG domains such as binding to nucleic acids, aggregation or interaction with other proteins. These multiple possibilities should be kept in mind when analyzing the effect of RGG methylation on nucleo-cytoplasmic localization.

8.2. Regulation of HuR import by caspase-dependent cleavage

Transportin-2-mediated import of HuR has been studied in the context of muscle cell differentiation and provides an interesting example of transport regulation. During myogenesis, a small fraction of HuR is cleaved into two fragments by caspase-3. The fragment that contains the NLS of HuR competes with full-length HuR for Transportin-2 binding. As a result, HuR accumulates in the cytoplasm, where it stabilizes pro-myogenic mRNAs [62,63].

Earlier observations had shown that the caspase-dependent cleavage of HuR contributes to apoptotic progression in cells exposed to lethal stresses [64]. Although the consequences of HuR cleavage on the expression of HuR-bound mRNAs have not been investigated in this study, one can speculate that known HuR mRNA ligands such as pro-apoptotic p53 [65] and cell growth inhibitor p21 [66] mRNAs might be stabilized and thereby contribute to the observed apoptotic phenotype.

8.3. ROS-dependent nuclear import of FOXO4/DAF-16 by Transportin-1

The nuclear import of the transcription factor FOXO4/DAF-16A seems to be governed by another original mechanism. Upon accumulation of reactive oxygen species (ROS), FOXO4 is translocated from the cytoplasm to the nucleus, where FOXO factors are known to stimulate the transcription of ROS-detoxifying enzymes. Recently, Putker et al. showed that ROS induce the formation of a disulfide bond between residue Cys239 of FOXO4 and TRN-1. Moreover, both knockdown of *Tnpo1* and replacement of Cys239 with serine reduce ROS-induced nuclear accumulation of FOXO4. These results suggest a model in which, under conditions of high ROS levels, FOXO4 and TRN-1 covalently associate in the cytoplasm, cross the NPC together, and dissociate in the nucleus where the redox potential is lower. This mechanism can account for the ROS-dependent nuclear import of FOXO4 [67].

8.4. Expression of Transportin-1 and Transportin-2

Nucleo-cytoplasmic transport can also be modulated through the regulation of karyopherin expression or through karyopherin post-translational modifications [50,51]; however, no examples have been described yet for Transportins, which seem ubiquitously expressed in mammals. Ubiquitous expression patterns of Tnpo1 and Tnpo2 mRNAs were obtained by microarrays experiments on human tissues [6], a result that tends to be confirmed by RNA-sequencing and EST data present in the Human Protein Atlas and Unigene databases (Twyffels et al., unpublished observation).

Similar data are recorded for cell lines in the Human Protein Atlas: nearly all cell lines examined seem to express Tnpo1 and Tnpo2 mRNAs, but TRN-2 protein is generally less well detected than TRN-1 (Twyffels et al., unpublished observation). An opposite result was reported for C2C12 cells. In these cells, TRN-2 protein was easily observed during all myogenic stages whereas TRN-1

signal increased from non-detectable to weak levels as C2C12 were differentiating into mature myotubes [62].

Interestingly, the splicing of *Tnpo2* seems to vary between cell lines. Indeed, while both Tnpo2A and Tnpo2B mRNAs are expressed in HeLa cells ([14,17,18] and our own observations), Tnpo2A mRNA was reported to be undetectable in HEK 293T cells [17]. Whether alterations in the relative expression levels of TRN-1, TRN-2A, and TRN-2B affects the nuclear import of their cargoes remains to be determined.

8.5. Transportin–nucleoporin interactions in interphase

All models explaining translocation through the NPC assume that the permeability barrier is mainly provided by a subset of nucleoporins that harbor domains rich in phenylalanine-glycine (FG) repeats. Karyopherins and other nuclear transport receptors cross the permeability barrier through direct and transient interactions with FG-nucleoporins [68,69]. No structural study of the interaction between Transportin-1 or-2 and FG-nups has been published; however, it is likely that these importins contact FG-nups in a manner parallel to that of Importin-β. Importin-β external surface contains multiple hydrophobic pockets appropriately spaced to enable binding from multiple phenylalanines from the same FG repeat region (see [70] and references therein).

In vitro assays show that each FG-nucleoporin can interact with several karyopherins. However, karyopherin-nucleoporin interactions are likely to be strongly modulated by interactions with Ran, cargoes and other nups in the environment of the NPC [70]. In yeast, it was shown that different karyopherin pathways were not equally disturbed by the deletion of specific nucleoporins [71]. Therefore, changes in the relative stoichiometry of nucleoporins in the NPC might result in the up- or down-regulation of specific karyopherin pathways. Interestingly, variations in NPC nucleoporin composition were recently described during cell differentiation and across tissues or cell lines [72–74].

Recombinant Transportin-1 was shown to pull down FG-nucleoporins Nup358, Nup214, Nup153, Nup98, Nup62, and Nup53, as well as nucleoporins belonging to the Nup107–160 subcomplex and associated nucleoporins ELYS and Centrin from *Xenopus* egg extracts (see [39,75] and references therein). These associations are either positively or negatively regulated by Ran-GTP [75]. Of course, some of them may be indirect, given that nucleoporins interact with each other to form subcomplexes (see [73] and references therein). All the nucleoporins mentioned above were also pulled down by recombinant Importin-β [75]. Interestingly, however, Importin-β and TRN-1 bind different sites of Nup153 [76]. In contrast to importin-β, TRN-1 does not seem to bind Nup50 [75]. Nup358 (Ran-binding protein 2) is described as necessary for both TRN-1- and importin-α/β-dependent nuclear import [77]. By contrast, in yeast, FG-domains from symmetric nucleoporins strongly disturbs Kap104p-mediated import of Nab2p but has limited effects on the classical NLS import pathway [71]. This suggests that a specific subset of nucleoporins might be required for the translocation of Transportin-1 or Importin-β, at least in yeast.

Several nucleoporins are targets of post-translational modifications, such as phosphorylation and O-GlcNAcylation [78], which might also affect specific karyopherin-mediated transports. For example, ERK-mediated phosphorylation of Nup50 has been shown to reduce its affinity for Importin-β or TRN-1 but not for CAS [79]. It remains to be demonstrated whether regulations at the level of nucleoporin expression or post-translational modifications are used by the cell to modulate Transportin-dependent pathways.

9. Potential roles of Tranportin-1 and -2 beyond protein nuclear import

9.1. Involvement of Transportin-1 in mitosis

The Ran GTPase is not only central to the regulation of nucleocytoplasmic transport during interphase: it is also a major regulator of numerous mitotic events, including mitotic spindle assembly, nuclear envelope assembly and nuclear pore complex assembly. Because the Ran guanine exchange factor RCC1 associates with chromatin, Ran-GTP is concentrated in the surroundings of chromosomes even after nuclear envelope breakdown. The Ran-GTP gradient works therefore as a genome-positioning system ("GPS") for mitotic cells [80]. Importin-β is a well-known effector of Ran mitotic functions. Alone or in combination with its adaptor Importin-α, Importin-β binds several spindle assembly factors and releases them upon Ran-GTP binding in the vicinity of chromatin, thereby ensuring a spatial regulation of spindle assembly [80]. A similar mechanism of localized release might explain how Importin-β spatially restricts nuclear envelope and nuclear pore complex assembly in a Ran-GTP-reversible manner [81–83]. An interesting question is whether other importins, and in particular Transportin-1 and -2, play a role in these mitotic processes.

Lau et al. and Bernis et al. studied the involvement of Transportin-1 in mitosis using mitotic and interphase cytosolic extracts derived from *Xenopus laevis* eggs. They observed that TRN-1 negatively regulated the assembly of a nuclear envelope around chromatin, as reported for Importin-β. TRN-1 also reduced the assembly of NPCs, first by inhibiting the initial recruitment of the nucleoporin ELYS/MEL-28 to AT-rich regions of the chromatin and second by inhibiting the incorporation of FG-nucleoporins into nascent nuclear pore complexes. Finally, TRN-1 negatively regulated the assembly of the mitotic spindle [75]; in particular, it bound ELYS/MEL-28 and the Nup107–160 complex and prevented their recruitment to the kinetochores [39]. All these effects were reverted by adding excess Ran-GTP [75], as described previously for those mediated by Importin-β [80–83], but also by adding the M9M inhibitor peptide [39]. Additionally, HeLa cells expressing M9M underwent defective mitosis or cytokinesis more frequently than control cells; in particular, they frequently formed disorganized mitotic spindles [39]. All together, these results argue in favor of a model in which Transportin-1 directly binds assembly factors used in spindle assembly, nuclear envelope assembly, and nuclear pore complex assembly to prevent the formation of these structures elsewhere than in the direct vicinity of chromatin.

9.2. Involvement of Transportin-1 in ciliary transport

Primary cilia are membrane-sheathed organelles that project from the surface of most eukaryotic cells and function as "cellular antennae" signaling chemo- or mechano-sensations [84]. They contain nine microtubule doublets, which form the primary cilium axoneme, and a distinct subset of proteins [85]. Recently, two studies suggested that Transportin-1 could be involved in the transport of specific proteins into the cilium.

The first study focused on the kinesin-2 motor KIF17, which plays a role in intra-flagellar transport. The authors reported that KIF17 interacts with TRN-1 through a basic motif named CLS. KIF17 CLS acted as an NLS when it was fused to a non-related reporter protein, but it appeared necessary and sufficient for the ciliary localization of KIF17 or another cytoplasmic kinesin. Moreover, replacement of KIF17 CLS by hnRNP A1 PY-NLS did not alter KIF17 ciliary localization [86]. The second study focused on Retinitis pigmentosa 2 (RP2), a ciliary membrane-associated protein. Transportin-1 was shown to bind acylated RP2 and knockdown

of *Tnpo1* reduced RP2 ciliary localization without affecting the formation of the cilium [87]. Finally, TRN-1 was found in the primary cilium [86,87], along with the GTP-bound form of Ran [86]. Several proteomics studies also suggested that Ran, β-karyopherins, and α-importins are present in the cilium and/or in its basal body [88–90].

This led the authors of the two aforementioned studies to investigate the hypothesis that ciliary transport might rely on the same functional components as nucleo-cytoplasmic transport – nuclear transport factors like importins, the Ran GTPase system, and nucleoporins. In that model, they imagined that a NPC-like structure might exist at the base of the cilium and constitute a ciliary permeability barrier. They did observe several nucleoporins located at the cilium base, and found that treatments known to block passage through the NPC also inhibited ciliary entry [91]. However, opposite results were recently obtained by another group [92]. Further work is therefore required to define the molecular basis of the ciliary permeability barrier and to evaluate the potential role of Transportin-1 in a mechanism of selective ciliary transport.

9.3. Involvement of Transportin-1 and -2 in RNA granules

The cellular response to acute stress includes a transient arrest of translation and the concomitant assembly of stress granules (SGs) in the cytoplasm. Core components of SGs are poly(A)+ mRNAs, the 40S ribosomal subunit and most of the translation initiation factors, which suggests that mRNPs in SGs resemble stalled initiation complexes. SGs also contain dozens of RNA-binding proteins (see for review [93–95]). While their exact role remains undefined, it seems clear that SGs are connected to post-transcriptional and translational regulations of gene expression during stress. Additional functions of SGs in modulating signaling pathways, including apoptosis signaling, have also been proposed [93–95]. mRNPs continuously shuttle in and out of SGs and can reassociate with polysomes or enter into P-bodies (PBs). These RNA granules comprise components of the general 5′–3′ mRNA decay machinery as well as of the non-sense-mediated decay (NMD) and RNAi pathways (see for review [94,96]). Unlike SGs, PBs are constitutively present and they do not contain ribosomal subunits. mRNAs found in P bodies are partially deadenylated, and several lines of evidence suggest that PBs are sites of mRNA degradation, although mRNA decay might be generally initiated and sometimes completed outside PBs [94,96].

Chang and Tarn observed that endogenous Transportin-1 is present in P-bodies and migrates into SGs when HeLa cells are exposed to various stresses. Importin-β and its adaptors Importin-α1, -α4 and –α5 [97–99] as well as Transportin-2B, but not -2A (our own unpublished observations), also localize into SGs induced by diverse experimental conditions. This property is nevertheless not shared by all karyopherins. Indeed, oxidative stress does not modify the localization of the CRM1 exportin; it induces a partial relocalization of Transportin-SR and Exportin-5 from the nucleus to the cytoplasm, but without any accumulation in SGs; and it causes the partial accumulation of Importin-13 into P-bodies but not into SGs [99]. Finally, the cytoplasmic pool of Ran, which represents only a small fraction of the total pool, is reported to accumulate into SGs in response to arsenite treatment [97].

Very little is known about the functional significance of the presence of Importin-α1, Importin-β, TRN-1, TRN-2 and possibly Ran in SGs. siRNA-mediated depletion of Importin-α1 was reported to delay the early stages of SG assembly [97] and likewise a RNAi screen identified Importin-β as necessary for SG assembly [100]. Knockdown of *Tnpo1* was reported to prevent localization of the RNA-binding protein TTP into SGs but not SG assembly [99]. Many cargoes of TRN-1 can localize into stress granules, including hnRNP A1 [101], hnRNP A2 [102], FUS [26,103], Sam68 [104], and the mRNA exporter NXF1 [105]. Even though Ran has been described in SGs [97], the hypothesis of a Transportin-mediated delivery of cargoes into SGs recapitulating the features of the nuclear import mechanism appears unlikely. Indeed, it has not been shown that the localization of any SG component into SGs requires its NLS. On the contrary, mutation of the PY-NLSs of accessory SG components hnRNP A1 and FUS rather favors the inclusion of these TRN-1 cargoes into SGs [26,101,103].

TRN-1 was found to localize not only in SGs but also in P-bodies. Furthermore, *Tnpo1* knockdown enhanced the formation of PBs and the accumulation of TTP in these structures [99]. This led the authors to propose that TRN-1 could promote the transfer of TTP from PBs to SGs. However, this would not suffice to explain the positive effect of *Tnpo1* knockdown on PB formation, which is observed in unstressed cells devoid of SGs. The possible role of Transportin-1 in SG and PB metabolism thus deserves further investigation.

Another question is whether TRN-1 and TRN-2-mediated import pathways are impaired upon stress because of the association of TRN-1 and -2B with SGs. GFP-TRN-1 appears to move in and out SGs rapidly [99], and a large fraction of TRN-1 stays outside of SGs. Therefore, the hypothesis of a "sequestration" of TRN-1 or TRN-2 in SGs is unlikely. In accordance with this, the nuclear localization of FUS, EWS and TAF15 appears preserved in stress conditions that do not induce their own accumulation into SGs at a significant level, such as upon oxidative stress or heat shock [38,106].

10. Conclusions and perspectives

Since the discovery of Importin-β and its family in the 1990s, β-karyopherins have proven to be responsible of an incessant ballet of macromolecules in and out of the nucleus. Collectively, they carry out the nucleo-cytoplasmic transport of thousands of proteins and RNAs, thereby ensuring that each of them is delivered to the right compartment for its proper function. Although we still lack an exact picture of the determinants required by most β-karyopherins to recognize their cargoes, recent structural studies of Transportin-1 have greatly contributed to the definition of important parameters for binding to the Transportin subfamily. As it could be expected, NLS recognized by Transportin-1 are modular allowing them to be very diverse in organization, structure and sequence. These characteristics prevent classical alignment-based methods from providing accurate and sensitive predictions. Therefore, the definition of the final repertoire of Transportin-1 cargoes remains an important challenge. The delimitation of the functional redundancies between Transportin-1 and -2 also awaits further investigation.

Beside their important role in nuclear import, Transportin-1 appear to be involved in several other cellular processes. These functions include the assembly of the mitotic spindle [39,75], ciliogenesis [86,87], and the formation of cytoplasmic RNA granules [99]. In conclusion, the research on this importinis livelier than ever and probably still reserves us exciting new discoveries.

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References

[1] Stewart, M. (2007) Molecular mechanism of the nuclear protein import cycle. Nat. Rev. Mol. Cell Biol. 8, 195–208.
[2] Güttler, T. and Görlich, D. (2011) Ran-dependent nuclear export mediators: a structural perspective. EMBO J. 30, 3457–3474.
[3] Cook, A.G. and Conti, E. (2010) Nuclear export complexes in the frame. Curr. Opin. Struct. Biol. 20, 247–252.
[4] Cook, A.G., Bono, F., Jinek, M. and Conti, E. (2007) Structural biology of nucleocytoplasmic transport. Annu. Rev. Biochem. 76, 647–671.
[5] Pemberton, L.F. and Paschal, B.M. (2005) Mechanisms of receptor-mediated nuclear import and nuclear export. Traffic 6, 187–198.
[6] Quan, Y., Ji, Z.-L., Wang, X., Tartakoff, A.M. and Tao, T. (2008) Evolutionary and transcriptional analysis of karyopherin beta superfamily proteins. Mol. Cell. Proteomics 7, 1254–1269.
[7] Wozniak, R.W., Rout, M.P. and Aitchison, J.D. (1998) Karyopherins and kissing cousins. Trends Cell Biol. 8, 184–188.
[8] Andrade, M.A., Petosa, C., O'Donoghue, S.I., Müller, C.W. and Bork, P. (2001) Comparison of ARM and HEAT protein repeats. J. Mol. Biol. 309, 1–18.
[9] Lange, A., Mills, R.E., Lange, C.J., Stewart, M., Devine, S.E. and Corbett, A.H. (2007) Classical nuclear localization signals: definition, function, and interaction with importin alpha. J. Biol. Chem. 282, 5101–5105.
[10] Pollard, V.W., Michael, W.M., Nakielny, S., Siomi, M.C., Wang, F. and Dreyfuss, G. (1996) A novel receptor-mediated nuclear protein import pathway. Cell 86, 985–994.
[11] Nakielny, S., Siomi, M.C., Siomi, H., Michael, W.M., Pollard, V. and Dreyfuss, G. (1996) Transportin: nuclear transport receptor of a novel nuclear protein import pathway. Exp. Cell Res. 229, 261–266.
[12] Fridell, R.A., Truant, R., Thorne, L., Benson, R.E. and Cullen, B.R. (1997) Nuclear import of hnRNP A1 is mediated by a novel cellular cofactor related to karyopherin-β. J. Cell Sci. 1331, 1325–1331.
[13] Bonifaci, N., Moroianu, J., Radu, A. and Blobel, G. (1997) Karyopherin β2 mediates nuclear import of a mRNA binding protein. Proc. Natl. Acad. Sci. USA 94, 5055–5060.
[14] Siomi, M.C., Eder, P.S., Kataoka, N., Wan, L., Liu, Q. and Dreyfuss, G. (1997) Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. J. Cell Biol. 138, 1181–1192.
[15] Izaurralde, E., Kutay, U., von Kobbe, C., Mattaj, I.W. and Görlich, D. (1997) The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. EMBO J. 16, 6535–6547.
[16] Shamsher, M.K., Ploski, J. and Radu, A. (2002) Karyopherin beta 2B participates in mRNA export from the nucleus. Proc. Natl. Acad. Sci. USA 99, 14195–14199.
[17] Rebane, A., Aab, A. and Steitz, J.A. (2004) Transportins 1 and 2 are redundant nuclear import factors for hnRNP A1 and HuR. RNA 10, 590–599.
[18] Güttinger, S., Mühlhäusser, P., Koller-Eichhorn, R., Brennecke, J. and Kutay, U. (2004) Transportin2 functions as importin and mediates nuclear import of HuR. Proc. Natl. Acad. Sci. USA 101, 2918–2923.
[19] Cansizoglu, A.E. and Chook, Y.M. (2007) Conformational heterogeneity of karyopherin beta2 is segmental. Structure 15, 1431–1441.
[20] Chook, Y.M. and Blobel, G. (1999) Structure of the nuclear transport complex karyopherin-beta2-Ran x GppNHp. Nature 399, 230–237.
[21] Chook, Y.M., Jung, A., Rosen, M.K. and Blobel, G. (2002) Uncoupling Kapbeta2 substrate dissociation and Ran binding. Biochemistry 41, 6955–6966.
[22] Lee, B.J., Cansizoglu, A.E., Süel, K.E., Louis, T.H., Zhang, Z.C. and Chook, Y.M. (2006) Rules for nuclear localization sequence recognition by Karyopherin beta 2. Cell 126, 543–558.
[23] Zhang, Z.C., Satterly, N., Fontoura, B.M. and Chook, Y.M. (2011) Evolutionary development of redundant nuclear localization signals in the mRNA export factor. Mol. Biol. Cell 22, 1–26.
[24] Cansizoglu, A.E., Lee, B.J., Zhang, Z.C., Fontoura, B.M. and Chook, Y.M. (2007) Structure-based design of a pathway-specific nuclear import inhibitor. Nat. Struct. Mol. Biol. 14, 452–454.
[25] Blanc, V., Kennedy, S. and Davidson, N.O. (2003) A novel nuclear localization signal in the auxiliary domain of apobec-1 complementation factor regulates nucleocytoplasmic import and shuttling. J. Biol. Chem. 278, 41198–41204.
[26] Dormann, D., Rodde, R., Edbauer, D., Bentmann, E., Fischer, I., Hruscha, A., Than, M.E., Mackenzie, I.R.A., Capell, A., Schmid, B., Neumann, M. and Haass, C. (2010) ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. EMBO J. 29, 2841–2857.
[27] Imasaki, T., Shimizu, T., Hashimoto, H., Hidaka, Y., Kose, S., Imamoto, N., Yamada, M. and Sato, M. (2007) Structural basis for substrate recognition and dissociation by human transportin 1. Mol. Cell 28, 57–67.
[28] Niu, C., Zhang, J., Gao, F., Yang, L., Jia, M., Zhu, H. and Gong, W. (2012) FUS-NLS/Transportin 1 complex structure provides insights into the nuclear targeting mechanism of FUS and the implications in ALS. PLoS One 7, e47056.
[29] Zhang, Z.C. and Chook, Y.M. (2012) Structural and energetic basis of ALS-causing mutations in the atypical proline–tyrosine nuclear localization signal of the fused in sarcoma protein (FUS). Proc. Natl. Acad. Sci. USA 109, 12017–12021.
[30] Süel, K.E., Gu, H. and Chook, Y.M. (2008) Modular organization and combinatorial energetics of proline–tyrosine nuclear localization signals. PLoS Biol. 6, e137.
[31] Desmond, C.R., Atwal, R.S., Xia, J. and Truant, R. (2012) Identification of a karyopherin β1/β2 proline–tyrosine nuclear localization signal in huntingtin protein. J. Biol. Chem. 287, 39626–39633.
[32] Arnold, M., Nath, A., Hauber, J. and Kehlenbach, R.H. (2006) Multiple importins function as nuclear transport receptors for the Rev protein of human immunodeficiency virus type 1. J. Biol. Chem. 281, 20883–20890.
[33] Darshan, M.S., Lucchi, J., Harding, E. and Moroianu, J. (2004) The l2 minor capsid protein of human papillomavirus type 16 interacts with a network of nuclear import receptors. J. Virol. 78, 12179–12188.
[34] Klucevsek, K., Daley, J., Darshan, M.S., Bordeaux, J. and Moroianu, J. (2006) Nuclear import strategies of high-risk HPV18 L2 minor capsid protein. Virology 352, 200–208.
[35] Jäkel, S. and Görlich, D. (1998) Importin β, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. EMBO J. 17, 4491–4502.
[36] Chook, Y.M. and Süel, K.E. (2011) Nuclear import by karyopherin-βs: recognition and inhibition. Biochim. Biophys. Acta 1813, 1593–1606.
[37] Suzuki, M., Iijima, M., Nishimura, A., Tomozoe, Y., Kamei, D. and Yamada, M. (2005) Two separate regions essential for nuclear import of the hnRNP D nucleocytoplasmic shuttling sequence. FEBS J. 272, 3975–3987.
[38] Neumann, M., Bentmann, E., Dormann, D., Jawaid, A., DeJesus-Hernandez, M., Ansorge, O., Roeber, S., Kretzschmar, H.A., Munoz, D.G., Kusaka, H., Yokota, O., Ang, L., Bilbao, J.M., Rademakers, R., Haass, C. and Mackenzie, I.R.A. (2011) FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. Brain 134, 2595–2609.
[39] Bernis, C., Swift-Taylor, B., Nord, M., Carmona, S., Chook, Y.M. and Forbes, D.J. (2014) Transportin acts to regulate mitotic assembly events by target binding rather than Ran sequestration. Mol. Biol. Cell 25, 992–1009.
[40] Aitchison, J.D., Blobel, G. and Rout, M.P. (1996) Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. Science 274, 624–627.
[41] Truant, R., Fridell, R.A., Benson, R.E., Bogerd, H.P. and Cullen, B.R. (1998) Identification and functional characterization of a novel nuclear localization signal present in the yeast Nab2 poly(A)+ RNA binding protein. Mol. Cell. Biol. 18, 1449–1458.
[42] Soniat, M., Sampathkumar, P., Collett, G., Gizzi, A.S., Banu, R.N., Bhosle, R.C., Chamala, S., Chowdhury, S., Fiser, A., Glenn, A.S., Hammonds, J., Hillerich, B., Khafizov, K., Love, J.D., Matikainen, B., Seidel, R.D., Toro, R., Rajesh Kumar, P., Bonanno, J.B., Chook, Y.M. and Almo, S.C. (2013) Crystal structure of human Karyopherin β2 bound to the PY-NLS of *Saccharomyces cerevisiae* Nab2. J. Struct. Funct. Genomics 14, 31–35.
[43] Lee, D.C. and Aitchison, J.D. (1999) Kap104p-mediated nuclear import. Nuclear localization signals in mRNA-binding proteins and the role of Ran and RNA. J. Biol. Chem. 274, 29031–29037.
[44] Lange, A., Mills, R.E., Devine, S.E. and Corbett, A.H. (2008) A PY-NLS nuclear targeting signal is required for nuclear localization and function of the *Saccharomyces cerevisiae* mRNA-binding protein Hrp1. J. Biol. Chem. 283, 12926–12934.
[45] Süel, K.E. and Chook, Y.M. (2009) Kap104p imports the PY-NLS-containing transcription factor Tfg2p into the nucleus. J. Biol. Chem. 284, 15416–15424.
[46] Mallet, P. and Bachand, F. (2013) A proline–tyrosine nuclear localization signal (PY-NLS) is required for the nuclear import of fission yeast PAB2, but not of human PABPN1. Traffic 14, 282–294.
[47] Kressler, D., Bange, G., Ogawa, Y., Stjepanovic, G., Bradatsch, B., Pratte, D., Amlacher, S., Strauß, D., Yoneda, Y., Katahira, J., Sinning, I. and Hurt, E. (2012) Synchronizing nuclear import of ribosomal proteins with ribosome assembly. Science 338, 666–671.
[48] Twyffels, L., Wauquier, C., Soin, R., Decaestecker, C., Gueydan, C. and Kruys, V. (2013) A masked PY-NLS in Drosophila TIS11 and its mammalian homolog Tristetraprolin. PLoS One 8, e71686.
[49] Shi, Q., Han, Y. and Jiang, J. (2014) Suppressor of fused impedes Ci/Gli nuclear import by opposing Trn/Kapβ2 in Hedgehog signaling. J. Cell Sci. 127, 1092–1103.
[50] Poon, I.K.H. and Jans, D.A. (2005) Regulation of nuclear transport: central role in development and transformation? Traffic 6, 173–186.
[51] Terry, L.J., Shows, E.B. and Wente, S.R. (2007) Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. Science 318, 1412–1416.
[52] Allemand, E., Guil, S., Myers, M., Moscat, J., Cáceres, J.F. and Krainer, A.R. (2005) Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. Proc. Natl. Acad. Sci. USA 102, 3605–3610.
[53] Lukong, K.E., Larocque, D., Tyner, A.L. and Richard, S. (2005) Tyrosine phosphorylation of sam68 by breast tumor kinase regulates intranuclear localization and cell cycle progression. J. Biol. Chem. 280, 38639–38647.
[54] Fronz, K., Güttinger, S., Burkert, K., Kühn, U., Stöhr, N., Schierhorn, A. and Wahle, E. (2011) Arginine methylation of the nuclear poly(a) binding protein weakens the interaction with its nuclear import receptor, transportin. J. Biol. Chem. 286, 32986–32994.
[55] Li, H., Park, S., Kilburn, B., Jelinek, M.A., Henschen-Edman, A., Aswad, D.W., Stallcup, M.R. and Laird-Offringa, I.A. (2002) Lipopolysaccharide-induced methylation of HuR, an mRNA-stabilizing protein by CARM1 coactivator-associated arginine methyltransferase. J. Biol. Chem. 277, 44623–44630.
[56] Ong, S.-E., Mittler, G. and Mann, M. (2004) Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. Nat. Methods 1, 119–126.
[57] Kwak, Y.T., Guo, J., Prajapati, S., Park, K.-J., Surabhi, R.M., Miller, B., Gehrig, P. and Gaynor, R.B. (2003) Methylation of SPT5 regulates its interaction with

RNA polymerase II and transcriptional elongation properties. Mol. Cell 11, 1055–1066.
[58] Thandapani, P., O'Connor, T.R., Bailey, T.L. and Richard, S. (2013) Defining the RGG/RG motif. Mol. Cell 50, 613–623.
[59] Yu, M.C. (2011) The role of protein arginine methylation in mRNP dynamics. Mol. Biol. Int. 2011, 163827.
[60] Dormann, D., Madl, T., Valori, C.F., Bentmann, E., Tahirovic, S., Abou-Ajram, C., Kremmer, E., Ansorge, O., Mackenzie, I.R.A., Neumann, M. and Haass, C. (2012) Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. EMBO J. 31, 4258–4275.
[61] Nichols, R.C., Wang, X.W., Tang, J., Hamilton, B.J., High, F.A., Herschman, H.R. and Rigby, W.F. (2000) The RGG domain in hnRNP A2 affects subcellular localization. Exp. Cell Res. 256, 522–532.
[62] Van der Giessen, K. and Gallouzi, I.-E. (2007) Involvement of transportin 2-mediated HuR import in muscle cell differentiation. Mol. Biol. Cell 18, 2619–2629.
[63] Beauchamp, P., Nassif, C., Hillock, S., van der Giessen, K., von Roretz, C., Jasmin, B.J. and Gallouzi, I.-E. (2010) The cleavage of HuR interferes with its transportin-2-mediated nuclear import and promotes muscle fiber formation. Cell Death Differ. 17, 1588–1599.
[64] Mazroui, R., Di Marco, S., Clair, E., von Roretz, C., Tenenbaum, S.A., Keene, J.D., Saleh, M. and Gallouzi, I.-E. (2008) Caspase-mediated cleavage of HuR in the cytoplasm contributes to pp32/PHAP-I regulation of apoptosis. J. Cell Biol. 180, 113–127.
[65] Mazan-Mamczarz, K., Galbán, S., López de Silanes, I., Martindale, J.L., Atasoy, U., Keene, J.D. and Gorospe, M. (2003) RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. Proc. Natl. Acad. Sci. USA 100, 8354–8359.
[66] Wang, W., Furneaux, H., Cheng, H., Caldwell, M.C., Hutter, D., Liu, Y., Holbrook, N. and Gorospe, M. (2000) HuR regulates p21 mRNA stabilization by UV light. Mol. Cell. Biol. 20, 760–769.
[67] Putker, M., Madl, T., Vos, H.R., de Ruiter, H., Visscher, M., van den Berg, M.C.W., Kaplan, M., Korswagen, H.C., Boelens, R., Vermeulen, M., Burgering, B.M.T. and Dansen, T.B. (2013) Redox-dependent control of FOXO/DAF-16 by transportin-1. Mol. Cell 49, 730–742.
[68] Kahms, M., Hüve, J., Wesselmann, R., Farr, J.C., Baumgärtel, V. and Peters, R. (2011) Lighting up the nuclear pore complex. Eur. J. Cell Biol. 90, 751–758.
[69] Grünwald, D. and Singer, R.H. (2012) Multiscale dynamics in nucleocytoplasmic transport. Curr. Opin. Cell Biol. 24, 100–106.
[70] Tetenbaum-Novatt, J., Hough, L.E., Mironska, R., McKenney, A.S. and Rout, M.P. (2012) Nucleocytoplasmic transport: a role for nonspecific competition in karyopherin–nucleoporin interactions. Mol. Cell. Proteomics 11, 31–46.
[71] Strawn, L.A., Shen, T., Shulga, N., Goldfarb, D.S. and Wente, S.R. (2004) Minimal nuclear pore complexes define FG repeat domains essential for transport. Nat. Cell Biol. 6, 197–206.
[72] D'Angelo, M.A., Gomez-Cavazos, J.S., Mei, A., Lackner, D.H. and Hetzer, M.W. (2012) A change in nuclear pore complex composition regulates cell differentiation. Dev. Cell 22, 446–458.
[73] Ori, A., Banterle, N., Iskar, M., Andrés-Pons, A., Escher, C., Khanh Bui, H., Sparks, L., Solis-Mezarino, V., Rinner, O., Bork, P., Lemke, E.A. and Beck, M. (2013) Cell type-specific nuclear pores: a case in point for context-dependent stoichiometry of molecular machines. Mol. Syst. Biol. 9, 648.
[74] Raices, M. and D'Angelo, M.A. (2012) Nuclear pore complex composition: a new regulator of tissue-specific and developmental functions. Nat. Rev. Mol. Cell Biol. 13, 687–699.
[75] Lau, C.K., Delmar, V.A., Chan, R.C., Phung, Q., Bernis, C., Fichtman, B., Rasala, B.A. and Forbes, D.J. (2009) Transportin regulates major mitotic assembly events: from spindle to nuclear pore assembly. Mol. Biol. Cell 20, 4043–4058.
[76] Shah, S. and Forbes, D.J. (1998) Separate nuclear import pathways converge on the nucleoporin Nup153 and can be dissected with dominant-negative inhibitors. Curr. Biol. 8, 1376–1386.
[77] Hutten, S., Wälde, S., Spillner, C., Hauber, J. and Kehlenbach, R.H. (2009) The nuclear pore component Nup358 promotes transportin-dependent nuclear import. J. Cell Sci. 122, 1100–1110.
[78] Yu, S.-H., Boyce, M., Wands, A.M., Bond, M.R., Bertozzi, C.R. and Kohler, J.J. (2012) Metabolic labeling enables selective photocrosslinking of O-GlcNAc-modified proteins to their binding partners. Proc. Natl. Acad. Sci. USA 109, 4834–4839.
[79] Kosako, H., Yamaguchi, N., Aranami, C., Ushiyama, M., Kose, S., Imamoto, N., Taniguchi, H., Nishida, E. and Hattori, S. (2009) Phosphoproteomics reveals new ERK MAP kinase targets and links ERK to nucleoporin-mediated nuclear transport. Nat. Struct. Mol. Biol. 16, 1026–1035.
[80] Kalab, P. and Heald, R. (2008) The RanGTP gradient – a GPS for the mitotic spindle. J. Cell Sci. 121, 1577–1586.
[81] Walther, T.C., Askjaer, P., Gentzel, M., Habermann, A., Griffiths, G., Wilm, M., Mattaj, I.W. and Hetzer, M. (2003) RanGTP mediates nuclear pore complex assembly. Nature 424, 689–694.
[82] D'Angelo, M.A., Anderson, D.J., Richard, E. and Hetzer, M.W. (2006) Nuclear pores form de novo from both sides of the nuclear envelope. Science 312, 440–443.
[83] Delmar, V.A., Chan, R.C. and Forbes, D.J. (2008) Xenopus importin beta validates human importin beta as a cell cycle negative regulator. BMC Cell Biol. 9, 14.
[84] Yuan, S. and Sun, Z. (2013) Expanding horizons: ciliary proteins reach beyond cilia. Annu. Rev. Genet. 47, 353–376.
[85] Inglis, P.N., Boroevich, K.A. and Leroux, M.R. (2006) Piecing together a ciliome. Trends Genet. 22, 491–500.
[86] Dishinger, J.F., Kee, H.L., Jenkins, P.M., Fan, S., Hurd, T.W., Hammond, J.W., Truong, Y.N.-T., Margolis, B.L., Martens, J.R. and Verhey, K.J. (2010) Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-β2 and RanGTP. Nat. Cell Biol. 12, 703–710.
[87] Hurd, T.W., Fan, S. and Margolis, B.L. (2011) Localization of retinitis pigmentosa 2 to cilia is regulated by Importin beta2. J. Cell Sci. 124, 718–726.
[88] Narita, K., Kozuka-Hata, H., Nonami, Y., Ao-Kondo, H., Suzuki, T., Nakamura, H., Yamakawa, K., Oyama, M., Inoue, T. and Takeda, S. (2012) Proteomic analysis of multiple primary cilia reveals a novel mode of ciliary development in mammals. Biol. Open 1, 815–825.
[89] Liu, Q., Tan, G., Levenkova, N., Li, T., Pugh, E.N., Rux, J.J., Speicher, D.W., Pierce, E.A. and Li$_{[}$, T. (2007) The proteome of the mouse photoreceptor sensory cilium complex. Mol. Cell. Proteomics 6, 1299–1317.
[90] Ishikawa, H., Thompson, J., Yates, J.R. and Marshall, W.F. (2012) Proteomic analysis of mammalian primary cilia. Curr. Biol. 22, 414–419.
[91] Kee, H.L., Dishinger, J.F., Blasius, T.L., Liu, C., Margolis, B.L. and Verhey, K.J. (2012) A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia. Nat. Cell Biol. 14, 431–437.
[92] Breslow, D.K., Koslover, E.F., Seydel, F., Spakowitz, A.J. and Nachury, M.V. (2013) An in vitro assay for entry into cilia reveals unique properties of the soluble diffusion barrier. J. Cell Biol. 203, 129–147.
[93] Anderson, P. and Kedersha, N. (2008) Stress granules: the Tao of RNA triage. Trends Biochem. Sci. 33, 141–150.
[94] Decker, C.J. and Parker, R. (2012) P-bodies and stress granules: possible roles in the control of translation and mRNA degradation. Cold Spring Harb. Perspect. Biol. 4, a012286.
[95] Anderson, P. and Kedersha, N. (2009) RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat. Rev. Mol. Cell Biol. 10, 430–436.
[96] Eulalio, A., Behm-Ansmant, I. and Izaurralde, E. (2007) P bodies: at the crossroads of post-transcriptional pathways. Nat. Rev. Mol. Cell Biol. 8, 9–22.
[97] Fujimura, K., Suzuki, T., Yasuda, Y., Murata, M., Katahira, J. and Yoneda, Y. (2010) Identification of importin alpha1 as a novel constituent of RNA stress granules. Biochim. Biophys. Acta 1803, 865–871.
[98] 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.
[99] Chang, W. and Tarn, W. (2009) A role for transportin in deposition of TTP to cytoplasmic RNA granules and mRNA decay. Nucleic Acids Res. 37, 6600–6612.
[100] Ohn, T., Kedersha, N., Hickman, T., Tisdale, S. and Anderson, P. (2008) A functional RNAi screen links O-GlcNAc modification of ribosomal proteins to stress granule and processing body assembly. Nat. Cell Biol. 10, 1224–1231.
[101] Guil, S., Long, J.C. and Cáceres, J.F. (2006) HnRNP A1 relocalization to the stress granules reflects a role in the stress response. Mol. Cell. Biol. 26, 5744–5758.
[102] McDonald, K.K., Aulas, A., Destroismaisons, L., Pickles, S., Beleac, E., Camu, W., Rouleau, G.A. and Vande Velde, C. (2011) TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via differential regulation of G3BP and TIA-1. Hum. Mol. Genet. 20, 1400–1410.
[103] Daigle, J.G., Lanson, N.A., Smith, R.B., Casci, I., Maltare, A., Monaghan, J., Nichols, C.D., Kryndushkin, D., Shewmaker, F. and Pandey, U.B. (2013) RNA-binding ability of FUS regulates neurodegeneration, cytoplasmic mislocalization and incorporation into stress granules associated with FUS carrying ALS-linked mutations. Hum. Mol. Genet. 22, 1193–1205.
[104] Henao-Mejia, J. and He, J.J. (2009) Sam68 relocalization into stress granules in response to oxidative stress through complexing with TIA-1. Exp. Cell Res. 315, 3381–3395.
[105] Katahira, J., Miki, T., Takano, K., Maruhashi, M., Uchikawa, M., Tachibana, T. and Yoneda, Y. (2008) Nuclear RNA export factor 7 is localized in processing bodies and neuronal RNA granules through interactions with shuttling hnRNPs. Nucleic Acids Res. 36, 616–628.
[106] Blechingberg, J., Luo, Y., Bolund, L., Damgaard, C.K. and Nielsen, A.L. (2012) Gene expression responses to FUS, EWS, and TAF15 reduction and stress granule sequestration analyses identifies FET-protein non-redundant functions. PLoS One 7, e46251.
[107] Maertens, G.N., Cook, N.J., Wang, W., Hare, S., Shree, S., Öztop, I., Lee, K., Pye, V.E., Cosnefroy, O., Snijders, P., Kewalramani, V.N., Fassati, A., Engelman, A., Gupta, S.S., Oztop, I., Snijders, A.P. and Cherepanov, P. (2014) Structural basis for nuclear import of splicing factors by human Transportin 3. Proc. Natl. Acad. Sci. USA, 2–7.
[108] Zakaryan, R.P. and Gehring, H. (2006) Identification and characterization of the nuclear localization/retention signal in the EWS proto-oncoprotein. J. Mol. Biol. 82, 27–38.
[109] Leemann-Zakaryan, R.P., Pahlich, S., Grossenbacher, D. and Gehring, H. (2011) Tyrosine phosphorylation in the C-terminal nuclear localization and retention signal (C-NLS) of the EWS protein. Sarcoma 2011, 218483.
[110] Wang, L., Li, M., Cai, M., Xing, J., Wang, S. and Zheng, C. (2012) A PY-nuclear localization signal is required for nuclear accumulation of HCMV UL79 protein. Med. Microbiol. Immunol. 201, 381–387.
[111] Nakielny, S., Shaikh, S., Burke, B. and Dreyfuss, G. (1999) Nup153 is an M9-containing mobile nucleoporin with a novel Ran-binding domain. EMBO J. 18, 1982–1995.

[112] Kimura, M., Kose, S., Okumura, N., Imai, K., Furuta, M., Sakiyama, N., Tomii, K., Horton, P., Takao, T. and Imamoto, N. (2013) Identification of cargo proteins specific for the nucleocytoplasmic transport carrier transportin by combination of an in vitro transport system and stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomics. Mol. Cell. Proteomics 12, 145–157.
[113] Siomi, M.C., Fromont, M., Rain, J.C., Wan, L., Wang, F., Legrain, P. and Dreyfuss, G. (1998) Functional conservation of the transportin nuclear import pathway in divergent organisms. Mol. Cell. Biol. 18, 4141–4148.
[114] Kawamura, H., Tomozoe, Y., Akagi, T., Kamei, D., Ochiai, M. and Yamada, M. (2002) Identification of the nucleocytoplasmic shuttling sequence of heterogeneous nuclear ribonucleoprotein D-like protein JKTBP and its interaction with mRNA. J. Biol. Chem. 277, 2732–2739.
[115] Van Dusen, C.M., Yee, L., McNally, L.M. and McNally, M.T. (2010) A glycine-rich domain of hnRNP H/F promotes nucleocytoplasmic shuttling and nuclear import through an interaction with transportin 1. Mol. Cell. Biol. 30, 2552–2562.
[116] Wang, W., Yang, X., Kawai, T., López de Silanes, I., Mazan-Mamczarz, K., Chen, P., Chook, Y.M., Quensel, C., Köhler, M. and Gorospe, M. (2004) AMP-activated protein kinase-regulated phosphorylation and acetylation of importin alpha1: involvement in the nuclear import of RNA-binding protein HuR. J. Biol. Chem. 279, 48376–48388.
[117] Truant, R., Kang, Y. and Cullen, B.R. (1999) The human TAP nuclear RNA export factor contains a novel Transportin-dependent nuclear localization signal that lacks nuclear export signal function. J. Biol. Chem. 274, 32167–32171.
[118] Calado, A., Kutay, U., Kühn, U., Wahle, E. and Carmo-Fonseca, M. (2000) Deciphering the cellular pathway for transport of poly (A)-binding protein II. RNA 6, 245–256.
[119] Wu, J., Zhou, L., Tonissen, K., Tee, R. and Artzt, K. (1999) The quaking I-5 protein (QKI-5) has a novel nuclear localization signal and shuttles between the nucleus and the cytoplasm. J. Biol. Chem. 274, 29202–29210.
[120] Hindley, C.E., Lawrence, F.J. and Matthews, D.A. (2007) A role for transportin in the nuclear import of adenovirus core proteins and DNA. Traffic 8, 1313–1322.
[121] Wodrich, H., Cassany, A., D'Angelo, M.A., Guan, T., Nemerow, G. and Gerace, L. (2006) Adenovirus core protein pVII is translocated into the nucleus by multiple import receptor pathways. J. Virol. 80, 9608–9618.
[122] Fritz, J., Strehblow, A., Taschner, A., Schopoff, S., Pasierbek, P. and Jantsch, M.F. (2009) RNA-regulated interaction of transportin-1 and exportin-5 with the double-stranded RNA-binding domain regulates nucleocytoplasmic shuttling of ADAR1. Mol. Cell. Biol. 29, 1487–1497.
[123] Janiszewska, M., De Vito, C., Le Bitoux, M.-A., Fusco, C. and Stamenkovic, I. (2010) Transportin regulates nuclear import of CD44. J. Biol. Chem. 285, 30548–30557.
[124] Arnold, M., Nath, A., Wohlwend, D. and Kehlenbach, R.H. (2006) Transportin is a major nuclear import receptor for c-Fos: a novel mode of cargo interaction. J. Biol. Chem. 281, 5492–5499.
[125] Malnou, C.E., Salem, T., Brockly, F., Wodrich, H., Piechaczyk, M. and Jariel-Encontre, I. (2007) Heterodimerization with Jun family members regulates c-Fos nucleocytoplasmic traffic. J. Biol. Chem. 282, 31046–31059.
[126] Baake, M., Bäuerle, M., Doenecke, D. and Albig, W. (2001) Core histones and linker histones are imported into the nucleus by different pathways. Eur. J. Cell Biol. 80, 669–677.
[127] Mühlhäusser, P., Müller, E.-C., Otto, A. and Kutay, U. (2001) Multiple pathways contribute to nuclear import of core histones. EMBO Rep. 2, 690–696.
[128] Henderson, B.R. and Percipalle, P. (1997) Interactions between HIV Rev and nuclear import and export factors: the Rev nuclear localisation signal mediates specific binding to human importin-beta. J. Mol. Biol. 274, 693–707.
[129] Le Roux, L.G. and Moroianu, J. (2003) Nuclear entry of high-risk human papillomavirus type 16 E6 oncoprotein occurs via several pathways. J. Virol. 77, 2330–2337.
[130] Huang, X., Zhang, L., Qi, H., Shao, J. and Shen, J. (2013) Identification and functional implication of nuclear localization signals in the N-terminal domain of JMJD5. Biochimie 95, 2114–2122.
[131] Waldmann, I., Wälde, S. and Kehlenbach, R.H. (2007) Nuclear import of c-Jun is mediated by multiple transport receptors. J. Biol. Chem. 282, 27685–27692.
[132] Wu, F., Wang, P., Young, L.C., Lai, R. and Li, L. (2009) Proteome-wide identification of novel binding partners to the oncogenic fusion gene protein, NPM-ALK, using tandem affinity purification and mass spectrometry. Am. J. Pathol. 174, 361–370.
[133] Lee, M.-S., Huang, Y.-H., Huang, S.-P., Lin, R.-I., Wu, S.-F. and Li, C. (2009) Identification of a nuclear localization signal in the polo box domain of Plk1. Biochim. Biophys. Acta 1793, 1571–1578.
[134] Dean, K.A., Von Ahsen, O., Görlich, D., Fried, H.M. and von Ahsen, O. (2001) Signal recognition particle protein 19 is imported into the nucleus by importin 8 (RanBP8) and transportin. J. Cell Sci. 114, 3479–3485.
[135] Tai, L.-R., Chou, C.-W., Lee, I.-F., Kirby, R. and Lin, A. (2013) The quantitative assessment of the role played by basic amino acid clusters in the nuclear uptake of human ribosomal protein L7. Exp. Cell Res. 319, 367–375.
[136] Dynes, J.L., Xu, S., Bothner, S., Lahti, J.M. and Hori, R.T. (2004) The carboxyl-terminus directs TAF(I)48 to the nucleus and nucleolus and associates with multiple nuclear import receptors. J. Biochem. 135, 429–438.
[137] Favre, N., Camps, M., Arod, C., Chabert, C., Rommel, C. and Pasquali, C. (2008) Chemokine receptor CCR2 undergoes transportin1-dependent nuclear translocation. Proteomics 8, 4560–4576.