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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.

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).

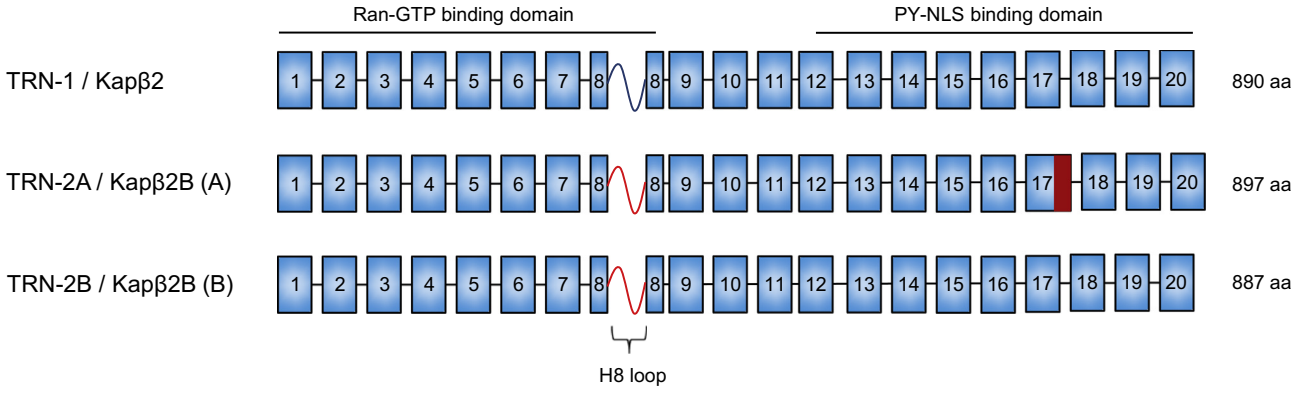

Fig. 1. A comparison between Transportin-1, -2A, and -2B. Each box represents a HEAT repeat. Transportins -2A and -2B are the products of two alternatively spliced isoforms of the *Tnpo2* gene [17]. The least conserved region between Transportin-1 and both isoforms of Transportin-2 is the loop connecting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