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REVIEW

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

On the asymmetric partitioning of nucleocytoplasmic transport – recent insights and open questions

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ABSTRACT

Macromolecular cargoes are asymmetrically partitioned in the nucleus or cytoplasm by nucleocytoplasmic transport (NCT). At the center of this activity lies the nuclear pore complex (NPC), through which soluble factors circulate to orchestrate NCT. These include cargo-carrying importin and exportin receptors from the β-karyopherin (Kapβ) family and the small GTPase Ran, which switches between guanosine triphosphate (GTP)- and guanosine diphosphate (GDP)-bound forms to regulate cargo delivery and compartmentalization. Ongoing efforts have shed considerable light on how these soluble factors traverse the NPC permeability barrier to sustain NCT. However, this does not explain how importins and exportins are partitioned in the cytoplasm and nucleus, respectively, nor how a steep RanGTP–RanGDP gradient is maintained across the nuclear envelope. In this Review, we peel away the multiple layers of control that regulate NCT and juxtapose unresolved features against known aspects of NPC function. Finally, we discuss how NPCs might function synergistically with Kapβs, cargoes and Ran to establish the asymmetry of NCT.

KEY WORDS: Nuclear transport receptor, Karyopherin, FG nucleoporin, Nuclear pore complex, Nucleocytoplasmic transport, Ran cycle

Introduction

Eukaryotic cells feature a protective double-layered membrane known as the nuclear envelope (NE) that encapsulates the nucleus within the cytoplasm. Segregating the genome from the protein synthesis machinery enables cells to exert control over transcription and translation in space and time. However, this requires key macromolecular cargoes, such as transcription factors and mRNA, to be selectively shuttled into or out of the cell nucleus. Understandably, neurodegeneration (Kim and Taylor, 2017), aging (Cho and Hetzer, 2020), cancer (Cagatay and Chook, 2018; Dickmanns et al., 2015) and viral pathogenesis (Fulcher and Jans, 2011; Miorin et al., 2020; Yarbrough et al., 2014) are associated with a dysregulation of this intracellular trafficking process, which is termed nucleocytoplasmic transport (NCT) (Stewart, 2007; Strambio-De-Castillia et al., 2010) and proceeds through nanoscale conduits in the NE known as nuclear pore complexes (NPCs) (Beck et al., 2007; Eibauer et al., 2015; Kim et al., 2018; von Appen et al., 2015).

NCT is unprecedentedly selective and efficient within the complex biological milieu. To appreciate its importance, range and complexity, at least 17% of all eukaryotic proteins are deemed to be imported into the nucleus (Cokol et al., 2000) with over 1000 cargoes being exchanged through each NPC every second (Ribbeck et al., 1998). In the past three decades, the key soluble factors that orchestrate NCT have been identified (Christie et al., 2016; Görlich and Kutay, 1999; Macara, 2001; Weis, 2003). Intensive efforts have also been devoted to understanding how these factors actively facilitate the speed, selectivity and direction of NCT through the permeability barrier of the NPC (Hoogenboom et al., 2021). These comprise members of the β-karyopherin (Kapβ) family, which include importins that usher diverse cargoes bearing nuclear localization signals (NLSs) into the nucleus (Boulikas, 1994; Cokol et al., 2000), and exportins, which escort cargoes bearing nuclear export signals (NESs) out of it (Xu et al., 2012), respectively (Baade and Kehlenbach, 2019). By convention, NES-containing cargoes are termed NES-cargo and NLS-containing cargoes are termed NLS-cargo. Another essential factor, the 25 kDa GTPase Ran, cooperates with Kapβs to regulate the delivery and accumulation of cargoes in an asymmetric, compartment-specific manner (Görlich et al., 1996; Moore and Blobel, 1993). This results from a steep gradient that separates its two nucleotide-bound forms, Ran-guanosine triphosphate (RanGTP) in the nucleus and Ran-guanosine diphosphate (RanGDP) in the cytoplasm. In this manner, NCT maintains essential functions within the nucleus and the cytoplasm without compromising the compositional integrity of either compartment (Terry et al., 2007).

However, several aspects of NCT function remain obscure. While both Kapβs and Ran freely traverse NPCs, an intriguing feature of NCT concerns how its opposing directional elements (importin versus exportin, and RanGTP versus RanGDP) remain asymmetrically partitioned across the NE to direct nuclear import and export processes (Fig. 1). For instance, exportins lack putative NLSs but yet accumulate in the nucleus. In this Review, we address the multiple layers of control that are centered around the NPC and regulate NCT. Thereafter, we highlight evidence that suggests how the asymmetry of NCT might be regulated by Kapβs based on their cellular, functional and structural properties. These include roles in reinforcing the NPC permeability barrier, preserving the steep Ran gradient, and the compartmentalization of small cargoes.

Nucleocytoplasmic transport is subject to multiple layers of control

NPCs exert the primary means of control over NCT as the exclusive sites of nucleocytoplasmic exchange. Each NPC is assembled from multiple proteins known as nucleoporins (Nups) (Cronshaw et al., 2002) that surround an aqueous central channel measuring ∼40–60 nm in diameter (Eibauer et al., 2015; Kim et al., 2018; von Appen et al., 2015). Each NPC is equipped with ∼200 intrinsically disordered, phenylalanine-glycine (FG)-rich Nups (or FG-Nups) that are tethered within its central channel. Collectively, the FG-Nups function as a filter-like permeability barrier that permits small molecules below ∼40 kDa (or 5 nm in diameter) to passively diffuse through the NPC, while suppressing the passage of larger non-specific cargoes, which

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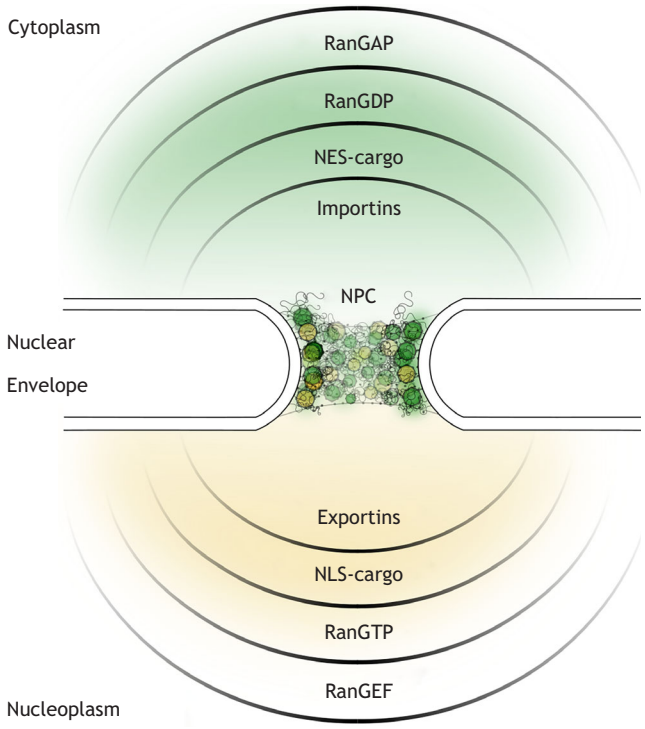

Fig. 1. Asymmetric partitioning of nucleocytoplasmic transport. Directionally opposed transport factors circulate through the NPC but remain asymmetrically partitioned across the NE at steady-state. Importins, NES-cargo, RanGDP and RanGAP are predominantly cytoplasmic. Exportins, NLS-cargo, RanGTP and RanGEF are localized in the nucleus.

are not recognized by Kapβs (Paine et al., 1975; Popken et al., 2015; Timney et al., 2016). Nevertheless, up to 50% of FG Nups can be deleted *in vivo* without a noticeable impact on NPC permeability (Strawn et al., 2004). However, the exact form of the NPC permeability barrier remains unclear (Huang and Szleifer, 2020; Lemke, 2016). This is due in part to the inherent flexibility and dynamic fluctuations of the FG-Nups (Sakiyama et al., 2016), which precludes structural characterization within NPCs. Consequently, NPC barrier models have mainly derived from studies with purified FG-repeat domains whose behavior can vary depending on length scale and experimental design (Hoogenboom et al., 2021).

A second layer of control is governed by importins, exportins and transportins (collectively termed 'Kapβs') (O'Reilly et al., 2011). Kapβs traverse the NPC permeability barrier in a matter of milliseconds (Dange et al., 2008) by engaging in multivalent interactions with the FG-repeats (Allen et al., 2001; Bayliss et al., 2000b; Kapinos et al., 2014; Port et al., 2015). As mentioned above, importins usher NLS-cargoes into the nucleus, whereas exportins deliver NES-cargoes out of it. Furthermore, transportins can exhibit both import and export functionalities (Twyffels et al., 2014). Altogether, 20 Kapβs are known in vertebrates and 14 in *Saccharomyces cerevisiae* (Chook and Suel, 2011; Kimura and Imamoto, 2014). This limits the number of cargoes assigned to each Kapβ to reduce potential errors during NCT. Although all Kapβs can bind to their cargoes directly, the 100 kDa canonical importin Kapβ1 (also known as importin β1, KPNB1) also recruits Kapα (importin α), which has seven isoforms (KPNA1–KPNA7) that function as cargo-adaptor proteins (Pumroy and Cingolani, 2015). Kapβ1 also recruits snurportin-1 (SPN1, also known as SNUPN) for the import of small nuclear ribonucleoproteins (Mitrousis et al., 2008). In both cases, SPN1 and Kapα bind to Kapβ1 through their N-terminal importin β-binding (IBB) domains (Lott and Cingolani, 2011).

Third, numerous NLSs and NESs greatly expand the repertoire of cargoes being recognized by each Kapβ. The best-characterized 'classical' nuclear import pathway consists of NLS-cargoes that typically form transport complexes with Kapα–Kapβ1, that is NLS-cargo–Kapα–Kapβ1 (Lange et al., 2007). Classical NLSs harbor multiple lysine (K) and arginine (R) residues as exemplified by the NLS of monopartite SV40 T-antigen (Kalderon et al., 1984) or the bipartite NLS of nucleoplasmin (Robbins et al., 1991). Nevertheless, substantial sequence variations exist across NLSs (Boulikas, 1994), both in cargoes that utilize the Kapα–Kapβ1 complex (Kosugi et al., 2009) and those that directly bind to Kapβ1 (Cokol et al., 2000; Lee et al., 2003). Some cargoes, such as myocardin-related transcription factors (MRTFs) (Pawlowski et al., 2010) may even harbor individual NLSs that are recognized by different Kapα isoforms (Goldfarb et al., 2004), although with varying affinities (Friedrich et al., 2006; Pumroy and Cingolani, 2015). Certain cargoes can also contain multiple NLSs that associate with different Kapαs or Kapβs, for instance hypoxia-inducible factors (HIFs) (Chachami et al., 2009; Depping et al., 2008). Other Kapβs such as transportin 1 (also termed Kapβ2) recognize cargoes via a consensus NLS-motif that contains proline (P) and tyrosine (Y) residues (termed PY-NLS cargoes) (Lee et al., 2006). In terms of exportins, chromosomal maintenance 1 (CRM1; also known as exportin 1, Exp1 or XPO1) recognizes a consensus leucine-rich NES (Kosugi et al., 2014). This clearly indicates that NLSs and NESs are diverse and that not all comply with consensus motifs (Cokol et al., 2000).

The Ran gradient constitutes a fourth layer of control that regulates NCT directionality, cargo partitioning and Kapβ recycling (Clarke and Zhang, 2008; Görlich et al., 1996; Izaurralde et al., 1997). RanGTP is ∼200 times more highly concentrated (i.e. partitioned) in the nucleus than in the cytoplasm (Görlich et al., 2003; Kalab et al., 2002; Smith et al., 2002). During import, NLS-cargo–importin complexes (including those that contain the adaptor protein; i.e. NLS-cargo–Kapα–Kapβ1) entering into the nucleus are disassembled upon binding of RanGTP to the importin (Jäkel and Görlich, 1998). This serves to retain the NLS-cargo in the nucleus as the NPC permeability barrier hinders its return to the cytoplasm. At the same time, the binding of RanGTP–importin complexes to the FG-Nups in the NPC facilitates its return to the cytoplasm. RanGTP is then hydrolyzed to RanGDP by SUMOylated RanGTPase-activating protein 1 (RanGAP1) together with Ran-binding protein 1 (RanBP1) and Ran-binding protein 2 (RanBP2, also known as Nup358), which constitute the eight cytoplasmic filaments surrounding the NPC cytoplasmic periphery (Koyama and Matsuura, 2010; Lounsbury and Macara, 1997; Monecke et al., 2013; Vetter et al., 1999). Thereafter, RanGDP frees the importin, which is then able to undertake another cargo import cycle (Stewart, 2007). Similarly, GTP hydrolysis mediated by RanGAP1 disassembles ternary NES-cargo–exportin–RanGTP complexes to complete their nuclear exit. RanGDP is then recycled back to the nucleus by its specific carrier nuclear transport factor 2 (NTF2; also known as NUTF2) (Ribbeck et al., 1998).

The Ran loop is finally closed by the chromatin-bound enzyme regulator of chromosome condensation 1 (RCC1; also known as RanGEF), which recharges RanGDP to RanGTP (Klebe et al., 1995b; Renault et al., 2001; Ribbeck et al., 1998). Hence, GTP is the energy source that powers NCT. Accordingly, the interconversion of RanGTP and RanGDP by RanGAP1 and RanGEF constitutes the fifth and final layer of NCT control.

How is asymmetry achieved in nucleocytoplasmic transport?

Each of the aforementioned layers constitutes key mechanistic steps of NCT that lead to the partitioning of NLS-cargoes in the nucleus

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and NES-cargoes in the cytoplasm. However, they do not sufficiently explain the steady-state partitioning of soluble, yet directionally opposed, transport factors (e.g. importin versus exportin) observed in living cells (Kirli et al., 2015) (Fig. 2). As a comparison, RanGEF contains an NLS (Nemergut and Macara, 2000), whereas RanGAP1 contains a single NLS and nine NESs (Matunis et al., 1998), which regulate their localization in the nucleus and cytoplasm, respectively. Clearly, their enzymatic activity dictates how much RanGTP and RanGDP are generated in each compartment (Görlich et al., 2003; Kalab et al., 2002; Smith et al., 2002). Nevertheless, it is not well understood how the inter-compartmental mixing of RanGTP and RanGDP is prevented to preserve the steep RanGTP–RanGDP gradient (Fig. 3A). There are also no known mechanisms that explain the asymmetric partitioning of importins and exportins. In the following section, we discuss potential factors that could influence the NPC to achieve Kapβ

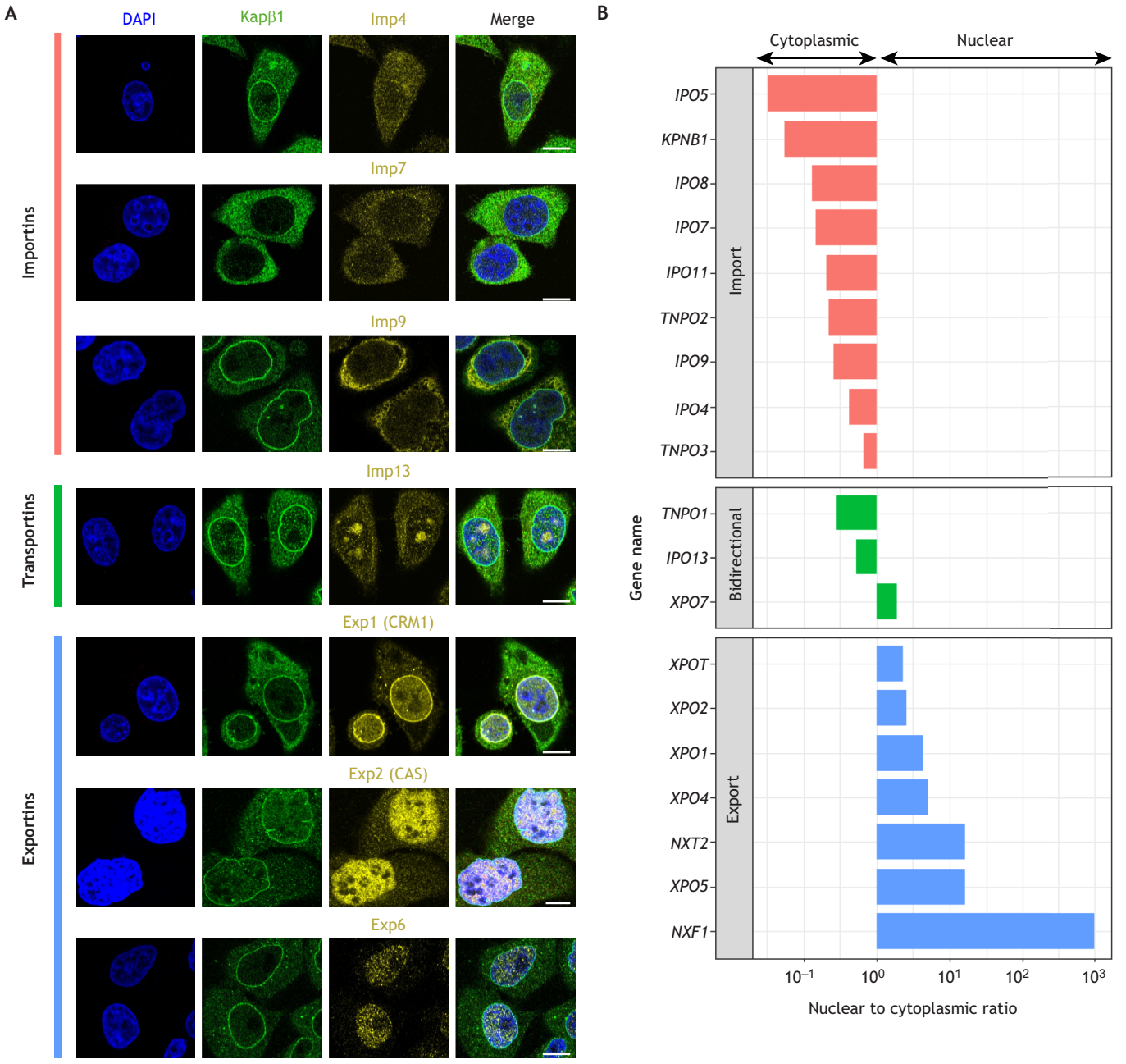

Fig. 2. Asymmetric partitioning of Kapβs and their enrichment at NPCs in vertebrate cells. (A) Immunofluorescence reveals that importins localize predominantly in the cytoplasm, while exportins are found in the nucleus. Nuclear rim stainings indicate that Kapβ1 and CRM1 are highly enriched at NPCs. Endogenous Kapβ1 was co-stained with Imp4, Imp7, Imp9, Imp13, Exp1 (CRM1), Exp2 (CAS) or Exp6 in HeLa cells by J. K. and L. E. K. using a standard protocol (Kapinos et al., 2017). The following antibodies were used: anti-Kapβ1 (abcam, Cat#ab2811), anti-Imp4 (abcam, Cat#ab181046), anti-Imp7 (abcam, Cat#ab15840), anti-Imp9 (abcam, Cat#ab52605), anti-Imp13 (abcam, Cat#ab95993), anti-CRM1 (abcam, Cat#ab24189), anti-CAS (abcam, Cat#ab96755), anti-Exp6 (Bethyl, A301-205A). Scale bars: 5 µm. (B) Nuclear to cytoplasmic ratios of Kapβs obtained from quantitative mass spectrometric analysis of fractionated *X. laevis* oocytes correlates with the immunofluorescence images shown in panel A. The bar plot was derived from the mass spectrometry data from Kirli et al. (2015) under a CC-BY 4.0 license. KPNB1, karyopherin β1; IPO, importins; TNPO, transportins; XPO, exportins; NXF, nuclear RNA export factor.

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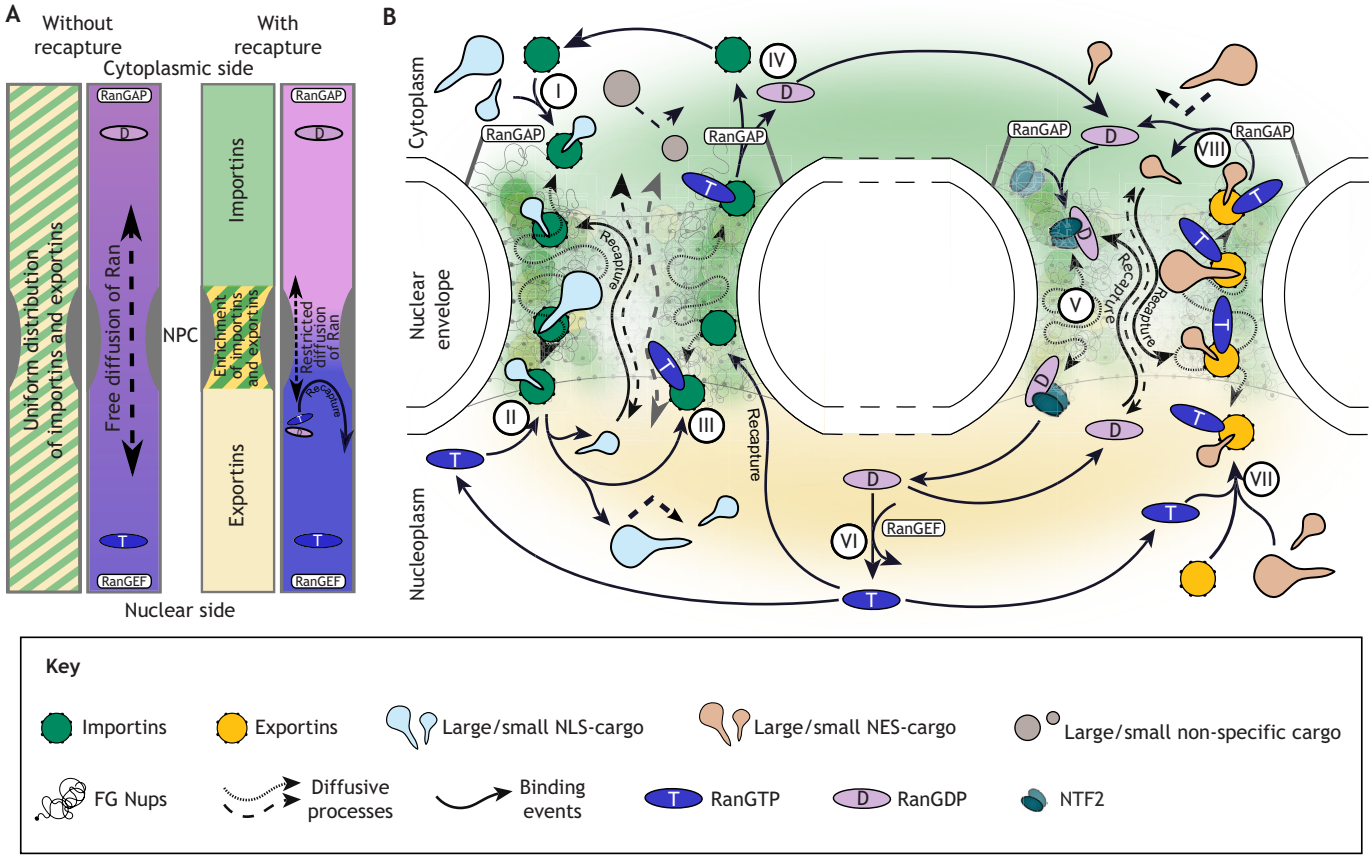

Fig. 3. Recapture at NPCs facilitates nucleocytoplasmic partitioning. (A) Left, RanGAP1 and RanGEF activity in the absence of enriched Kapβs at NPCs results in a poor Ran gradient due to the leakage of RanGTP and RanGDP between compartments. Right, an enrichment of Kapβs at the NPC facilitates the recapture of RanGTP to minimize leakage and preserve the steep Ran gradient. (B) The mechanistic steps necessary for maintaining nucleocytoplasmic partitioning are as follows. (I) Importins partition in the cytoplasm and shuttle NLS-cargoes through NPCs into the nucleus. (II) NLS-cargo is released into the nucleus following RanGTP–importin binding at the NPC exit. (III) RanGTP–importin complexes traverse NPCs to return to the cytoplasm. (IV) RanGAP hydrolyzes RanGTP into RanGDP, which frees the importin in the cytoplasm for another import cycle. (V) RanGDP is returned to the nucleus through NPCs by NTF2. (VI) RanGEF converts RanGDP back into RanGTP. (VII) RanGTP enables the formation of NES-cargo–exportin–RanGTP complexes that circulate back through the NPC. (VIII) Upon reaching the cytoplasm, RanGAP again hydrolyzes RanGTP into RanGDP, which disassembles the NES-cargo–exportin–RanGTP complex. Additional remarks: (1) the partitioning of exportins in the nucleus results from an as yet unknown mechanism; (2) both importins and exportins enrich at NPCs; (3) RanGDP, RanGTP and other small cargoes are recaptured by Kapβs that are enriched inside the NPC to minimize non-specific leakage between compartments; (4) NCT translocation processes are diffusive; (5) NCT directionality is conferred by the RanGTP gradient; (6) nucleocytoplasmic exchanges might occur in close spatial proximity to the NE so that Kapβs are rapidly re-circulated back through the NPC; (7) large non-specific cargoes are repelled from the NPC.

partitioning, maintenance of the steep Ran gradient and the partitioning of other small cargoes between the nucleus and cytoplasm (Fig. 3B).

Nature of the permeability barrier

Currently, the NPC permeability barrier is largely modeled after the behaviors of FG-Nups observed *in vitro*. This ranges from tethered molecular layers (Eisele et al., 2012, 2010; Kapinos et al., 2014; Schleicher et al., 2014; Schoch et al., 2012; Zahn et al., 2016), liquid droplets (Celetti et al., 2020), and gel-like (Frey et al., 2018; Schmidt and Görlich, 2015) to more solid-like hydrogels (Frey and Görlich, 2007, 2009; Milles et al., 2013). Nevertheless, all of the above studies report permeability barrier properties that facilitate Kapβ passage, but exclude non-specific cargoes irrespective of their different material characteristics. The so-called 'selective phase' model postulates that the FG-Nups form a cross-linked gel-like meshwork within the NPC. Here, passive diffusion is determined by the mesh size, whereas selective transport occurs through binding of Kapβ to FG repeats that might effectively break individual cross-links (Frey and Görlich, 2007; Hülsmann et al., 2012). Based on the dynamic behavior of the FG Nups (Sakiyama et al., 2016), the 'polymer brush' or 'virtual gating' model suggests that the entropic fluctuations of surface-tethered FG-Nups excludes non-specific cargoes from the NPC (Lim et al., 2007, 2006; Rout et al., 2003). Finally, the 'two-gate' model envisages the central channel to be occupied by a cohesive meshwork, whereas peripheral FG-Nups are brush-like, thus providing spatially distinct pathways for the cargo molecules to translocate in the NPC central channel (Yamada et al., 2010).

However, Kapβs such as Kapβ1 and CRM1 exhibit a marked enrichment at the NPCs, which is visible as a distinct nuclear rim staining (Heaton et al., 2019; Kapinos et al., 2017; Lim et al., 2015; Lowe et al., 2015) (Fig. 2). On this basis, the NPC permeability barrier might resemble a mixed ternary phase, comprising Kapβs, FG-Nups and water (Zilman, 2018). Thus, Kapβ-binding to FG-Nups could modulate their biophysical behavior to impact on NPC barrier function in a manner that remains incompletely understood (Kapinos et al., 2014; Vovk et al., 2016; Zahn et al., 2016). Indeed, depleting Kapβ1 *ex vivo* abrogates NPC barrier function against

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non-specific cargoes, whereas adding back Kapβ1 rescues it (Kapinos et al., 2017). Hence, enrichment of Kapβ might reinforce the barrier-forming qualities of the FG-Nups (Fig. 3B) (Lim et al., 2015). It remains to be seen whether and how different Kapβs might regulate the permeability barrier as integral components of the pore.

Kapβ transport kinetics within NPCs

Depending on their cargoes, the dwell times of Kapβs in the NPC are between 5 and 20 ms (Kubitscheck et al., 2005; Tu et al., 2013; Yang et al., 2004), but can reach 180 ms for mRNA (Grünwald and Singer, 2010). Moreover, increasing the concentration of Kapβ1 enhances cargo transport efficiency through the NPC and decreases cargo dwell time at the NPC (Yang and Musser, 2006). The latter might be due to a reduction of available FG repeats and the frequency of their interactions with individual Kapβs (Aramburu and Lemke, 2017), which decreases the avidity of Kapβ–FG-Nup binding (Kapinos et al., 2017, 2014; Lowe et al., 2015; Schleicher et al., 2014; Wagner et al., 2015). Nevertheless, import cargo dwell times also depend on the binding of RanGTP to importins and are not *a priori* equivalent to Kapβ residence times. Thus, successful import depends on the accessibility of RanGTP to importin–cargo complexes on the nuclear side of the NPC, whereas successful export depends on GTP hydrolysis by RanGAP1 on the cytoplasmic side.

Within the NPC, Kapβ complexes exhibit Brownian diffusion that is facilitated by interactions with the FG-repeats, also termed facilitated diffusion, which seems to expedite their translocation through the central channel (Cardarelli et al., 2011; Yang et al., 2004). However, whether and how a crowding of Kapβs within the NPC affects their kinetic interactions with the FG-Nups and ensuing dynamic movements within the pore remains unclear. To gain a physical understanding of such effects, the behavior of Kapβ1-functionalized colloidal beads was studied on surface-tethered FG-Nup layers. The beads transitioned from being immobile to exhibiting two-dimensional diffusion when the amount of soluble Kapβ1 was raised from low to physiologically relevant concentrations, which resulted in an enrichment of soluble Kapβ1 within the FG-Nup layer (Schleicher et al., 2014). In contrast, non-specific control beads exhibited three-dimensional diffusion that transiently impinged on the FG-Nup layer without binding (Schleicher et al., 2014). It remains to be determined how Kapβ complexes can exhibit rapid movements in the NPC while reinforcing the permeability barrier at the same time.

Cellular abundance of Kapβs

Although not all Kapβ–FG-Nup interactions have been characterized, their known apparent dissociation constants (K_D) typically fall in the sub-micromolar range (Kapinos et al., 2014; Schoch et al., 2012; Tan et al., 2018; Tetenbaum-Novatt et al., 2012). Hence, the amount of each Kapβ that populates the NPC will depend on its cellular concentration, which varies from the nanomolar to micromolar range (Nguyen et al., 2019; Wühr et al., 2015). Indeed, the four most abundant Kapβs are Kapβ1, importin 5 (Imp5, also known as IPO5 or RANBP5), CRM1 and exportin 2 (Exp2 or Xpo2; also known as CAS or CSE1L) (Kirli et al., 2015; Wang et al., 2015) (Fig. 4). Given that Kapβ1 and CRM1 colocalize at NPCs (Fig. 2), this suggests that their presence might modulate the multivalent interactions between the FG-Nups and other Kapβs. Indeed, this so-called binding promiscuity is relevant to how intrinsically disordered proteins interact with multiple partners simultaneously (Uversky, 2013), as has been shown for the binding of Kapβ1 and NTF2 to the FG Nups (Wagner et al., 2015).

Conformational flexibility of Kapβs

The secondary and tertiary structures of Kapβs are highly conserved across subfamilies and species despite their low sequence similarity (Conti et al., 2006). Kapβs comprise 19 to 21 consecutive HEAT repeats that are arranged as a pair of amphiphilic α-helices. Thus, Kapβs constitute highly flexible right-handed solenoids that vary in curvature, diameter and pitch (Conti et al., 2006; Fukuhara et al., 2004). By this means, Kapβs exhibit a conformational versatility to bind to different ligands, such as NLS-cargoes, NES-cargoes, Kapα and RanGTP (Cingolani et al., 2000; Fukuhara et al., 2004; Kappel et al., 2010; Monecke et al., 2013; Port et al., 2015; Yoshimura et al., 2014). In addition, adjacent HEAT motifs harbor several hydrophobic

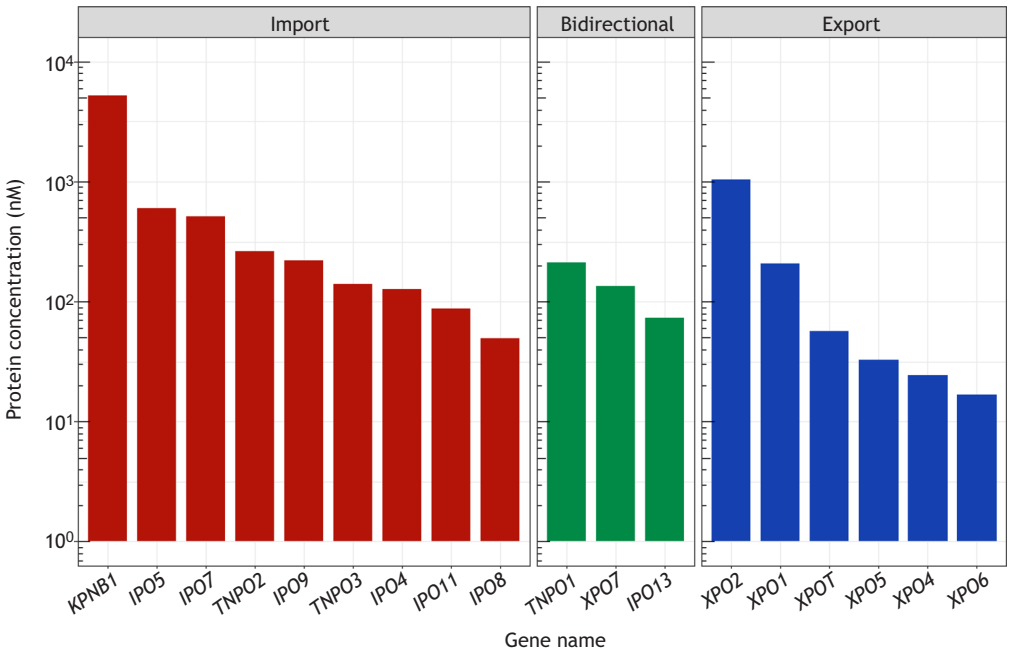

Fig. 4. Absolute abundance of Kapβs in *X. laevis* oocytes. Cellular concentration of Kapβs obtained via mass spectroscopy-based proteomics. The bar plot was derived from the supplementary data from Wühr et al. (2015) with permission from Elsevier. KPNB1, karyopherin β1; IPO, importins; TNPO, transportins; XPO, exportins.

pockets that facilitate multivalent binding interactions with FG repeats (Bayliss et al., 2000a; Isgro and Schulten, 2005; Port et al., 2015). Taken together, this suggests that the apparent binding affinity (i.e. binding avidity) of Kapβs to FG-Nups may depend on the resulting conformation that each respective Kapβ adopts during cargo loading. Indeed, FG-Nup binding is stronger for NLS-cargo–Kapα–Kapβ1 complexes than for RanGTP–Kapβ1 and Kapβ1 alone (Kapinos et al., 2017, 2014). The binding of CRM1 to FG-Nups also appears to be enhanced in the presence of RanGTP and NES-cargo (Koyama et al., 2017; Port et al., 2015; Roloff et al., 2013). Accordingly, Kapβ–cargo complexes may populate NPCs more than either Kapβ1 alone or RanGTP–Kapβ1 (Kapinos et al., 2017).

Maintenance of the Ran gradient and partitioning of small cargoes

GTP- and GDP-bound states of Ran are continuously interchanged between the nucleus and the cytoplasm (Görlich et al., 1996; Moore and Blobel, 1993). However, neither RanGTP nor RanGDP interacts directly with the FG-Nups (Rexach and Blobel, 1995). Moreover, Ran is smaller in size than the estimated 40 kDa passive exclusion limit of the NPC. Yet, RanGTP is ∼200 times more concentrated in the nucleus than the cytoplasm (Görlich et al., 2003; Kalab et al., 2002; Smith et al., 2002). Thus, it is puzzling how an uncontrolled mixing of RanGTP and RanGDP is minimized at the NPC level. Indeed, interfering with this steep gradient impairs NCT directionality (Nachury and Weis, 1999), can alter the cellular distribution of Kapβs (Kuersten et al., 2002), and is associated with apoptosis (Wong et al., 2009), stress (Chan et al., 2010; Kelley and Paschal, 2007) and neurological disease (Eftekharzadeh et al., 2018). Clearly, RanGTP is generated in the nucleus by RanGEF and is hydrolyzed to RanGDP in the cytoplasm by RanGAP1, which, together, form the basis of the Ran gradient (Kalab and Heald, 2008) (Fig. 3A, left panel). This is further enhanced by the action of NTF2, which facilitates the return of RanGDP into the nucleus (Ribbeck et al., 1998). Thus far, the *in vitro* reaction rates of RanGEF to produce RanGTP and its hydrolysis to RanGDP by RanGAP1 have been determined to be 2.1 s^{-1} and 5.0 s^{-1}, respectively (Klebe et al., 1995a). However, it remains to be experimentally verified whether these enzymatic reactions alone are sufficient to maintain the observed steep Ran gradient *in vivo* (Görlich et al., 2003; Kalab et al., 2002; Smith et al., 2002).

To preserve the steep Ran gradient, we hypothesized that nuclear leakage of RanGTP is mediated through binding to the enriched pool of Kapβ1 at NPCs (Fig. 3A) (Barbato et al., 2020). Indeed, we observed a substantial leakage of Ran from the nucleus when NPCs lacked Kapβ1 enrichment. In comparison, we found that enriched Kapβ1 provides a retention mechanism at the pore that is biochemically specific for RanGTP, as passive molecules of comparable size, such as GFP, could still traverse the NPC (Barbato et al., 2020). Such a retention mechanism might further explain the steady-state accumulation of Ran at NPCs (Abu-Arish et al., 2009; Smith et al., 2002; Yang and Musser, 2006). Besides its retention at the NPC, we could also show that the efflux of RanGTP depends on its hydrolysis to RanGDP by RanGAP1 by comparing it to a non-hydrolyzable RanQ69L-GTP mutant that was unable to depart from the NPC (Barbato et al., 2020). Finally, we rationalized that NTF2 is required to provide a separate pathway to shuttle RanGDP back into the nucleus (Barbato et al., 2020) because the binding of RanGTP to Kapβ1 (K_d≈35 nM) (Kapinos et al., 2017) is significantly stronger than RanGDP to Kapβ1 (K_d=2 µM) (Forwood et al., 2008). Taken together, this is consistent with simulations, which show that the Ran gradient is sensitive to changes in the permeability of the NPC (Becskei and Mattaj, 2003; Görlich et al., 2003). More generally, we hypothesize that Kapβ1 enrichment at the NPCs increases the efficiency of NCT by minimizing RanGTP losses from the nucleus.

A similar retention mechanism may also apply to other small NLS-cargoes that accumulate in the nucleus. For example, most histones and ribosomal proteins (Table 1) have molecular masses that lie below the NPC size-exclusion limit. In this manner, small NLS-cargoes may be prevented from returning to the cytoplasm by binding to importins that are enriched within the NPC.

Asymmetric partitioning of Kapβs

Another striking and, perhaps least-understood hallmark, of NCT concerns the asymmetric partitioning of Kapβs themselves. Kapβs lack NLS or NES signals, yet most importins tend to localize in the cytoplasm [with the exception of importin-11 (Imp11, also known as IPO11)], whereas exportins reside in the nucleus, and transportins can be evenly distributed in the nucleus or cytoplasm, depending on their function (Fig. 2). Quantitative analysis by compartment-based mass spectrometry of *Xenopus laevis* oocytes revealed that the nuclear-to-cytoplasmic ratio (N:C) of Kapβ1 is ∼1:10, while the N:C ratio for both CAS and CRM1 is almost 2:1 (Fig. 2B) (Kirli et al., 2015).

As a case in point, the mechanism(s) that regulates the partitioning of exportins in the nucleus remains elusive despite noted associations between exportins and cancer (Cagatay and Chook, 2018). For example, CRM1 is involved in the export of NES-cargos (Johnson et al., 2002) including mRNA complexes and ribosomal subunits (Chao et al., 2012; Jäkel and Görlich, 1998; Spits et al., 2019; Sutherland et al., 2015), as well as tumor suppressor and regulatory proteins such as BRCA1 (Brodie and Henderson, 2012) and p53 (Kanai et al., 2007). In cancer, CRM1 overexpression enhances the nuclear export of such tumor suppressor proteins, resulting in their mislocalization and functional inactivation in the cytoplasm (Azmi et al., 2021). This has led to the development of selective inhibitors of nuclear export (SINE) that prevent the binding of such NES-cargoes to CRM1 (Azizian and Li, 2020; Parikh et al., 2014; Sun et al., 2016). CAS, whose role is to export Kapα back to the cytoplasm to sustain nuclear import, is another exportin that is overexpressed during cancer progression and metastasis (Jiang, 2016). It is therefore pertinent to account for how exportins are asymmetrically partitioned in the nucleus and to address how interfering with this behavior leads to downstream defects in NCT with relevance to disease. Thus far, only one study has linked the nuclear localization of exportin-T (Xpo-t) to the RanGTP gradient (Kuersten et al., 2002) whereby Xpo-t was mislocalized when its interactions with RanGTP was impaired. Evidently, the lack of any further explanation underscores the little we know about the mechanism(s) that regulates the accumulation of importins and exportins in the cytoplasm and nucleus, respectively. For now, we hypothesize that the dissociation of Kapβ-cargo complexes at the peripheries of the NPC allows for the Kapβs to be rapidly re-captured and circulated back through the NPC (Fig. 3B).

Conclusion

The asymmetric partitioning of NCT and its directionally opposed transport factors (NLS-cargo versus NES-cargo, importin versus exportin and RanGTP versus RanGDP) is achieved by a complex interplay between: (1) the nature of the permeability barrier, (2) cellular abundance of each Kapβ, (3) binding to FG-Nups, and (4) the Ran gradient. We hypothesize that this is further mediated by

Table 1. Function and cellular localization of Kapβs

Vertebrate	Yeast	Function in NCT/cargoes	References (Kapβ function)	Cellular localization*	References (immunofluorescence data)**
Nuclear import					
Kapβ1 (KPNB1)	KAP95	Import of IBB, NLS-cargo and Kapα isoforms	Chi et al., 1995; Görlich et al., 1995	C/NE	Guttinger et al., 2004; Mingot et al., 2001
Imp4 (IPO4, RANBP4, ` IMP4B)	KAP123	Import of histone H3/H4·Asf1a complex, ribosomal protein S3a, the vitamin D receptor, hypoxia inducible factor-α, epididymis protein4	Yoon et al., 2018	C	n.d.
Imp5 (IPO5, RANBP5, IMP5B)	KAP121	Import of ribosomal proteins, such as RPL23A (17.7 kDa), RPS7 (17.7 kDa) and RPL5 (34.4 kDa), and histones: H2A (14 kDa), H2B (14 kDa), H3 (11.4 kDa) and H4 (11.4 kDa)	Jäkel and Görlich, 1998	C	Guttinger et al., 2004; Spits et al., 2019; Zhang et al., 2019
Imp7 (IPO7, RANBP7)	KAP114	Import of ribosomal proteins (RPL23A, RPS7 and RPL5) and histones (H1, H2A, H2B, H3 and H4) together with Kapβ1	Jäkel and Görlich, 1998	C/NE	Görlich et al., 1997; Mingot et al., 2001; Wei et al., 2014
Imp8 (IPO8, RANBP8)	KAP120	Import of SRP19 (signal recognizing particle 19)	Dean et al., 2001	C/NE	Görlich et al., 1997; Hu et al., 2018; Wei et al., 2014
Imp9 (IPO9*, IMP9, KIAA1192, RANBP9, HSPC273)	KAP114	Import of ribosomal proteins [RPS7, RPL18A (20.8 kDa), RPL6 (32.7 kDa)], histones (H2A, H2B) and actin (by similarity); Prevents the cytoplasmic aggregation of RPS7 and RPL18A by shielding exposed basic domains	Jäkel and Görlich, 1998	C	Guttinger et al., 2004; Padavannil et al., 2019
Imp11 (IPO11, RANBP11)	KAP120	Import of UBE2E3 and RPL12	Strambio-De-Castillia et al., 2010	N	Plafker and Macara, 2000
Transportin-2 (Kapβ_2B, TNPO2)	KAP104	Import of NLS-cargoes (by similarity)/n.d.	Gaudet et al., 2011	N	Guttinger et al., 2004
Transportin-3 (Imp12, TNPO3, IPO12, TNP-SR)	–	Import of splicing factor SR proteins RBM4, SFRS1 and SFRS2 (recognized by phosphorylated RS domains, i.e. arginine-serine rich domains).	Lai et al., 2003; Lai et al., 2001; Maertens et al., 2014	n.d.	n.d.
Nuclear export					
Exp1 (CRM1, XPO1)	CRM1	Export of NES cargoes	Fornerod et al., 1997; Haasen et al., 1999; Ossareh-Nazari et al., 1997; Stade et al., 1997	N/NE	Stade et al., 1997
Exp2 (CAS, XPO2, CSE1L)	CSE1	Export of Kapα	Kutay et al., 1997	N	Kutay et al., 1997
Exp4 (XPO4 KIAA1721)	–	Export of Smad3	Boulikas, 1994; Cokol et al., 2000	N	Lipowsky et al., 2000
Exp5 (XPO5, RANBP21)	MSN5	Export of micro-RNA precursors, synthetic short hairpin RNAs and specific dsRNAs	Allen et al., 2001; Gwizdek et al., 2004; Lund et al., 2004; Okada et al., 2009; Xu et al., 2012; Yi et al., 2005	N	n.d.
Exp6 (XPO6, KIAA0370, RANBP20)	–	Export of actin and profilin-actin complexes in somatic cells	Görlich et al., 2003	n.d.	n.d.
Exportin-T (XPOT)	LOS1	Export of tRNA	Kuersten et al., 2002; Kutay et al., 1998	N	Kuersten et al., 2002
Bidirectional transport (import and export)					
Transportin-1 (TNPO1, KPNB2, MIP1, TRN)	KAP104	Import of M9-containing proteins; Binds to a beta-like import receptor binding (BIB) domain. Export of hnRNP A1/A2	Arnold et al., 2006	N	Guttinger et al., 2004; Siomi et al., 1997
Imp13 (IPO13* (KIAA0724, RANBP13)	MTR10	Import of UBC9, the RBM8A/MAGOH complex, PAX6; Export of eIF-1A (release is triggered by IPO13)	Mingot et al., 2001; Ploski et al., 2004	C/nucleoli	Mingot et al., 2001
Exp7 (XPO7, KIAA0745, RANBP16)	KAP123	Export of ∼200 cargoes, e.g. RhoGAP1 and 14-3-3σ, α- and β-tubulin and import of ∼30 cargoes	Aksu et al., 2018	n.d.	n.d.
Ran-binding protein 17 (RANBP17)	–	May function as transporter (by similarity)	–	n.d.	n.d.
–	sal3	Import of Cdc25	Chua et al., 2002	n.d.	n.d.

*Based on immunofluorescence data; **see also Human Cell Atlas (https://www.proteinatlas.org/humanproteome/cell). N, nucleus; C, cytoplasm; NE, nuclear envelope (i.e. localization at NPCs); n.d., no data.

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Box 1. Unresolved questions with respect to nucleocytoplasmic transport

- What is the nuclear versus cytoplasmic concentration of each Kapβ in different cell types?
- What is the steady state occupancy of different Kapβs in NPCs?
- Is there a prioritization of transport for different Kapβs through the NPC?
- How might functional redundancy between Kapβs and cargoes impact on NCT?
- How does cargo loading influence structural changes in Kapβs to modulate Kapβ-FG Nup binding interactions and occupancy within the NPC?
- How does enrichment within the NPC impact on Kapβ transport kinetics?
- How far do Kapβs penetrate past the NPC?
- How do Kapβs assist in maintaining the steep Ran gradient?
- What mechanisms regulate the asymmetric partitioning of Kapβs?

Kapβ enrichment at the NPC permeability barrier (Fig. 3), which serves to (1) facilitate signal-specific cargo transport, (2) prevent the unsolicited entry of non-specific cargoes, and (3) prevent the leakage of Ran and other small specific cargoes between compartments. Moreover, we speculate that the release of both NLS- and NES-cargo occurs in close proximity to the NPCs so that Kapβs are rapidly re-captured by the FG-Nups and can be circulated back through the NPC. Taken together, these attributes constitute a puzzling causal dilemma – what forms the underlying basis for asymmetry during NCT, transport or partitioning? Certainly, this along with several open questions motivate further basic research in NCT (Box 1). Moving forward, future studies would benefit from adopting a more systems-based approach (Becskei and Mattaj, 2003; Görlich et al., 2003; Kopito and Elbaum, 2009; Smith et al., 2002) to resolve the fascinating complexities of NCT asymmetry and partitioning behavior.

Competing interests
The authors declare no competing or financial interests.

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References

Abu-Arish, A., Kalab, P., Ng-Kamstra, J., Weis, K. and Fradin, C. (2009). Spatial distribution and mobility of the Ran GTPase in live interphase cells. *Biophys. J.* **97**, 2164-2178. doi:10.1016/j.bpj.2009.07.055

Aksu, M., Pleiner, T., Karaca, S., Kappert, C., Dehne, H. J., Seibel, K., Urlaub, H., Bohnsack, M. T. and Gorlich, D. (2018). Xpo7 is a broad-spectrum exportin and a nuclear import receptor. *J. Cell Biol.* **217**, 2329-2340. doi:10.1083/jcb.201712013

Allen, N. P. C., Huang, L., Burlingame, A. and Rexach, M. (2001). Proteomic analysis of nucleoporin interacting proteins. *J. Biol. Chem.* **276**, 29268-29274. doi:10.1074/jbc.M102629200

Aramburu, I. V. and Lemke, E. A. (2017). Floppy but not sloppy: Interaction mechanism of FG-nucleoporins and nuclear transport receptors. *Semin. Cell Dev. Biol.* **68**, 34-41. doi:10.1016/j.semcdb.2017.06.026

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. doi:10.1074/jbc.M513281200

Azizian, N. G. and Li, Y. L. (2020). XPO1-dependent nuclear export as a target for cancer therapy. *J. Hematol. Oncol.* **13**, 61. doi:10.1186/s13045-020-00903-4

Azmi, A. S., Uddin, M. H. and Mohammad, R. M. (2021). The nuclear export protein XPO1-from biology to targeted therapy. *Nat. Rev. Clin. Oncol.* **18**, 152-169. doi:10.1038/s41571-020-00442-4

Baade, I. and Kehlenbach, R. H. (2019). The cargo spectrum of nuclear transport receptors. *Curr. Opin. Cell Biol.* **58**, 1-7. doi:10.1016/j.ceb.2018.11.004

Barbato, S., Kapinos, L. E., Rencurel, C. and Lim, R. Y. H. (2020). Karyopherin enrichment at the nuclear pore complex attenuates Ran permeability. *J. Cell Sci.* **133**, jcs238121. doi:10.1242/jcs.238121

Bayliss, R., Kent, H. M., Corbett, A. H. and Stewart, M. (2000a). Crystallization and initial X-ray diffraction characterization of complexes of FxFG nucleoporin repeats with nuclear transport factors. *J. Struct. Biol.* **131**, 240-247. doi:10.1006/jsbi.2000.4297

Bayliss, R., Littlewood, T. and Stewart, M. (2000b). Structural basis for the interaction between FxFG nucleoporin repeats and importin-beta in nuclear trafficking. *Cell* **102**, 99-108. doi:10.1016/S0092-8674(00)00014-3

Beck, M., Lucic, V., Forster, F., Baumeister, W. and Medalia, O. (2007). Snapshots of nuclear pore complexes in action captured by cryo-electron tomography. *Nature* **449**, 611-615. doi:10.1038/nature06170

Becskei, A. and Mattaj, L. W. (2003). The strategy for coupling the RanGTP gradient to nuclear protein export. *Proc. Natl. Acad. Sci. USA* **100**, 1717-1722. doi:10.1073/pnas.252766999

Boulikas, T. (1994). Putative nuclear-localization signals (NLS) in protein transcription factors. *J. Cell. Biochem.* **55**, 32-58. doi:10.1002/jcb.240550106

Brodie, K. M. and Henderson, B. R. (2012). Characterization of BRCA1 protein targeting, dynamics, and function at the centrosome: a role for the nuclear export signal, CRM1, and Aurora A kinase. *J. Biol. Chem.* **287**, 7701-7716. doi:10.1074/jbc.M111.327296

Cagatay, T. and Chook, Y. M. (2018). Karyopherins in cancer. *Curr. Opin. Cell Biol.* **52**, 30-42. doi:10.1016/j.ceb.2018.01.006

Cardarelli, F., Lanzano, L. and Gratton, E. (2011). Fluorescence correlation spectroscopy of intact nuclear pore complexes. *Biophys. J.* **101**, L27-L29. doi:10.1016/j.bpj.2011.04.057

Celetti, G., Paci, G., Caria, J., VanDelinder, V., Bachand, G. and Lemke, E. A. (2020). The liquid state of FG-nucleoporins mimics permeability barrier properties of nuclear pore complexes. *J. Cell Biol.* **219**, e201907157. doi:10.1083/jcb.201907157

Chachami, G., Paraskeva, E., Mingot, J. M., Braliou, G. G., Gorlich, D. and Simos, G. (2009). Transport of hypoxia-inducible factor HIF-1 alpha into the nucleus involves importins 4 and 7. *Biochem. Biophys. Res. Commun.* **390**, 235-240. doi:10.1016/j.bbrc.2009.09.093

Chan, K. S., Wong, C. H., Huang, Y. F. and Li, H. Y. (2010). Survivin withdrawal by nuclear export failure as a physiological switch to commit cells to apoptosis. *Cell Death Dis.* **1**, e57. doi:10.1038/cddis.2010.34

Chao, H. W., Lai, Y. T., Lu, Y. L., Lin, C. L., Mai, W. and Huang, Y. S. (2012). NMDAR signaling facilitates the IPO5-mediated nuclear import of CPEB3. *Nucleic Acids Res.* **40**, 8484-8498. doi:10.1093/nar/gks598

Chi, N. C., Adam, E. J. H. and Adam, S. A. (1995). Sequence and characterization of cytoplasmic nuclear-protein import factor p97. *J. Cell Biol.* **130**, 265-274. doi:10.1083/jcb.130.2.265

Cho, U. H. and Hetzer, M. W. (2020). Nuclear Periphery Takes Center Stage: The Role of Nuclear Pore Complexes in Cell Identity and Aging. *Neuron* **106**, 899-911. doi:10.1016/j.neuron.2020.05.031

Chook, Y. M. and Suel, K. E. (2011). Nuclear import by karyopherin-betas: recognition and inhibition. *Biochim. Biophys. Acta* **1813**, 1593-1606. doi:10.1016/j.bbamcr.2010.10.014

Christie, M., Chang, C. W., Rona, G., Smith, K. M., Stewart, A. G., Takeda, A. A., Fontes, M. R., Stewart, M., Vertessy, B. G., Forwood, J. K. et al. (2016). Structural biology and regulation of protein import into the nucleus. *J. Mol. Biol.* **428**, 2060-2090. doi:10.1016/j.jmb.2015.10.023

Chua, G., Lingner, C., Frazer, C. and Young, P. G. (2002). The sal3(+) gene encodes an importin-beta implicated in the nuclear import of Cdc25 in Schizosaccharomyces pombe. *Genetics* **162**, 689-703.

Cingolani, G., Lashuel, H. A., Gerace, L. and Muller, C. W. (2000). Nuclear import factors importin alpha and importin beta undergo mutually induced conformational changes upon association. *FEBS Lett.* **484**, 291-298. doi:10.1016/s0014-5793(00)02154-2

Clarke, P. R. and Zhang, C. M. (2008). Spatial and temporal coordination of mitosis by Ran GTPase. *Nat. Rev. Mol. Cell Biol.* **9**, 464-477. doi:10.1038/nrm2410

Cokol, M., Nair, R. and Rost, B. (2000). Finding nuclear localization signals. *EMBO Rep.* **1**, 411-415. doi:10.1093/embo-reports/kvd092

Conti, E., Muller, C. W. and Stewart, M. (2006). Karyopherin flexibility in nucleocytoplasmic transport. *Curr. Opin. Struct. Biol.* **16**, 237-244. doi:10.1016/j.sbi.2006.03.010

Cronshaw, J. A., Krutchinsky, A. N., Zhang, W. Z., Chait, B. T. and Matunis, M. J. (2002). Proteomic analysis of the mammalian nuclear pore complex. *J. Cell Biol.* **158**, 915-927. doi:10.1083/Jcb.200206106

Dange, T., Grünwald, D., Grünwald, A., Peters, R. and Kubitscheck, U. (2008). Autonomy and robustness of translocation through the nuclear pore complex: A single-molecule study. *J. Cell Biol.* **183**, 77-86. doi:10.1083/jcb.200806173

Dean, K. A., von Ahsen, O., Gorlich, D. and Fried, H. M. (2001). Signal recognition particle protein 19 is imported into the nucleus by importin 8 (RanBP8) and transportin. *J. Cell Sci.* **114**, 3479-3485.

Depping, R., Steinhoff, A., Schindler, S. G., Friedrich, B., Fagerlund, R., Metzen, E., Hartmann, E. and Kohler, M. (2008). Nuclear translocation of hypoxia-inducible factors (HIFs): Involvement of the classical importin alpha/beta

pathway. *Biochim. Biophys. Acta* **1783**, 394-404. doi:10.1016/j.bbamcr.2007.12.006

Dickmanns, A., Kehlenbach, R. H. and Fahrenkrog, B. (2015). Nuclear Pore Complexes and Nucleocytoplasmic Transport: From Structure to Function to Disease. In *International Review of Cell and Molecular Biology*, vol. 320 (ed. K. W. Jeon), pp. 171-233, Elsevier.

Eftekharzadeh, B., Daigle, J. G., Kapinos, L. E., Coyne, A., Schiantarelli, J., Carlomagno, Y., Cook, C., Miller, S. J., Dujardin, S., Amaral, A. S. et al. (2018). Tau Protein Disrupts Nucleocytoplasmic Transport in Alzheimer's Disease. *Neuron* **99**, 925-940e7. doi:10.1016/j.neuron.2018.07.039

Eibauer, M., Pellanda, M., Turgay, Y., Dubrovsky, A., Wild, A. and Medalia, O. (2015). Structure and gating of the nuclear pore complex. *Nat. Commun.* **6**, 7532. doi:10.1038/ncomms8532

Eisele, N. B., Frey, S., Piehler, J., Görlich, D. and Richter, R. P. (2010). Ultrathin nucleoporin phenylalanine-glycine repeat films and their interaction with nuclear transport receptors. *EMBO Rep.* **11**, 366-372. doi:10.1038/embor.2010.34

Eisele, N. B., Andersson, F. I., Frey, S. and Richter, R. P. (2012). Viscoelasticity of thin biomolecular films: a case study on nucleoporin phenylalanine-glycine repeats grafted to a histidine-tag capturing QCM-D sensor. *Biomacromolecules* **13**, 2322-2332. doi:10.1021/bm300577s

Fornerod, M., Ohno, M., Yoshida, M. and Mattaj, I. W. (1997). CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* **90**, 1051-1060. doi:10.1016/s0092-8674(00)80371-2

Forwood, J. K., Lonhienne, T. G., Marfori, M., Robin, G., Meng, W. N., Guncar, G., Liu, S. M., Stewart, M., Carroll, B. J. and Kobe, B. (2008). Kap95p Binding Induces the Switch Loops of RanGDP to Adopt the GTP-Bound Conformation: Implications for Nuclear Import Complex Assembly Dynamics. *J. Mol. Biol.* **383**, 772-782. doi:10.1016/j.jmb.2008.07.090

Frey, S. and Görlich, D. (2007). A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. *Cell* **130**, 512-523. doi:10.1016/J.Cell.2007.06.024

Frey, S. and Görlich, D. (2009). FG/FxFG as well as GLFG repeats form a selective permeability barrier with self-healing properties. *EMBO J.* **28**, 2554-2567. doi:10.1038/Emboj.2009.199

Frey, S., Rees, R., Schunemann, J., Ng, S. C., Funfgeld, K., Huyton, T. and Görlich, D. (2018). Surface properties determining passage rates of proteins through nuclear pores. *Cell* **174**, 202-217e9. doi:10.1016/j.cell.2018.05.045

Friedrich, B., Quensel, C., Sommer, T., Hartmann, E. and Kohler, M. (2006). Nuclear localization signal and protein context both mediate importin at specificity of nuclear import substrates. *Mol. Cell. Biol.* **26**, 8697-8709. doi:10.1128/mcb.00708-06

Fukuhara, N., Fernandez, E., Ebert, J., Conti, E. and Svergun, D. (2004). Conformational variability of nucleo-cytoplasmic transport factors. *J. Biol. Chem.* **279**, 2176-2181. doi:10.1074/jbc.M309112200

Fulcher, A. J. and Jans, D. A. (2011). Regulation of nucleocytoplasmic trafficking of viral proteins: An integral role in pathogenesis? *Biochim. Biophys. Acta* **1813**, 2176-2190. doi:10.1016/j.bbamcr.2011.03.019

Gaudet, P., Livstone, M. S., Lewis, S. E. and Thomas, P. D. (2011). Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Brief. Bioinform.* **12**, 449-462. doi:10.1093/bib/bbr042

Goldfarb, D. S., Corbett, A. H., Mason, D. A., Harreman, M. T. and Adam, S. A. (2004). Importin alpha: a multipurpose nuclear-transport receptor. *Trends Cell Biol.* **14**, 505-514. doi:10.1016/j.tcb.2004.07.016

Görlich, D. and Kutay, U. (1999). Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell. Dev. Biol.* **15**, 607-660. doi:10.1146/annurev.cellbio.15.1.607

Görlich, D., Vogel, F., Mills, A. D., Hartmann, E. and Laskey, R. A. (1995). Distinct functions for the 2 importin subunits in nuclear-protein import. *Nature* **377**, 246-248. doi:10.1038/377246a0

Görlich, D., Pante, N., Kutay, U., Aebi, U. and Bischoff, F. R. (1996). Identification of different roles for RanGDP and RanGTP in nuclear protein import. *EMBO J.* **15**, 5584-5594. doi:10.1002/j.1460-2075.1996.tb00943.x

Görlich, D., Dabrowski, M., Bischoff, F. R., Kutay, U., Bork, P., Hartmann, E., Prehn, S. and Izaurralde, E. (1997). A novel class of RanGTP binding proteins. *J. Cell Biol.* **138**, 65-80. doi:10.1083/jcb.138.1.65

Görlich, D., Seewald, M. J. and Ribbeck, K. (2003). Characterization of Ran-driven cargo transport and the RanGTPase system by kinetic measurements and computer simulation. *EMBO J.* **22**, 1088-1100. doi:10.1093/emboj/cdg113

Grünwald, D. and Singer, R. H. (2010). In vivo imaging of labelled endogenous b-actin mRNA during nucleocytoplasmic transport. *Nature* **467**, 604-609. doi:10.1038/nature09438

Guttinger, S., Muhlhausser, 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. doi:10.1073/pnas.0400342101

Gwizdek, C., Ossareh-Nazari, B., Brownawell, A. M., Evers, S., Macara, I. G. and Dargemont, C. (2004). Minihelix-containing RNAs mediate exportin-5-dependent nuclear export of the double-stranded RNA-binding protein ILF3. *J. Biol. Chem.* **279**, 884-891. doi:10.1074/jbc.M306808200

Haasen, D., Kohler, C., Neuhaus, G. and Merkle, T. (1999). Nuclear export of proteins in plants: AtXPO1 is the export receptor for leucine-rich nuclear export signals in Arabidopsis thaliana. *Plant J.* **20**, 695-705. doi:10.1046/j.1365-313x.1999.00644.x

Heaton, S. M., Atkinson, S. C., Sweeney, M. N., Yang, S. N. Y., Jans, D. A. and Borg, N. A. (2019). Exportin-1-Dependent Nuclear Export of DEAD-box Helicase DDX3X is Central to its Role in Antiviral Immunity. *Cells* **8**, 1181. doi:10.3390/cells8101181

Hoogenboom, B. W., Hough, L. E., Lemke, E. A., Lim, R. Y. H., Onck, P. R. and Zilman, A. (2021). Physics of the nuclear pore complex: theory, modeling and experiment. *Phys. Rep.* doi:10.1016/j.physrep.2021.03.003

Hu, X. P., Kan, H. W., Boye, A., Jiang, Y. F., Wu, C. and Yang, Y. (2018). Mitogen-activated protein kinase inhibitors reduce the nuclear accumulation of phosphorylated Smads by inhibiting Imp 7 or Imp 8 in HepG2 cells. *Oncology Letters* **15**, 4867-4872. doi:10.3892/ol.2018.7926

Huang, K. and Szleifer, I. (2020). Modeling the nucleoporins that form the hairy pores. *Biochem. Soc. Trans.* **48**, 1447-1461. doi:10.1042/BST20190941

Hülsmann, B. B., Labokha, A. A. and Görlich, D. (2012). The permeability of reconstituted nuclear pores provides direct evidence for the selective phase model. *Cell* **150**, 738-751. doi:10.1016/j.cell.2012.07.019

Isgro, T. A. and Schulten, K. (2005). Binding dynamics of isolated nucleoporin repeat regions to importin-beta. *Structure* **13**, 1869-1879. doi:10.1016/j.str.2005.09.007

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. doi:10.1093/emboj/16.21.6535

Jäkel, S. and Görlich, D. (1998). Importin beta, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. *EMBO J.* **17**, 4491-4502. doi:10.1093/emboj/17.15.4491

Jiang, M. C. (2016). CAS (CSE1L) signaling pathway in tumor progression and its potential as a biomarker and target for targeted therapy. *Tumor Biol.* **37**, 13077-13090. doi:10.1007/s13277-016-5301-x

Johnson, A. W., Lund, E. and Dahlberg, J. (2002). Nuclear export of ribosomal subunits. *Trends Biochem. Sci.* **27**, 580-585. doi:10.1016/s0968-0004(02)02208-9

Kalab, P. and Heald, R. (2008). The RanGTP gradient - a GPS for the mitotic spindle. *J. Cell Sci.* **121**, 1577-1586. doi:10.1242/jcs.005959

Kalab, P., Weis, K. and Heald, R. (2002). Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts. *Science* **295**, 2452-2456. doi:10.1126/science.1068798

Kalderon, D., Richardson, W. D., Markham, A. F. and Smith, A. E. (1984). Sequence requirements for nuclear location of simian virus-40 large t-antigen. *Nature* **311**, 33-38. doi:10.1038/311033a0

Kanai, M., Hanashiro, K., Kim, S. H., Hanai, S., Boulares, A. H., Miwa, M. and Fukasawa, K. (2007). Inhibition of Crm1-p53 interaction and nuclear export of p53 by poly(ADP-ribosyl)ation. *Nat. Cell Biol.* **9**, 1175-1183. doi:10.1038/ncb1638

Kapinos, L. E., Schoch, R. L., Wagner, R. S., Schleicher, K. D. and Lim, R. Y. H. (2014). Karyopherin-centric control of nuclear pores based on molecular occupancy and kinetic analysis of multivalent binding with FG nucleoporins. *Biophys. J.* **106**, 1751-1762. doi:10.1016/j.bpj.2014.02.021

Kapinos, L. E., Huang, B., Rencurel, C. and Lim, R. Y. H. (2017). Karyopherins regulate nuclear pore complex barrier and transport function. *J. Cell Biol.* **216**, 3609-3624. doi:10.1083/jcb.201702092

Kappel, C., Zachariae, U., Dolker, N. and Grubmuller, H. (2010). An unusual hydrophobic core confers extreme flexibility to HEAT repeat proteins. *Biophys. J.* **99**, 1596-1603. doi:10.1016/j.bpj.2010.06.032

Kelley, J. B. and Paschal, B. M. (2007). Hyperosmotic stress signaling to the nucleus disrupts the Ran gradient and the production of RanGTP. *Mol. Biol. Cell* **18**, 4365-4376. doi:10.1091/mbc.e07-01-0089

Kim, H. J. and Taylor, J. P. (2017). Lost in Transportation: Nucleocytoplasmic Transport Defects in ALS and Other Neurodegenerative Diseases. *Neuron* **96**, 285-297. doi:10.1016/j.neuron.2017.07.029

Kim, S. J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W. Z., Raveh, B., Herricks, T., Slaughter, B. D., Hogan, J. A., Upla, P. et al. (2018). Integrative structure and functional anatomy of a nuclear pore complex. *Nature* **555**, 475. doi:10.1038/nature26003

Kimura, M. and Imamoto, N. (2014). Biological significance of the importin-beta family-dependent nucleocytoplasmic transport pathways. *Traffic* **15**, 727-748. doi:10.1111/tra.12174

Kirli, K., Karaca, S., Dehne, H. J., Samwer, M., Pan, K. T., Lenz, C., Urlaub, H. and Görlich, D. (2015). A deep proteomics perspective on CRM1-mediated nuclear export and nucleocytoplasmic partitioning. *eLife* **4**, e11466. doi:10.7554/eLife.11466

Klebe, C., Bischoff, F. R., Ponstingl, H. and Wittinghofer, A. (1995a). Interaction of the nuclear GTP-binding protein Ran with its regulatory proteins RCC1 and RanGAP1. *Biochemistry* **34**, 639-647. doi:10.1021/bi00002a031

Klebe, C., Prinz, H., Wittinghofer, A. and Goody, R. S. (1995b). The kinetic mechanism of Ran–nucleotide exchange catalyzed by RCC1. *Biochemistry* **34**, 12543-12552. doi:10.1021/bi00039a008

Kopito, R. B. and Elbaum, M. (2009). Nucleocytoplasmic transport: a thermodynamic mechanism. *HFSP J.* **3**, 130-141. doi:10.2976/1.3080807

Kosugi, S., Hasebe, M., Matsumura, N., Takashima, H., Miyamoto-Sato, E., Tomita, M. and Yanagawa, H. (2009). Six Classes of Nuclear Localization Signals Specific to Different Binding Grooves of Importin alpha. *J. Biol. Chem.* **284**, 478-485. doi:10.1074/jbc.M807017200

Kosugi, S., Yanagawa, H., Terauchi, R. and Tabata, S. (2014). NESmapper: Accurate Prediction of Leucine-Rich Nuclear Export Signals Using Activity-Based Profiles. *PLoS Comp. Biol.* **10**, e1003841. doi:10.1371/journal.pcbi.1003841

Koyama, M. and Matsuura, Y. (2010). An allosteric mechanism to displace nuclear export cargo from CRM1 and RanGTP by RanBP1. *EMBO J.* **29**, 2002-2013. doi:10.1038/emboj.2010.89

Koyama, M., Hirano, H., Shirai, N. and Matsuura, Y. (2017). Crystal structure of the Xpo1p nuclear export complex bound to the SxFG/PxFG repeats of the nucleoporin Nup42p. *Genes Cells* **22**, 861-875. doi:10.1111/gtc.12520

Kubitscheck, U., Grünwald, D., Hoekstra, A., Rohleder, D., Kues, T., Siebrasse, J. P. and Peters, R. (2005). Nuclear transport of single molecules: dwell times at the nuclear pore complex. *J. Cell Biol.* **168**, 233-243. doi:10.1083/jcb.200411005

Kuersten, S., Arts, G. J., Walther, T. C., Englmeier, L. and Mattaj, I. W. (2002). Steady-state nuclear localization of exportin-t involves RanGTP binding and two distinct nuclear pore complex interaction domains. *Mol. Cell. Biol.* **22**, 5708-5720. doi:10.1128/mcb.22.16.5708-5720.2002

Kutay, U., Bischoff, F. R., Kostka, S., Kraft, R. and Görlich, D. (1997). Export of importin alpha from the nucleus is mediated by a specific nuclear transport factor. *Cell* **90**, 1061-1071. doi:10.1016/s0092-8674(00)80372-4

Kutay, U., Lipowsky, G., Izaurralde, E., Bischoff, F. R., Schwarzmaier, P., Hartmann, E. and Gorlich, D. (1998). Identification of a tRNA-specific nuclear export receptor. *Mol. Cell* **1**, 359-369. doi:10.1016/s1097-2765(00)80036-2

Lai, M. C., Lin, R. I. and Tarn, W. Y. (2001). Transportin-SR2 mediates nuclear import of phosphorylated SR proteins. *Proc. Natl. Acad. Sci. USA* **98**, 10154-10159. doi:10.1073/pnas.181354098

Lai, M. C., Kuo, H. W., Chang, W. C. and Tarn, W. Y. (2003). A novel splicing regulator shares a nuclear import pathway with SR proteins. *EMBO J.* **22**, 1359-1369. doi:10.1093/emboj/cdg126

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. doi:10.1074/jbc.R600026200

Lee, S. J., Sekimoto, T., Yamashita, E., Nagoshi, E., Nakagawa, A., Imamoto, N., Yoshimura, M., Sakai, H., Chong, K. T., Tsukihara, T. et al. (2003). The structure of importin-beta bound to SREBP-2: Nuclear import of a transcription factor. *Science* **302**, 1571-1575. doi:10.1126/science.1088372

Lee, B. J., Cansizoglu, A. E., Suel, 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. doi:10.1016/j.cell.2006.05.049

Lemke, E. A. (2016). The Multiple Faces of Disordered Nucleoporins. *J. Mol. Biol.* **428**, 2011-2024. doi:10.1016/j.jmb.2016.01.002

Lim, R. Y. H., Huang, N. P., Koser, J., Deng, J., Lau, K. H. A., Schwarz-Herion, K., Fahrenkrog, B. and Aebi, U. (2006). Flexible phenylalanine-glycine nucleoporins as entropic barriers to nucleocytoplasmic transport. *Proc. Natl. Acad. Sci. USA* **103**, 9512-9517. doi:10.1073/pnas.0603521103

Lim, R. Y. H., Fahrenkrog, B., Koser, J., Schwarz-Herion, K., Deng, J. and Aebi, U. (2007). Nanomechanical basis of selective gating by the nuclear pore complex. *Science* **318**, 640-643. doi:10.1126/science.1145980

Lim, R. Y. H., Huang, B. and Kapinos, L. E. (2015). How to operate a nuclear pore complex by Kap-centric control. *Nucleus* **6**, 366-372. doi:10.1080/19491034.2015.1090061

Lipowsky, G., Bischoff, F. R., Schwarzmaier, P., Kraft, R., Kostka, S., Hartmann, E., Kutay, U. and Görlich, D. (2000). Exportin 4: a mediator of a novel nuclear export pathway in higher eukaryotes. *EMBO J.* **19**, 4362-4371. doi:10.1093/emboj/19.16.4362

Lott, K. and Cingolani, G. (2011). The importin beta binding domain as a master regulator of nucleocytoplasmic transport. *Biochim. Biophys. Acta* **1813**, 1578-1592. doi:10.1016/j.bbamcr.2010.10.012

Lounsbury, K. M. and Macara, I. G. (1997). Ran-binding protein 1 (RanBP1) forms a ternary complex with Ran and karyopherin beta and reduces Ran GTPase-activating protein (RanGAP) inhibition by karyopherin beta. *Journal of Biological Chemisry* **272**, 551-555. doi:10.1074/jbc.272.1.551

Lowe, A. R., Tang, J. H., Yassif, J., Graf, M., Huang, W. Y., Groves, J. T., Weis, K. and Liphardt, J. T. (2015). Importin-beta modulates the permeability of the nuclear pore complex in a Ran-dependent manner. *eLife* **4**, e04052. doi:10.7554/eLife.04052

Lund, E., Guttinger, S., Calado, A., Dahlberg, J. E. and Kutay, U. (2004). Nuclear export of microRNA precursors. *Science* **303**, 95-98. doi:10.1126/science.1090599

Macara, I. G. (2001). Transport into and out of the nucleus. *Microbiol. Mol. Biol. Rev.* **65**, 570. doi:10.1128/mmbr.65.4.570-594.2001

Maertens, G. N., Cook, N. J., Wang, W., Hare, S., Gupta, S. S., Oztop, I., Lee, K., Pye, V. E., Cosnefroy, O., Snijders, A. P. et al. (2014). Structural basis for nuclear import of splicing factors by human Transportin 3. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 2728-2733. doi:10.1073/pnas.1320755111

Matunis, M. J., Wu, J. A. and Blobel, G. (1998). SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. *J. Cell Biol.* **140**, 499-509. doi:10.1083/jcb.140.3.499

Milles, S., Bui, K. H., Koehler, C., Eltsov, M., Beck, M. and Lemke, E. A. (2013). Facilitated aggregation of FG nucleoporins under molecular crowding conditions. *EMBO Rep.* **14**, 178-183. doi:10.1038/embor.2012.204

Mingot, J. M., Kostka, S., Kraft, R., Hartmann, E. and Görlich, D. (2001). Importin 13: a novel mediator of nuclear import and export. *EMBO J.* **20**, 3685-3694. doi:10.1093/emboj/20.14.3685

Miorin, L., Kehrer, T., Sanchez-Aparicio, M. T., Zhang, K., Cohen, P., Patel, R. S., Cupic, A., Makio, T., Mei, M. H., Moreno, E. et al. (2020). SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *Proc. Natl. Acad. Sci. USA* **117**, 28344-28354. doi:10.1073/pnas.2016650117

Mitrousis, G., Olia, A. S., Walker-Kopp, N. and Cingolani, G. (2008). Molecular basis for the recognition of snurportin 1 by importin beta. *J. Biol. Chem.* **283**, 7877-7884. doi:10.1074/jbc.M709093200

Monecke, T., Haselbach, D., Voss, B., Russek, A., Neumann, P., Thomson, E., Hurt, E., Zachariae, U., Stark, H., Grubmuller, H. et al. (2013). Structural basis for cooperativity of CRM1 export complex formation. *Proc. Natl. Acad. Sci. USA* **110**, 960-965. doi:10.1073/pnas.1215214110

Moore, M. S. and Blobel, G. (1993). The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. *Nature* **365**, 661-663. doi:10.1038/365661a0

Nachury, M. V. and Weis, K. (1999). The direction of transport through the nuclear pore can be inverted. *Proc. Natl. Acad. Sci. USA* **96**, 9622-9627. doi:10.1073/pnas.96.17.9622

Nemergut, M. E. and Macara, I. G. (2000). Nuclear import of the Ran exchange factor, RCC1, is mediated by at least two distinct mechanisms. *J. Cell Biol.* **149**, 835-849. doi:10.1083/jcb.149.4.835

Nguyen, T., Pappireddi, N. and Wühr, M. (2019). Proteomics of nucleocytoplasmic partitioning. *Curr. Opin. Chem. Biol.* **48**, 55-63. doi:10.1016/j.cbpa.2018.10.027

Okada, C., Yamashita, E., Lee, S. J., Shibata, S., Katahira, J., Nakagawa, A., Yoneda, Y. and Tsukihara, T. (2009). A high-resolution structure of the pre-microRNA nuclear export machinery. *Science* **326**, 1275-1279. doi:10.1126/science.1178705

O'Reilly, A. J., Dacks, J. B. and Field, M. C. (2011). Evolution of the karyopherin-beta family of nucleocytoplasmic transport factors; ancient origins and continued specialization. *PLoS One* **6**, e19308. doi:10.1371/journal.pone.0019308

Ossareh-Nazari, B., Bachelerie, F. and Dargemont, C. (1997). Evidence for a role of CRM1 in signal-mediated nuclear protein export. *Science* **278**, 141-144. doi:10.1126/science.278.5335.141

Padavannil, A., Sarkar, P., Kim, S. J., Cagatay, T., Jiou, J., Brautigam, C. A., Tomchick, D. R., Sali, A., D'Arcy, S. and Chook, Y. M. (2019). Importin-9 wraps around the H2A-H2B core to act as nuclear importer and histone chperone. *eLife* **8**, e43630. doi:10.7554/eLife.43630

Paine, P. L., Moore, L. C. and Horowitz, S. B. (1975). Nuclear envelope permeability. *Nature* **254**, 109-114. doi:10.1038/254109a0

Parikh, K., Cang, S. D., Sekhri, A. and Liu, D. L. (2014). Selective inhibitors of nuclear export (SINE)- a novel class of anti-cancer agents. *J. Hematol. Oncol.* **7**, 78. doi:10.1186/s13045-014-0078-0

Pawlowski, R., Rajakyla, E. K., Vartiainen, M. K. and Treisman, R. (2010). An actin-regulated importin alpha/beta-dependent extended bipartite NLS directs nuclear import of MRTF-A. *EMBO J.* **29**, 3448-3458. doi:10.1038/emboj.2010.216

Plafker, S. M. and Macara, I. G. (2000). Importin-11, a nuclear import receptor for the ubiquitin-conjugating enzyme, UbcM2. *EMBO J.* **19**, 5502-5513. doi:10.1093/emboj/19.20.5502

Ploski, J. E., Shamsher, M. K. and Radu, A. (2004). Paired-type homeodomain transcription factors are imported into the nucleus by karyopherin 13. *Mol. Cell. Biol.* **24**, 4824-4834. doi:10.1128/MCB.24.11.4824-4834.2004

Popken, P., Ghavami, A., Onck, P. R., Poolman, B. and Veenhoff, L. M. (2015). Size-dependent leak of soluble and membrane proteins through the yeast nuclear pore complex. *Mol. Biol. Cell* **26**, 1386-1394. doi:10.1091/mbc.E14-07-1175

Port, S. A., Monecke, T., Dickmanns, A., Spillner, C., Hofele, R., Urlaub, H., Ficner, R. and Kehlenbach, R. H. (2015). Structural and Functional Characterization of CRM1-Nup214 Interactions Reveals Multiple FG-Binding Sites Involved in Nuclear Export. *Cell Rep.* **13**, 690-702. doi:10.1016/j.celrep.2015.09.042

Pumroy, R. A. and Cingolani, G. (2015). Diversification of importin-alpha isoforms in cellular trafficking and disease states. *Biochem. J.* **466**, 13-28. doi:10.1042/bj20141186

Renault, L., Kuhlmann, J., Henkel, A. and Wittinghofer, A. (2001). Structural basis for guanine nucleotide exchange on Ran by the regulator of chromosome condensation (RCC1). *Cell* **105**, 245-255. doi:10.1016/s0092-8674(01)00315-4

Rexach, M. and Blobel, G. (1995). Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell* **83**, 683-692. doi:10.1016/0092-8674(95)90181-7

Ribbeck, K., Lipowsky, G., Kent, H. M., Stewart, M. and Görlich, D. (1998). NTF2 mediates nuclear import of Ran. *EMBO J.* **17**, 6587-6598. doi:10.1093/emboj/17.22.6587

Robbins, J., Dilworth, S. M., Laskey, R. A. and Dingwall, C. (1991). 2 interdependent basic domains in nucleoplasmin nuclear targeting sequence –

indentification of a class of bipartite nuclear targeting sequence. *Cell* **64**, 615-623. doi:10.1016/0092-8674(91)90245-t

Roloff, S., Spillner, C. and Kehlenbach, R. H. (2013). Several Phenylalanine-Glycine Motives in the Nucleoporin Nup214 Are Essential for Binding of the Nuclear Export Receptor CRM1. *J. Biol. Chem.* **288**, 3952-3963. doi:10.1074/jbc.M112.433243

Rout, M. P., Aitchison, J. D., Magnasco, M. O. and Chait, B. T. (2003). Virtual gating and nuclear transport: the hole picture. *Trends Cell Biol.* **13**, 622-628. doi:10.1016/j.tcb.2003.10.007

Sakiyama, Y., Mazur, A., Kapinos, L. E. and Lim, R. Y. H. (2016). Spatiotemporal dynamics of the nuclear pore complex transport barrier resolved by high-speed atomic force microscopy. *Nat. Nanotechnol.* **11**, 719-723. doi:10.1038/nnano.2016.62

Schleicher, K. D., Dettmer, S. L., Kapinos, L. E., Pagliara, S., Keyser, U. F., Jeney, S. and Lim, R. Y. H. (2014). Selective transport control on molecular velcro made from intrinsically disordered proteins. *Nat. Nanotechnol.* **9**, 525-530. doi:10.1038/nnano.2014.103

Schmidt, H. B. and Görlich, D. (2015). Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear pore-like permselectivity. *eLife* **4**, e04251. doi:10.7554/eLife.04251

Schoch, R. L., Kapinos, L. E. and Lim, R. Y. H. (2012). Nuclear transport receptor binding avidity triggers a self-healing collapse transition in FG-nucleoporin molecular brushes. *Proc. Natl. Acad. Sci. USA* **109**, 16911-16916. doi:10.1073/pnas.1208440109

Siomi, M. C., Eder, P. S., Kataoka, N., Wan, L. L., Liu, Q. and Dreyfuss, G. (1997). Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. *J. Cell Biol.* **138**, 1181-1192. doi:10.1083/jcb.138.6.1181

Smith, A. E., Slepchenko, B. M., Schaff, J. C., Loew, L. M. and Macara, I. G. (2002). Systems analysis of Ran transport. *Science* **295**, 488-491. doi:10.1126/science.1064732

Spits, M., Janssen, L. J., Voortman, L. M., Kooij, R., Neefjes, A. C. M., Ovaa, H. and Neefjes, J. (2019). Homeostasis of soluble proteins and the proteasome post nuclear envelope reformation in mitosis. *J. Cell Sci.* **132**, jcs225524. doi:10.1242/jcs.225524

Stade, K., Ford, C. S., Guthrie, C. and Weis, K. (1997). Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell* **90**, 1041-1050. doi:10.1016/s0092-8674(00)80370-0

Stewart, M. (2007). Molecular mechanism of the nuclear protein import cycle. *Nat. Rev. Mol. Cell Biol.* **8**, 195-208. doi:10.1038/nrm2114

Strambio-De-Castillia, C., Niepel, M. and Rout, M. P. (2010). The nuclear pore complex: bridging nuclear transport and gene regulation. *Nat. Rev. Mol. Cell Biol.* **11**, 490-501. doi:10.1038/nrm2928

Strawn, L. A., Shen, T. X., 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. doi:10.1038/Ncb10097

Sun, Q. X., Chen, X. Q., Zhou, Q., Burstein, E., Yang, S. Y. and Jia, D. (2016). Inhibiting cancer cell hallmark features through nuclear export inhibition. *Signal Transduct. Target. Ther.* **1**, 16010. doi:10.1038/sigtrans.2016.10

Sutherland, J. M., Sobinoff, A. P., Fraser, B. A., Redgrove, K. A., Davidson, T. L., Siddall, N. A., Koopman, P., Hime, G. R. and McLaughlin, E. A. (2015). RNA binding protein Musashi-1 directly targets Msi2 and Erh during early testis germ cell development and interacts with IPO5 upon translocation to the nucleus. *FASEB J.* **29**, 2759-2768. doi:10.1096/fj.14-265868

Tan, P. S., Aramburu, I. V., Mercadante, D., Tyagi, S., Chowdhury, A., Spitz, D., Shammas, S. L., Grater, F. and Lemke, E. A. (2018). Two Differential Binding Mechanisms of FG-Nucleoporins and Nuclear Transport Receptors. *Cell Rep.* **22**, 3660-3671. doi:10.1016/j.celrep.2018.03.022

Terry, L. J., Shows, E. B. and Wente, S. R. (2007). Crossing the nuclear envelope: Hierarchical regulation of nucleocytoplasmic transport. *Science* **318**, 1412-1416. doi:10.1126/science.1142204

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. doi:10.1074/mcp.M111.013656

Timney, B. L., Raveh, B., Mironska, R., Trivedi, J. M., Kim, S. J., Russel, D., Wente, S. R., Sali, A. and Rout, M. P. (2016). Simple rules for passive diffusion through the nuclear pore complex. *J. Cell Biol.* **215**, 57-76. doi:10.1083/jcb.201601004

Tu, L. C., Fu, G., Zilman, A. and Musser, S. M. (2013). Large cargo transport by nuclear pores: implications for the spatial organization of FG-nucleoporins. *EMBO J.* **32**, 3220-3230. doi:10.1038/emboj.2013.239

Twyffels, L., Gueydan, C. and Kruys, V. (2014). Transportin-1 and Transportin-2: protein nuclear import and beyond. *FEBS Lett.* **588**, 1857-1868. doi:10.1016/j.febslet.2014.04.023

Uversky, V. N. (2013). Unusual biophysics of intrinsically disordered proteins. *Biochimica Et Biophysica Acta-Proteins and Proteomics* **1834**, 932-951. doi:10.1016/j.bbapap.2012.12.008

Vetter, I. R., Nowak, C., Nishimoto, T., Kuhlmann, J. and Wittinghofer, A. (1999). Structure of a Ran-binding domain complexed with Ran bound to a GTP analogue: implications for nuclear transport. *Nature* **398**, 39-46. doi:10.1038/17969

von Appen, A., Kosinski, J., Sparks, L., Ori, A., DiGuilio, A. L., Vollmer, B., Mackmull, M. T., Banterle, N., Parca, L., Kastritis, P. et al. (2015). In situ structural analysis of the human nuclear pore complex. *Nature* **526**, 140. doi:10.1038/nature15381

Vovk, A., Gu, C., Opferman, M. G., Kapinos, L. E., Lim, R. Y. H., Coalson, R. D., Jasnow, D. and Zilman, A. (2016). Simple biophysics underpins collective conformations of the intrinsically disordered proteins of the Nuclear Pore Complex. *eLife* **5**, e10785. doi:10.7554/eLife.10785

Wagner, R. S., Kapinos, L. E., Marshall, N. J., Stewart, M. and Lim, R. Y. H. (2015). Promiscuous binding of Karyopherinbeta1 modulates FG nucleoporin barrier function and expedites NTF2 transport kinetics. *Biophys. J.* **108**, 918-927. doi:10.1016/j.bpj.2014.12.041

Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. and von Mering, C. (2015). Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168. doi:10.1002/pmic.201400441

Wei, Y., Li, L. M., Wang, D., Zhang, C. Y. and Zen, K. (2014). Importin 8 Regulates the Transport of Mature MicroRNAs into the Cell Nucleus. *J. Biol. Chem.* **289**, 10270-10275. doi:10.1074/jbc.C113.541417

Weis, K. (2003). Regulating access to the genome: Nucleocytoplasmic transport throughout the cell cycle. *Cell* **112**, 441-451. doi:10.1016/s0092-8674(03)00082-5

Wong, C. H., Chan, H., Ho, C. Y., Lai, S. K., Chan, K. S., Koh, C. G. and Li, H. Y. (2009). Apoptotic histone modification inhibits nuclear transport by regulating RCC1. *Nat. Cell Biol.* **11**, 36-45. doi:10.1038/ncb1810

Wühr, M., Guttler, T., Peshkin, L., McAlister, G. C., Sonnett, M., Ishihara, K., Groen, A. C., Presler, M., Erickson, B. K., Mitchison, T. J. et al. (2015). The Nuclear Proteome of a Vertebrate. *Curr. Biol.* **25**, 2663-2671. doi:10.1016/j.cub.2015.08.047

Xu, D. R., Farmer, A., Collett, G., Grishin, N. V. and Chook, Y. M. (2012). Sequence and structural analyses of nuclear export signals in the NESdb database. *Mol. Biol. Cell* **23**, 3677-3693. doi:10.1091/mbc.E12-01-0046

Yamada, J., Phillips, J. L., Patel, S., Goldfien, G., Calestagne-Morelli, A., Huang, H., Reza, R., Acheson, J., Krishnan, V. V., Newsam, S. et al. (2010). A bimodal distribution of two distinct categories of intrinsically disordered structures with separate functions in FG nucleoporins. *Mol. Cell. Proteomics* **9**, 2205-2224. doi:10.1074/mcp.M000035-MCP201

Yang, W. and Musser, S. M. (2006). Nuclear import time and transport efficiency depend on importin beta concentration. *J. Cell Biol.* **174**, 951-961. doi:10.1083/jcb.200605053

Yang, W., Gelles, J. and Musser, S. M. (2004). Imaging of single-molecule translocation through nuclear pore complexes. *Proc. Natl. Acad. Sci. USA* **101**, 12887-12892. doi:10.1073/pnas.0403675101

Yarbrough, M. L., Mata, M. A., Sakthivel, R. and Fontoura, B. M. (2014). Viral subversion of nucleocytoplasmic trafficking. *Traffic* **15**, 127-140. doi:10.1111/tra.12137

Yi, R., Doehle, B. P., Qin, Y., Macara, I. G. and Cullen, B. R. (2005). Overexpression of exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. *RNA* **11**, 220-226. doi:10.1261/rna.7233305

Yoon, J., Kim, S. J., An, S., Cho, S., Leitner, A., Jung, T., Aebersold, R., Hebert, H., Cho, U. S. and Song, J. J. (2018). Integrative Structural Investigation on the Architecture of Human Importin4_Histone H3/1-14_Asf1a Complex and Its Histone H3 Tail Binding. *J. Mol. Biol.* **430**, 822-841. doi:10.1016/j.jmb.2018.01.015

Yoshimura, S. H., Kumeta, M. and Takeyasu, K. (2014). Structural mechanism of nuclear transport mediated by importin beta and flexible amphiphilic proteins. *Structure* **22**, 1699-1710. doi:10.1016/j.str.2014.10.009

Zahn, R., Osmanovic, D., Ehret, S., Callis, C. A., Frey, S., Stewart, M., You, C. J., Goerlich, D., Hoogenboom, B. W. and Richter, R. P. (2016). A physical model describing the interaction of nuclear transport receptors with FG nucleoporin domain assemblies. *eLife* **5**, e14119. doi:10.7554/eLife.14119

Zhang, W., Lu, Y., Li, X., Zhang, J., Lin, W., Zhang, W., Zheng, L. and Li, X. (2019). IPO5 promotes the proliferation and tumourigenicity of colorectal cancer cells by mediating RASAL2 nuclear transportation. *J. Exp. Clin. Cancer Res.* **38**, 296. doi:10.1186/s13046-019-1290-0

Zilman, A. (2018). Aggregation, Phase Separation and Spatial Morphologies of the Assemblies of FG Nucleoporins. *J. Mol. Biol.* **430**, 4730-4740. doi:10.1016/j.jmb.2018.07.011