nature portfolio

https://doi.org/10.1038/s41580-021-00446-7

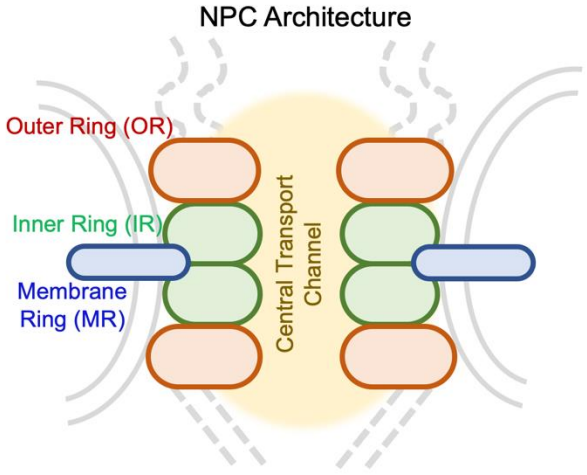
Supplementary information

Karyopherin-mediated nucleocytoplasmic transport

In the format provided by the authors and unedited

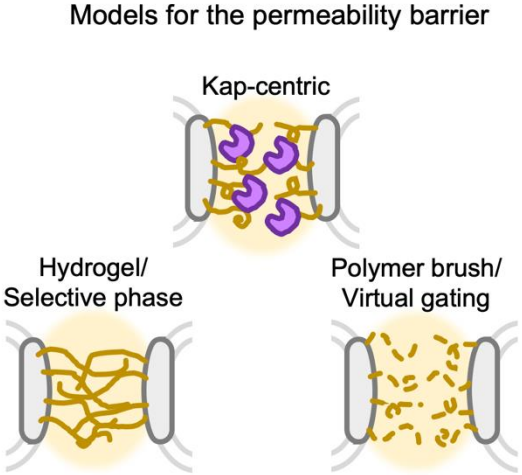
Supplementary Box 1| The nuclear pore complex: permeability barrier and Kap-cargo translocation

An NPC contains ~30 different nuclear pore proteins or nucleoporins (nups) that occur in multiples of eight to form a massive eight-fold symmetric protein assembly (~1000 Å wide, ~400 Å high, >500 nup subunits) that is embedded in the double membrane nuclear envelope (NE). Many crystal structures of nups and nup complexes along with cryo-electron microscopy (CryoEM), cryo-electron tomography (cryo-ET) and integrative structural studies of isolated and *in situ* NPCs have resulted in structural models of NPCs from multiple organisms, all showing a common organization of several conserved modules even though the architectures are distinct in different organisms (recently reviewed[1-4]). All NPCs contain a NE-embedded core scaffold that forms the 400-600 Å diameter central transport channel (CTC) with attached cytoplasmic and nucleoplasmic peripheral regions. The core scaffold has – 1) The membrane ring (MR) which contains integral membrane nups with transmembrane domains in the NE membrane and some MR nup domains in the NE lumen that likely form the path for transport of INM proteins. 2) Two approximately symmetric inner rings (IRs) that contact both the MR and the NE membrane, and extends all the way to the CTC where phenylalanine-glycine nups (FG Nups) attach to the inner face of IRs to form the permeability barrier and sites of Kap-cargo translocation. 3) Outer rings (ORs) connect to both the cytoplasmic and nuclear sides of the IRs. One OR is made up of eight Y-complexes, each with 6-9 different nups, and the numbers of ORs vary in NPCs from different organisms. ORs contact the NE membrane and the IRs. Peripheral cytoplasmic modules carrying mRNA disassembly/export machineries attach to the cytoplasmic ORs, while the highly flexible and mostly undefined nuclear peripheral modules



(e.g. the nuclear basket) attach to the nuclear ORs.

Central and peripheral FG Nups populate the CTC to form the selective permeability barrier that allows passive diffusion of solutes and small molecules but not macromolecules. Early NPC permeability studies using polyvinylpyrrolidone-coated colloidal gold particles of different diameters suggested a 40 kDa cutoff for passive diffusion[5], but diffusion/active transport of macromolecules in cells is more complicated. Assembly states and surface properties may be as or more important than molecular weights in determining the ability to traverse the NPC barrier. It is clear that the FG repeats in the CTC form the permeability barrier and that FG-nuclear transport receptor interactions allow the receptor-cargo complexes to enter/traverse the barrier but the exact nature/material properties of the FG barrier and thus Kap-cargo translocation mechanism are still heavily debated (recently reviewed[6-10]). FG Nups have been shown to form phase-separated liquids[11], hydrogels (heavily hydrated solids)[12], amyloid fibers[13], dynamic polymer brushes[14] and films[15], but their states *in situ* within the CTC remain unknown. A resident Impβ population may also be a key part of the permeability barrier[16]. Models for receptor-cargo translocation are equally varied and include receptors binding FG repeats: 1) to break the selective phase formed by FG-FG meshworks, 2) to overcome the energetic penalty of entropic exclusion of the highly dynamic fluctuating FG-nups, and 3) to break barriers with combined modes and properties of 1) and 2).



Supplementary Box 2 | Import of inner nuclear membrane proteins

Integral membrane proteins of the inner nuclear membrane (INM) are thought to diffuse from the ER membrane to the outer nuclear membrane (ONM) and then most are transported to the INM

through the NPC (previously reviewed[17-20], see Supplementary Box 1). We briefly describe the mechanisms INM transport, focusing on Kap-mediated mechanisms. Several INM proteins such as yeast HEH1, HEH2 and human POM121 are imported by IMPα/β binding to their extralumenal bipartite cNLSs, which are separated from their transmembrane regions by long extralumenal IDRs (>~20 – 25 nm)[21]. The long IDRs allow the importin-bound NLS to reach through the NPC scaffolding into the central channel[22,23]. The cNLSs of HEH1 (^{173}RKKRK^{177}-linker-^{189}SKENK^{193}), HEH2 (^{101}NKRKR^{105}-linker-^{125}KKKRK^{129}) and POM121 (^{294}KKKR^{297}-linker-^{313}KRRR^{316}) seem more densely basic than typical cNLSs and they bind very tightly to IMPα alone (low nM affinity) and displace the IBB domain without IMPβ[24,25]. Interestingly, the long linkers of HEH2 and POM121 cNLSs structurally resemble the IBB bound to IMPα in its autoinhibited state but do not confer binding to directly to IMPβ, suggesting that they do not behave entirely like IMPα IBB[24,25]. Several other INM proteins contain predicted cNLSs in their extraluminal domains but use of these putative signals have not been tested in the context of the full-length proteins[17,18,26]. It is unclear at this time if other importins can mediate INM protein transport.

Many INM proteins do not have extraluminal cNLSs or long IDRs and are likely transported through the NPC via different mechanisms. For proteins with small enough extralumenal domains to fit (<~60 kDa), another path for translocation may be through the NPC peripheral channels (~9 nm diameter)[20,27]. This mode of transport is either mediated by an INM sorting motif (INM-SM) binding to a truncated IMPα4-ARM repeat isoform or by a diffusion-retention mechanism[28-30]. The INM-SM has been reported in several INM proteins and consists of a hydrophobic 18 – 20 residue transmembrane domain and an adjacent extralumenal cluster of basic residues[17,29]. The diffusion-retention mechanism involves INM proteins passively diffusing from the ONM through the NPC to the INM where they are retained by interactions with lamins and/or chromatin[27,31]. Finally, theoretical (no experimental support) NPC-independent models of INM protein import include vesicle fusion between the ONM and INM[17,19] in a manner similar to nuclear egress by

herpesvirus[32] or in Wnt signaling[33], an autophagy-like mechanism of nuclear membrane rupture[19], or channel-mediated insertion similar to that in mitochondria[19].

Supplementary Box 3 | Conservation, characteristics and cellular levels of Kaps

Conservation and characteristics of Kaps

Kaps are conserved across eukaryotes and share common physical and chemical characteristics. The number of Kap family members in different organisms vary from 14 in *S. cerevisiae* (yeast) to 20 in human cells (Table 1). Homologous human and yeast Kaps share 18 – 47% sequence identity and function similarly as importins or exportins. Model organisms such as *D. melanogaster*, *C. elegans*, *X. laevis*, *S. pombe*, and pathogenic eukaryotes like trypanosomes have most of the human Kap homologs but are missing some Kaps or have additional Kaps. Drosophila is missing homologs of IPO8, XPO4, XPOT, RANBP6 and RANBP17, but has multiple TNPO3 and IPO4 homologs[34]. Plants also have many human Kap homologs, with a few exceptions: 1) plants have multiple IMPβ, TNPO3 and IPO5 homologs, 2) land plants have a novel PLANTKAP that is distantly related to IPO8, and 3) XPO6 is absent. Even Kaps in trypanosomes, which diverged early in eukaryotic evolution, share significant homology with human Kaps except they are missing TNPO3, XPO6 and IPO13. Conservation of Kaps parallels high conservation of NPC components[35-37].

Sequence similarities within a Kap family are generally low (~10 – 20% sequence identity) with the exception of a few highly related (~65 – 85% identical) Kap pairs like KAPβ2/KAPβ2b, IPO5/RANBP6, IPO7/IPO8 and XPO7/RANBP17 (Table 1). All Kaps are negatively charged, with importins carrying more negative charges (isoelectric points 4.5 – 5.4 for importins and 5.0 – 6.0 for exportins and biportins). Kaps also have many surface-accessible hydrophobic residues as well as arginine, histidine and cysteine residues, all of which can interact with aromatic residues

such as those in the FG repeats of the NPC. The arginine and histidine side chains are also important for maintaining solubility of Kaps in the cytoplasm and the nucleus[38].

Cellular levels of Kaps

Gene expression microarray data showed that most Kap genes are expressed in most human tissues, but expression levels do vary somewhat[39]. For example, 12 of the 20 human Kaps listed in the Gene Expression Omnibus or GEO appear highly expressed in proliferative tissues like stem cells, lymphocytes, and testis. Kap levels and localization also vary during development; for example, 10 Kaps (KAPβ2, TNPO3, IPO4, IPO5, IPO7, IPO11, CRM1, CAS, XPOT and IPO13) are consistently highly expressed during mouse embryo development[39], IPO13 is highly expressed in brain and spinal cord tissue and its expression and subcellular localization appears to be regulated in mouse brain development[39,40], and several Kaps (IMPβ, IPO4, IPO5, IPO9, CRM1, CAS and XPO6) are highly expressed in various stages of spermatogenesis[39,41,42].

Western blotting or Coomassie staining studies with protein standards and Ran concentrations (~7 μM in HeLa cells[43]) as references estimated the concentrations of several Kaps to be ~1 – 3 μM in HeLa cells and Xenopus extracts[44,45]. A recent multi-omic study on the heterogeneity of HeLa cell lines revealed only small variations in Kap levels across 14 different HeLa cell lines, with Kap concentration ranges estimated to be 0.06 – 2 μM and similar concentrations of Kaps were also estimated in budding yeast[46-48].

Supplementary Box 4 | Chaperoning activity of Kaps

Many Kaps also function as aggregation chaperones for their cargoes. A few importins (IMPβ, IPO4, IPO5, IPO7, IMPβ/IPO7, IPO9) were found to shield highly basic cargoes, such as

ribosomal proteins and histones, from inappropriate interactions with polyanions such as RNAs in the cytoplasm[49-51]. IPO9 wraps around the H2A-H2B histone dimer and shields its nucleosomal interfaces from inappropriate and nonspecific interactions in the cytoplasm and nucleus. The IPO9•H2A-H2B complex is not dissociated by RAN-GTP but rather forms a 4-protein complex, possibly to prevent premature histone release in the nucleus before reaching assembling nucleosomes on replicating DNA[52]. The IMPβ/IPO7 dimer also chaperones linker histone H1, with IMPβ cradling the H1 globular domain and the IMPβ/IPO7 interface protecting the highly basic C-terminal H1 tail[53].

Importins also shield RNA-binding proteins that are prone to self-association/aggregation; many of these cargoes are involved in neurodegenerative diseases. Studies on KAPβ2 chaperoning and inhibiting phase separation/gelation/aggregation of cargoes FUS, EWS, and TAF15, as well as on IMPα/β chaperoning TDP-43, were discussed in several recent reviews[54-57]. Additionally, nuclear import of SUMO-E2 conjugating enzyme UBC9 by IPO13/PDR6 occurs in a chaperone-like manner where biportin-binding blocks the E1, E3, and substrate docking site on UBC9, ensuring its import in the inactive state to prevent off-target SUMOylation[58,59].

Examples of exportins acting as chaperones include CSE1 binding autoinhibited IMPα to prevent export of previously imported cargoes[60,61]. Additionally, the 17 kDa export cargo eIF5A does not have a known nuclear function but passively leaks into the nucleus/nucleoli due to off-target interactions with RNAs[62]. XPO4 or PDR6 shields the RNA-binding interfaces of eIF5A and re-exports it to prevent nucleoli accumulation[59,63].

Supplementary Table 1 | **Importin-α family members**

Importin-α subtype in humans	**Other Names**	**Yeast homolog**	**Mode(s) cargo of binding**	**References[a]**
Imporin-α1 (IMPα1)	KPNA2, hSRPα, RCH1, QIP2, NPI-3	KAP60/SRP1	cNLS	[64]
Imporin-α3 (IMPα3)	KPNA4, QIP1		cNLS, ARM domain flexibility, weak IBB autoinhibition, extra-cNLS binding sites	[65-68]
Imporin-α4 (IMPα4)	KPNA3, hSRP1γ, QIP2		cNLS	[64]
Imporin-α5 (IMPα5)	KPNA1, NPI-1, SRP1, hSRP1		cNLS, extra-cNLS binding sites, weak IBB autoinhibition	[69,70]
Imporin-α6 (IMPα6)	KPNA5		cNLS	[64]
Imporin-α7 (IMPα7)	KPNA6		cNLS, weak IBB autoinhibition	[66]
Imporin-α8 (IMPα8)	KPNA7, NPI-2		cNLS	[64]

[a]For lists of specific cargoes, see review [64]

Supplementary Table 2 | **Putative MSN5 cargoes**

Putative cargo	Regions required for export	Phospho-site required for MSN5 binding	References
Nutrient and stress response			
Aft1	147-270, 304-498	S210, S224	[71]
Crz1	186-279	Not mapped	[72]
Haa1	Not mapped	Not mapped	[73]
Maf1	Not mapped	Not mapped	[74]
Msn2	1-516	Not mapped	[75]
Mig1	244-340	Not mapped	[76]
Pho4	Not mapped	S114, S128	[77,78]
Rtg1/Rtg3 dimer	2-279 in Rtg3	Not mapped	[79,80]
Ssa4	16-236	Not mapped	[81]
Cell-cycle regulation			
Cdh1	1-50	Not mapped	[82]
Cln2	225-299	Not mapped	[83]
Far1	285-390	Not mapped	[84]
Ho	248-311	Not mapped	[85,86]
Ste5	24-55, 160-218	T4, S11, T29, S43, S69, S71, S81, T102	[87-89]
Swi5	1-325 (224-285 is necessary but insufficient)	Not mapped	[90]
Swi6/Swi4	Not mapped	Not mapped	[91]
Whi5	Not mapped	Not mapped	[92]

References

1 Fernandez-Martinez, J. & Rout, M. P. One Ring to Rule them All? Structural and Functional Diversity in the Nuclear Pore Complex. *Trends Biochem Sci* **46**, 595-607, doi:10.1016/j.tibs.2021.01.003 (2021).

2 Hampoelz, B., Andres-Pons, A., Kastritis, P. & Beck, M. Structure and Assembly of the Nuclear Pore Complex. *Annu Rev Biophys* **48**, 515-536, doi:10.1146/annurev-biophys-052118-115308 (2019).

3 Lin, D. H. & Hoelz, A. The Structure of the Nuclear Pore Complex (An Update). *Annu Rev Biochem* **88**, 725-783, doi:10.1146/annurev-biochem-062917-011901 (2019).

4 Beck, M. & Hurt, E. The nuclear pore complex: understanding its function through structural insight. *Nat Rev Mol Cell Biol* **18**, 73-89, doi:10.1038/nrm.2016.147 (2017).

5 Feldherr, C. M. Evidence for changes in nuclear permeability during different physiological states. *Tissue Cell* **3**, 1-8, doi:10.1016/s0040-8166(71)80027-7 (1971).

6 Kalita, J., Kapinos, L. E. & Lim, R. Y. H. On the asymmetric partitioning of nucleocytoplasmic transport - recent insights and open questions. *J Cell Sci* **134**, doi:10.1242/jcs.240382 (2021).

7 Paci, G., Zheng, T., Caria, J., Zilman, A. & Lemke, E. A. Molecular determinants of large cargo transport into the nucleus. *Elife* **9**, doi:10.7554/eLife.55963 (2020).

8 Heinss, N., Sushkin, M., Yu, M. & Lemke, E. A. Multifunctionality of F-rich nucleoporins. *Biochem Soc Trans* **48**, 2603-2614, doi:10.1042/BST20200357 (2020).

9 Hayama, R., Rout, M. P. & Fernandez-Martinez, J. The nuclear pore complex core scaffold and permeability barrier: variations of a common theme. *Curr Opin Cell Biol* **46**, 110-118, doi:10.1016/j.ceb.2017.05.003 (2017).

10 Schmidt, H. B. & Gorlich, D. Transport Selectivity of Nuclear Pores, Phase Separation, and Membraneless Organelles. *Trends Biochem Sci* **41**, 46-61, doi:10.1016/j.tibs.2015.11.001 (2016).

11 Celetti, G. *et al.* The liquid state of FG-nucleoporins mimics permeability barrier properties of nuclear pore complexes. *J Cell Biol* **219**, doi:10.1083/jcb.201907157 (2020).

12 Petri, M., Frey, S., Menzel, A., Gorlich, D. & Techert, S. Structural characterization of nanoscale meshworks within a nucleoporin FG hydrogel. *Biomacromolecules* **13**, 1882-1889, doi:10.1021/bm300412q (2012).

13 Ader, C. *et al.* Amyloid-like interactions within nucleoporin FG hydrogels. *Proc Natl Acad Sci U S A* **107**, 6281-6285, doi:10.1073/pnas.0910163107 (2010).

14 Sakiyama, Y., Mazur, A., Kapinos, L. E. & Lim, R. Y. 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 (2016).

15 Zahn, R. *et al.* A physical model describing the interaction of nuclear transport receptors with FG nucleoporin domain assemblies. *Elife* **5**, doi:10.7554/eLife.14119 (2016).

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

17 Katta, S. S., Smoyer, C. J. & Jaspersen, S. L. Destination: inner nuclear membrane. *Trends Cell Biol* **24**, 221-229, doi:10.1016/j.tcb.2013.10.006 (2014).

18 Lusk, C. P., Blobel, G. & King, M. C. Highway to the inner nuclear membrane: rules for the road. *Nat Rev Mol Cell Biol* **8**, 414-420, doi:10.1038/nrm2165 (2007).

19 Zuleger, N., Kerr, A. R. & Schirmer, E. C. Many mechanisms, one entrance: membrane protein translocation into the nucleus. *Cell Mol Life Sci* **69**, 2205-2216, doi:10.1007/s00018-012-0929-1 (2012).

20 Mudumbi, K. C. *et al.* Nucleoplasmic signals promote directed transmembrane protein import simultaneously via multiple channels of nuclear pores. *Nat Commun* **11**, 2184, doi:10.1038/s41467-020-16033-x (2020).
21 King, M. C., Lusk, C. P. & Blobel, G. Karyopherin-mediated import of integral inner nuclear membrane proteins. *Nature* **442**, 1003-1007, doi:10.1038/nature05075 (2006).
22 Meinema, A. C. *et al.* Long unfolded linkers facilitate membrane protein import through the nuclear pore complex. *Science* **333**, 90-93, doi:10.1126/science.1205741 (2011).
23 Rempel, I. L. *et al.* Flexible and Extended Linker Domains Support Efficient Targeting of Heh2 to the Inner Nuclear Membrane. *Structure* **28**, 185-195 e185, doi:10.1016/j.str.2019.11.003 (2020).
24 Lokareddy, R. K. *et al.* Distinctive Properties of the Nuclear Localization Signals of Inner Nuclear Membrane Proteins Heh1 and Heh2. *Structure* **23**, 1305-1316, doi:10.1016/j.str.2015.04.017 (2015).
25 Kralt, A. *et al.* Conservation of inner nuclear membrane targeting sequences in mammalian Pom121 and yeast Heh2 membrane proteins. *Mol Biol Cell* **26**, 3301-3312, doi:10.1091/mbc.E15-03-0184 (2015).
26 Soullam, B. & Worman, H. J. Signals and structural features involved in integral membrane protein targeting to the inner nuclear membrane. *J Cell Biol* **130**, 15-27, doi:10.1083/jcb.130.1.15 (1995).
27 Wu, W., Lin, F. & Worman, H. J. Intracellular trafficking of MAN1, an integral protein of the nuclear envelope inner membrane. *J Cell Sci* **115**, 1361-1371, doi:10.1242/jcs.115.7.1361 (2002).
28 Ungricht, R., Klann, M., Horvath, P. & Kutay, U. Diffusion and retention are major determinants of protein targeting to the inner nuclear membrane. *J Cell Biol* **209**, 687-703, doi:10.1083/jcb.201409127 (2015).
29 Saksena, S., Summers, M. D., Burks, J. K., Johnson, A. E. & Braunagel, S. C. Importin-alpha-16 is a translocon-associated protein involved in sorting membrane proteins to the nuclear envelope. *Nat Struct Mol Biol* **13**, 500-508, doi:10.1038/nsmb1098 (2006).
30 Liu, D. *et al.* Truncated isoforms of Kap60 facilitate trafficking of Heh2 to the nuclear envelope. *Traffic* **11**, 1506-1518, doi:10.1111/j.1600-0854.2010.01119.x (2010).
31 Furukawa, K., Fritze, C. E. & Gerace, L. The major nuclear envelope targeting domain of LAP2 coincides with its lamin binding region but is distinct from its chromatin interaction domain. *J Biol Chem* **273**, 4213-4219, doi:10.1074/jbc.273.7.4213 (1998).
32 Johnson, D. C. & Baines, J. D. Herpesviruses remodel host membranes for virus egress. *Nat Rev Microbiol* **9**, 382-394, doi:10.1038/nrmicro2559 (2011).
33 Speese, S. D. *et al.* Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. *Cell* **149**, 832-846, doi:10.1016/j.cell.2012.03.032 (2012).
34 O'Reilly, A. J., Dacks, J. B. & Field, M. C. 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 (2011).
35 DeGrasse, J. A. *et al.* Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor. *Mol Cell Proteomics* **8**, 2119-2130, doi:10.1074/mcp.M900038-MCP200 (2009).
36 Neumann, N., Lundin, D. & Poole, A. M. Comparative genomic evidence for a complete nuclear pore complex in the last eukaryotic common ancestor. *PLoS One* **5**, e13241, doi:10.1371/journal.pone.0013241 (2010).
37 Obado, S. O. *et al.* Interactome Mapping Reveals the Evolutionary History of the Nuclear Pore Complex. *PLoS Biol* **14**, e1002365, doi:10.1371/journal.pbio.1002365 (2016).
38 Frey, S. *et al.* Surface Properties Determining Passage Rates of Proteins through Nuclear Pores. *Cell* **174**, 202-217 e209, doi:10.1016/j.cell.2018.05.045 (2018).

39 Quan, Y., Ji, Z. L., Wang, X., Tartakoff, A. M. & Tao, T. Evolutionary and transcriptional analysis of karyopherin beta superfamily proteins. *Mol Cell Proteomics* **7**, 1254-1269, doi:10.1074/mcp.M700511-MCP200 (2008).
40 You, P., Peng, Z., Wang, Y. & Tao, T. Expression and subcellular distribution of imp13 are regulated in brain development. *In Vitro Cell Dev Biol Anim* **49**, 346-353, doi:10.1007/s11626-013-9599-z (2013).
41 Pradeepa, M. M., Manjunatha, S., Sathish, V., Agrawal, S. & Rao, M. R. Involvement of importin-4 in the transport of transition protein 2 into the spermatid nucleus. *Mol Cell Biol* **28**, 4331-4341, doi:10.1128/MCB.00519-07 (2008).
42 Major, A. T., Whiley, P. A. & Loveland, K. L. Expression of nucleocytoplasmic transport machinery: clues to regulation of spermatogenic development. *Biochim Biophys Acta* **1813**, 1668-1688, doi:10.1016/j.bbamcr.2011.03.008 (2011).
43 Bischoff, F. R. & Ponstingl, H. Mitotic regulator protein RCC1 is complexed with a nuclear ras-related polypeptide. *Proc Natl Acad Sci U S A* **88**, 10830-10834, doi:10.1073/pnas.88.23.10830 (1991).
44 Ribbeck, K., Lipowsky, G., Kent, H. M., Stewart, M. & Gorlich, D. NTF2 mediates nuclear import of Ran. *EMBO J* **17**, 6587-6598, doi:10.1093/emboj/17.22.6587 (1998).
45 Riddick, G. & Macara, I. G. The adapter importin-alpha provides flexible control of nuclear import at the expense of efficiency. *Mol Syst Biol* **3**, 118, doi:10.1038/msb4100160 (2007).
46 Ghaemmaghami, S. *et al.* Global analysis of protein expression in yeast. *Nature* **425**, 737-741, doi:10.1038/nature02046 (2003).
47 Timney, B. L. *et al.* Simple kinetic relationships and nonspecific competition govern nuclear import rates in vivo. *J Cell Biol* **175**, 579-593, doi:10.1083/jcb.200608141 (2006).
48 Liu, Y. *et al.* Multi-omic measurements of heterogeneity in HeLa cells across laboratories. *Nat Biotechnol* **37**, 314-322, doi:10.1038/s41587-019-0037-y (2019).
49 Jakel, S., Mingot, J. M., Schwarzmaier, P., Hartmann, E. & Gorlich, D. Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains. *EMBO J* **21**, 377-386, doi:10.1093/emboj/21.3.377 (2002).
50 Bernardes, N. E. & Chook, Y. M. Nuclear import of histones. *Biochem Soc Trans* **48**, 2753-2767, doi:10.1042/BST20200572 (2020).
51 Soniat, M., Cagatay, T. & Chook, Y. M. Recognition Elements in the Histone H3 and H4 Tails for Seven Different Importins. *J Biol Chem* **291**, 21171-21183, doi:10.1074/jbc.M116.730218 (2016).
52 Padavannil, A. *et al.* Importin-9 wraps around the H2A-H2B core to act as nuclear importer and histone chaperone. *Elife* **8**, doi:10.7554/eLife.43630 (2019).
53 Ivic, N. *et al.* Fuzzy Interactions Form and Shape the Histone Transport Complex. *Molecular Cell*, doi:10.1016/j.molcel.2019.01.032 (2019).
54 Springhower, C. E., Rosen, M. K. & Chook, Y. M. Karyopherins and condensates. *Curr Opin Cell Biol* **64**, 112-123, doi:10.1016/j.ceb.2020.04.003 (2020).
55 Yoshizawa, T. & Matsumura, H. Effect of nuclear import receptors on liquid-liquid phase separation. *Biophys Physicobiol* **17**, 25-29, doi:10.2142/biophysico.BSJ-2019052 (2020).
56 Pasha, T. *et al.* Karyopherin abnormalities in neurodegenerative proteinopathies. *Brain*, doi:10.1093/brain/awab201 (2021).
57 Guo, L., Fare, C. M. & Shorter, J. Therapeutic Dissolution of Aberrant Phases by Nuclear-Import Receptors. *Trends Cell Biol* **29**, 308-322, doi:10.1016/j.tcb.2018.12.004 (2019).
58 Grunwald, M. & Bono, F. Structure of Importin13-Ubc9 complex: nuclear import and release of a key regulator of sumoylation. *EMBO J* **30**, 427-438, doi:10.1038/emboj.2010.320 (2011).

59 Aksu, M., Trakhanov, S., Vera Rodriguez, A. & Gorlich, D. Structural basis for the nuclear import and export functions of the biportin Pdr6/Kap122. *J Cell Biol* **218**, 1839-1852, doi:10.1083/jcb.201812093 (2019).
60 Kutay, U., Bischoff, F. R., Kostka, S., Kraft, R. & Gorlich, D. 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 (1997).
61 Matsuura, Y. & Stewart, M. Structural basis for the assembly of a nuclear export complex. *Nature* **432**, 872-877, doi:10.1038/nature03144 (2004).
62 Lipowsky, G. *et al.* 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 (2000).
63 Aksu, M., Trakhanov, S. & Gorlich, D. Structure of the exportin Xpo4 in complex with RanGTP and the hypusine-containing translation factor eIF5A. *Nat Commun* **7**, 11952, doi:10.1038/ncomms11952 (2016).
64 Pumroy, R. A. & Cingolani, G. Diversification of importin-alpha isoforms in cellular trafficking and disease states. *Biochem J* **466**, 13-28, doi:10.1042/BJ20141186 (2015).
65 Friedrich, B., Quensel, C., Sommer, T., Hartmann, E. & Kohler, M. Nuclear localization signal and protein context both mediate importin alpha specificity of nuclear import substrates. *Mol Cell Biol* **26**, 8697-8709, doi:10.1128/MCB.00708-06 (2006).
66 Pumroy, R. A., Ke, S., Hart, D. J., Zachariae, U. & Cingolani, G. Molecular determinants for nuclear import of influenza A PB2 by importin alpha isoforms 3 and 7. *Structure* **23**, 374-384, doi:10.1016/j.str.2014.11.015 (2015).
67 Smith, K. M. *et al.* Structural basis for importin alpha 3 specificity of W proteins in Hendra and Nipah viruses. *Nat Commun* **9**, 3703, doi:10.1038/s41467-018-05928-5 (2018).
68 Jagga, B. *et al.* Structural basis for nuclear import selectivity of pioneer transcription factor SOX2. *Nat Commun* **12**, 28, doi:10.1038/s41467-020-20194-0 (2021).
69 Nardozzi, J., Wenta, N., Yasuhara, N., Vinkemeier, U. & Cingolani, G. Molecular basis for the recognition of phosphorylated STAT1 by importin alpha5. *J Mol Biol* **402**, 83-100, doi:10.1016/j.jmb.2010.07.013 (2010).
70 Xu, W. *et al.* Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1. *Cell Host Microbe* **16**, 187-200, doi:10.1016/j.chom.2014.07.008 (2014).
71 Ueta, R., Fujiwara, N., Iwai, K. & Yamaguchi-Iwai, Y. Mechanism underlying the iron-dependent nuclear export of the iron-responsive transcription factor Aft1p in Saccharomyces cerevisiae. *Mol Biol Cell* **18**, 2980-2990, doi:10.1091/mbc.e06-11-1054 (2007).
72 Boustany, L. M. & Cyert, M. S. Calcineurin-dependent regulation of Crz1p nuclear export requires Msn5p and a conserved calcineurin docking site. *Genes Dev* **16**, 608-619, doi:10.1101/gad.967602 (2002).
73 Sugiyama, M. *et al.* Nuclear localization of Haa1, which is linked to its phosphorylation status, mediates lactic acid tolerance in Saccharomyces cerevisiae. *Appl Environ Microbiol* **80**, 3488-3495, doi:10.1128/AEM.04241-13 (2014).
74 Towpik, J., Graczyk, D., Gajda, A., Lefebvre, O. & Boguta, M. Derepression of RNA polymerase III transcription by phosphorylation and nuclear export of its negative regulator, Maf1. *J Biol Chem* **283**, 17168-17174, doi:10.1074/jbc.M709157200 (2008).
75 Gorner, W. *et al.* Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. *EMBO J* **21**, 135-144, doi:10.1093/emboj/21.1.135 (2002).
76 DeVit, M. J. & Johnston, M. The nuclear exportin Msn5 is required for nuclear export of the Mig1 glucose repressor of Saccharomyces cerevisiae. *Curr Biol* **9**, 1231-1241, doi:10.1016/s0960-9822(99)80503-x (1999).

77 Kaffman, A., Rank, N. M., O'Neill, E. M., Huang, L. S. & O'Shea, E. K. The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. *Nature* **396**, 482-486, doi:10.1038/24898 (1998).
78 Komeili, A. & O'Shea, E. K. Roles of phosphorylation sites in regulating activity of the transcription factor Pho4. *Science* **284**, 977-980, doi:10.1126/science.284.5416.977 (1999).
79 Komeili, A., Wedaman, K. P., O'Shea, E. K. & Powers, T. Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. *J Cell Biol* **151**, 863-878, doi:10.1083/jcb.151.4.863 (2000).
80 Sekito, T., Thornton, J. & Butow, R. A. Mitochondria-to-nuclear signaling is regulated by the subcellular localization of the transcription factors Rtg1p and Rtg3p. *Mol Biol Cell* **11**, 2103-2115, doi:10.1091/mbc.11.6.2103 (2000).
81 Quan, X., Tsoulos, P., Kuritzky, A., Zhang, R. & Stochaj, U. The carrier Msn5p/Kap142p promotes nuclear export of the hsp70 Ssa4p and relocates in response to stress. *Mol Microbiol* **62**, 592-609, doi:10.1111/j.1365-2958.2006.05395.x (2006).
82 Jaquenoud, M., van Drogen, F. & Peter, M. Cell cycle-dependent nuclear export of Cdh1p may contribute to the inactivation of APC/C(Cdh1). *EMBO J* **21**, 6515-6526, doi:10.1093/emboj/cdf634 (2002).
83 Quilis, I. & Igual, J. C. Molecular basis of the functional distinction between Cln1 and Cln2 cyclins. *Cell Cycle* **11**, 3117-3131, doi:10.4161/cc.21505 (2012).
84 Blondel, M. *et al.* Nuclear export of Far1p in response to pheromones requires the export receptor Msn5p/Ste21p. *Genes Dev* **13**, 2284-2300, doi:10.1101/gad.13.17.2284 (1999).
85 Bakhrat, A. *et al.* Nuclear export of Ho endonuclease of yeast via Msn5. *Curr Genet* **54**, 271-281, doi:10.1007/s00294-008-0216-8 (2008).
86 Kaplun, L., Ivantsiv, Y., Bakhrat, A. & Raveh, D. DNA damage response-mediated degradation of Ho endonuclease via the ubiquitin system involves its nuclear export. *J Biol Chem* **278**, 48727-48734, doi:10.1074/jbc.M308671200 (2003).
87 Hu, Z. *et al.* Mapping regions in Ste5 that support Msn5-dependent and -independent nuclear export. *Biochem Cell Biol* **94**, 109-128, doi:10.1139/bcb-2015-0101 (2016).
88 Mahanty, S. K., Wang, Y., Farley, F. W. & Elion, E. A. Nuclear shuttling of yeast scaffold Ste5 is required for its recruitment to the plasma membrane and activation of the mating MAPK cascade. *Cell* **98**, 501-512, doi:10.1016/s0092-8674(00)81978-9 (1999).
89 Wang, Y., Chen, W., Simpson, D. M. & Elion, E. A. Cdc24 regulates nuclear shuttling and recruitment of the Ste5 scaffold to a heterotrimeric G protein in Saccharomyces cerevisiae. *J Biol Chem* **280**, 13084-13096, doi:10.1074/jbc.M410461200 (2005).
90 Taberner, F. J., Quilis, I., Sendra, J., Bano, M. C. & Igual, J. C. Regulation of cell cycle transcription factor Swi5 by karyopherin Msn5. *Biochim Biophys Acta* **1823**, 959-970, doi:10.1016/j.bbamcr.2012.02.009 (2012).
91 Queralt, E. & Igual, J. C. Cell cycle activation of the Swi6p transcription factor is linked to nucleocytoplasmic shuttling. *Mol Cell Biol* **23**, 3126-3140, doi:10.1128/MCB.23.9.3126-3140.2003 (2003).
92 Wagner, M. V. *et al.* Whi5 regulation by site specific CDK-phosphorylation in Saccharomyces cerevisiae. *PLoS One* **4**, e4300, doi:10.1371/journal.pone.0004300 (2009).