© 2021. Published by The Company of Biologists Ltd | Journal of Cell Science (2021) 134, jcs247874. doi:10.1242/jcs.247874

CELL SCIENCE AT A GLANCE

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

Cargo transport through the nuclear pore complex at a glance

Giulia Paci[1,2,3,*], Joana Caria[1,2,3,*] and Edward A. Lemke[1,2,3,‡]

ABSTRACT

Bidirectional transport of macromolecules across the nuclear envelope is a hallmark of eukaryotic cells, in which the genetic material is compartmentalized inside the nucleus. The nuclear pore complex (NPC) is the major gateway to the nucleus and it regulates nucleocytoplasmic transport, which is key to processes including transcriptional regulation and cell cycle control. Accordingly, components of the nuclear transport machinery are often found to be dysregulated or hijacked in diseases. In this Cell Science at a Glance article and accompanying poster, we provide an overview of our current understanding of cargo transport through the NPC, from the basic transport signals and machinery to more emerging aspects, all from a 'cargo perspective'. Among these, we discuss the transport of large cargoes (>15 nm), as well as the roles of different cargo properties to nuclear transport, from size and number of bound nuclear transport receptors (NTRs), to surface and mechanical properties.

KEY WORDS: Large cargoes, Nuclear pore complex, Nuclear transport, Nuclear transport receptors, Multivalent binding, Virus, Gene therapy

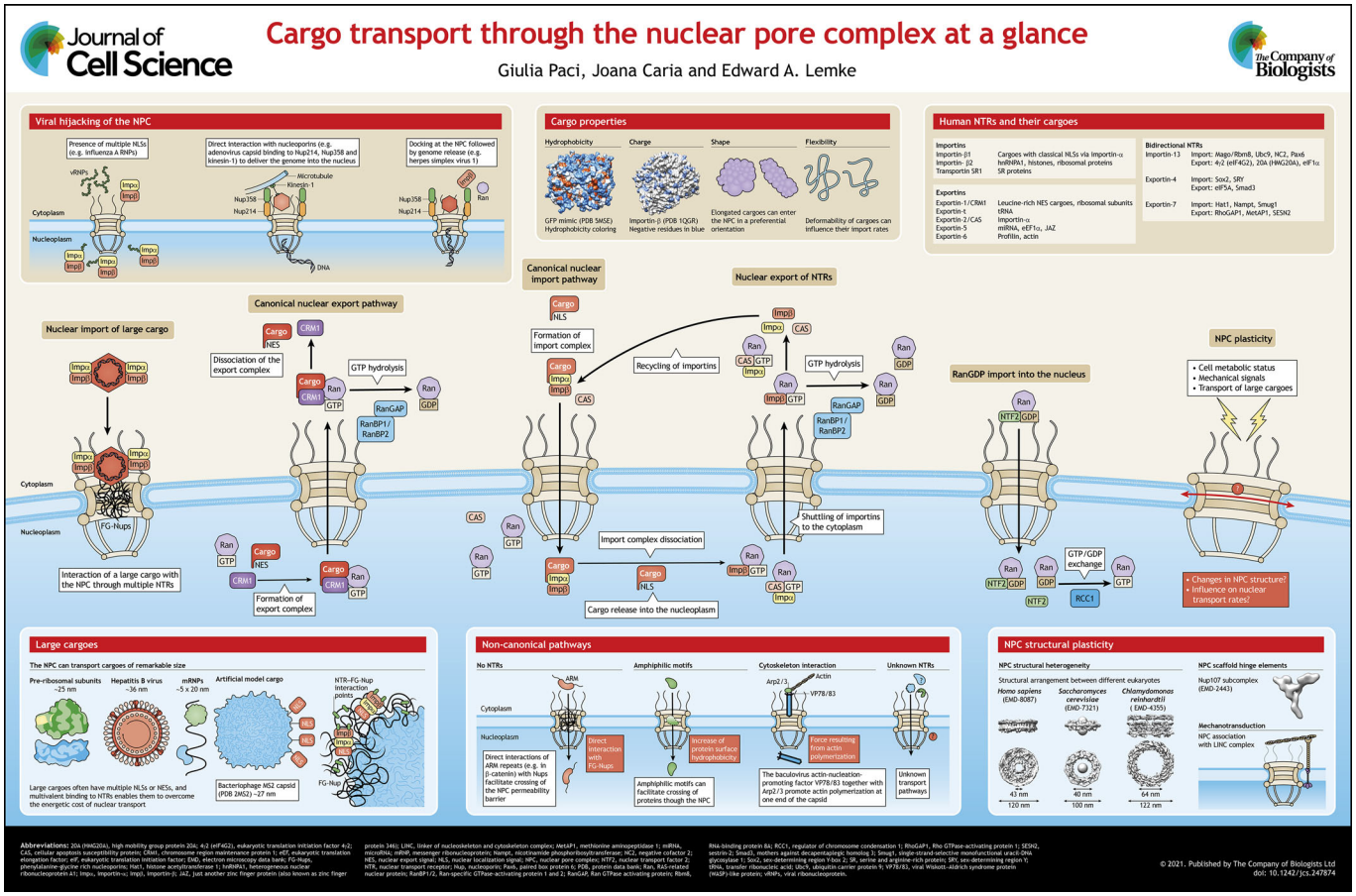
[1]Biocentre, Johannes Gutenberg-University Mainz, Hans-Dieter-Hüsch-Weg 15, 555128 Mainz, Germany. [2]Institute of Molecular Biology, Ackermannweg 4, 55128 Mainz, Germany. [3]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany.
*These authors contributed equally to this work

‡Author for correspondence (edlemke@uni-mainz.de)

G.P., 0000-0003-0565-4356; J.C., 0000-0002-8791-4766; E.A.L., 0000-0002-0634-0503

Introduction

In eukaryotic cells, the genetic material is enclosed inside the nucleus and most communication between this compartment and the cytoplasm occurs through large protein channels that span the nuclear envelope, called nuclear pore complexes (NPCs) (Hampoelz et al., 2019). The continuous transport of proteins,



Journal of Cell Science (2021) 134, jcs247874. doi:10.1242/jcs.247874

RNA and metabolites across NPCs is key to processes indispensable for cellular viability including transcriptional regulation and cell cycle control, just to name a few examples. The central role of nuclear transport is also highlighted by the fact that many of its components are dysregulated in aging and in several diseases including cancer, autoimmune diseases and viral infection (Capelson and Hetzer, 2009; Sakuma and D'Angelo, 2017).

The structure of the NPC is assembled by multiple copies of ∼30 nucleoporins (Nups); approximately two thirds of these proteins are folded and form the donut-shaped NPC scaffold, whose structure has been elucidated by intensive structural studies using both electron tomography and X-ray crystallography (Szymborska et al., 2013; Von Appen et al., 2015; Kosinski et al., 2016; Lin et al., 2016). The scaffold is filled with intrinsically disordered proteins, known as FG nucleoporins (FG-Nups) that form the NPC permeability barrier (for reviews discussing proposed models of the still elusive barrier architecture see, for example, Wälde and Kehlenbach, 2010; Lim et al., 2015; Lemke, 2016; Schmidt and Görlich, 2016). The main function of the permeability barrier is to block entry of undesired molecules, while allowing selective transport of up to 1000 cargoes per second (Ribbeck and Görlich, 2001). There are two main mechanisms of transport through the NPC, passive and facilitated diffusion. Small cargoes (typically up to ∼5 nm) can freely diffuse through the NPC; however, the passage of larger molecules is hindered. Recent work has suggested that passive transport through the NPC might not have a strict size cut-off but rather act as a 'soft' permeability barrier (Timney et al., 2016). Larger cargoes can be efficiently transported via facilitated diffusion by binding to nuclear transport receptors (NTRs), which recognize the nuclear localization signals (NLSs) or nuclear export signals (NESs) displayed by the cargo.

In this Cell Science at a Glance article and accompanying poster, we focus on our current understanding of nuclear transport from a 'cargo perspective'. We provide a brief overview of the classical transport machinery, as well as alternative mechanisms of entry, and refer to more detailed reviews on specific aspects of NPC structure and function. We discuss the emerging role of different cargo properties on their transport and highlight recent advances in our understanding of multivalent binding to NTRs, focusing especially on large cargoes. In this context, we also review current hypotheses and supporting evidences for the potential dynamics of the NPC structure during cargo transport. Finally, we highlight how the advancements in our basic understanding of nuclear transport can be translated into biomedical fields including viral infection, gene therapy and drug delivery (Boxes 1 and 2).

Box 1. Hijacking of the NPC by viral cargoes

Most DNA viruses and some RNA viruses replicate inside the nucleus of the host cell, and they have developed a diverse set of strategies to overcome the barrier posed by the nuclear envelope and NPCs (see poster). Most viruses uncoat, that is release their genome from the capsid, in the cytoplasm, and only their genome translocates into the nucleus, as the intact capsid is too large to cross the NPC.

Two of the main strategies adopted involve viral factors (e.g. RNPs and capsid proteins) carrying NLSs to hijack the host-cell nuclear import machinery or their direct binding to Nups. Among important human viruses that adopt the first strategy are the influenza A virus, whose viral RNP complexes present several NLS motifs (Martin and Helenius, 1991; Ozawa et al., 2007; Eisfeld et al., 2015). The hepatitis B virus encodes multiple NLSs in the C-termini of its capsid core proteins, which are buried inside the capsid and only exposed upon capsid phosphorylation, which occurs concomitantly to reverse transcription and virus maturation. Recognition of these NLSs by importin-α–importin-β enables binding of the capsid to the NPC, but only mature capsids are released into the nucleoplasm (Yeh et al., 1990; Eckhardt et al., 1991; Kann et al., 1999), which might also depend on capsid destabilization (Cui et al., 2013). It is worth noting that an important aspect of viral import is the presence of multiple NLSs and potential multimeric binding to several importins, which is also a hallmark of large cargo transport (see the section on large cargo). Aside from factors associated with the viral genome, several other viral proteins, such as the simian virus SV40 large tumor antigen and the human papilloma virus E1 protein, exploit the nuclear-import machinery of the host cell to shuttle into or out of the nucleus and perform different functions in pathogenesis and replication (reviewed in Fulcher and Jans, 2011).

Direct interaction of viral components with Nups is an alternative entry strategy, often used in combination with NTR-mediated transport (see poster). Several viruses dock their capsids at the cytoplasmic side of the NPC; for instance, adenoviruses bind to Nup214 (Trotman et al., 2001), a key component of the flexible filaments located on the cytoplasmic side of the NPC, and exploit kinesin-1 binding to both capsid and Nup358 to disassemble the capsid (Strunze et al., 2011) and subsequently deliver their genome to the nucleus owing to a combination of NTR-mediated transport and increased NPC permeability (Wodrich et al., 2006; Hindley et al., 2007). The capsid of herpes simplex virus 1 also docks to the cytoplasmic side of the NPC by binding to the Nup214–Nup358 complex, which is importin-β and Ran dependent (Ojala et al., 2000; Copeland et al., 2009). The viral DNA is then ejected from the capsid and translocated through the NPC via a yet unknown mechanism that might be driven by the pressure stored in the capsid during packaging (Liashkovich et al., 2011a).

Some viruses exploit multiple of the above-described strategies to overcome the NPC barrier throughout their life cycle. For instance, the human immunodeficiency virus type 1 (HIV-1) integrase protein has an NLS that likely mediates the import of the HIV-1 pre-integration complex (Whittaker et al., 2000), and its reverse transcriptase contains an NES that is recognized by CRM1 (Neville et al., 1997), while the HIV-1 capsid can also directly bind to several Nups including Nup153 (Whittaker et al., 2000; Di Nunzio, 2013).

Regardless of the specific delivery strategy, a large body of work has highlighted that nuclear entry through the NPC can often be a bottleneck for viral infection (reviewed in Flatt and Greber, 2015); therefore, a better understanding of how nuclear transport is hijacked by viruses will be key to developing effective antiviral strategies.

Targeting signals for import and export complexes

Cargoes targeted for nuclear import and/or export present, on their surface, short amino acid sequences that can be classified as NLSs or NESs. Classical NLSs are characterized by the presence of stretches of basic amino acids and can be monopartite, such as in the SV40 large T antigen NLS (PKKKRRV), with a loose consensus sequence K-(K/R)-X-(K/R) (Kalderon et al., 1984; Dang and Lee, 1988), or bipartite (two motifs separated by a variable spacer), such as in the nucleoplasmin NLS (KRPAATKKAGQAKKKK), with a loose consensus sequence $(K/R)(K/R)X_{10–12}(K/R)_{3/5}$ (Robbins et al., 1991; Lange et al., 2007). Further non-classical NLSs have been described (Kosugi et al., 2009); these require other importin-mediated transport, such as the PY-NLSs, recognized by transportin-1 (Tnpo1) and generally characterized by a hydrophobic or basic motif followed by a C-terminal consensus sequence $R/K/HX_{(2–5)}PY$ (Lee et al., 2006). One famous example is the PY-NLS (also known as M9 NLS) from human hnRNPA1 (Pollard et al., 1996; Bonifaci et al., 1997). Recent studies have also shown different non-classical NLSs for RNA-binding proteins whose transport is mediated by Tnpo1 and Tnpo3 (Bourgeois et al., 2020). Interestingly, phosphorylation of NLSs can both enhance and disrupt binding to NTRs, allowing an additional layer of regulation on import (Nardozzi et al., 2010). NESs are typically composed of short sequences enriched in hydrophobic conserved amino acids, showing a loose consensus sequence (L/I/V/

Journal of Cell Science (2021) 134, jcs247874. doi:10.1242/jcs.247874

Box 2. Gene therapy applications

Gene therapy and targeted drug-delivery systems have been one of the greatest promises in the biomedical field as a potential treatment for several human diseases, including cancer and genetic disorders. However, their broad application has been hindered by a number of application challenges. These systems all need to overcome the various biological barriers of the human body, reach their target cell and deliver the gene or drug, with minimal off-target effects and effective bioaccumulation.

Delivery systems are generally classified into viral or non-viral vectors. Viral vectors are derived from human pathogens; here, adenovirus, adeno-associated virus and retroviruses (lentiviruses) are the most widely used (Waehler et al., 2007). Viral systems take advantage of their evolution into highly efficient gene transfer systems, with strategies to evade the immune system, enter cells and gain access to the nucleus (see also Box 1). However, their costs of production are high, and viruses have a broad tropism requiring extensive research to make cell-type-specific targeting possible. Non-viral delivery systems pose a versatile, biocompatible and chemically stable alternative. These systems comprise different types of nanoparticles that are easily synthetized and can be surface modified (Blanco et al., 2015; Fortuni et al., 2019). Unlike viruses, synthetic nanoparticles have to be functionalized with different signals in order to be internalized and efficiently targeted.

As the nucleus is the ultimate functional target for gene therapy applications, especially in the case of non-viral delivery systems where nuclear transport is still the major obstacle, understanding how to successfully target and accumulate cargoes in the nucleus is of great interest. Several strategies have been developed in order to functionalize nanoparticles for nuclear import (Yao et al., 2013), and coupling a NLS sequence is one of the most common. Despite this, the nuclear accumulation of most these nanoparticles remains inefficient, mainly because of their large size. In fact, nanoparticles can exceed the diameter of the nuclear pore (Jana et al., 2013; Mackey et al., 2013). Besides their large size, the stiffness and/or deformability of the nanoparticles is also an important aspect to consider, as deformable cargoes can cross the permeability barrier more easily (Zelmer et al., 2020).

Since successful therapies rely on the accumulation of an effective concentration of genetic material in the nucleus, a low import rate is a major setback. Given the importance of multivalent binding to NTRs, understanding how to enhance NLS coverage in relation to nanoparticle size to optimize nuclear accumulation and, more generally, the determinants that are involved in large cargo transport may help in the appropriate design of such delivery systems (see also the related discussion in the main text).

F/M)-$X_{2–3}$-(L/I/V/F/M)-$X_{2–3}$-(L/I/V/F/M)-X-(L/I/V/F/M) (Kosugi et al., 2008). The classical NESs are leucine-rich NESs, which were first described in the HIV Rev and protein kinase A inhibitor (Fischer et al., 1995; Wen et al., 1995) and whose recognition is mediated by exportin-1 (also known as chromosomal maintenance 1, CRM1) (Fornerod et al., 1997; Fukuda et al., 1997; Askjaer et al., 1998).

NTRs bind to the NLSs and NESs on the cargoes to form import and export complexes (see poster). One of the largest family of NTRs are β-karyopherins, which include both import and export receptors (importins and exportins) and bidirectional receptors (Kimura and Imamoto, 2014). In humans, there are at least 20 different β-karyopherins; they are typically large proteins (90–150 kDa), characterized by an N-terminal Ran-binding domain and the presence of multiple HEAT motifs (Lott and Cingolani, 2011). These create a superhelical architecture that confers high flexibility to these molecules, enabling them to recognize different cargoes (Yuh and Blobel, 2001; Cook et al., 2007). Possibly the most famous member of this family is importin-β (Harel and Forbes, 2004), which like most β-karyopherins can recognize its cargoes directly, or by binding to an adaptor molecule. Importin-α family proteins (in humans importin-α1 to -α8) act as such adaptors, recognizing NLSs on cargoes via their NLS-binding domain formed by ten armadillo (ARM) repeats (Goldfarb et al., 2004) and binding to importin-β via their importin-β-binding domain (IBB). Other NTRs include exportin-t (Arts et al., 1998; Kutay et al., 1998), exportin-2 (also known as cellular apoptosis susceptibility gene, CAS) (Brinkmann et al., 1995; Kutay et al., 1997), transportin-3 (also known as transportin-SR2) (Maertens et al., 2014) and bidirectional NTRs, such as exportin-4 (Aksu et al., 2016), exportin-7 (Aksu et al., 2018) and exportin-13 (Mingot et al., 2001; Baade et al., 2018). One key property of NTRs is their ability to engage in multivalent interactions with the FG-Nups that form the NPC permeability barrier, thereby enabling cargo transport. Recent studies have shown that this interaction is based on multiple low-affinity contacts between NTRs and FG-Nups that allow an ultrafast binding and unbinding, enabling a rapid exchange of FG-motifs from the different NTR binding pockets. This mechanism is crucial for the fast and selective transport of the NTR–cargo complex through the NPC (Hough et al., 2015; Milles et al., 2015; Raveh et al., 2016).

In the transport of NLS- or NES-containing cargoes, the first step is the formation of a complex in the cytoplasm (importin-β–importin-α–NLS-cargo complex), or in the nucleoplasm (CRM1-GTP bound to Ran–NES-cargo complex). The complex then crosses the NPC and dissociates; in the nucleoplasm, binding of RAS-related nuclear protein (Ran) in the GTP-bound form (RanGTP) to importin-β triggers the dissociation of the import complex, while a second RanGTP molecule together with CAS frees the NLS-cargo by binding to importin-α. In the cytoplasm, hydrolysis of RanGTP to RanGDP leads to dissociation of the export complex (see poster). Differentially localized molecules are responsible for GTP hydrolysis: in the cytoplasm, Ran-binding protein 1 (RanBP1), in its soluble form, or RanBP2 (also known as Nup358) catalyzes the activity of Ran GTPase-activating protein (RanGAP1), which converts RanGTP into RanGDP. In the nucleus, regulator of chromosome condensation 1 (RCC1) catalyzes the GTP/GDP exchange in RanGDP, which is imported into the nucleus by NTF2 (also known as NUTF2) (Görlich and Kutay, 1999). This creates an asymmetric distribution of RanGTP and RanGDP between the nucleus and cytoplasm (high RanGDP in the cytoplasm and high RanGTP in the nucleus), and this gradient is essential to maintain the directionality of nucleocytoplasmic transport (Lui and Huang, 2009).

Besides the recognition of classical NLS and NES signals, NTRs can recognize their cargo through their surface properties and folded domains (see poster). For instance, importin-9 wraps preferentially around the globular core domain of the histone H2A–H2B dimer, having only weak and dynamic interactions with the H2A–H2B tails that harbor NLS-like sequences (Padavannil et al., 2019). Similar mechanisms have been also shown for exportin-5 (Okada et al., 2009), exportin-4 (Aksu et al., 2016) and importin-13 (Bono et al., 2010; Grünwald et al., 2013). These studies not only show a flexibility of NTRs in cargo recognition, but also provide insight into how some can act as bidirectional NTRs or have other cellular functions (e.g. as chaperones).

Non-canonical import pathways

The canonical import pathway described in the previous section is not sufficient to explain the localization of every nuclear protein, as for many proteins a classical NLS has not been identified. This could be because NLS prediction has been difficult, or the NTRs for

Journal of Cell Science (2021) 134, jcs247874. doi:10.1242/jcs.247874

certain proteins have not been yet identified (Marfori et al., 2011; Bernhofer et al., 2018); however, there is also growing evidence for the existence of alternative, non-canonical transport pathways (see poster).

Cytoskeletal-assisted nuclear transport is often associated with viral entry as an efficient way of achieving nuclear targeting (see Box 1). Generally, the cytoskeleton is not required for nuclear transport, although microtubules play a role in the nuclear import of some cancer regulatory proteins (Roth et al., 2007), and alterations to actin by mutant profilin 1 impact nucleocytoplasmic transport, leading to motor neuron dysfunction in amyotrophic lateral sclerosis (Giampetruzzi et al., 2019). Cytoskeletal-assisted nuclear transport typically acts as an enhancer and functions together with a NTR-dependent pathway for efficient import. In some cases, it might even act as a propulsive force to enable cargoes to cross the NPC. Such a mechanism has been suggested for baculovirus nucleocapsids, which contain VP78/83, a protein that activates the Arp2/3 complex and induces actin polymerization at one end of the nucleocapsid (Au et al., 2016). Indeed, nuclear import of nucleocapsids could be reconstituted in purified nuclei supplemented with G-actin and Arp2/3 under actin polymerization conditions (Au et al., 2016).

Similar to NTRs, other proteins can interact directly with FG-Nups and translocate through the NPC. For example, β-catenin (a member of the armadillo family, like importin-α) has several ARM repeats, which are structurally similar to HEAT repeats (Fagotto et al., 1998; Yokoya et al., 1999) and support its nuclear import through direct binding to Nups. Additionally, Importin-β can inhibit nuclear accumulation of β-catenin, pointing to a competition for NPC-binding sites (Fagotto et al., 1998). Recent studies argue that β-catenin might also have a second independent transport pathway, mediated by RAPGEF5 (Griffin et al., 2018).

Proteins that possess amphiphilic motifs have also been shown to cross the NPC in an NTR-independent manner. In hydrophobic environments, the amphiphilic region undergoes a conformational change and exposes its hydrophobic amino acids to the outer surface of the protein. This increase in protein surface hydrophobicity facilitates the transport through the permeability barrier (Kumeta et al., 2012).

While these are just a few examples of alternative pathways, other mechanisms have also been reported (Wagstaff and Jans, 2009). Examples include for instance molecules that can 'piggyback' on proteins with a functional NLS (Thompson, 2010), or ribonucleoprotein (RNP) particles that can be exported from the nucleus by budding from the nuclear envelope (Speese et al., 2012). Moreover, many proteins appear to exploit multiple nuclear import pathways, which might provide additional robustness to the system or allow adaptation to different cues.

Emerging roles for cargo properties in transport regulation

In addition to the presence of NLS or NES signals, other cargo properties play an important role in the passage through the NPC (see poster). Surface charges have been proposed to affect cargo transport, as NTRs (which are capable of fast translocation through NPCs) are characterized by a high number of negative charges on their surface, whereas Nups have several positively charged linkers between their FG repeats (Colwell et al., 2010). A recent study systematically characterized the effect of surface properties on the passage of proteins through the NPC (Frey et al., 2018), using GFP as a model cargo. By introducing mutations that altered the surface properties, the authors could obtain transport-competent GFP variants that translocated across the NPC on their own and faster than the NTR NTF2 and 10,000-fold faster than the slowest GFP variant. To test the effect of cargo surface properties on NTR-mediated transport, they then analyzed the larger IBB–3XEGFP variants (consisting of a tandem of three GFP variants with a single IBB domain), and they observed that a 'super-inert' GFP variant failed to import efficiently, despite the presence of an IBB (Frey et al., 2018). We will return to this point in the section dedicated to large cargo transport.

In addition to surface properties, cargo shape can also affect nuclear transport – studies of passive transport have found that elongated molecules enter the nucleus faster than spherical ones of similar mass (Mohr et al., 2009), likely because they enter the channel in a preferred orientation. Finally, the mechanical stability of cargoes can also influence their transport across the NPC. Recent work on the nuclear import of myocardin-related transcription factor A (MRTFA) found that concatenating MRTFA with protein domains of varying mechanical stability resulted in import rates that are inversely correlated with the stability of the domains (Infante et al., 2019). This might be relevant in the transport of large deformable synthetic cargoes, such as polymer vesicles (Discher et al., 1999). Deformability and cargo remodeling are likely to also play an important role in the transport of RNPs, which can be of remarkable size, for example, the Balbiani ring mRNA particles, which are 50 nm in diameter. Indeed, high-resolution studies using single-particle tracking have shown that mRNPs can be restructured and unfolded during translocation (Mor et al., 2010).

Large cargoes and multivalent NTR binding

The NPC can transport cargoes of remarkable size, including viral capsids, components of the proteasome machinery, pre-ribosomal subunits and mRNA complexes (see poster). Synthetic vectors for the delivery of drugs or gene therapy also fall into this category (see Box 2). The transport of these large cargoes (>15 nm) is especially striking if we consider that substantial amounts of FG-Nups must be displaced in order for it to occur. Considering a spherical cargo of 27 nm in diameter, for example, the MS2 bacteriophage capsid, an artificial model system which has been recently characterized in Paci et al. (2020), and assuming a FG-Nup concentration of 2 mM in the central channel of the NPC forming the permeability barrier (Frey and Görlich, 2007), a mass of ∼1 MDa of FG-Nups would need to be displaced. A key feature of large cargo transport is the requirement for binding to multiple NTRs – for instance, nuclear export of pre-ribosomal subunits depends on several transport receptors (Tschochner and Hurt, 2003), mRNA export depends on the heterodimeric transport receptor Tap–p15 (also known as NXF1 and NXT, respectively) (Köhler and Hurt, 2007) and hepatitis B virus (HBV) capsids can contain up to 240 NLSs. However, as the surface exposure of the NLS is modulated by viral packaging and its phosphorylation status, the precise number of simultaneously exposed NLSs or bound NTRs is not known (Yeh et al., 1990; Eckhardt et al., 1991).

Enhancement of cargo transport by multivalent binding of NTRs had already been observed in early experiments with pentameric nucleoplasmin (Dingwall et al., 1982). In another study, simultaneous binding of a cargo to two different NTRs (importin-β and transportin-1), but not to only one of these, enabled efficient import (Ribbeck and Görlich, 2002). Similar results were also found in studies employing larger cargoes – IBB-coated quantum dots, which bound to up to 40 importin-β molecules (Lowe et al., 2010), and β-galactosidase carrying four NLSs (Tu et al., 2013). However, a comprehensive view on the respective roles of cargo size and multivalent NTR-binding was lacking. Our recent study approached this question systematically by employing a large cargo toolkit

Journal of Cell Science (2021) 134, jcs247874. doi:10.1242/jcs.247874

based on capsid-like cargoes of a size range of 17 to 36 nm and containing a tuneable number of NLSs on the surface (Paci et al., 2020). Combining biophysical characterization and quantitative imaging in permeabilized cells for over 30 unique cargo samples, we found that the requirements for nuclear import scaled non-linearly with cargo size and could be recapitulated with a simple biophysical model linking the import flux to the energetic requirements for nuclear transport, that is the cost of cargo insertion into the FG assembly and energetic gain from FG-NTR binding (Paci et al., 2020). A similar framework has also been employed previously to explain the NTR requirement for the import of a β-galactosidase cargo (Tu et al., 2013). Both models take into account the potential presence of regions with lower density of FG Nups ('vestibules') on the cytoplasmic and nuclear sides of the NPC (Paci et al., 2020; Tu et al., 2013). Such structural differences in the NPC permeability barrier are consistent with observations of single-particle transport occurring through multiple steps – two slow docking/undocking phases at the NPC periphery and a fast channel-crossing step (Grünwald and Singer, 2010; Lowe et al., 2010). Finally, it is important to note that, in the presence of multivalent binding of NTRs, the cargo surface properties discussed above appear to play a lesser role; this is likely due to a substantial shielding of any local surface properties by the high coverage of NTRs (Paci et al., 2020).

Structural implications of large cargo transport – pore dilation

An additional important facet of large cargo transport through the NPC relates to the question of whether flexibility of the permeability barrier and/or scaffold is required. Dilation of the NPC scaffold is a question that has long been debated. Although conclusive *in vivo* experiments regarding the timescale of structural changes are lacking, we discuss here several lines of evidence that support the possibility of NPC plasticity within cells.

An important line of evidence comes from studies investigating NPC structural heterogeneity. The extent to which the oligomeric composition of the NPC and its architecture are conserved between eukaryotes branches still remains unclear (Field et al., 2014; Beck et al., 2018; Field and Rout, 2019). Nonetheless, there is heterogeneity between the structural arrangements of NPCs in different species and even between different cell types (see poster). For example, the early branching eukaryote *Chlamydomonas reinhardtii* presents an asymmetric oligomeric state and a larger inner-ring diameter compared to the human NPC (Mosalaganti et al., 2018). A recent study in *Saccharomyces cerevisiae* cells under starvation and exponential growth conditions found an inner-ring diameter 20 nm larger than previously described (Allegretti et al., 2020), while another study reported that *Schizosaccharomyces pombe* NPCs have a constricted conformation in response to energy depletion, together with a reduced passive diffusion and no active nuclear transport (Zimmerli et al., 2020 preprint). Moreover, in intact HeLa cells, dilated conformations of the NPC have been observed (Mahamid et al., 2016), and studies using *Xenopus* oocyte nuclei found rotational symmetries that diverge from the traditional eight-fold symmetry (Hinshaw and Milligan, 2003; Stanley et al., 2018). Finally, with regard to cell-type differences, a proteomic study revealed that Nups can have a variable subunit stoichiometry (Ori et al., 2013). In addition to this intrinsic structural plasticity, several studies have reported reversible NPC dilation after treatment with chemicals, including steroids, hexanediol and *trans*-cyclohexane-1,2-diol, which can collapse the permeability barrier (Jäggi et al., 2003; Shahin et al., 2005; Liashkovich et al., 2011b).

From a structural perspective, the question arises of how exactly dilation of the scaffold could occur. In human NPCs, one striking example is the Nup107 subcomplex, composed of ten different nucleoporins (Nup-160, -133, -107, -96, -85, -43, and -37, Seh1, Sec13 and ELYS); studies using the isolated complex revealed it is highly flexible in specific positions and can adopt numerous conformations, possibly acting as a hinge component on both sides of the nuclear pore (Bui et al., 2013).

Mechanical forces are another potential means of altering the structure of the NPC. The linker of nucleoskeleton and cytoskeleton (LINC) complex provides a direct pathway to transmit mechanical signals through the nuclear envelope, interacting with the cytoskeleton on one side and with nuclear lamina and chromatin on the other. Forces transmitted through the LINC complex have been shown to cause nuclear distortion and alter the NPC size, with an impact on cargo transport rates (Jahed et al., 2016; Donnaloja et al., 2019). For example, the transcriptional regulator YAP1 has an increased transport rate in cells under mechanical stress, and blocking the LINC complex impairs its nuclear accumulation (Elosegui-Artola et al., 2017). In the future, further investigating the role of mechanotransduction in tuning nuclear transport through changes in the NPC conformation could provide important insight into the regulation of gene expression.

Perspectives

The regulation of nucleocytoplasmic transport is crucial to countless cellular processes. While our understanding of the basic transport mechanisms has greatly improved in recent years, many open questions remain, especially regarding the details of the mutual NPC–cargo interaction *in vivo*. High-resolution imaging technologies, for example focused ion-beam scanning electron microscopy (cryo FIB-SEM) in cells, combined with high-throughput automated image analysis pipelines based on machine learning, could enable new insights into NPC plasticity and capture its different conformational states. Fluorescence-based methods, such as super-resolution microscopy and single-particle tracking, will be an essential complementary approach that could help to dissect the detailed dynamics and kinetics of the import and export pathways in live cells. Furthermore, elegant *in vitro* NPC systems based on DNA origami are being developed to enable a bottom-up understanding of nuclear transport from minimal components (Fisher et al., 2018; Ketterer et al., 2018). In addition, recent methods could enable the rapid isolation of native yeast NPCs (Kim et al., 2018), enabling *in vitro* structural and functional studies on intact NPCs. Finally, further research into non-canonical pathways through the NPC and physiological cargoes that exploit several import or export mechanisms could shed light on the robustness and regulation of nuclear transport, and how it goes awry in aging and during disease. Ultimately, this will allow the development of improved gene therapy and targeted drug-delivery systems (see Box 2) as potential treatment for several human diseases, including cancer and genetic disorders.

Acknowledgements
We thank all members of the Lemke group for critical discussion.

Competing interests
The authors declare no competing or financial interests.

Funding
We thank SFB 1129 (project number 240245660 funded by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for generous funding of our laboratory.

Journal of Cell Science (2021) 134, jcs247874. doi:10.1242/jcs.247874

Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.247874.supplemental

References

Aksu, M., Trakhanov, S. and Görlich, D. (2016). Structure of the exportin Xpo4 in complex with RanGTP and the hypusine-containing translation factor eIF5A. *Nat. Commun.* **7**, 11952. doi:10.1038/ncomms11952

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

Allegretti, M., Zimmerli, C. E., Rantos, V., Wilfling, F., Ronchi, P., Fung, H., Lee, C. W., Hagen, W., Turoňová, B., Karius, K. et al. (2020). In-cell architecture of the nuclear pore and snapshots of its turnover. *Nature* **586**, 796-800. doi:10.1038/s41586-020-2670-5

Arts, G.-J., Kuersten, S., Romby, P., Ehresmann, B. and Mattaj, I. W. (1998). The role of exportin-t in selective nuclear export of mature tRNAs. *EMBO J.* **17**, 7430-7441. doi:10.1093/emboj/17.24.7430

Askjaer, P., Jensen, T. H., Nilsson, J., Englmeier, L. and Kjems, J. (1998). The specificity of the CRM1-Rev nuclear export signal interaction is mediated by RanGTP. *J. Biol. Chem.* **273**, 33414-33422. doi:10.1074/jbc.273.50.33414

Au, S., Wu, W., Zhou, L., Theilmann, D. A. and Panté, N. (2016). A new mechanism for nuclear import by actin-based propulsion used by a baculovirus nucleocapsid. *J. Cell Sci.* **129**, 2905-2911. doi:10.1242/jcs.191668

Baade, I., Spillner, C., Schmitt, K., Valerius, O. and Kehlenbach, R. H. (2018). Extensive identification and in-depth validation of importin 13 cargoes. *Mol. Cell. Proteomics.* **17**, 1337-1353. doi:10.1074/mcp.RA118.000623

Beck, M., Mosalaganti, S. and Kosinski, J. (2018). From the resolution revolution to evolution: structural insights into the evolutionary relationships between vesicle coats and the nuclear pore. *Curr. Opin. Struct. Biol.* **52**, 32-40. doi:10.1016/j.sbi.2018.07.012

Bernhofer, M., Goldberg, T., Wolf, S., Ahmed, M., Zaugg, J., Boden, M. and Rost, B. (2018). NLSdb—major update for database of nuclear localization signals and nuclear export signals. *Nucleic Acids Res.* **46**, D503-D508. doi:10.1093/nar/gkx1021

Blanco, E., Shen, H. and Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **33**, 941-951. doi:10.1038/nbt.3330

Bonifaci, N., Moroianu, J., Radu, A. and Blobel, G. (1997). Karyopherin β2 mediates nuclear import of a mRNA binding protein. *Proc. Natl. Acad. Sci. USA* **94**, 5055-5060. doi:10.1073/pnas.94.10.5055

Bono, F., Cook, A. G., Grünwald, M., Ebert, J. and Conti, E. (2010). Nuclear import mechanism of the EJC component Mago-Y14 revealed by structural studies of importin 13. *Mol. Cell* **37**, 211-222. doi:10.1016/j.molcel.2010.01.007

Bourgeois, B., Hutten, S., Gottschalk, B., Hofweber, M., Richter, G., Sternat, J., Abou-Ajram, C., Göbl, C., Leitinger, G., Graier, W. F. et al. (2020). Nonclassical nuclear localization signals mediate nuclear import of CIRBP. *Proc. Natl. Acad. Sci. USA* **117**, 8503-8514. doi:10.1073/pnas.1918944117

Brinkmann, U., Brinkmann, E., Gallo, M. and Pastan, I. (1995). Cloning and characterization of a cellular apoptosis susceptibility gene, the human homologue to the yeast chromosome segregation gene CSE1. *Proc. Natl. Acad. Sci. USA* **92**, 10427-10431. doi:10.1073/pnas.92.22.10427

Bui, K. H., Von Appen, A., Diguilio, A. L., Ori, A., Sparks, L., Mackmull, M.-T., Bock, T., Hagen, W., Andrés-Pons, A., Glavy, J. S. et al. (2013). Integrated structural analysis of the human nuclear pore complex scaffold. *Cell* **155**, 1233-1243. doi:10.1016/j.cell.2013.10.055

Capelson, M. and Hetzer, M. W. (2009). The role of nuclear pores in gene regulation, development and disease. *EMBO Rep.* **10**, 697-705. doi:10.1038/embor.2009.147

Colwell, L. J., Brenner, M. P. and Ribbeck, K. (2010). Charge as a selection criterion for translocation through the nuclear pore complex. *PLoS Comput. Biol.* **6**, e1000747. doi:10.1371/journal.pcbi.1000747

Cook, A., Bono, F., Jinek, M. and Conti, E. (2007). Structural biology of nucleocytoplasmic transport. *Annu. Rev. Biochem.* **76**, 647-671. doi:10.1146/annurev.biochem.76.052705.161529

Copeland, A. M., Newcomb, W. W. and Brown, J. C. (2009). Herpes simplex virus replication: roles of viral proteins and nucleoporins in capsid-nucleus attachment. *J. Virol.* **83**, 1660-1668. doi:10.1128/JVI.01139-08

Cui, X., Ludgate, L., Ning, X. and Hu, J. (2013). Maturation-associated destabilization of hepatitis B virus nucleocapsid. *J. Virol.* **87**, 11494-11503. doi:10.1128/JVI.01912-13

Dang, C. V. and Lee, W. M. (1988). Identification of the human c-myc protein nuclear translocation signal. *Mol. Cell. Biol.* **8**, 4048-4054. doi:10.1128/MCB.8.10.4048

Dingwall, C., Sharnick, S. V. and Laskey, R. A. (1982). A polypeptide domain that specifies migration of nucleoplasmin into the nucleus. *Cell* **30**, 449-458. doi:10.1016/0092-8674(82)90242-2

Di Nunzio, F. (2013). New insights in the role of nucleoporins: a bridge leading to concerted steps from HIV-1 nuclear entry until integration. *Virus Res.* **178**, 187-196. doi:10.1016/j.virusres.2013.09.003

Discher, B. M., Won, Y.-Y., Ege, D. S., Lee, J. C.-M., Bates, F. S., Discher, D. E. and Hammer, D. A. (1999). Polymersomes: tough vesicles made from diblock copolymers. *Science* **284**, 1143-1146. doi:10.1126/science.284.5417.1143

Donnaloja, F., Jacchetti, E., Soncini, M. and Raimondi, M. T. (2019). Mechanosensing at the nuclear envelope by nuclear pore complex stretch activation and its effect in physiology and pathology. *Front. Physiol.* **10**, 896. doi:10.3389/fphys.2019.00896

Eckhardt, S. G., Milich, D. R. and Mclachlan, A. (1991). Hepatitis B virus core antigen has two nuclear localization sequences in the arginine-rich carboxyl terminus. *J. Virol.* **65**, 575-582. doi:10.1128/JVI.65.2.575-582.1991

Eisfeld, A. J., Neumann, G. and Kawaoka, Y. (2015). At the centre: influenza A virus ribonucleoproteins. *Nat. Rev. Microbiol.* **13**, 28-41. doi:10.1038/nrmicro3367

Elosegui-Artola, A., Andreu, I., Beedle, A. E. M., Lezamiz, A., Uroz, M., Kosmalska, A. J., Oria, R., Kechagia, J. Z., Rico-Lastres, P., Le Roux, A.-L. et al. (2017). Force triggers YAP nuclear entry by regulating transport across nuclear pores. *Cell* **171**, 1397-1410.e14. doi:10.1016/j.cell.2017.10.008

Fagotto, F., Glück, U. and Gumbiner, B. M. (1998). Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of β-catenin. *Curr. Biol.* **8**, 181-190. doi:10.1016/s0960-9822(98)70082-x

Field, M. C. and Rout, M. P. (2019). Pore timing: the evolutionary origins of the nucleus and nuclear pore complex. *F1000Res.* **8**, F1000, doi:10.12688/F1000RESEARCH.16402.1

Field, M. C., Koreny, L. and Rout, M. P. (2014). Enriching the pore: splendid complexity from humble origins. *Traffic* **15**, 141-156. doi:10.1111/tra.12141

Fischer, U., Huber, J., Boelens, W. C., Mattajt, L. W. and Lührmann, R. (1995). The HIV-1 rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell* **82**, 475-483. doi:10.1016/0092-8674(95)90436-0

Fisher, P. D. E., Shen, Q., Akpinar, B., Davis, L. K., Chung, K. K. H., Baddeley, D., Šarić, A., Melia, T. J., Hoogenboom, B. W., Lin, C. et al. (2018). A programmable DNA origami platform for organizing intrinsically disordered nucleoporins within nanopore confinement. *ACS Nano* **12**, 1508-1518. doi:10.1021/acsnano.7b08044

Flatt, J. and Greber, U. (2015). Misdelivery at the nuclear pore complex—stopping a virus dead in its tracks. *Cells* **4**, 277-296. doi:10.3390/cells4030277

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

Fortuni, B., Inose, T., Ricci, M., Fujita, Y., Van Zundert, I., Masuhara, A., Fron, E., Mizuno, H., Latterini, L., Rocha, S. et al. (2019). Polymeric engineering of nanoparticles for highly efficient multifunctional drug delivery systems. *Sci. Rep.* **9**, 2666. doi:10.1038/s41598-019-39107-3

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

Fukuda, M., Asano, S., Nakamura, T., Adachi, M., Yoshida, M., Yanagida, M. and Nishida, E. (1997). CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature* **390**, 308-311. doi:10.1038/36894

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

Giampetruzzi, A., Danielson, E. W., Gumina, V., Jeon, M., Boopathy, S., Brown, R. H., Ratti, A., Landers, J. E. and Fallini, C. (2019). Modulation of actin polymerization affects nucleocytoplasmic transport in multiple forms of amyotrophic lateral sclerosis. *Nat. Commun.* **10**, 3827. doi:10.1038/s41467-019-11837-y

Goldfarb, D. S., Corbett, A. H., Mason, D. A., Harreman, M. T. and Adam, S. A. (2004). Importin α: A multipurpose nuclear-transport receptor. *Trends Cell Biol.* **14**, 505-514. 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

Griffin, J. N., Del Viso, F., Duncan, A. R., Robson, A., Hwang, W., Kulkarni, S., Liu, K. J. and Khokha, M. K. (2018). RAPGEF5 regulates nuclear translocation of β-catenin. *Dev. Cell* **44**, 248-260.e4. doi:10.1016/j.devcel.2017.12.001

Grünwald, D. and Singer, R. H. (2010). In vivo imaging of labelled endogenous β-actin mRNA during nucleocytoplasmic transport. *Nature* **467**, 604-607. doi:10.1038/nature09438

Grünwald, M., Lazzaretti, D. and Bono, F. (2013). Structural basis for the nuclear export activity of Importin13. *EMBO J.* **32**, 899-913. doi:10.1038/emboj.2013.29

Hampoelz, B., Andres-Pons, A., Kastritis, P. and Beck, M. (2019). Structure and assembly of the nuclear pore complex. *Annu. Rev. Biophys.* **48**, 515-536. doi:10.1146/annurev-biophys-052118-115308

Harel, A. and Forbes, D. J. (2004). Importin beta: conducting a much larger cellular symphony. *Mol. Cell* **16**, 319-330. doi:10.1016/j.molcel.2004.10.026

Hindley, C. E., Lawrence, F. J. and Matthews, D. A. (2007). A role for transportin in the nuclear import of adenovirus core proteins and DNA. *Traffic* **8**, 1313-1322. doi:10.1111/j.1600-0854.2007.00618.x

Hinshaw, J. E. and Milligan, R. A. (2003). Nuclear pore complexes exceeding eightfold rotational symmetry. *J. Struct. Biol.* **141**, 259-268. doi:10.1016/S1047-8477(02)00626-3

Hough, L. E., Dutta, K., Sparks, S., Temel, D. B., Kamal, A., Tetenbaum-Novatt, J., Rout, M. P. and Cowburn, D. (2015). The molecular mechanism of nuclear transport revealed by atomic-scale measurements. *eLife*, **4**, e10027. doi:10.7554/eLife.10027

Infante, E., Stannard, A., Board, S. J., Rico-Lastres, P., Rostkova, E., Beedle, A. E. M., Lezamiz, A., Wang, Y. J., Gulaidi Breen, S., Panagaki, F. et al. (2019). The mechanical stability of proteins regulates their translocation rate into the cell nucleus. *Nat. Phys.* **15**, 973-981. doi:10.1038/s41567-019-0551-3

Jäggi, R. D., Franco-Obregón, A., Mühlhäusser, P., Thomas, F., Kutay, U. and Ensslin, K. (2003). Modulation of nuclear pore topology by transport modifiers. *Biophys. J* **84**, 665-670. doi:10.1016/S0006-3495(03)74886-3

Jahed, Z., Soheilypour, M., Peyro, M. and Mofrad, M. R. K. (2016). The LINC and NPC relationship - it's complicated! *J. Cell Sci.* **129**, 3219-3229. doi:10.1242/jcs.184184

Jana, A., Saha, B., Banerjee, D. R., Ghosh, S. K., Nguyen, K. T., Ma, X., Qu, Q., Zhao, Y. and Singh, N. D. P. (2013). Photocontrolled nuclear-targeted drug delivery by single component photoresponsive fluorescent organic nanoparticles of acridin-9-methanol. *Bioconjug. Chem.* **24**, 1828-1839. doi:10.1021/bc400170r

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

Kann, M., Sodeik, B., Vlachou, A., Gerlich, W. H. and Helenius, A. (1999). Phosphorylation-dependent binding of Hepatitis B virus core particles to the nuclear pore complex. *J. Cell Biol.* **145**, 45-55. doi:10.1083/jcb.145.1.45

Ketterer, P., Ananth, A. N., Laman Trip, D. S., Mishra, A., Bertosin, E., Ganji, M., van der Torre, J., Onck, P., Dietz, H., Dekker, C. et al. (2018). DNA origami scaffold for studying intrinsically disordered proteins of the nuclear pore complex. *Nat. Commun.* **9**, 902. doi:10.1038/s41467-018-03313-w

Kim, S. J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W., 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**, 475-482. doi:10.1038/nature26003

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

Köhler, A. and Hurt, E. (2007). Exporting RNA from the nucleus to the cytoplasm. *Nat. Rev. Mol. Cell Biol.* **8**, 761-773. doi:10.1038/nrm2255

Kosinski, J., Mosalaganti, S., Von Appen, A., Teimer, R., Diguilio, A. L., Wan, W., Bui, K. H., Hagen, W. J. H., Briggs, J. A. G., Glavy, J. S. et al. (2016). Molecular architecture of the inner ring scaffold of the human nuclear pore complex. *Science* **352**, 363-365. doi:10.1126/science.aaf0643

Kosugi, S., Hasebe, M., Tomita, M. and Yanagawa, H. (2008). Nuclear export signal consensus sequences defined using a localization-based yeast selection system. *Traffic* **9**, 2053-2062. doi:10.1111/j.1600-0854.2008.00825.x

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 α. *J. Biol. Chem.* **284**, 478-485. doi:10.1074/jbc.M807017200

Kumeta, M., Yamaguchi, H., Yoshimura, S. H. and Takeyasu, K. (2012). Karyopherin-independent spontaneous transport of amphiphilic proteins through the nuclear pore. *J. Cell Sci.* **125**, 4979-4984. doi:10.1242/jcs.109520

Kutay, U., Bischoff, F. R., Kostka, S., Kraft, R. and Görlich, D. (1997). Export of importin α 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 Görlich, D. (1998). Identification of a tRNA-specific nuclear export receptor. *Mol. Cell* **1**, 359-369. doi:10.1016/S1097-2765(00)80036-2

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 α. *J. Biol. Chem.* **282**, 5101-5105. doi:10.1074/jbc.R600026200

Lee, B. J., Cansizoglu, A. E., Süel, K. E., Louis, T. H., Zhang, Z. and Chook, Y. M. (2006). Rules for nuclear localization sequence recognition by Karyopherinβ2. *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

Liashkovich, I., Hafezi, W., Kühn, J. M., Oberleithner, H. and Shahin, V. (2011a). Nuclear delivery mechanism of herpes simplex virus type 1 genome. *J. Mol. Recognit.* **24**, 414-421. doi:10.1002/jmr.1120

Liashkovich, I., Meyring, A., Kramer, A. and Shahin, V. (2011b). Exceptional structural and mechanical flexibility of the nuclear pore complex. *J. Cell. Physiol.* **226**, 675-682. doi:10.1002/jcp.22382

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

Lin, D. H., Stuwe, T., Schilbach, S., Rundlet, E. J., Perriches, T., Mobbs, G., Fan, Y., Thierbach, K., Huber, F. M., Collins, L. N. et al. (2016). Architecture of the symmetric core of the nuclear pore. *Science* **352**, aaf1015. doi:10.1126/science.aaf1015

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

Lowe, A. R., Siegel, J. J., Kalab, P., Siu, M., Weis, K. and Liphardt, J. T. (2010). Selectivity mechanism of the nuclear pore complex characterized by single cargo tracking. *Nature* **467**, 600-603. doi:10.1038/nature09285

Lui, K. and Huang, Y. (2009). RanGTPase: a key regulator of nucleocytoplasmic trafficking. *Mol. Cell. Pharmacol.* **1**, 148-156. doi:10.4255/mcpharmacol.09.19

Mackey, M. A., Saira, F., Mahmoud, M. A. and El-Sayed, M. A. (2013). Inducing cancer cell death by targeting its nucleus: Solid gold nanospheres versus hollow gold nanocages. *Bioconjug. Chem.* **24**, 897-906. doi:10.1021/bc300592d

Maertens, G. N., Cook, N. J., Wang, W., Hare, S., Gupta, S. S., Öztop, I., Lee, K. E., 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. USA* **111**, 2728-2733. doi:10.1073/pnas.1320755111

Mahamid, J., Pfeffer, S., Schaffer, M., Villa, E., Danev, R., Kuhn Cuellar, L., Forster, F., Hyman, A. A., Plitzko, J. M. and Baumeister, W. (2016). Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**, 969-972. doi:10.1126/science.aad8857

Marfori, M., Mynott, A., Ellis, J. J., Mehdi, A. M., Saunders, N. F. W., Curmi, P. M., Forwood, J. K., Bodén, M. and Kobe, B. (2011). Molecular basis for specificity of nuclear import and prediction of nuclear localization. *Biochim. Biophys.Acta (BBA) Mol. Cell Res.* **1813**, 1562-1577. doi:10.1016/j.bbamcr.2010.10.013

Martin, K. and Helenius, A. (1991). Transport of incoming influenza virus nucleocapsids into the nucleus. *J. Virol.* **65**, 232-244. doi:10.1128/JVI.65.1.232-244.1991

Milles, S., Mercadante, D., Aramburu, I. V., Jensen, M. R., Banterle, N., Koehler, C., Tyagi, S., Clarke, J., Shammas, S. L., Blackledge, M. et al. (2015). Plasticity of an ultrafast interaction between nucleoporins and nuclear transport receptors. *Cell* **163**, 734-745. doi:10.1016/j.cell.2015.09.047

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

Mohr, D., Frey, S., Fischer, T., Güttler, T. and Görlich, D. (2009). Characterisation of the passive permeability barrier of nuclear pore complexes. *EMBO J.* **28**, 2541-2553. doi:10.1038/emboj.2009.200

Mor, A., Suliman, S., Ben-Yishay, R., Yunger, S., Brody, Y. and Shav-Tal, Y. (2010). Dynamics of single mRNP nucleocytoplasmic transport and export through the nuclear pore in living cells. *Nat. Cell Biol.* **12**, 543-552. doi:10.1038/ncb2056

Mosalaganti, S., Kosinski, J., Albert, S., Schaffer, M., Strenkert, D., Salomé, P. A., Merchant, S. S., Plitzko, J. M., Baumeister, W., Engel, B. D. et al. (2018). In situ architecture of the algal nuclear pore complex. *Nat. Commun.* **9**, . 2361. doi:10.1038/s41467-018-04739-y

Nardozzi, J. D., Lott, K. and Cingolani, G. (2010). Phosphorylation meets nuclear import: a review. *Cell Commun. Signal.* **8**, 32. doi:10.1186/1478-811X-8-32

Neville, M., Stutz, F., Lee, L., Davis, L. I. and Rosbash, M. (1997). The importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export. *Curr. Biol.* **7**, 767-775. doi:10.1016/S0960-9822(06)00335-6

Ojala, P. M., Sodeik, B., Ebersold, M. W., Kutay, U. and Helenius, A. (2000). Herpes simplex virus type 1 entry into host cells: reconstitution of capsid binding and uncoating at the nuclear pore complex in vitro. *Mol. Cell. Biol.* **20**, 4922-4931. doi:10.1128/MCB.20.13.4922-4931.2000

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

Ori, A., Banterle, N., Iskar, M., Andrés-Pons, A., Escher, C., Khanh Bui, H., Sparks, L., Solis-Mezarino, V., Rinner, O., Bork, P. et al. (2013). Cell type-specific nuclear pores: a case in point for context-dependent stoichiometry of molecular machines. *Mol. Syst. Biol.* **9**, 648. doi:10.1038/msb.2013.4

Ozawa, M., Fujii, K., Muramoto, Y., Yamada, S., Yamayoshi, S., Takada, A., Goto, H., Horimoto, T. and Kawaoka, Y. (2007). Contributions of two nuclear localization signals of influenza a virus nucleoprotein to viral replication. *J. Virol.* **81**, 30-41. doi:10.1128/JVI.01434-06

Paci, G., Zheng, T., Caria, J., Zilman, A., and Lemke, E. A. (2020). Molecular determinants of large cargo transport into the nucleus. *Elife* **9**, e55963. doi:10.7554/eLife.55963

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

Pollard, V. W., Michael, W. M., Nakielny, S., Siomi, M. C., Wang, F. and Dreyfuss, G. (1996). A novel receptor-mediated nuclear protein import pathway. *Cell* **86**, 985-994. doi:10.1016/S0092-8674(00)80173-7

Raveh, B., Karp, J. M., Sparks, S., Dutta, K., Rout, M. P., Sali, A. and Cowburn, D. (2016). Slide-and-exchange mechanism for rapid and selective transport through the nuclear pore complex. *Proc. Natl. Acad. Sci. USA* **113**, E2489-E2497. doi:10.1073/pnas.1522663113

Ribbeck, K. and Görlich, D. (2001). Kinetic analysis of translocation through nuclear pore complexes. *EMBO J.* **20**, 1320-1330. doi:10.1093/emboj/20.6.1320

Ribbeck, K. and Görlich, D. (2002). The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. *EMBO J.* **21**, 2664-2671. doi:10.1093/emboj/21.11.2664

Robbins, J., Dilwortht, S. M., Laskey, R. A. and Dingwall, C. (1991). Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell* **64**, 615-623. doi:10.1016/0092-8674(91)90245-T

Roth, D. M., Moseley, G. W., Glover, D., Pouton, C. W. and Jans, D. A. (2007). A microtubule-facilitated nuclear import pathway for cancer regulatory proteins. *Traffic* **8**, 673-686. doi:10.1111/j.1600-0854.2007.00564.x

Sakuma, S. and D'angelo, M. A. (2017). The roles of the nuclear pore complex in cellular dysfunction, aging and disease. *Semin. Cell Dev. Biol.* **68**, 72-84. doi:10.1016/j.semcdb.2017.05.006

Schmidt, H. B. and Görlich, D. (2016). Transport selectivity of nuclear pores, phase separation, and membraneless organelles. *Trends Biochem. Sci.* **41**, 46-61. doi:10.1016/j.tibs.2015.11.001

Shahin, V., Albermann, L., Schillers, H., Kastrup, L., Schäfer, C., Ludwig, Y., Stock, C. and Oberleithner, H. (2005). Steroids dilate nuclear pores imaged with atomic force microscopy. *J. Cell. Physiol.* **202**, 591-601. doi:10.1002/jcp.20152

Speese, S. D., Ashley, J., Jokhi, V., Nunnari, J., Barria, R., Li, Y., Ataman, B., Koon, A., Chang, Y.-T., Li, Q. et al. (2012). Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. *Cell* **149**, 832-846. doi:10.1016/j.cell.2012.03.032

Stanley, G. J., Fassati, A. and Hoogenboom, B. W. (2018). Atomic force microscopy reveals structural variability amongst nuclear pore complexes. *Life Sci. Alliance* **1**. e201800142. doi:10.26508/lsa.201800142

Strunze, S., Engelke, M. F., Wang, I.-H., Puntener, D., Boucke, K., Schleich, S., Way, M., Schoenenberger, P., Burckhardt, C. J., Greber, U. F. et al. (2011). Kinesin-1-mediated capsid disassembly and disruption of the nuclear pore complex promote virus infection. *Cell Host Microbe* **10**, 210-223. doi:10.1016/j.chom.2011.08.010

Szymborska, A., De Marco, A., Daigle, N., Cordes, V. C., Briggs, J. A. G. and Ellenberg, J. (2013). Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging. *Science* **341**, 655-658. doi:10.1126/science.1240672

Thompson, M. E. (2010). BRCA1 16 years later: nuclear import and export processes. *FEBS J.* **277**, 3072-3078. doi:10.1111/j.1742-4658.2010.07733.x

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

Trotman, L. C., Mosberger, N., Fornerod, M., Stidwill, R. P. and Greber, U. F. (2001). Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1. *Nat. Cell Biol.* **3**, 1092-1100. doi:10.1038/ncb1201-1092

Tschochner, H. and Hurt, E. (2003). Pre-ribosomes on the road from the nucleolus to the cytoplasm. *Trends Cell Biol.* **13**, 255-263. doi:10.1016/S0962-8924(03)00054-0

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

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**, 140-143. doi:10.1038/nature15381

Waehler, R., Russell, S. J. and Curiel, D. T. (2007). Engineering targeted viral vectors for gene therapy. *Nat. Rev. Genet.* **8**, 573-587. doi:10.1038/nrg2141

Wagstaff, K. M. and Jans, D. A. (2009). Importins and beyond: non-conventional nuclear transport mechanisms. *Traffic* **10**, 1188-1198. doi:10.1111/j.1600-0854.2009.00937.x

Wälde, S. and Kehlenbach, R. H. (2010). The part and the whole: functions of nucleoporins in nucleocytoplasmic transport. *Trends Cell Biol.* **20**, 461-469. doi:10.1016/j.tcb.2010.05.001

Wen, W., Meinkotht, J. L., Tsien, R. Y. and Taylor, S. S. (1995). Identification of a signal for rapid export of proteins from the nucleus. *Cell* **82**, 463-473. doi:10.1016/0092-8674(95)90435-2

Whittaker, G. R., Kann, M. and Helenius, A. (2000). Viral entry into the nucleus. *Annu. Rev. Cell Dev. Biol.* **16**, 627-651. doi:10.1146/annurev.cellbio.16.1.627

Wodrich, H., Cassany, A., D'angelo, M. A., Guan, T., Nemerow, G. and Gerace, L. (2006). Adenovirus core protein pVII is translocated into the nucleus by multiple import receptor pathways. *J. Virol.* **80**, 9608-9618. doi:10.1128/JVI.00850-06

Yao, J., Fan, Y., Li, Y. and Huang, L. (2013). Strategies on the nuclear-targeted delivery of genes. *J. Drug Target* **21**, 926-939. doi:10.3109/1061186X.2013.830310

Yeh, C. T., Liaw, Y. F. and Ou, J. H. (1990). The arginine-rich domain of hepatitis B virus precore and core proteins contains a signal for nuclear transport. *J. Virol.* **64**, 6141-6147. doi:10.1128/JVI.64.12.6141-6147.1990

Yokoya, F., Imamoto, N., Tachibana, T. and Yoneda, Y. (1999). β-Catenin can be transported into the nucleus in a ran-unassisted manner. *Mol. Biol. Cell* **10**, 1119-1131. doi:10.1091/mbc.10.4.1119

Yuh, M. C. and Blobel, G. (2001). Karyopherins and nuclear import. *Curr. Opin. Struct. Biol.* **11**, 703-715. doi:10.1016/S0959-440X(01)00264-0

Zelmer, C., Zweifel, L. P., Kapinos, L. E., Craciun, I., Güven, Z. P., Palivan, C. G. and Lim, R. Y. H. (2020). Organelle-specific targeting of polymersomes into the cell nucleus. *Proc. Natl. Acad. Sci. USA* **117**, 2770-2778. doi:10.1073/pnas.1916395117

Zimmerli, C. E., Allegretti, M., Rantos, V., Goetz, S. K., Obarska-Kosinska, A., Zagoriy, I., Halavatyi, A., Mahamid, J., Kosinski, J. and Beck, M. (2020). Nuclear pores constrict upon energy depletion. *bioRxiv* 2020.07.30.228585. doi:10.1101/2020.07.30.228585