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Protein Transport by the Nuclear Pore Complex: Simple Biophysics of a Complex Biomachine

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ABSTRACT In eukaryotic cells, transport of molecules between the nucleus and the cytoplasm is facilitated by highly selective and efficient biomachines known as nuclear pore complexes (NPCs). The structural details of NPCs vary across species, with many of their constituent proteins exhibiting relatively low sequence conservation; yet the NPC as a whole retains its general architecture and mechanism of action in all eukaryotes from yeast to humans. This functional conservation in the absence of precise molecular conservation suggests that many aspects of the NPC transport mechanism may be understood based on general biophysical considerations. Accordingly, some aspects of NPC function have been recapitulated in artificial nanochannel mimics, even though they lack certain molecular elements of the endogenous NPC. Herein, we review biophysical aspects of NPC architecture and function and cover recent progress in the field. We also review recent advances in man-made molecular filters inspired by NPCs, and their applications in nanotechnology. We conclude the review with an outlook on outstanding questions in the field and biomedical aspects of NPC transport.

What is a nuclear pore complex?

In eukaryotic cells, genetic material is compartmentalized inside the nucleus, which is separated from the cytoplasm by the double membrane nuclear envelope (NE) (1). The two compartments can communicate through gateways embedded in the NE, known as nuclear pore complexes (NPCs), which form passageways through the NE to connect the nucleus and the cytoplasm (1). These highly selective and efficient conduits facilitate the bidirectional nucleocytoplasmic transport of a wide range of cargo molecules and regulate a diverse array of fundamental cellular processes. Below we discuss the advances in understanding NPC organization and function—from a brief historical introduction to current research. For further details and original historical articles, we refer the reader to the following reviews (2–5).

In the 1950s, electron microscopy (EM) was combined with advanced preparation techniques and applied to amphibian cells, providing the first glimpses of both the structure of the NE and the existence of NPCs (6). In the next two decades, extensive EM studies in eukaryotic cells determined the overall shape and dimensions of the NPC, and showed that it has an eightfold radial symmetry (Fig. 1). Several critical tools—including the purification of NE from rat liver, production of monoclonal antibodies against NPC components, and advanced proteomics approaches—enabled identification of the molecular constituents of the NPC in multiple species, and their approximate locations within the complex (7,8).

Currently, the NPC has been established as the cell's largest macromolecular complex, with a molecular mass ranging from ~50 MDa in yeast to ~112 MDa in vertebrates (5,9–11). It is composed of multiple copies of ~30 different proteins known as "nucleoporins" (3,4). Transmembrane nucleoporins anchor the NPC in the NE, whereas structural nucleoporins form a scaffold that shapes an hourglass passageway with a diameter ranging from 35 nm in yeast to 50 nm in vertebrates (5,9–11). The lumen of this passageway is filled with an assembly of intrinsically disordered nucleoporins harboring repeats of hydrophobic phenylalanine (F) and glycine (G) motifs—hence known as FG nucleoporins or FG nups. FG nup assembly is a critical component of the NPC transport mechanism (3,4). Recently, localizations of different types of nucleoporins within the NPC have been refined using a combination of biochemistry/molecular biology techniques, powerful biophysical approaches (e.g., (cryo)EM, x-ray crystallography, and superresolution microscopy), and integrative computational biology methods (9,10,12–16). These approaches have provided structural maps of the NPC. However, details about FG nup localizations and morphologies are still

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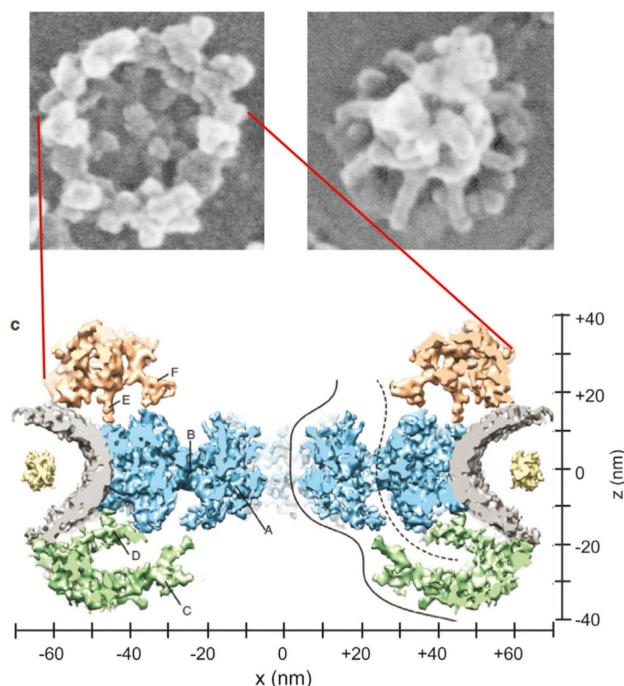

FIGURE 1 Shown here is the electron microscopy structure of the NPC. (*Upper panel*) Given here is the electron microscopy image of the cytoplasmic (*left*) and the nucleocytoplasmic (*right*) openings of the NPC imaged in *Xenopus* oocyte; adapted from Goldberg et al. (15) (Springer-Verlag with permission). (*Lower panel*) Given here is a cryo-TEM reconstruction of the NPC vertical cross-section in *Xenopus* oocyte (nuclear basket is not shown) (16). The nuclear envelope is shown in gray. Structural proteins forming the central ring are shown in blue, whereas those forming the distal rings are shown in beige and green. To see this figure in color, go online.

limited because the FG nups are disordered in nature, and few experimental methods can probe their conformations at relevant length and timescales.

In parallel with investigating NPC organization and structure, significant effort has been made to understand its function as a nucleocytoplasmic transporter. Research in the 1970s using *Xenopus* oocytes established that NPCs allow small molecules (up to ~40 kDa) to pass through without discrimination but translocate large molecules with high selectivity. Subsequent studies in the 1980s and 1990s identified specific signal sequences within cargo molecules allowing them to enter or exit the nucleus—known as nuclear localization sequences (NLS) and nuclear export sequences (NES) (2,4). Around this time, the newly developed digitonin-permeabilized cell assay in vertebrate cells (17) was used to identify transport proteins—also known as Karyopherins, Importins, Exportins, and Transportins—that bind cargo molecules via NLS/NES and ferry them through the NPC (reviewed in (4); see also (18)). It was established that this directional transport depends on GTP hydrolysis and the nucleocytoplasmic gradient of the small GTPase Ran (4,19). The RanGTP/GDP gradient is maintained by the guanine nucleotide exchange factor RanGEF (localized in the nucleus), GTPase-activating protein RanGAP1 (localized in the cytoplasm), and a soluble transport protein Nuclear Transport Factor 2 (NTF2) (3,4).

Strikingly, both the general mechanism of NPC function and the blueprint of its overall architecture are mainly conserved among species, from yeast to humans (8). In particular, NPCs in investigated species contain passageways lined with FG nups. However, molecular details exhibit considerable interspecies variation. This includes the yeast NPC being significantly smaller than the human NPC (5,9–11), and the relatively low sequence conservation of FG nups (8). Nevertheless, the degree of functional conservation is so high that: 1) transport proteins (NTF2) from one species (humans) are operational in other species (yeast) (20), and 2) chimeras containing yeast FG nups can restore selective barrier function in *Xenopus* FG nup deletion mutants (21). This suggests that many aspects of NPC function may be understood based on general biophysical considerations. The following discussion reviews such biophysical aspects of NPC function with an emphasis on protein import, which has served as a test bed for uncovering the biophysical transport mechanisms.

Simple biophysics of the NPC

What drives directional transport through the NPC?

Macroscopically, NPCs can efficiently import and export macromolecular cargoes into and out of the nucleus against the cargoes' apparent concentration gradients. From the thermodynamic standpoint, this requires energy input, which is provided by GTP hydrolysis during the transport cycle (4,22). In many other types of transporters (such as ion exchangers or proton pumps), GTP/ATP hydrolysis is directly coupled to directional substrate transport via conformational changes of the transporter (1,23). By contrast, NPCs do not appear to possess gates transitioning from open to closed states (Fig. 2). Translocation of individual transport protein-cargo complexes is not directly coupled to GTP hydrolysis (4) and occurs by thermal diffusion that is modulated by the interactions with the FG nups (24–27). Accordingly, transport protein-cargo complexes can move in both directions through the NPC, which often results in abortive translocations, as observed by single-molecule fluorescence microscopy and bulk flux measurements (28–30). Although the main function of the transport proteins is to ferry cargo, they can also translocate through the NPC without cargo. Multiple copies of cargo-bound and cargo-free transport proteins have been found in the NPC; they may play a role in shaping the NPC structure (4,28,31–35).

In the canonical import pathway (Fig. 2), transport proteins bind their cargoes in the cytoplasm and carry them through the NPC passageway. Once the transport protein-cargo complex reaches the nucleus, nuclear factor RanGTP

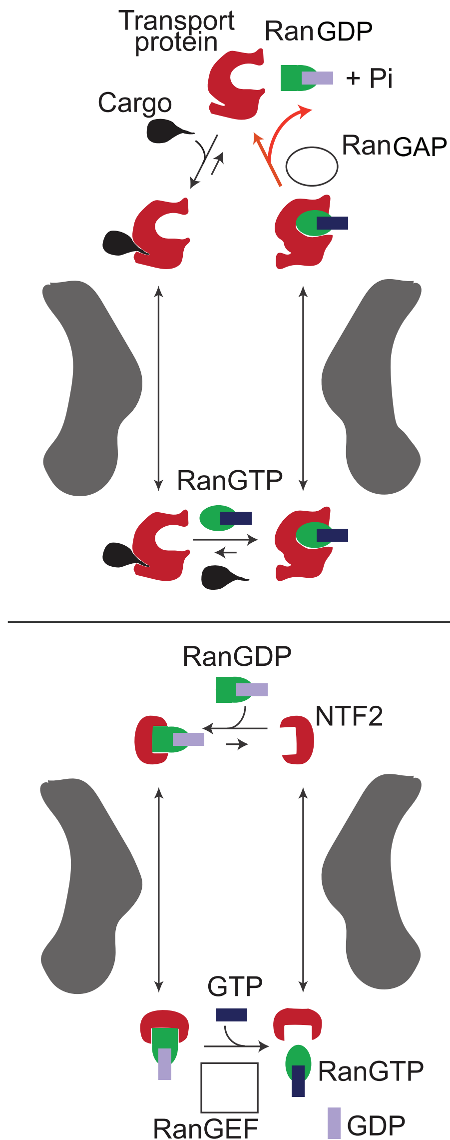

FIGURE 2 Given here are simplified schematics of the NPC operating cycle. The NPC import process comprises two interlinked cycles. The first, shown in the upper panel, uses the energy released by the hydrolysis of one GTP molecule (catalyzed by RanGAP) to import one cargo molecule into the nucleus and relies on the higher concentration of RanGTP in the nucleus relative to the cytoplasm. The red line is the only nonequilibrium step of the import cycle that depends on the metabolic energy release in the form of GTP hydrolysis. The nucleocytoplasmic RanGTP/RanGDP gradient is maintained by the second cycle, shown in the lower panel, which relies on the high concentration of RanGEF in the nucleus due to its association with chromatin. To see this figure in color, go online.

binds the transport protein; as a result, the cargo is released and sequestered in the nucleus. The resulting RanGTP-transport protein complex is capable of translocating through the NPC back into the cytoplasm, where GTP is hydrolyzed by the cytoplasmic factor RanGAP1. Energy released by the conversion of RanGTP to RanGDP leads to a conformational change that sets RanGDP free from the transport protein. The cycle is completed through the import of RanGDP back into the nucleus by a specialized transport protein NTF2. In the nucleus, RanGDP is converted to RanGTP by a chromatin-associated guanine exchange factor RanGEF. Overall, directional transport is driven by the energy released from irreversible GTP hydrolysis in the cytoplasm (22) and maintained by the localization gradient of RanGTP; RanGTP concentration is higher in the nucleus relative to the cytoplasm. In turn, this gradient relies on the constitutive localizations of RanGEF in the nucleus, RanGAP1 in the cytoplasm, and the NTF2-mediated transport of RanGDP (4). The canonical NPC protein export cycle operates in a similar fashion and is reviewed in Cautain et al. (4); see Grünwald et al. (36) for the review of mRNA export.

Why is NPC selective?

Small molecules, such as ATP and ions, freely diffuse through the NPC. In contrast, the ability of macromolecules (or similarly sized nanoparticles) to cross the NPC unaided decreases as their size increases: those $>\sim$40 kDa can efficiently translocate though the NPC only when they are bound to a transport protein or can interact directly with the FG nups (4,37,38). The NPC is able to recognize these cognate transport proteins and selectively translocate them despite the vast numbers of other macromolecules present in the cell that they could potentially interact with the NPC non-specifically. There are two main tiers of recognition: 1) the first tier is based on binding of the cargoes (directly or via adaptor proteins) to the transport proteins through a recognition sequence—NLS for import or NES for export; and 2) the second tier relies on the interactions between the transport proteins and the FG nups (3,4). The NPC is not unique in this regard—molecular recognition commonly relies on the interactions between the transported molecules and the transporter (1,23). However, unlike the specificity mechanisms of many other transporters, which are commonly conferred by strong lock-and-key interactions, the selectivity of the NPC is based on multiple weak interactions between the transport proteins and the intrinsically disordered and dynamic FG nups, whose conformations likely undergo significant thermal fluctuations (24–27). The binding of the transport proteins to the FG nups relies mostly on the hydrophobic interactions between the FG motifs and the hydrophobic pockets on the transport proteins. However, electrostatic and possibly other interactions are likely involved as well (39–41). Although the exact number of binding sites on the transport proteins is not completely established, experimental and computational estimates range from 2–6 for NTF2 to 4–10 for Importins (42,43).

Many ideas have been discussed in the last two decades regarding the mechanisms of NPC transport and selectivity (for recent reviews, see (3,4)). Although the proposed mechanisms often differ on the conformational dynamics of FG nups, most of them agree on the fundamental physical

picture of transport. The FG nups set up a permeability barrier for the inert molecules and serve as the template for the transient binding of transport proteins. Translocation of the transport protein-cargo complexes relies on Brownian diffusion through the NPC passageway, modulated by the interactions with the FG nups. This fundamental physical picture can be encapsulated in the "Honorary Enzyme" model, which was first coined in the context of cell membrane channels and transporters (23). According to this picture (Fig. 3), the binding of the transport proteins to the FG nups lowers the free energy permeability barrier, thus enabling their partitioning into and translocation through the NPC (21,39,44–46).

These ideas can be mathematically quantified by formulating the translocation of transport proteins within the NPC as 1D diffusion in an effective potential arising from the interactions with the FG nups (44,47) (Fig. 3). Similarly, by formulating the translocation as hopping between discrete sites within the NPC, one can account for molecular crowding inside the NPC (44). Mathematical modeling suggests that the binding of the transport proteins to the FG nups can enhance transport flux because binding increases the probability of a transport protein molecule to permeate and translocate through the NPC. However, this happens at the expense of increased individual translocation times, and proteins that bind the NPC very tightly can block the passageway. Consequently, only the transport proteins that bind the FG nups in a particular range of affinities are transported efficiently, providing a natural mechanism of specificity (44,47,48). Application of these ideas to single-molecule tracking experiments enables estimation of effective diffusivities of transport proteins and the local free energies within the NPC, arising from different FG nup distributions (49).

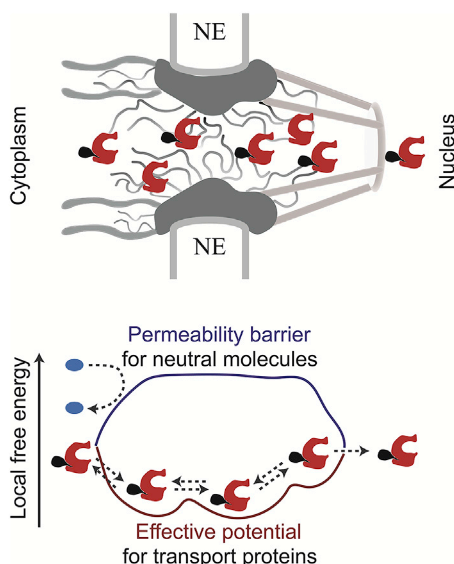

FIGURE 3 Shown here are schematics of NPC structure and function. (*Upper panel*) Schematic rendering of the NPC structure in the vertical cross-section is given. The wavy lines denote FG nups whereas the transport proteins and their cargo are shown in red and black, respectively. (*Lower panel*) Given here is the "Honorary Enzyme" picture of the NPC. The inert molecules (*blue*) encounter a high entry barrier provided by the FG nups (*dark blue*) whereas the binding of the transport proteins (*red*) to the FG nups lowers this barrier, so that they experience an attractive effective potential (*burgundy*), catalyzing their translocation through the NPC. The dashed lines indicate transport protein-cargo complex entry and diffusion through, and exit from, the NPC. To see this figure in color, go online.

These simple ideas are sufficient to explain how the NPC retains selectivity despite the nonspecific competition that is always present in the cell. Through a nonequilibrium kinetic mechanism, strongly bound transport proteins filter out nonspecific competitors—even if competitors are present in orders-of-magnitude excess (48). These ideas have been tested and verified in artificial NPC mimics (33). They highlight NPC transport as a collective process, whereby the crowding of multiple copies of the transport proteins inside the NPC plays a crucial role in its transport mechanism (discussed in more detail below) (31,33–35,48,50).

Biophysics of the NPC building blocks

Many features of NPC transport have been successfully explained by the simple models of facilitated diffusion described above. Yet comprehensive understanding requires a better picture of the spatial architecture and dynamics of assemblies formed by FG nups and transport proteins. A number of ideas regarding the conformational dynamics of these assemblies during transport have been put forward (3,35,47,51). However, even using cutting-edge experimental techniques (27,31,32,49,52), it is challenging to directly probe the collective dynamics of the FG nups inside intact NPCs on relevant time (milliseconds) and length (several nanometers) scales. Thus, much of our biophysical knowledge about FG nup and transport protein assemblies has been derived from in vitro experiments augmented by computational and theoretical modeling.

In vitro, individual disordered FG nup chains behave in many ways as conventional polymers. They exhibit a persistence length of a few Ångstroms, a radius of gyration and hydrodynamic radius of several nanometers, and wormlike chain entropic elasticity (24,51,53). As shown by atomic force microscopy (AFM) experiments, grafted assemblies of multiple FG nups have nanomechanical properties resembling those of a polymer brush: a layer of grafted polymers whose spatial conformations are stabilized by entropic repulsion between the chains, which arise from their thermal fluctuations (35). Whereas these layers are impenetrable for inert molecules, binding of transport proteins allows them to penetrate FG nup assemblies (35). Penetration of the transport proteins modulates the layer morphology and, depending on the conditions, can either swell the layer or cause its compaction (35,54,58). These results underscored the importance of entropic effects in determining the conformations of FG nup assemblies and suggest that the

permeability barrier is at least in part entropic, encapsulated in the brush model (3,35,55).

The intra- and interchain cohesiveness of the FG nups has also been implicated in shaping their spatial morphology and selective permeability of their assemblies (47,51,56). The cohesiveness predominantly arises from hydrophobic interactions between the FG motifs and can be modulated by electrostatic interactions between the charged amino acids and other interactions between the chains (3,4,39,41,51,57). The magnitude of the cohesiveness depends on the amino acid sequence of the specific FG nups (51,58). For some FG nups, notably the centrally located Nup100/116 in yeast and its functional homolog Nup98 in vertebrates, the cohesiveness is strong enough to promote formation of large aggregates in bulk solutions, observed by microscopy and light scattering (21,25,47,56). Similar to the surface-grafted assemblies mentioned above, 3D assemblies of FG nups also possess rudimentary selectivity properties. Although these assemblies are impenetrable for inert molecules, transport proteins are able to penetrate them (56). These results gave rise to the selective phase model known as the "gel" model. The model postulates that disruption of cross-linked FG motifs is energetically unfavorable for inert molecules, and their passage is thus prevented, resulting in the permeability barrier. In contrast, transport proteins that bind to FG motifs can open the cross-links, which allows their passage through the NPC (21,47).

Although gel-versus-brush models have generated considerable controversy in the past, it is becoming clear that effects from both models, as well as the spatial heterogeneities of the FG nup assembly, play a role in the NPC organization and transport mechanisms. It has also become apparent that the transport proteins play a considerable role in shaping NPC architecture (31,33–35,54,58). Despite the diversity of observed morphologies of FG nup and transport protein assemblies and their nanomechanical and permeability properties, their main features can be understood based on the statistical thermodynamics of the fundamental interplay between enthalpic and entropic effects (51,54,58,59). Cohesive interactions between the transport proteins and the FG nups favor more compact and less dynamic structures whereas the configurational entropy of the chains favors more diffuse and plastic morphologies (58). Interplay between these factors, combined with different cohesiveness and lengths of individual FG nups and the intrachain sequence heterogeneity, might allow formation of regions of different physical and nanomechanical properties that enable robust selectivity for a wide range of cargoes (32,39,49,51,57,58,67–69). Overall, good agreement with in vitro results has been obtained with physical models of FG nup and transport factor assemblies that rely on the statistical physics of polymers using coarse-grained FG nup descriptions (39,54,57–59). Such models provide a theoretical underpinning for further understanding of FG nup assemblies.

Crucially, FG nups remain highly dynamic even within dense assemblies. Both the cohesive interactions between the FG nups and the interactions between the FG nups and the transport proteins result from transient contacts that break and reform on very short time scales (24–26). This is consistent with the agile diffusion of transport proteins observed in the gel-like FG nup aggregates and the short (millisecond) transport times observed in the NPC by single-molecule fluorescence (21,29,30). These findings emphasize the fact that both FG-FG and FG-transport protein binding likely rely on multiple weak interactions. On the other hand, other in vitro (35) and in vivo (29,31) results indicate that some transport proteins are relatively immobile. Thus, the picture of transport protein mobility within FG nup assemblies still remains incomplete.

Spatial architecture of the NPC passageway

In vitro experiments have provided significant insight into the spatial morphologies of FG nup-transport protein assemblies. However, in vitro findings do not directly translate in vivo, where NPCs contain multiple copies of diverse FG nups at different locations within the NPC passageway. Cutting-edge experimental methods, such as superresolution microscopy and high-speed AFM, have started to provide insight into the internal organization and dynamics of intact NPCs (27,31,32). These methods can 1) quantify the number of transport proteins in the pore (31), 2) determine regions where they preferentially accumulate (32,52), and 3) visualize individual FG nup chain dynamics (27). The interpretation of these experiments is substantially assisted by computational modeling, which has become a major tool for describing the NPC spatial architecture, with parameterizations inferred from in vitro measurements. Most models use coarse-grained representations of the flexible FG nups as chains of monomers with appropriate physical attributes, such as charge, hydrophobicity, and degree of interaction with other monomers; the models are typically calibrated by comparison with in vitro data. These models vary in some of their assumptions: parameterization of molecular interactions, implementation of electrostatic and water-mediated interactions, and the degree of coarse-graining (26,39,40,50,54,57–61). Although they differ in some of their predictions regarding FG nup distribution in the pore, these studies have generally produced a set of robust predictions about the expected FG nup organization in the pore. In the absence of transport proteins, FG nups typically appear to form a toroidal cloud of relatively high density near the pore walls; a region of lower FG nup density lies along the pore axis (Fig. 4). Transport proteins that either translocate through or reside within the NPC can partition into the FG nup cloud and significantly change the density distribution within the pore. Some predictions of the coarse-grained models agree with the experimentally observed size dependence of inert particle transport (37,38), but a consensus has

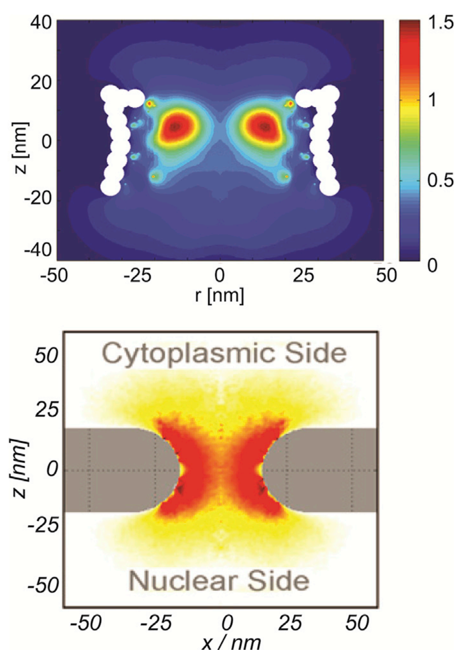

FIGURE 4 Given here are computational models of FG nup distribution within the NPC passageway. Upper and lower panels show the results of two different computational models of the FG nup distribution within the NPC passageway, reproduced from Ghavami et al. (57) and Tagliazucchi et al. (39), respectively. Each panel shows the vertical cross-section of the FG nup density distribution within the NPC passageway; red indicates higher local density. The results in the upper panel are obtained using Brownian dynamics simulations; the results in the lower panel are based on the density functional theory. Although using different parameterizations and computational techniques, the models agree in the qualitative predictions of the FG nup density distribution, which also agrees with the more coarse-grained models (50,60). To see this figure in color, go online.

not been reached regarding the spatial distribution of different types of FG nups and transport proteins, and their dynamics within the NPC.

Selective artificial nanochannels and NPC mimics

Many aspects of NPC transport can be recapitulated in vitro using artificial mimics. Because these mimics can be customized with NPC components, they allow us to test aspects of different models in a controlled environment. For example, they can be used to study the impact of pore geometry and environment as well as protein density and composition. NPC mimics are not only excellent tools for understanding basic physical principles of NPC organization and function, but also have potential to advance the design of artificial biomolecular sorters.

The forerunners to the NPC mimics were functionalized nanopores designed to separate molecules based on charge, affinity, hydrophobicity, and size (62,63). In parallel, nuclei isolated from *Xenopus* oocytes were attached to nanoporous membranes; this setup was used to investigate NPC transport by optical single-transporter recording method (64). Building upon these approaches, several devices were engineered with NPC components to mimic transport selectivity in the last decade.

Nucleocytoplasmic transport is robustly selective, which is conferred by three essential features: 1) flexible FG nups line the NPC channel and create a permeability barrier; 2) transport proteins transiently bind to the FG nups; and 3) translocating molecules are confined within the transport channel, creating crowding. Artificial nanochannel-based devices with these basic elements reproduce many transport properties of native NPCs. In one example (33), nanopores in polycarbonate membranes were functionalized with two different yeast FG nups, Nsp1 or Nup100, which are involved in forming the selectivity barrier in native NPCs. These FG nup-functionalized pores sustained the efficient passage of transport proteins and transport protein-cargo complexes, whereas the transport of control nonspecific molecules (such as bovine serum albumine) was significantly inhibited. Binding of transport proteins to FG nups was critical for transport selectivity: control pores functionalized with the neutral polymer PEG were not selective, in accord with theoretical understanding (48).

Further insights into transport at the single-molecule level were provided by another mimic, which was developed by functionalizing single SiN solid-state nanopores with vertebrate FG nups (Nup98 or Nup153) (65). Through ionic current measurements with submillisecond temporal resolution, transport proteins were shown to efficiently cross the pore, whereas nonspecific molecules of similar size (bovine serum albumine) were significantly blocked. Furthermore, the data largely supported a model wherein FG nups are densely concentrated around the pore walls, with a relatively open channel along the pore axis. The size of the opening could be changed by varying the pore radius. This agrees with the current theoretical models of the spatial distribution of the polymers in the pore (39,50,57,60).

NPC-like selectivity can be also recapitulated in entirely synthetic, nonbiological nanochannels. In one example, polycarbonate nanoporous membranes were functionalized with polyisopropyl-acrylamide (PNIPAM), whose intra- and interchain cohesiveness can be controlled by temperature (66). In this system, receptor-mediated selective diffusion was modeled through weak interactions between PNIPAM chains lining the nanochannel and soluble PNIPAM segments, which served as the carrier molecules for single-stranded DNA cargo molecules.

Current questions and outlook

Despite the rapid progress of recent years in understanding NPC structure and function, we still do not fully understand the heterogeneity of its spatial organization. Likewise, the dynamics of FG nup and transport protein assemblies is not well understood on the nanoscale level. Notably, the NPC maintains fast and selective transport in both directions whereas its passageway appears to be densely packed with

intercalated FG nups and transport proteins. According to recent studies, separating different types of import and export traffic may rely on spatial segregation of different regions within the FG meshwork in the pore (32,67–69). To investigate this aspect further, it will be important to integrate in vivo imaging tools and advanced labeling methods with the insights arising from artificial in vitro mimics and computational studies. Higher resolution imaging and microscopy techniques, such as superresolution microscopy, FRET, ultrafast AFM, and fluorescence anisotropy, will be crucial for mapping the locations, mobility, and interactions of individual FG nups and their cooperative dynamics during transport. On the computational side, interpretation of such data will require models with enhanced parameterization and improved descriptions of molecular interactions. This will allow us to further clarify the fundamental concepts and pinpoint the critical molecular factors contributing to the import and export traffic into and out of the nucleus.

As a part of this effort, it will be important to advance our understanding of the biophysical mechanisms of the transport of large cargoes through the NPC. One example is the efficient translocation of very large mRNA particles through the dense environment of the NPC and coordination of their transport with other NPC transport pathways (36,70). Understanding nuclear translocation of viruses, another class of large cargoes, is an emerging topic with important biomedical applications (71). Of paramount importance in their own right, these studies will provide additional insights into NPC organization and dynamics.

Although most of the studies of NPC selectivity have been focused on FG nups, it has been proposed that conformational changes and dynamics of the scaffold might contribute to the transport mechanism (72). Integrating structural studies using cryo-EM (5,9,16), crystallography (11–13), superresolution imaging (13), and integrative biology (10,14) with computational models of scaffold dynamics (73) may yield further insights into NPC transport selectivity and answer the ultimate questions about its transport dynamics.

In a broader biomedical context, defects in nucleocytoplasmic transport have been implicated in a number of diseases. In particular, expression levels of certain nucleoporins and transport proteins are commonly altered in cancer and other diseases (74,75). This makes nuclear trafficking of therapeutic interest; targeting NPC translocation may open new avenues toward inhibiting disease processes. Detailed understanding of the molecular underpinnings of the NPC transport can lead to novel molecular targets for rational drug development.

Given the high effectiveness of the NPC in molecular separation, new insights into the NPC transport mechanism may accelerate nanotechnological advances. We expect that future NPC mimics will incorporate multiple FG nups, include active transport through a RanGTP gradient, support bidirectional transport, and lead to new nanodevices with active molecular separation.

AUTHOR CONTRIBUTIONS

Both authors conceived the idea and wrote the manuscript.

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