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# Multiscale dynamics in nucleocytoplasmic transport

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The nuclear pore complex (NPC) has long been viewed as a point-like entry and exit channel between the nucleus and the cytoplasm. New data support a different view whereby the complex displays distinct spatial dynamics of variable duration ranging from milliseconds to events spanning the entire cell cycle. Discrete interaction sites outside the central channel become apparent, and transport regulation at these sites seems to be of greater importance than currently thought. Nuclear pore components are highly active outside the NPC or impact the fate of cargo transport away from the nuclear pore. The NPC is a highly dynamic, crowded environment — constantly loaded with cargo while providing selectivity based on unfolded proteins. Taken together, this comprises a new paradigm in how we view import/export dynamics and emphasizes the multiscale nature of NPC-mediated cellular transport.

## Addresses

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## Introduction

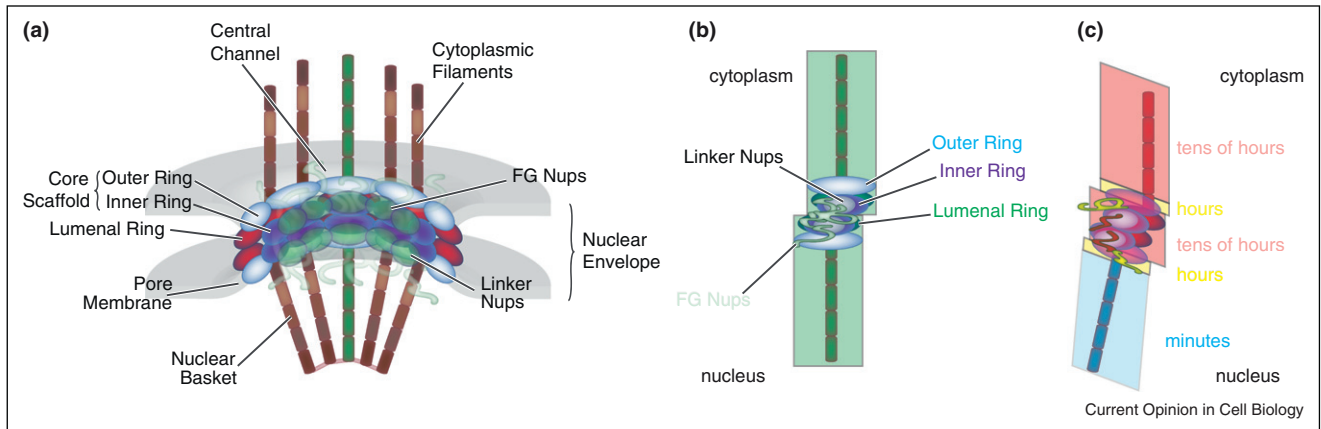
Compartmentalization is a uniform principle of cellular function in higher evolved organisms. The nuclear pore complex (NPC) mediates the exchange of matter, energy, and information between the two major compartments in eukaryotic cells, the nucleus and the cytoplasm [1]. Traffic between these two compartments is heavy; transport rates of individual NPCs have been found to be as high as 1000 transport complexes with cargo per second [2,3]. That such numbers are physiologically relevant can be ascertained by estimating the number of histones and ribosomal proteins that need to enter the nucleus every cell cycle. A cell with an average number of NPCs (~3000) requires a transport rate of approximately 150 import events per NPC per second for histones and

ribosomal proteins and two export events per NPC per second for ribosomal subunits [4]. This calculation, however, does not account for general mRNA export or proteins needed to maintain transcription, replication, or other nuclear processes. Interestingly, the transport capacity of NPCs is not rate limiting *in vivo* [5], although formation of transport complexes might very well be [6]. While small molecules below ~60 kDa can passively diffuse through the central channel without transport receptor adaptation [7], however, a smaller cutoff size of 40 kDa was also reported [8]. Many small nuclear proteins possess a NLS and use NPC-mediated transport to shuttle into the nucleus, although they are below the size limit for passive diffusion [9]. At the same time NPCs can export cargo of substantial size such as mRNAs and ribosomal subunits, which range from ~15 nm (ribosomal subunits) to ~50 nm (Balbiani Ring mRNP) [4,10<sup>\*\*</sup>,11,12]. Using gold particles bound to transport receptors, channel diameters of 24 nm were found to be readily accessible [13], and even ~30 nm Qdot transport complexes were located within the central channel [14<sup>\*\*</sup>]. Importantly, Balbiani Ring particles unfold for transport across the NPC, and the transport of flexible mRNAs is much faster than that of rigid artificial cargo of similar size [10<sup>\*\*</sup>,11,12,14<sup>\*\*</sup>]. Unlike other channels, however, the NPC does not mandatorily require unfolding of its cargo, like it is the case, for example, for transport about the membrane of the endoplasmic reticulum.

## Spatial symmetry within the NPC

NPCs are superprotein complexes with an overall diameter of ~120 nm and a channel diameter of ~50 nm and up to 90 nm in length [15–17]. Electron microscopic images of the NPC reveal a strict symmetry composed of eight spokes extending to the nuclear and cytoplasmic surfaces [16]. These spokes have three distinct regions: cytoplasmic filaments, a core structure, and the nuclear basket (Figure 1a). This, together with its shear mass (up to 120 MDa in frog oocytes and ~60 MDa in yeast), makes NPCs arguably the largest nanomachine in the eukaryotic cell. Interestingly however, the NPC is built from a relative small number of parts. The NPC is built from approximately 30 different proteins comprising six structural motives, among them the natively unfolded phenylalanine–glycine (FG) repeat domains (concentrating toward the central channel) that form the permeability barrier [18]. While the radial symmetry of the NPC spokes is clearly maintained on the surfaces, the scaffold forming the actual pore in the membrane is a four-layered ring with the base number of eight and multiples thereof being maintained (Figure 1b) [19]. A transmembrane domain anchors the NPC in the nuclear

Figure 1



NPC architecture — structural and dynamic features. **(a)** General structure of the NPC. An NPC sliced through its center is shown revealing the octameric symmetry of its major features. The ring stack in the center extends outwards into the nucleus and cytoplasm by means of filaments resulting in eight spokes transversing the nuclear membrane. There is possibly a slight shift between the upper and lower rings as shown in **(b)**. The FG-Nups, anchored in the center, form the permeability barrier. **(c)** Nucleoporins display a wide range of turnover rates within the NPC ranging from seconds to tens of hours. Core components are generally more stably associated compared to the asymmetric nucleoporins. Nup98 (yellow), the nucleoporin with the most FG repeats, has a medium association time (hours), while gp210 (see text), although part of the luminal ring shows fast turnover at the minutes time scale. Adapted from Grünwald *et al.* [40].

membrane, and three layers of rings, classified as coat, adaptor, and channel nucleoporins form the scaffold that hosts the FG repeat in its center. While the exact orientation of subcomplexes in these rings is a matter of debate (two alternative models are termed fence pole and lattice [21,22]), it is clear that the radial spoke symmetry of NPCs is at least supplemented by a rotational symmetric ring structure of the different layers in a central channel [20–22]. A large-scale modeling approach combining available structural data on NPC subcomplexes as well as biochemical interaction data suggests that the radial symmetry might be partly broken by the ring-like symmetry of nucleoporins in the central scaffold (Figure 1b) [19].

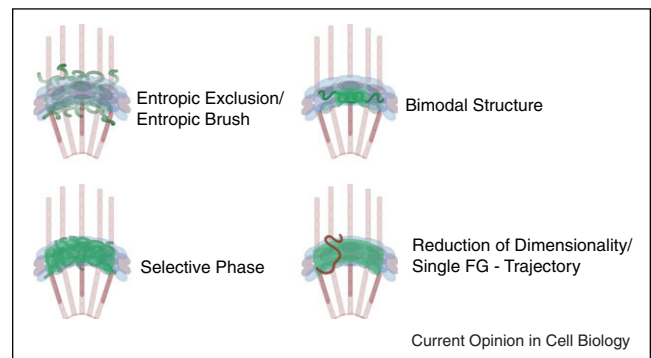
With respect to the transport of large cargo, the structure of the different ring layers in the central channel is of outstanding interest [23]. This is because ‘breathing’ of the core structure could contribute to the translocation of large cargo. This concept has fueled the discussion about the existence of specialized pores; however, experimental evidence is limited [24–27].

### The current transport models need to account for activity outside the central channel

Cargo localizes to and from the nucleus across the NPC via a ‘ticket sequence’, that is, the nuclear localization sequence (NLS) or nuclear export sequence (NES) [28]. This is mediated by a specific class of proteins and pathways (transport factors termed importins/exportins or karyopherins). Since the discovery of NLS and NES, different transport models have been discussed

(Figure 2). While the translocation step does not require energy *per se*, gradients of specific metabolic energy are maintained across the nuclear membrane [29,30]. For example, the RanGTPase gradient, by means of GTP

Figure 2



Nuclear pore complex transport models. In the entropic exclusion/entropic brush model the FG-Nups organize as a repulsing entropic barrier against nonspecific cargo. Transport receptors mediate interaction of cargo complexes with this barrier thereby facilitating transport. In the selective phase model the FG-Nups form a physical gel-like barrier by dense FG–FG interaction. Transport receptors locally ‘melt’ the gel allowing entrance and transition. In the bimodal structure model FG-Nups fold into different zones within the central channel resulting in a more gel-like center with less dense peripheries. This model predicts different spatial routes for different cargo translocation. The reduction of dimensionality model predicts that FG-Nups coat the wall of the central channel allowing for transport receptors to ‘slide’ on a two-dimensional surface across the NPC. The single trajectory hypothesis can be easily pictured as a discreet path across such a landscape but applies to all models presented.

to GDP dephosphorylation disassembles the transport complex, thereby rendering translocation irreversible for the cargo [31]. Transport receptors are recycled by RanGDP (and other factors) thereby maintaining the Ran gradient [32–34]. On the basis of the selectivity of transport in model systems like frog oocytes and digitonin permeabilized cells [35,36], combined with evidence of multiple transport pathways [37–39], models for the function of the NPC have evolved over time [1,40]. All current transport models postulate that a specific class of nucleoporins, rich in intrinsically unfolded FG-repeat domains, provide an energy landscape that reduces the energy costs of translocation, while not interfering with cargo release from the NPC [41,42]. The NPC acts as a molecular pump, which in its steady state supports a balanced flux of cargo resulting in a continuous back and forth of cargo across the NPC [43]. Net directionality of transport is the result of cargo release from the NPC resulting in an effective enrichment of concentration on one side of the NPC [29]. Unfolded nucleoporins, localized within the center of the central scaffold of the NPC form a permeability barrier sufficient for the sorting capability of the NPC [44,45]. Interestingly, most models explicitly assume a transient interaction between transport complex and FG repeats, based on modeling of stochastic transport through a narrow channel. This would be sufficient to explain exclusion and enrichment of molecules [46]. This core structure of FG repeats has been a center of attention, based on groundbreaking work on the physical properties of the filaments that form the permeability barrier located in the central channel [19,47–50]. Accordingly, the focus for differences in the current models is on the physical behavior of nucleoporins in the permeability barrier. These models (Figure 2) include: first, ‘entropic exclusion’ based on volume occupied by FG-repeat domains [41]; second, ‘entropic brush’ like collapse of the FG repeats upon interaction with transport molecules [50]; third, formation of a ‘selective gel phase’ by polymerization of FG repeats [48,49]; fourth, a ‘bimodal structure’ of FG repeats resulting in distinct transport regions within the central channel [51]; fifth, a ‘reduction of dimensionality’ of transport by sliding of transport receptors and transport complexes along the channel wall coated with collapsed FG-repeat domains [52]; and sixth, a ‘single FG-repeat trajectory’ of transport molecules along the NPC [53]. A key argument of the ‘reduction of dimensionality’ model is that within the living cell it is likely that the NPC is always loaded with transport receptors, which may or may not be loaded with cargo [52].

While the NPC has long been viewed as a static, stable installation within the nuclear membrane, it is now clear that there is stratification among the NPC composed of different binding constants and binding times [54]. Interestingly, this dynamic nature is found not only for cargo in transit but also for nucleoporins themselves (Figure 1c).

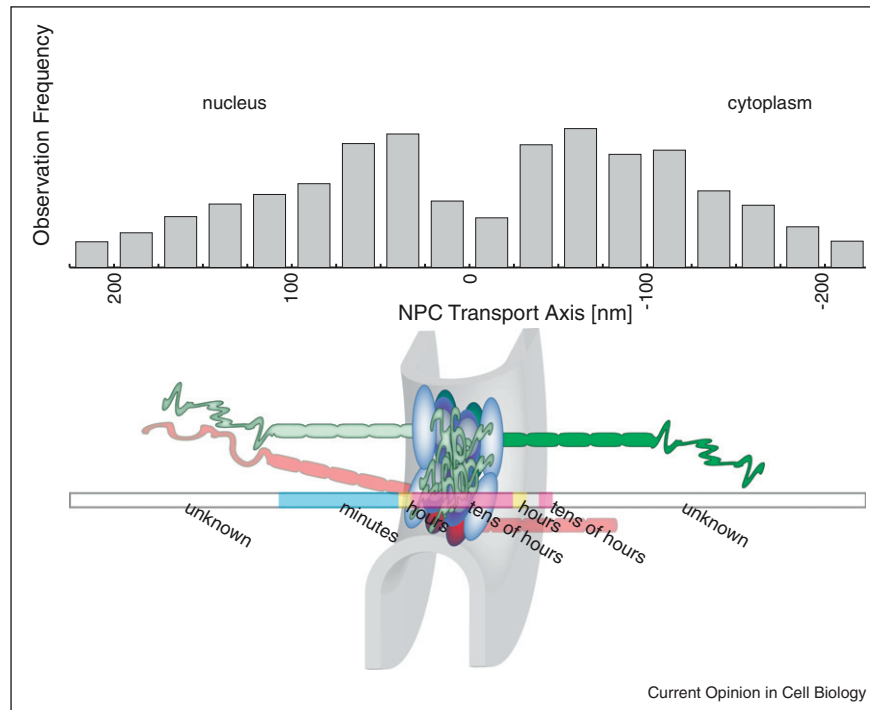
The building blocks of the NPC show turnover times covering five orders of magnitude ranging from seconds to days. In general, scaffold nucleoporins were found to be associated relatively stably (~10 hours to three days) with the NPC (with the exception of gb210, an anchoring protein with a residence time of ~4 min) while peripheral nucleoporins exhibited shorter interaction times in the range of seconds to ~10 min [54]. The interaction times of cargo with the NPC are generally short; dwell times at the NPC of import complexes were between 1 and 100 ms [5,55,56], while the dwell time of  $\beta$ -actin mRNA was between 180 ms and more than 2 s [10]. The dwell time of mRNA from an engineered dystrophin gene was 5 to ~40 min [57], while quantum dots used as cargo exhibited dwell times from 2 s to 15 min [14]. The dynamic presence of mobile nucleoporins, transport complexes, and transport factors adds substantial mass to an already crowded environment [52]. Crowding has effects on the physical properties of the FG-repeat domains [58], such as entropic changes caused for instance by ‘depletion attraction’ a change in free volume due to molecular interactions of transport complexes, receptors, and FG-repeat domains [59,60]. While current transport models are elegant and account for the available data regarding transport selectivity, newly emerging spatial information on cargo interaction sites with the NPC in the living cell makes it necessary to rethink how transport is mediated outside the core structure of the central channel.

### Differences between the dynamic profile of proteins and mRNA indicate cargo-specialization within the NPC topography

The extensions of the NPC into the nucleus and cytoplasm have been described as places of cargo modification [61,62]. When artificial, fluorescently tagged protein cargos, Qdots, and transport receptors were tracked from the cytoplasm to the nucleus, a single-peak distribution was found corresponding to the central channel (30–70 nm depending on the substrate) [5,14,56,63]. Import times ranged from a few milliseconds to tens of milliseconds leading to the interpretation that the central channel is the determinant of the translocation process [3,5,14,55,56,63,64]. These data led to the widely held notion that cargo modification is largely uncoupled from translocation with the permeability barrier located in the central channel.

A very different picture, both spatially and dynamically, emerged when export of an endogenously tagged mRNA was followed from the nucleus to the cytoplasm [10]. A bimodal distribution with peaks located at the nuclear surface of the NPC and in the cytoplasm toward the outer edge of the nuclear filaments was observed (Figure 3). These sites, docking within the nucleus and release at the cytoplasm, showed kinetics of ~100 ms for the majority of exporting mRNAs. A subpopulation of mRNAs was found at these sites for at least a few seconds. Interestingly, in

Figure 3



NPC-mediated mRNA export. mRNA docks ( $\sim 80$  ms), translocates ( $>20$  ms), and releases ( $\sim 80$  ms) in a three-step kinetic process across the NPC. The gray bars indicate the binding site distribution of exporting mRNA along the NPC axis and are combined with the turnover rates shown in Figure 1. The roles of individual nucleoporins on nuclear and cytoplasmic processes away from the NPC add to a multiscale space and time topography of NPC function. Addition of more data on import cargos, genetic mutants, and disease-related transport defects will open a new view on many subcellular processes in the future.

both cases the transit through the central channel was very fast ( $\sim 5$ – $20$  ms) and comparable to the times for protein import. Using a modeling approach, a bimodal distribution of cargo was also predicted for import, but the separation between the peaks was found to be narrower than the experimental data on mRNA export [53]. The existence of translocation-limiting binding sites outside of the central channel for mRNA exports extends the transport process well beyond the permeability barrier in the central channel. A time-consuming release step is well in agreement with speculation that mRNA needs to be loaded with multiple transport factors that need to be removed to prevent re-import after export from the nucleus [65]. Structure data showing the interaction between DBP5 (DEAD box helicase active in RNA export), Nup214 (cytoplasmic nucleoporin), and mRNA are consistent with the low number of import events found for  $\beta$ -actin mRNA [10,66,67]. A common picture is emerging that the release of DBP5 from the mRNA functions in an ATP-dependent manner in conjunction with Gle1 (mRNA export factor) and Nup214 as a de facto ratchet for mRNA export [68,69,70]. The bimodal binding site distribution contrasts with the interaction of

transport receptors with the NPC, which has been found to center closely on the central channel of the NPC. A symmetric binding site distribution is presumed based on 2D imaging of translocation of transport receptors through NPC in the equatorial plane of the nucleus and 3D interpolation of tracing data [5,71]. Interestingly, an EM study based on ultrafast freezing of cells showed a distinct spatial distribution of cargo within the central channel [72]. Deletion of nucleoporin sections and labeling of different transport markers (either primary transport receptors or cofactors like DBP5 or Gle1) were used to determine if cargo traveled closer to the rim or the center of the central channel. These data strongly supported spatially separate transport pathways within the NPC; mRNA is likely to travel more centrally while smaller cargo might travel more toward the periphery of the central channel [72]. A similar prediction was made based on the biophysical interpretation of hydrodynamic diameters of nucleoporins contributing to the permeability barrier [51].

Understanding of transport regulation will rely on how the extended binding site distribution along the transport axis of the NPC and the spatial organization of transport zones

orthogonal to the transport axis interact for different cargo types. This is especially interesting as the formation of the receptor–cargo complex and access of the complex to the central channel are two rate-limiting steps for the transport process.

### The life of nucleoporins away from the NPC

Multiple nucleoporins have been found to have additional functions outside the NPC. The nucleoporins Sec13, Nup88 (which is overexpressed in tumors [73]), and Nup98 were shown to localize to chromatin in the absence of NPCs in *Drosophila*; Nup98 and Sec13 were also identified as transcription factors [74]. Among ciliates (a unicellular organism) Nup98 distinguishes the transcriptionally active macronucleus from the inactive micronucleus, and is responsible for differences in import specificity of the NPC in the two nuclei [75]. Also, in *Drosophila*, Nup153 was found to be involved in transcription [76]. Nup98 together with Nup50 and Nup62 were shown to impact gene expression in S2 cells by acting as transcription factors [77]. In yeast the TREX2 complex links transcription of at least a subset of genes to the NPC [78]. Various other aspects of the relationship of nucleoporins to the nuclear structure of the genome have recently been reviewed (cell differentiation [79]; regulatory functions [79]; cancer and nuclear structure [80]). Tpr, the major constituent of the nuclear basket, has been reported to function not only as a docking site in import and export, but also to be required for the formation of heterochromatin-free areas close to NPCs, which are thought to be needed to regulate accessibility of NPCs to cargo [81<sup>••</sup>,82]. The interaction of nuclear Nup60 with localized mRNA is, however, necessary to maintain the proper delivery of the mRNA (shown for ASH1 and IST2 mRNA) to the bud of the yeast cell, placing nucleoporins not only in a context of nuclear interactions but also extending their reign into the cytoplasm [83].

### Summary

Trafficking of cargo between nucleus and cytoplasm mediated by the NPC spans time scales ranging from milliseconds to the entire duration of the cell cycle and beyond, length scales from 50 nm for the permeability barrier to multiple 100 nm for the docking and release sites, and localizations from an individual pore at the transcription site and the cytoplasm covering micrometer distances (Figure 3). These features make the NPC a multiscale player on the cellular level. The ability to follow individual proteins and RNA complexes in real time in the living cell, and superimposing spectrally resolved single molecule signals with high resolution, has opened a window on the functional details of cargo translocation. Extending this technology toward single mRNA imaging in yeast promises to reveal further insights into the mechanisms underlying nucleocytoplasmic transport.

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