Insights into the gate of the nuclear pore complex

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ABSTRACT
Nuclear pore complexes (NPCs) serve as the gateway of the cell nucleus. These macromolecular assemblies form selective aqueous translocation channels permitting the free diffusion of small molecules, as well as receptor-mediated transport of large cargoes. Over the past decade, major progress has been made in both the structural determination of individual nucleoporins and subcomplexes by X-ray crystallography and in the structural analysis of the entire NPC by cryo-electron tomography (cryo-ET). The metazoan NPC structure from Xenopus laevis oocytes was recently resolved up to 20 Å by combining cryo-ET with advanced image processing techniques, revealing for the first time the architecture of the central channel. Here, we discuss the structure of the Xenopus laevis NPC and consider future perspectives that will eventually allow reconstructing the scaffold and gate of the NPC with higher resolution and identifying its transport-relevant regions. This will eventually allow us to describe the structure of the NPC ‘in action’.

KEYWORDS
Cryo-electron tomography; FG-repeat domains; nuclear pore complex; nucleoporins; oocytes; Xenopus laevis

Introduction

The NPC enables the exchange of molecules between the cytoplasm and the nucleus. It consists of multiple copies of ~30 different proteins termed nucleoporins (Nups). Some of these Nups form scaffold elements while others occupy the central channel and constitute the permeability barrier of the pore (FG-repeat Nups). Based on the pore’s eightfold symmetry, each of the Nups is thought to be present in multiples of 8 copies.1-4 It has been shown that NPCs are composed of a scaffold consisting of 3 ring moieties: the nucleoplasmic ring (NPR), the cytoplasmic ring (CPR), and the spoke ring (SR). The SR is sandwiched between the NPR and the CPR and harbors the central channel of the NPC, which is the active part in selective transport of the pore complex.

Despite intensive research on NPCs, it is not fully understood how the NPC enables the bidirectional transport of cargo complexes at very high rates, while solidly keeping up the barrier for larger proteins without a nuclear localization sequence.5,6 Several models have been proposed describing how transport through the NPC could work, but the precise transport mechanism is still controversial.

Different approaches are currently utilized to resolve the mechanism of transport through the NPC, including cutting-edge fluorescence-based approaches7-12 and biophysical analyses of FG-repeat Nups.13,14 However, structural analysis of the FG-repeat domains and the FG-rich network that constitute the NPC gate is challenging due to the complexity and the flexibility of these elements. Nevertheless, a major step forward in the understanding of the NPC scaffold has been achieved by determining the structure of individual Nups as well as subcomplexes by X-ray crystallography.15-17 In addition, the structural analysis of the entire NPC by cryo-ET in conjunction with 3D averaging provided new insights into the scaffold of the human18,19 and Xenopus laevis NPC20 at unprecedented resolutions. In the latter study, the central channel of the NPC was reconstructed at different levels of transport activity, leading to a better understanding of the structural basis of transport through the pore.

Structural investigation of intact NPCs

Structural analysis of the NPC is challenging due to its sheer size and the dynamic nature of its components. Therefore, extensive purification procedures may lead...
to the loss of some of its constituents and may also induce conformational changes in some of its substructures. NPCs from different species were previously studied by cryo-ET in intact cells, or using fast and stepwise purification strategies, and displayed mostly the pore’s scaffold. Studying the scaffold at the highest possible resolution is of great importance for understanding the NPC’s function and assembly.

For obtaining a high resolution structure large data-sets of NPCs have to be acquired and, in a first step, the entire NPC volumes are averaged. In this initial 3D averaging an eightfold rotational symmetry is applied, resulting in a structure with a low resolution of up to 6 nm, due to the deviations of the protomers from their putative symmetric positions. By averaging the structure of individual protomers that were computationally dissected from tomograms (in silico) the resolution of the NPC structure can be increased extensively. This procedure was previously applied in order to resolve the NPC scaffold, although it relies on the assumption that all protomers are identical, which awaits further experimental validation.

The structure of the NPC from *Xenopus laevis* presented by Eibauer et al. was obtained by refining the above-described approach, which led to a major increase in the final resolution and provided new insight into the pore’s architecture (Fig. 1). First, we demonstrated that the cytoplasmic ring and the nucleoplasmic ring structurally differ from one another (Fig. 2B and 3A). This was possible because C2 symmetry was not applied in the averaging process, meaning that the cytoplasmic and the nuclear half of the pore were not considered identical. This is in line with the fact that certain Nups only reside at the cytoplasmic ring, while others reside only at the nucleoplasmic face of the pore, e.g. Nup358, Tpr, and Nup50. Despite reaching a resolution that allowed us to identify distinct structural motifs (e.g., β-propellers), we applied a modest interpretation of the structure in order to avoid over-interpretation and false identification of individual proteins. We believe that a map with a resolution better than 10 Å is required to be able to accurately map individual Nups within the NPC’s structure.

The second major progress in the structural analysis of the *Xenopus laevis* NPC was the determination of the central channel of the NPC. Previous structures from cryo-ET studies of the NPC usually excluded the central channel from averaging because of its structural heterogeneity and the flexibility of its components that reduced the resolution of the overall NPC structure. Although the inner most ~17 nm were still masked due to technical reasons (Fig. 1, dotted circle), a larger portion of the central channel, including the FG-repeat Nups, was structurally resolved in the current model. In contrast to the prevalent view that there is no higher structural order within the central channel, the refined model showed filamentous protrusions extending into the central channel of the NPC (Fig. 1). This indicates that FG-repeat Nups, despite their intrinsically disordered nature, constitute a highly structured framework. It is worth mentioning that nuclear transport receptors (NTRs) were likely to stay bound to the FG-repeat meshwork since native NPCs (i.e. ‘NPCs in action’) were analyzed. It is possible that the NTR-FG-repeat-Nup interactions increased the electron density of the structures within the central channel, thereby strengthening the signal and helping to visualize the FG-repeat network within the final averaged structure.

**Arresting pores at different states**

In order to learn more about conformational and structural changes occurring within the NPC’s scaffold
and the architecture of its gate, 2 different states of the NPC were studied: the active-transport state (wild type-NPC, Fig. 2A, left) and a state in which mRNA transcription was blocked using actinomycin D (ActD-NPC, Fig. 2A, right). Interestingly, ActD-NPCs are still transport competent despite the significant decrease in the levels of RNA being transcribed and transported into the cytoplasm, and therefore lowered

![Figure 2](image-url)

**Figure 2.** Structural differences of the NPC at different transport states. (A) Schematic representation of cargo being transported through the NPC in the wildtype state (wild type-NPC, left) and after actinomycin D treatment (ActD-NPC, right). Ribosomal subunits are depicted in green colors, RNA in blue colors, and proteins in red colors. (B and C) A view of the 25-nm thick central nucleocytoplasmic section of the wild type-NPC (left) and the ActD-NPC (right) demonstrates the structural differences of the 2 states of the NPC. (B) The local resolution of the structures is depicted by surface coloring. (C) The local cross-resolution values of the structures are visualized by surface coloring and reveal regions where structural changes occur due to altered transport activity (red color). (B and C) Resolution values are given by the color key. The figure was modified from.20
synthesis of novel proteins potentially including NPC components. As shown in Fig. 2B, both structures were resolved to a similar resolution enabling us to identify non-flexible scaffold parts of the NPC as well as conformational changes in regions that are likely to be involved in cargo transport. While the structure of the cytoplasmic ring is very similar in both NPCs, at least at the current resolution, major structural changes are seen in the organization of the nuclear ring as well as within the central channel (Fig. 2C). This indicates a very high level of structural flexibility and/or variability in the protein composition within these regions.

Several lines of evidence indicate that the central channel of the pore is highly flexible and can undergo structural changes to allow different cargo complexes to be transported through it. It was shown that even complexes with a diameter of ~39 nm could be translocated through the central channel of the NPC. No empty space as large as this diameter was ever detected within the pore, nevertheless large assemblies such as pre-ribosomal complexes are able to traverse the barrier. This was taken as an indirect proof of structural rearrangements in the central pore of the NPC. The present study provides a direct proof for such structural changes occurring within the central channel.

**Nuclear transport mechanism**

Several models have been proposed to describe the passage of receptor-cargo molecules through the pore and their interactions with the FG-network. Some of these models rely on studies performed using whole FG-repeat Nups or parts of FG-repeat Nups and the investigation of their biochemical or biophysical properties. Görlich and coworkers demonstrated that several FG-repeat Nups are capable of forming a homogenous FG-hydrogel through hydrophobic interactions. Interestingly, these hydrogels displayed selective properties reminiscent of the gating behavior of NPCs. They concluded that the interactions with NTRs enabled local dissolving of hydrophobic interactions between the FG-repeat Nups and cargo passage through the pore. In another study, Lim, Aebi, and coworkers described that in vitro purified FG-repeat domains of the human Nup153 collapse into a compact molecular conformation upon NTR binding. They suggested that FG-repeat Nups act as a selective entropic barrier for large molecules. Yamada and coworkers investigated the structure and properties of all FG-repeat Nups and combined both of the afore mentioned concepts in a single model. This “forest” model of NPC architecture proposes that some FG-repeat Nups adopt a globular, collapsed-coil configuration, while others have more dynamic, extended-coil conformations, with a third group that features both types of structures. These Nups are arranged in a non-random distribution along the pore and constitute 2 separate zones of traffic across the NPC: one zone in the interior of the central transporter structure, which may be one homogeneous meshwork, as proposed for a hydrogel, and a second zone with Nups that collapse upon transport receptor binding.

How can a static model generated by averaging many NPCs help understand the mode of nuclear transport, or support one model over another? It is the comparison of the 2 structures at different transport states that may provide some hints into the dynamic nature of nuclear transport (Fig. 2). As mentioned above, structural changes were observed between those 2 states especially at the NPR, with filamentous structures protruding more into the central channel upon reduction of transport events. This observation indeed indicates that Nups in this region function as a repulsive entropic barrier at the nuclear plasmic NPC entrance.

**Central and peripheral channels**

The analysis of the densities within the central channel of the NPC reveals an organized framework of proteins arranged into a ring-like assembly. These central densities are attached to the scaffold of the NPC by a porous interface located at a distance of ~23 nm from the center of the channel. Thus, cargo complexes may translocate through the NPC barrier along 2 putative paths (Fig. 3, orange and purple tubes): through the central region of the NPC and along peripheral channels located at a radius of ~23 nm. Interestingly, independent studies using single-point edge excitation sub-diffraction (SPEED) microscopy showed that importin β1-cargo complexes were predominantly located at the periphery of the central channel, ~23 nm from its center, while small cargos may be transported through the middle of the central channel. FG-repeat domains of the central channel Nups are likely to be heterogeneously organized within the
Moreover, it was suggested that the innermost part of the central channel hosts a lower density of FG-repeat domains or that mediated transport events are even absent in this region. The densities that were detected using cryo-ET within the central channel are heterogeneous in their distribution (Fig. 3). These structures undergo substantial structural changes when the transcription is reduced. However, the innermost part of the central channel was still masked in the current NPC structural map (Fig. 1, dotted circle). Future cryo-ET studies on the NPC will therefore focus on analyzing this region under different conditions, such as in the wild type state, in a reduced-transport state as well as in the absence of transport events. We hope that with these studies we will be able to decipher the organization of proteins and their FG-repeat domains, as well as the localization of cargo and NTRs within the central channel.

Conclusions and Outlook

The complexity of the NPC and its gigantic size requires the application of hybrid experimental techniques, involving computational, biochemical, and structural approaches. Cryo-ET is likely to play an important role in this field, especially due to major ongoing advancements in the cryo-EM field. Novel developments, e.g. voltage phase plates and direct electron detectors, are expected to increase the resolution of NPC structural maps and along with this, help refine our understanding of the nuclear transport processes. The analysis described here was still conducted using CCD-type of detectors that are limited in retrieving high resolution information. It is therefore likely that similar analyses using direct-electron detectors will improve the structural determination of the NPC. This will hopefully result in a structure in the sub-nanometer resolution range that is needed for providing a complete map of the protein localization and organization within the NPC.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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