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Perspective on the metazoan nuclear pore complex

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Fusing the inner and outer membranes of the nucleus, the nuclear pore complex (NPC) forms a selective portal which serves as the sole gateway of the nucleus. These aqueous translocation channels allow free diffusion of small molecules and ions, as well as receptor-mediated transport of large macromolecules. Over the last several years major progress has been made in both structural determination of individual nucleopurins (Nups) and their complexes by X-ray crystallography and in structural analysis of the entire assembly by means of cryo-electron tomography. By combining cryo-electron tomography with advanced image processing techniques, the metazoan NPC structure from Xenopus oocytes was resolved to medium resolution, revealing novel details. Here, we discuss new features of the Xenopus NPC and consider future perspectives that will eventually allow resolution of the structure and function of NPCs with high accuracy.

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Introduction

Macromolecular transport across the nuclear envelope (NE) is mediated by the nuclear pore complex (NPC). These channels fuse the outer nuclear membrane with the inner nuclear membrane to form an aqueous translocation channel that allows free passage of small molecules and ions, while mediating transport of larger molecules in an energy-dependent fashion.[1] The NPC is composed of ~30 different proteins, termed nucleoporins (Nups), suggested as appearing in multiples of eights,[2-4] due to the pseudo-eightfold rotational symmetric structure observed. Thus, the NPC is composed of eight protomers, each with a similar composition of Nups. The complex composition of the NPC, its sheer size, i.e., over 100 MDa of the vertebrate NPC[5] and the high degree of dynamics it shows, imposes a major challenge for structural determination.

With technical advances in automated cryo-electron microscopy and 3D averaging procedures,[6] cryo-electron tomography (Cryo-ET) has become the method of choice for dissecting the structure of the intact NPC.[7,8] In particular, Cryo-ET was used to reconstruct the canonical components of the entire NPC structure from *Xenopus laevis* and *Dictyostelium discoideum* to ~6 nm resolution.[9,10] Such resolution was achieved by using symmetry-independent averaging procedures to compensate for deviation of protomers from perfect eight-fold rotational symmetry. These deviations are due to NPC structural plasticity and the different residence time of some Nups, as compared to others.[11]

Architecture, Composition and Dynamics

The conserved structure of the NPC is revealed in its tripartite architecture, consisting of central scaffold ring (SR) complex flanked by the cytoplasmic and nucleoplasmic rings (CR & NR). From the CR emanate eight cytoplasmic filaments which are thought to interact with the cargo passing through the NPC. In the same manner, eight filaments are connected to the NR which join into a distal ring to form the nuclear basket (see **Fig. 1C**). On the inner nuclear membrane (INM) side, the NPC is found in close proximity to the nuclear lamina and is thought to interact with it. The NPC structure is generally conserved from yeast to man,[12]

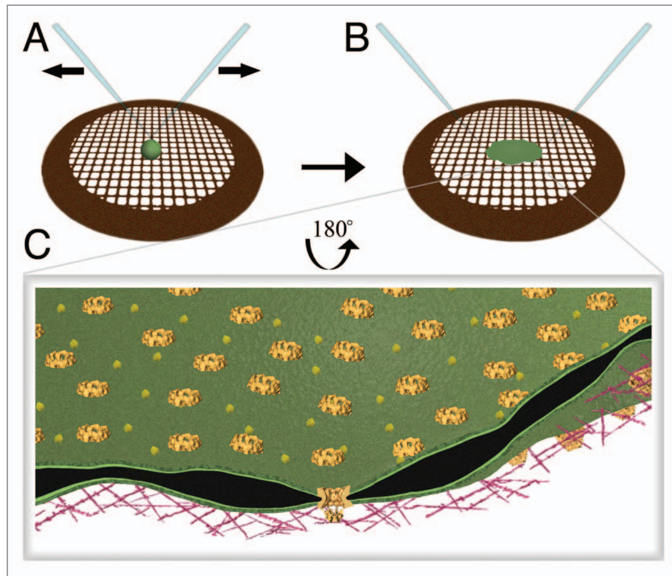

Figure 1. Schematic representation of a preparation of an isolated Xenopus oocyte nucleus for Cryo-ET. (A) The nucleus is isolated manually and being placed on an EM grid, (B) sliced across using fine glass needles and then washed. (C) Upon magnification and rotation of a specific area of the NE, the dense population of NPCs scattered across the NE can be seen. Ribosomes decorate the outer nuclear membrane, while the nuclear lamina can be seen attached to the inner nuclear membrane and is found in close proximity to the nuclear baskets.

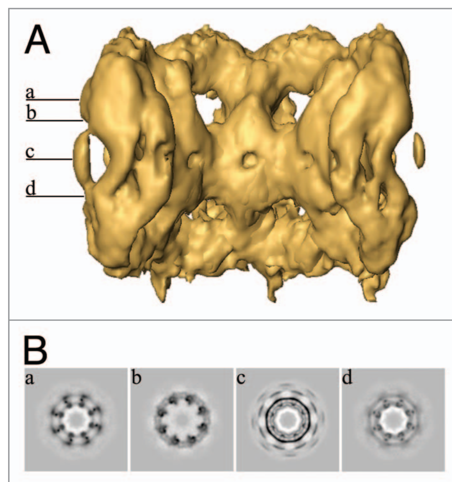

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Figure 2. Architecture of the Xenopus NPC. (A) A cut-away view of the Xenopus NPC. (B) Ten-nm-thick *x-y* slices along the z axis of the NPC structure at positions shown schematically in (A). The sections computed at the levels indicated in (A) were: (a) The outer nuclear membrane, (b) the peripheral channels, (c) the scaffold ring complex in which eight high-density connections of the two concentric rings can be seen and (d) the inner nuclear membrane.

yet there are differences. The structure of the *Xenopus laevis* NPC is taller than that of the *Dictyostelium discoideum*, estimated to be ~60 nm and ~95 nm, respectively. However, the narrowest diameter of the NPC in both species is localized to the center of the channel and is found to be ~50 nm, giving the pore its hourglass shape. The outer diameter of the NPC is ~125 nm, including the membrane-bound and luminal components.

The Metazoan NPC

Nuclear transport within *Xenopus laevis* oocytes is well characterized, both biochemically and structurally. The *Xenopus laevis* NPC exhibits high homology in terms of protein component sequence to the mammalian NPC, making it ideal for understanding NPC function. Additionally, the number of NPCs per cell varies greatly, depending on cell type and species. The number of NPCs in the mature Xenopus oocyte is roughly ~60 NPCs/μm^2 and ~5 x 10^7 NPCs/nucleus, in total.[13,14] Such a dense population of NPCs is presumably due to the gene expression needed for proliferative activity, thus making Xenopus oocyte an excellent system for structural analysis of NPCs by Cryo-ET.[15,16]

Nuclei thickness hampers the implementing of Cryo-ET techniques on intact nuclei of many cell types, including *Xenopus laevis* oocyte nuclei exhibiting a diameter of ~0.2 mm. This large size renders them however amenable to manual manipulation. By extracting the nucleus from the oocyte, placing it on an EM grid, manually opening the nucleus and washing out the genetic material[17] (**Fig. 1**), it is possible to obtain thin samples in which the NE is spread across the EM grid with the NPCs are intact, without the use of detergents or chemicals (**Fig. 1C**). However, since the NPCs within the spread NE exhibit similar orientations, the sample cannot be viewed from all possible directions. As a result, the averaged structure of the NPC suffers from ~10–20% artificial elongation along the nucleocytoplasmic axis.

In the study by Frenkiel et al.[10] we applied symmetry-independent analysis of the NPC protomers.[9] Thus, the resolution of the Xenopus NPC was improved to about 6 nm in resolution, revealing novel features of the Xenopus NPC and offering better insight into the connection of the NPC to the NE (**Fig. 2**). This study revealed densities in the envelope which presumably span the lumen and connect the INM with the ONM (**Fig. 2Ac**). The overall organization of the NPC presents an hourglass shape, maximizing the docking volume at the cytoplasmic phase of the pore where import would be initiated. This architecture is apparent in the cut-away view where careful investigation of the reconstructed volume indicating structural differences between the cytoplasmic

and the nuclear sides of the NPC (**Fig. 2Ba and d**), suggesting an asymmetric organization along the nucleocytoplasmic axis. Such differences may be attributed to the different composition of Nups in the cytoplasmic and nuclear aspects of the pore complex.

The central SR exhibit a concentric ring architecture, with the rings being fused at the center of the protomer, within a high density region. A lower density region between these rings was revealed, which may serve as a 'mass buffer zone', allowing plastic deformation and structural changes of the main channel. Although such a concentric ring architecture was previously suggested,[18] we have only detected two of the four predicted rings. It is conceivable that visualization of additional ring structures will require higher resolution.

As for the *D. discoideum* NPC, symmetry-independent averaging applied to spread envelopes indicated deviations of individual protomer from an eight-fold symmetrical structure. This emphasizes the plasticity properties of the structure and the possibility of individual protomers moving with respect to each other, within individual pore. Interestingly, coarse-grained structural model based analysis conducted on the yeast NPC had suggested similar deviations as those later found experimentally, indicating that such flexibility is a physical characteristic of such a complex structure.[19] Additional variability in protomer structure may be the result of variations in protein composition within individual asymmetric units, due to the high flexibility and short residence time of some Nups.[11] Such structural changes may accompany the different functional states of translocation. It is expected that future developments in 3D classification will eventually enable us to distinguish between fine structural changes in individual protomers and, therefore, shed light on structural variations within the nuclear pore complex.

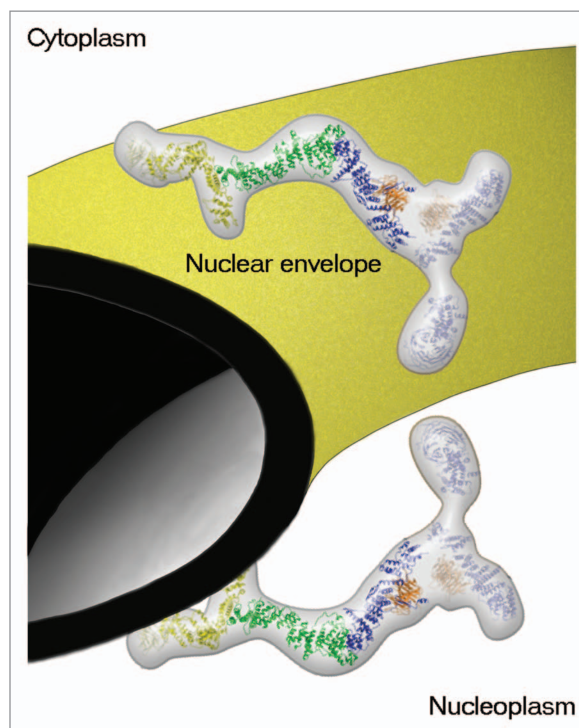

Figure 3. Composite atomic model of the heptameric Y complex. The long arm of the Y complex model is composed from the crystal structures of Nup145C (blue), Sec13 (orange), Nup84 (green) and Nup133 (yellow). The half transparent shorter arms components, Nup120 (blue) Nup85-Seh1 (blue-orange) and the N-terminal propeller of Nup133 (yellow), are tentatively oriented in relative position to the other Nups composing the structure. The position of the Y complex, relative to the nuclear envelope, is based on analogy to the Sec13-Sec31 edge element in the COPII coat and is not predicted to directly contact the nuclear envelope. Adapted from Brohawn et al.[21]

Outlook

To unravel the exact mode of action of the NPC, a high resolution model is needed. However, since crystallographic analysis of the entire structure is unforeseen, due to the sheer size and dynamic nature of the complex, the use of integrative approaches[4] relying on information obtained from a large number of disciplines will eventually lead to a high degree of understanding of these multi-protein complexes. In particular, by combining X-ray crystallography of individual Nups, high-resolution electron microscopy of sub-complexes and a better resolved structure of the entire NPC by means of Cryo-ET, it will be possible to generate 3D models of the pore at pseudo-atomic resolution to yield a high degree of understanding of these essential protein assemblies. A model of the heptameric yeast Nup84 Y-shaped complex[20] in which the atomic structures of individual components were fitted onto the nuclear membrane[21] is shown in **Figure 3**. A better resolved, intact NPC structure would eventually confirm the position and orientation of this Y-shaped complex. Thus, an increase in the number of structural analyses providing substantial steps forward in understanding the complex structure of nuclear pores is to be expected in the near future.

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