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Structural Analysis of a Metazoan Nuclear Pore Complex Reveals a Fused Concentric Ring Architecture

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The sole gateway for molecular exchange between the cytoplasm and the nucleus is the nuclear pore complex (NPC). This large supramolecular assembly mediates transport of cargo into and out of the nucleus and fuse the inner and outer nuclear membranes to form an aqueous translocation channel. The NPC is composed of eight proteinaceous asymmetric units forming a pseudo-8-fold symmetric passage. Due to its shear size, complexity, and plastic nature, dissecting the high-resolution three-dimensional structure of the NPC in its hydrated state is a formidable challenge. Toward this goal, we applied cryo-electron tomography to spread nuclear envelopes from *Xenopus* oocytes. To compensate for perturbations of the 8-fold symmetry of individual NPCs, we performed symmetry-independent asymmetric unit averaging of three-dimensional tomographic NPC volumes to eventually yield a refined model at 6.4 nm resolution. This approach revealed novel structural features, particularly in the spoke–ring complex and luminal domains. Fused concentric ring architecture of the spoke–ring complex was found along the translocation channel. Additionally, a comparison of the refined *Xenopus* model to that of its *Dictyostelium* homologue yielded similar pore diameters at the level of the three canonical rings, although the *Xenopus* NPC was found to be 30% taller than the *Dictyostelium* pore. This discrepancy is attributed primarily to the relatively low homology and different organization of some nucleoporins in the *Dictyostelium* genome as compared to that of vertebrates. Nevertheless, the experimental conditions impose a preferred axial orientation of the NPCs within spread *Xenopus* oocyte nuclear envelopes. This may at least in part explain the increased height of the reconstructed vertebrate NPCs compared to those obtained from tomographic reconstruction of intact *Dictyostelium* nuclei.

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Introduction

Transport of macromolecules across the double-membrane nuclear envelope (NE) is mediated by nuclear pore complexes (NPCs). NPCs allow the free diffusion of ions and small molecules across the NE and, at the same time, facilitate receptor-mediated nucleocytoplasmic transport of proteins, RNAs, and ribonucleoprotein particles.[1,2] The consensus model describing the overall three-dimensional (3D) structure of the NPC, which is based on previous electron microscopy (EM) studies,[3–6] reveals a distinct tripartite architecture. The NPC spoke–ring (SR) complex exhibits 8-fold rotational symmetry and forms the main translocation channel through which bidirec-

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Abbreviations used: NPC, nuclear pore complex; NE, nuclear envelope; 3D, three-dimensional; EM, electron microscopy; SR, spoke–ring; CR, cytoplasmic ring; NR, nuclear ring; NM, nuclear membrane; INM, inner nuclear membrane; ONM, outer nuclear membrane; Nup, nucleoporin; FG-repeat domain, phenylalanine–glycine-rich nucleoporin repeat domain; cryo-ET, cryo-electron tomography; ALD, additional luminal density; LSB, low-salt buffer.

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tional transport occurs.[7,8] The SR complex is sandwiched between a cytoplasmic ring (CR) and a nuclear ring (NR) moiety. Eight flexible filaments, thought to interact with cargo via bound transport receptors, are anchored to the CR. Similarly, eight filaments emanate from the NR and are joined by a distal ring to yield a distinct basket-like structure protruding into the nucleoplasm from the inner nuclear membrane (INM). The molecular mass of the vertebrate NPC has been measured to be ~125 MDa,[9] making it one of the largest and most complex cellular nano-machines and, hence, a major challenge to structural biologists.

While small ions and molecules traverse the NPC by simple diffusion, most macromolecular cargos harbor specific nuclear localization signals or nuclear export signals that are recognized by transport receptors, which, in turn, mediate cargo passage through the NPC. Such receptors, referred to as karyopherins, chaperone cargo during transport across the NPC by means of hydrophobic interactions with phenylalanine–glycine-rich nucleoporin (Nup) repeat domains (FG-repeat domains). Although the exact mode of translocation has remained controversial, recent experiments showed that the FG-repeat domains form a polymer brush-like barrier that is traversed by the cargo upon transient and local "melting" by the transport receptors complexed to the cargo.[10]

Despite its size, the NPC was shown to comprise only 30 different Nups, present in multiple copies of eight.[11,12] The vertebrate NPC is mainly anchored in the nuclear membrane (NM) by three transmembrane Nups: POM121, POM210, and NDC1. The Nups share common structural motifs, namely, α-helical domains (e.g., Nup205, Nup188, Nup93, and Nup155), coiled-coil segments (e.g., Nup58/45 and TPR), and β-propellers (e.g., Nup37, Nup43, SEH1, and SEC13).[13] About 30% of the Nups harbor natively unstructured FG-repeat domains (called FG-Nups) that are assumed to surround or span the central translocation channel.[12,14] While these FG-repeat domains play an important role in attenuating passive and accelerating cargo-mediated translocation (i.e., "selective gating"; cf., Ref. 15), they escape depiction in averaged 3D reconstructions of NPCs due to their flexible and unstructured nature.

The 8-fold symmetry of the NPC is often perturbed[16] due to its pronounced structural plasticity.[17,18] In addition, the dynamic properties of the NPC were also documented by the mean residence time of various GFP-tagged Nups within a given NPC as measured by iFRAP.[19] The Nups, which were suggested to preserve the structure of the NPC over time, were shown to reside within one and the same NPC for hours, with the exception of POM121. Interestingly, all WD-repeat-containing Nups also yielded long resident times, as demonstrated by iFRAP, suggesting a fundamental role for this sequence motif in establishing protein–protein interactions required for the structural integrity/maintenance of the NPCs. On the other hand, short residence times, for example, as short as a few seconds, were measured for most of the FG-Nups analyzed. This, together with the natively unstructured nature of the FG-repeat domains, implies that FG-repeats have no specific function in NPC assembly and/or structural integrity but instead play a crucial role in nucleocytoplasmic transport.

Structural dissection of the NPC is a prerequisite for a mechanistic understanding of its function at the molecular level. To date, high-resolution structural analysis of the NPC has been hampered by its complex multi-subunit composition and spatial arrangement, as well as its plastic nature.[16,18] Hence, cryo-electron tomography (cryo-ET) has become the method of choice for investigating the 3D structure of the NPC preserved in a close-to-native environment, that is, without exposing it to chemical fixation or detergent treatment. Accordingly, cryo-ET was employed to determine the 3D structure of the NPC from intact nuclei of the amoeboid *Dictyostelium discoideum*.[20,21] Similarly, cryo-ET was performed in vertebrates on intact NPCs that were kept embedded in native NEs spread on EM grids after manual dissection from *Xenopus* oocytes.[5] This well-characterized vertebrate system, which exhibits a high level of homology to mammalian NPCs, serves as an ideal platform for such studies. A spread NE can be kept relatively thin and free of nuclear contents so that higher-resolution tomographic data may be collected than from an NE of an intact nucleus. On the other hand, 3D tomographic reconstruction of spread NEs suffers from anisotropic resolution due to their preferred, that is, parallel, orientation on the EM grid. Previous cryo-ET performed with spread NEs only revealed the overall NPC architecture to a resolution of ~12 nm.[5] Obtaining higher-resolution data is mandatory to gain a deeper, structure-based insight into the transport mechanism of the NPC.

In the present study, the 3D structure of the NPC from spread NEs prepared from *Xenopus laevis* oocytes was determined by cryo-ET, in combination with symmetry-independent asymmetric unit averaging of 3D tomographic NPC volumes.[20] As a result, the in-plane resolution was improved from 12 to 6.4 nm, thereby identifying novel structural features in the refined 3D NPC model. In particular, a better insight into the 3D architecture of the SR complex was obtained, and its connectivity to the NM, including a novel luminal connection, was identified.

Results

The structure of amorphous ice-embedded NPCs residing in spread NEs prepared from *Xenopus* oocytes has previously been determined to a resolution of 10–12 nm.[5,22] With the recent advancements made in cryo-sample preparation, instrumentation, and image processing, it is now possible to preserve, record, and extract structural information of such large, morphologically complex and mechanically plastic supramolecular assemblies at higher resolution.[20,21,23]

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