

Article Addendum

A lattice model of the nuclear pore complex

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The nuclear pore complex (NPC) is one of the largest protein machines in the cell and forms the sole conduit for nucleocytoplasmic transport in eukaryotes. The NPC is composed of an eightfold radially symmetric scaffold of architectural proteins that anchor a set of phenylalanine-glycine (FG) repeat proteins that form the transport barrier. As a step toward elucidating the molecular architecture of the NPC, we solved the structure of nucleoporin 85 (Nup85) in complex with Seh1, a module in the heptameric Nup84 subcomplex. We define a new tripartite protein element, the ancestral coatomer element ACE1, which Nup85 specifically shares with several other nucleoporins and vesicle coat proteins. We predicted and verified functional sites on nucleoporin ACE1 members based on analogy to ACE1 interactions that propagate the COPII vesicle coat. Thus, we provide the first experimental evidence for evolution of the NPC and vesicle coats from a common ancestor. We propose that the NPC structural scaffold, like vesicle coats, is a polygonal network composed of vertex and edge elements that forms a molecular lattice upon which additional nucleoporins assemble. Here we further discuss our findings and elaborate on our lattice model of the nuclear pore complex.

All nucleocytoplasmic transport in a cell proceeds through nuclear pore complexes (NPCs). NPCs are composed of an eightfold radially symmetric structural scaffold that anchors a group of FG-repeat-containing proteins that form the transport barrier.^{1–3} Elucidating the three dimensional structure of the NPC is critical for understanding its roles in nucleocytoplasmic transport and cellular homeostasis. A path towards an atomic resolution structure of the 40–60 MDa NPC is made possible by the realization that the NPC is a modular assembly.⁴ The NPC is composed of ~30 proteins (Nups) that are arranged into distinct subcomplexes, each present in multiple copies.⁵ The structural scaffold contains the most stably attached nups and comprises two subcomplexes in yeast: the heptameric Nup84 subcomplex (composed of Nup133, Nup84,

Nup145C, Sec13, Nup85, Seh1 and Nup120) and the heteromeric Nic96 subcomplex (likely composed of Nic96, Nup192, Nup188, Nup157/170, Nup59 and Nup53).⁶

In an attempt to better understand the architecture of the NPC, we solved the crystal structure of Nup85 in complex with Seh1.⁷ Nup85 interacts with Seh1 via insertion of an N-terminal blade into the open six-bladed β -propeller of Seh1. The remainder of Nup85 forms a uniquely arrayed J-shaped α -helical block with two distinct units we term “crown” and “trunk”. The fold is notably different from the regular α -helical solenoid that was predicted.^{8,9}

We found that this fold is shared in four other proteins of known structure: the nucleoporins Nup145C, Nup84 and Nic96 and the COPII vesicle coatomer Sec31.^{10–13} While a related architecture between the NPC and vesicle coats has been proposed based on similar fold composition,⁸ we provide the first experimental and structural evidence of a common ancestry.⁷ Comparison of the structures shows a shared core composed of three modules: the crown, trunk and C-terminal tail. We termed this tripartite fold the ancestral coatomer element 1 (ACE1). While the overall organization and topology is identical, there are significant differences in the relative orientation of the modules between members. This suggests the boundaries between ACE1 modules may function as hinges. This relationship was not previously predicted due to low sequence conservation and was initially obscured at the structural level by differences in relative orientation of the modules.¹²

We used characterized interactions of ACE1 proteins to predict functional sites on other members. First, in COPII vesicle coats, Sec31-Sec13 dimers form edge elements through Sec31 crown-crown homodimerization (Fig. 1A).¹¹ We predicted and demonstrated that Nup145C likewise interacts crown-crown with its binding partner in the Nup84 complex, Nup84. Second, we predicted and verified that Nup85 and Nup145C tails interact with Nup120 as the Nup84 tail module interacts with Nup133.¹⁰ These and other data were used to construct an improved model of the Nup84 subcomplex (Fig. 1B).

We propose that the nuclear pore complex scaffold has a lattice structure assembled from vertex and edge elements similar in principle to vesicle coats. We can envision at least two alternative models for this lattice. In the first (Fig. 1B(i)), two rings of the Nup84 subcomplex sandwich an inner ring of the Nic96 subcomplex. This arrangement would generate a scaffold of ~50–80 nm diameter, consistent with the observed pore size in yeast.^{14,15} Alternatively (Fig. 1B(ii)), the scaffold may consist of two stacked rings of the Nup84 subcomplex without an intervening Nic96 subcomplex ring, which may be sufficient to traverse the ~30–50 nm pore height. Uncertainty about the exact arrangement arises from still incompletely understood

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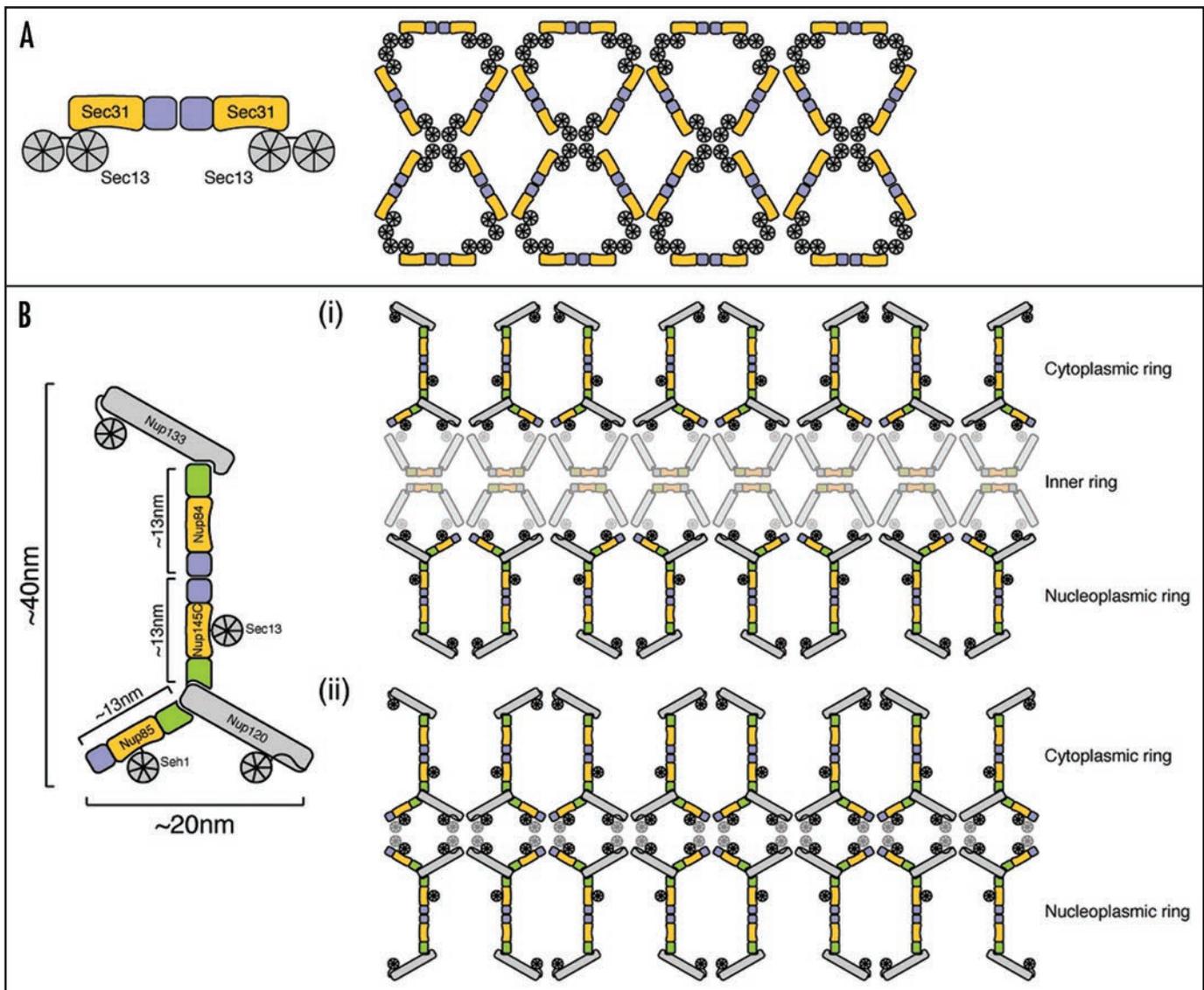


Figure 1. A lattice model of the NPC. Sec31, Nup85, Nup145C, Nup84 and Nic96 ACE1 proteins are colored with crowns blue, trunks orange and tail modules green. Other protein folds are shown in grey. (A) Schematic organization of the COPII outer vesicle coat. On the left, an edge element consisting of two Sec31-Sec13 heterodimers is shown. Two Sec31 molecules interact crown-crown. On the right, an entire COPII cuboctahedron coat composed of 24 edge elements is shown unwrapped and laid flat.¹¹ Vertex elements are formed where two Sec31 and two Sec13 β -propellers interact. (B) Alternative organizations of the NPC lattice. On the left, the Nup84 subcomplex is shown in schematic fashion illustrating how ACE1 interactions organize the Y-shaped structure. Nup145C and Nup84 also interact crown-crown. On the right, the entire NPC structural scaffold is shown unwrapped and laid flat. Two rings of the Nup84 subcomplex form the lattice of the NPC scaffold either with an intervening ring of the Nic96 subcomplex (i) or alone (ii). Both the identity and organization of the vertex elements and the Nic96 subcomplex in the pore lattice are unknown and are shown half-transparent. The presented organization is not meant to predict relative positions of proteins or the structure per se, but rather emphasizes the principally similar lattice organization of NPCs and vesicle coats.

stoichiometries of components and limited information about overall NPC size. Future clarification of the connectivity between scaffold subcomplexes will additionally help to discern the possible lattice arrangements. Homology to COPII coatomers suggests ACE1-containing subcomplexes will be edge elements in the NPC lattice. The nature of the vertex elements in the NPC is less clear, though it may well also involve β -propeller- β -propeller interactions.

Our lattice model of the NPC prompts a number of additional potential parallels to vesicle coats. First, vesicle coatomers do not directly contact membranes, but use adapter protein complexes to span the ~8 nm gap and recruit cargo.¹⁶ Consistently, a ~8 nm gap

has been observed between the structural scaffold of the NPC and the nuclear membrane.¹⁷ Conceivably, other nups fill corresponding adapter complex roles by linking the lattice to transmembrane nups, or transmembrane nups could act directly as adapters. Second, COPII vesicle coats size flexibility is made possible largely by hinges at coat vertices.¹⁸ It may be that analogous hinges as well as those between ACE1 modules in the NPC lattice confer plasticity that may be used for pore dilation or NPC (dis)assembly.

Recent work has produced two conflicting models of the molecular organization of the NPC. A computationally generated model that integrates a wealth of localization, interaction and other primary

data similarly places the Nup84 subcomplex in two peripheral NPC rings flanking an inner ring composed of the Nic96 subcomplex.^{19,20} In contrast, a model based on crystal packing interactions in Nup145C-Sec13 places the Nup84 subcomplex in four stacked rings organized by hetero-octameric poles of Nup145C-Sec13 and Nup85-Seh1 units.¹² A tube of 32 Nup84 subcomplexes was proposed to envelope inner cylinders of the Nic96 subcomplex and FG Nups generating a “concentric cylinder” model of the NPC.^{12,21}

Our model is incompatible with the “concentric cylinder” model for the NPC. Specifically, the demonstrated crown-crown interaction between Nup84 and Nup145C overlaps with Nup145C crystal contacts necessary for the propagation of the models hetero-octameric poles. Exposed hydrophobic surfaces tend to form crystal-packing contacts; whether or not they are physiologically relevant needs to be addressed by additional experiments.²² Careful analysis of packing interactions in crystals can unveil biologically important protein interfaces, especially if the crystallized proteins are part of a higher-order assembly *in vivo*, as nups are. Crystal contacts with at least some hydrophobic character are also observed in the structures of Nup85-Seh1, Nup107·Nup133 and Nic96,^{7,10,13,23} which are all fragments of larger assemblies. While some of these interactions can likely be ruled out as crystal artifacts, *i.e.*, because they involve surfaces created by the use of truncated proteins that would otherwise be buried in the hydrophobic core of the protein, others may be indicative of real functional sites. Weak interactions observed in crystals may point to inter-subcomplex contact areas that are important for self-assembly and to date have not been observed in solution.

We have provided the first structural evidence of a common ancestry of vesicle coats and the nuclear pore complex and provide a lattice model of the NPC based on this commonality. Our lattice model is generally consistent with the computational model of the NPC,²⁰ though the absence of additional structural knowledge precludes a detailed comparison. Our model provides a framework upon which further structural and cell biological studies can be placed in an effort to more fully understand the assembly principles and function of the NPC.

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