Membrane-coating lattice scaffolds in the nuclear pore and vesicle coats
Commonalities, differences, challenges

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The nuclear pore complex (NPC) regulates all traffic between the cytoplasm and the nucleus. It is a large protein assembly composed of multiple copies of ~30 nucleoporins (nups). Structural studies of the NPC have been limited by its considerable size and complexity. Progress toward understanding the structure of this nanomachine has benefited from its modular nature, which allows for this 40–60 MDa assembly to be broken down into subcomplexes that can be studied individually. While recent work by both crystallographers and electron microscopists has greatly enhanced our model of the NPC, the resolution gap between crystal and EM structures remains too large to confidently place individual proteins within the context of the fully assembled NPC. In an effort to arrive at a veritable model of the NPC, we solved the structure of several scaffold nups and defined the ancestral coatomer element (ACE1) common to a set of nucleoporins and COPII vesicle coat proteins. Subsequently, we proposed a lattice-like model of the NPC, analogous to the COPII lattice, in which ACE1 proteins form the edge elements and β-propellers form the vertex elements. Here, we review our recent studies, speculate on how interactions between subcomplexes of the NPC are mediated, and outline the steps and challenges that lay ahead on the path to understanding this enormous assembly in molecular detail.

Introduction

Nuclear pore complexes (NPCs) perforate the nuclear envelope (NE) and serve as the sole conduit for nucleocytoplasmic transport.[1,2] They are essential for maintaining cellular homeostasis and, consequently, the survival of the eukaryotic cell. Architecturally, the NPC is composed of ~30 nucleoporins (nups) which assemble into a set of subcomplexes that are repeated around a central axis with eight-fold rotational symmetry.[3] The heptameric Nup84 and the heteromeric Nic96 complexes form the stable structural scaffold,[4] which anchors the phenylalanine glycine (FG) repeat nups responsible for translocation of transport receptor-cargo complexes across the NE. The 575 kDa Nup84 complex is composed of Nup145C, Nup133, Nup120, Nup85, Nup84, Sec13 and Seh1 in *S. cerevisiae,* while in metazoa additional members have been reported. The Nup84 complex assembles into a branched Y-shaped structure[5,6] and thus has been dubbed the 'Y-complex' (**Fig. 1**). The Nic96 complex is less well understood but is thought to be composed of Nic96, Nup192, Nup188, Nup157/Nup170 and Nup53/Nup59.[7-9]

ACE1 Domain

Recently, much progress has been made in deciphering the interactions mediating complex formation as well as solving structures of nucleoporins within the Y- and Nic96 subcomplexes.[4] In an effort to extend the repertoire of nup structures and to form a better understanding of the overall architecture of the NPC, we solved the structure of the Nup85•Seh1 heterodimer, one of the two short arms of the Y-complex.[10] The structure shows that Seh1 forms an open six-bladed β-propeller that is completed in trans by a three-stranded insertion blade at

Figure 1. Schematic of ACE1 and the Y-complex. (A) The three modules of the ACE1 domain—crown, trunk and tail—are shown in blue, orange and green, respectively. The positions of the N and C termini are indicated. (B) The relative positions of the nups within the Y complex are shown. ACE1 nups are colored as in (A), all others in gray.

the N-terminus of Nup85. After the insertion blade, Nup85 is entirely helical. Many scaffold proteins contain large helical domains that often group into certain helical repeat classes, such as TPR- or HEAT repeats.[11] In Nup85 the helices are also stacked, but with an arrangement that was initially surprising.

Structural comparison of nup and vesicle coat proteins allowed us to characterize the ancestral coatomer element (ACE1), shared by Nup85, Nup145C and Nup84 of the Y- complex, the nucleoporin Nic96, and Sec31 of the COPII coat—a discovery which had previously been precluded by low sequence similarity (6–10%) between these proteins.[10] The 60 kDa tripartite ACE1 element is characterized by three distinct helical modules—crown, trunk and tail (**Fig. 1**). Between all ACE1-containing proteins, each of these three modules is structurally conserved, but they differ noticeably in their relative orientations to one another indicating restrained flexibility. A common ancestry between nucleoporins and vesicle coat proteins was proposed based on common fold composition, the so-called 'protocoatomer hypothesis'.[12,13] Solving the

structure of the Nup85•Seh1 heterodimer and comparing it to other experimental structures, notably that of the COPII coatomer unit Sec31•Sec13,[14] helped to specify the commonalities and differences between the NPC and vesicle coats.

Lattice Model of the NPC

Upon discovery of ACE1, we proposed a model of the NPC scaffold based on and analogous to the lattice-like assembly of COPII vesicle coats (**Fig. 2**). COPII vesicle coats are composed of two layers. The membrane-proximal or inner layer is composed of the three proteins Sar1, Sec23 and Sec24, while the membrane-distal or outer layer can be exclusively constructed from the ACE1-protein Sec31 and the β-propeller protein Sec13.[15] In the COPII outer coat, one assembly unit is composed of two Sec31•Sec13 heterodimers that interact in a homotypic fashion via the crowns of Sec31's ACE1 domain, positioning the N-terminal β-propeller of Sec31 and the adjacent β-propeller of Sec13 as a tandem pair at either end of this extended rod-shaped structure (**Fig. 2**).[14] Thus,

ACE1 domains form the symmetrical edge elements of the lattice, while four Sec31-β-propellers from adjacent edges form the vertex elements in the assembled COPII coat. Beyond spacing the N-terminal Sec31 β-propeller and ACE1-domain, the role of the Sec13 β-propeller is still largely unclear. In our lattice model of the NPC, we predicted that the ACE1 domains of Nup84 and Nup145C form a heterotypic edge element analogous to the homotypic edge element of the COPII coat, indicating that COPII and the NPC share not only building blocks, but also assemble at least in part by similar mechanisms.[10] We were able to provide the evidence for this edge element in the Nup84•Nup145C•Sec13 crystal structure.[16] While Sec31•Sec13 is the principal building block of the COPII outer coat, the Y-complex is equally essential for the construction of the NPC coat. Biochemical and structural studies have characterized the interactions between nucleoporins within the Y-complex.[4] Interactions between the stacked helical domains of the components Nup133, Nup84, Nup145C, Nup120 and Nup85 appear sufficient to assemble its branched Y-shaped structure. The β-propeller domains, of which there are four (N-terminal domains of Nup133 and Nup120, Seh1 and Sec13), are not central to these interactions, leaving them as candidates for mediating interactions outside of the Y.

Interestingly, structural analogs to the inner COPII coat components Sec23, Sec24 and Sar1 have not been observed in the NPC. Yet, we speculate that the NPC lattice built from Y- and Nic96 complexes is connected to the pore membrane via adaptors, consistent with the ~8 nm gap between the scaffold and the pore membrane observed in cryo-tomography of the NPC.[17] We speculate, that this gap could serve as a conduit for the transport of inner nuclear membrane proteins to their destination after insertion into the endoplasmic reticulum upon synthesis. In contrast to COPII vesicles, which need to be assembled and disassembled quickly and repeatedly, the NPC is a very stable entity, virtually without turnover in the non-dividing cell.[18] Thus the adaptor components may be very different, with Ndc1 and its direct interactors being strong candidates.[19] Besides the difference in membrane-anchorage,
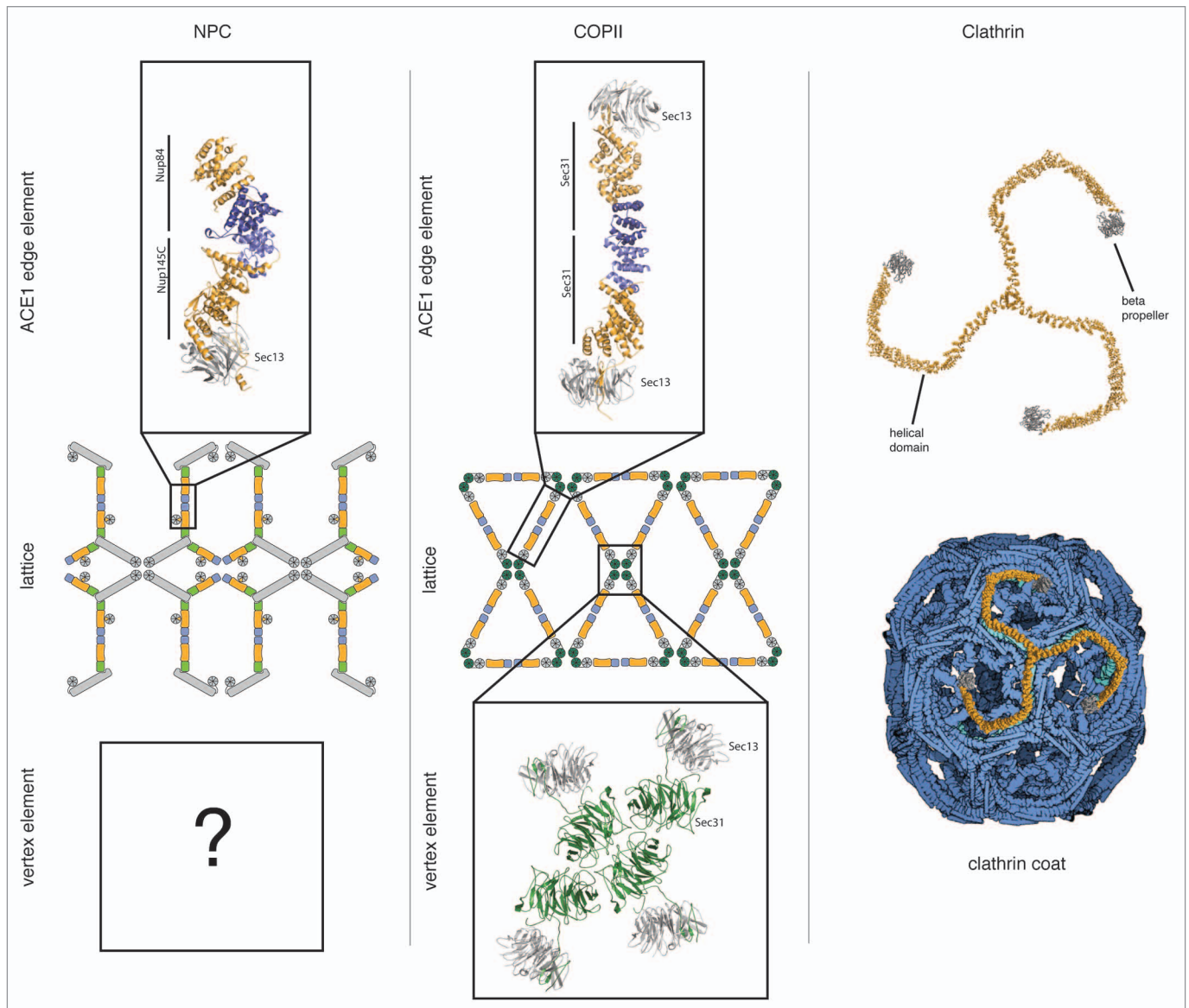
Figure 2. Membrane-coating scaffolds of the NPC and vesicle coats. Schematics of the lattice like assemblies of the NPC and COPII scaffolds are shown, with ACE1 modules colored as in Figure 1. Enlarged views of the ACE1 edge elements are shown for both the NPC and COPII, in addition to the vertex element of the COPII lattice (with Sec31 shown in green and Sec13 shown in gray). The assembly of the vertex element, of the NPC, including which β-propellers are used, is currently unknown. While the COPII ACE1 interaction is homotypic, the analogous ACE1 interaction between Nup145C and Nup84 is heterotypic. A clathrin triskelion and the entire clathrin coat are shown in the right panel. While the clathrin coat is similarly composed of helical and β-propeller domains (colored in orange and gray, respectively), overall structure and assembly differ significantly from the NPC and COPII lattices.

the NPC-COPII analogy also differs in another way. While both systems coat highly curved membranes, the pore membrane has a decidedly different topology from a spherical vesicle. The COPII coat only needs to stabilize a contiguous, positively curved (convex) membrane. The nuclear pore membrane, like the hole in a doughnut, is convex in the longitudinal direction, but negatively curved (concave) in the latitudinal direction.[20] While

the vesicle membrane has neither end nor beginning, the pore membrane ends at the perimeter of the opening. Thus, the NPC likely has elements that are specific to the distinct pore membrane topology. In the lattice model, the Y-complex is oriented relative to the pore membrane such that the Nup145C•Nup84 edge element follows the positive curvature, positioning Nup133 at the periphery of the NPC.[16] However, how the Y complexes are connected is unclear.

While the interactions may be direct, they may also be mediated by Nic96 complex components, or other factors.

β-propellers and the Assembly of the NPC

Regarding the higher-order assembly of the NPC from its subcomplexes, the β-propeller proteins need to be looked at closely. In COPII, the vertices are formed
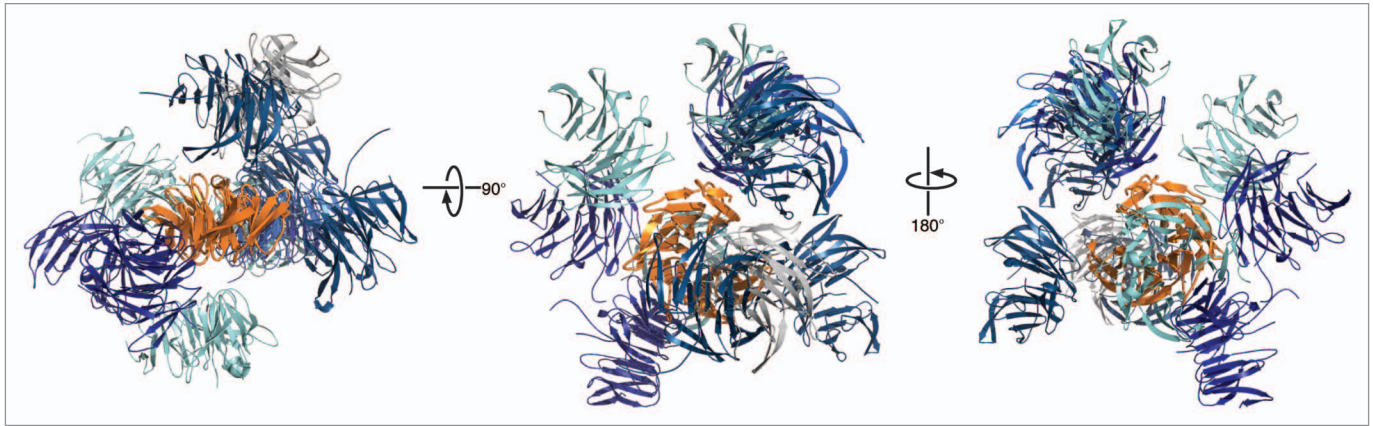
Figure 3. Crystal contacts of the Sec13 β-propeller. A Sec13 reference molecule is shown in orange, with neighboring molecules from five different crystal structures (PDB accession codes 2PM6, 2PM9, 3BG1, 3JRO, 3JRP) superimposed to indicate the number and variety of β-propeller-β-propeller contacts that the Sec13 molecule can make in the crystal. Neighboring molecules from the same crystal are colored in the same shade of blue.

from Sec31 β-propellers exclusively.[14,21] Although there is no direct equivalent to the Sec31 β-propeller in the NPC, the β-propellers of Sec31, Seh1 (including the insertion blade of Nup85) and Sec13 (including the Nup145C insertion blade) are in fact quite similar. Thus, it is tempting to speculate that these two nucleoporins, Sec13 and Seh1, play a major role in the higher-order assembly of the NPC. Propeller-propeller interactions have several intrinsic features. First, they tend to be less hydrophobic than helix-mediated interactions. Neighboring helices largely interact via exposed side chains, typically establishing a hydrophobic core. The β-propeller fold, however, exposes the peptide backbone on every outermost strand of the blades, allowing for a velcro-like interaction with a neighboring β-propeller. Since such interactions are backbone-mediated and thus largely sequence-independent, they are consequently very difficult to detect by sequence conservation, which is otherwise a useful tool for such predictive analysis.

The prospect of β-propellers mediating intersubcomplex interactions within the NPC is likely. Throughout eukaryotes β-propellers are prominent mediators of protein-protein interactions and serve as versatile binding platforms.[22,23] Sometimes, a single β-propeller can interact with different partners using a variety of binding modes. For instance, the Gemin5 protein contains two β-propellers and interacts with at least 5 different proteins to assemble the SMN (survival of

motor neuron) complex.[24] This complex is composed of the SMN protein and Gemin2-7 and coordinates the assembly of small nuclear ribonucleoproteins (snRNPS) important to motor neurons. Another example is the Tup1 protein found in yeast, which together with Cyc8 forms a complex that functions as a general repressor of transcription mediated by a diverse collection of repressor proteins.[25] While the β-propeller domain of Tup1 is not necessary for interaction with Cyc8, it interacts directly with several other proteins. In the COPII lattice, the N-terminal β-propeller domains of Sec31 interact with one another via three different contacts to form the vertices.[14,21] If β-propellers are also involved in lattice formation in the NPC and the interactions are equally homotypic as in the COPII coat one can ask whether the numerous crystal structures of β-propeller nups may provide clues. Lattice contacts in crystals sometimes mimic physiologically relevant interactions. The prime interaction candidate Sec13 has been crystallized in six different crystal forms.[14,16,26,27] Superposition of all neighboring molecules on Sec13 reveals a remarkably diverse ensemble of arrangements, not showing a strongly preferred orientation (**Fig. 3**). Thus, it may be indicative of a flexible arrangement of neighboring subcomplexes.

Challenges

Intersubcomplex interactions are yet poorly defined, including contacts required to

propagate eight copies of the Y-complex around the circumference of the pore. For instance, how does the Y-complex interact with the Nic96 complex? Is this interaction direct or indirect, and if it is indirect, are there other players that we have not yet identified? Does the Y-complex interact with the Ndc1 complex? It seems unlikely that the Y-complex contacts the membrane directly in an extensive fashion. EM studies have also shown an ~8 nm gap between the NPC structural scaffold and the nuclear membrane.[17] Is this indicative of an adaptor layer that links the membrane and the Y-complex, similar to the adaptor layer found in COPII? Thus far, we have taken advantage of the modular nature of the NPC[28] and the analogy to COPII vesicle coats to study its subcomplexes in molecular detail and to suggest a lattice-like model of the NPC structural scaffold,[10] respectively. One of the next steps in understanding the structure of the NPC in its entirety is to determine the specific interactions linking all of its constituent subcomplexes together.

Not only are the intersubcomplex interactions within the NPC numerous, but they are likely weaker and more transient in nature than intrasubcomplex interactions. This is especially evident in higher eukaryotes, where NPCs disassemble into their constituent subcomplexes in mitosis. Establishing similar interactions mediating assembly of the Sec31•Sec13 heterotetramers, which are stable in solution, into vesicle coats has challenged researchers studying the assembly of the COPII

outer coat. However, due to the possibility to reconstitute the COPII coat in vitro, a model for the assembled COPII coat was inferred with the help of an EM-map of high enough resolution (3 nm)[21] for the crystal structures of Sec31•Sec13 to be confidently placed.[14] Without better-resolved EM structures of the NPC, solving its overall structure will be difficult. Recently, Kampmann and Blobel have provided an EM map of the Y-complex at 3.5 nm resolution, allowing for the tentative placement of currently solved structures into the Y-shaped density.[6,16] while such single-particle reconstructions are not yet abundant, they will be key in understanding the NPC assembly.

Additionally, studying NPC structure and assembly in vivo is likewise challenging due to the possibility of redundant interactions obscuring the function of an individual protein. While mutational and deletion analyses can be useful in studying unknown protein functions or interactions in vivo, the ability of proteins coevolved from common ancestors (i.e., ACE1 and β-propellers of the NPC) to substitute for one another may be sufficient to preserve the integrity of the scaffold. Even without such substitution, enough interactions may still remain to withstand the omission of one individual nucleoporin and may result in no apparent phenotype. For instance, we recently showed that truncation of the C-terminal 280 residues from Nup120 results in the loss of NPC localization of Nup120.[29] Despite severing Nup120 integration, however, the NPC appears to be largely functional and intact. Similarly, the dual function of architectural nups and membrane coat proteins is also not uncommon, ranging from the role of Sec13 in the NPC and COPII, to COPI coatomers playing an essential role in nuclear disassembly[30] and Y-complex members being recruited to kinetochores during mitosis.[31] In this case, mutational and deletion analyses can result in difficult to interpret phenotypes.

Outlook

It is apparent that there is a multiplicity of challenges that must be overcome to dissect and define the intersubcomplex contacts that mediate NPC assembly.

The structural relationship between the NPC and vesicle coat proteins has given us a good starting point from which to formulate a likely, yet tentative, model of the NPC structural scaffold. Building on this analogy, it seems that the β-propellers of the Y-complex will likely prove to be important players in mediating the inter-subcomplex interactions necessary for NPC assembly. Simultaneously, we must keep in mind that there are some key differences between the NPC and the COPII coat, a necessity of the different membrane topologies that are stabilized in each case. There are still a number of obstacles to overcome on the path to gaining a detailed, molecular understanding of how the individual subcomplexes come together and interact with the membrane to form the intact NPC, and it will require the continued use of multidisciplinary efforts to reach this goal. But if the progress of the last few years gives any indication of what is to come, it will not be too long before a basic experimental model of the NPC scaffold will be available.

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