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Nuclear pore complex – a coat specifically tailored for the nuclear envelope

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Abstract

Nuclear Pore Complexes (NPCs) are highly selective transport gates that enable the bidirectional traffic of macromolecules across the nuclear envelope (NE). NPCs are located at the fusion pores between the inner and outer membranes of the NE and are built from a common set of ~30 different proteins, nucleoporins. Remarkably, recent proteomic, bioinformatic and structural studies have provided firm evidence that key structural nucleoporins share common ancestry with elements of coated vesicles, indicating an evolutionary link between these structures. This has provided novel insight into the origin of NPCs and may help us to functionally characterize these fundamental components of eukaryotic cells.

Introduction

NPCs are uniquely eukaryotic structures required for the functional separation of the cytoplasm and the nucleus by the NE. Analyses of NPCs from a range of species revealed that they are assembled from ~30 nucleoporins, the majority of which are highly conserved [1–4•,5]. Due to an inherent 8-fold rotational symmetry of NPCs all nucleoporins are present in multiple copies accounting for ~ 500 individual polypeptides per pore. The general organization of NPCs in various organisms is very similar consisting of a ring-like channel with 8 identical subunits or “spokes” [6–8]. The NPC ring has an outer diameter of ~ 120 nm and is almost symmetrical with respect to the NE (Fig 1A). The central transport channel is filled with unstructured material often referred to as the “central plug” or “transporter” [9,10•] (Fig 1A left panel, Fig 1B). In addition, the NPC contains asymmetrical nuclear and cytoplasmic extensions, known as “nuclear basket” and “cytoplasmic filaments” [8,9,11–13] (Fig 1B).

NPC components can be broadly grouped into three categories: (a) membrane nucleoporins, which are integrated in the pore membrane; (b) scaffold nucleoporins involved in forming the NPC framework; and (c) barrier nucleoporins critical for the selective permeability of NPCs. These three classes appear to occupy distinct zones or layers within the NPC [14••, 15••]. The first layer is formed by membrane nucleoporins, which help to anchor the scaffold layer nucleoporins. The scaffold layer in turn attaches barrier nucleoporins that face the central channel and form the asymmetric NPC extensions (Fig. 2 and 1A–B). Although this classification is somewhat arbitrary, it reflects the general functional specialization observed among nucleoporins.

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It is an intriguing problem how a highly complex structure, such as the NPC, co-evolved with the nuclear envelope. Interestingly, Sec13, a key component of the COPII membrane coat, involved in ER-to-Golgi transport, is also found to be a stable NPC component [16]. Moreover, scaffold nucleoporins generally display a simple and characteristic structural composition containing either a beta-propeller fold (Sec13 and Seh1), a predominantly alpha-helical fold (Nup84, Nup85, Nup145(C), Nic96, Nup188, Nup192) or a combination of these two fold types (Nup170/157, Nup120 and Nup133) [2,17]. The same features also characterize components of the evolutionary related endomembrane trafficking coats, COPI, COPII and clathrin, leading to the hypothesis that the NPC scaffold and these membrane coats originated from a common ancestral structure during the evolution of the endomembrane system [2,18]. In this review, we highlight recent progress in our understanding of NPC architecture and assembly, and discuss parallels in the organization of NPCs and COP/clathrin coats.

Structural similarities between nuclear pore complex and membrane coat components

In the last several years, considerable progress has been made in determining the atomic structures of multiple scaffold nucleoporins. Remarkably, at least six of them display similarities in their structural organization with elements of the COPII coat. Despite extremely limited sequence homology, Nup84, Nup85, Nup145(C) and Nic96 share a common U-shaped architecture with Sec31, a component of the Sec13/31 COPII coatomer complex [19• •,20•,21–24] (Fig 3). Furthermore, a characteristic in-trans insertion of Sec31 into the propeller blades of Sec13 is found in both Nup145(C)-Sec13 and Nup85-Seh1 hetero-dimers [19••,24–26] (Fig 3). Finally, the anti-parallel arrangement of the Nup84-Nup145(C) complex also resembles the organization of a Sec31 dimer within the Sec13/31 structure [23,26] (Fig 3). Therefore it seems likely that at least six scaffold nucleoporins (corresponding to ~60% of the scaffold layer) share ancestry with the COPII coatomer element.

General comparison of nuclear pore complex and membrane coat organization

The structural similarities between some of the scaffold nucleoporins and membrane coat elements raise the intriguing question of how much resemblance these multiprotein structures have to one another? It is well established that COP and clathrin-type coats form through polymerization of coatomer subunits and package membrane cargos into transport vesicles. All these coats are comprised of a membrane layer, which contains cargos and regulators of coat assembly, followed by a double layer formed by adaptor proteins and outer coat proteins [27] (Fig 4A). The adaptor proteins couple coat assembly with cargo packaging by direct interactions with membrane cargos. In contrast, the outer coat is primarily responsible for polymerization into a higher-order structure, which curves the membrane and shapes the transport vesicle [28]. At first glance, this arrangement looks remarkably similar to the layered NPC organization described above. Whereas, the membrane layer of the NPC can be compared to the membrane layer of the COP/clathrin coats, the NPC scaffold layer corresponds to the adaptor/outer coat layer (Fig 2 and Fig 4A–B).

However, in spite of these parallels, the endomembrane coating systems and NPCs also exhibit significantly different properties. For example, endomembrane coats have a spherical geometry stabilizing positive membrane curvatures (Fig 1C), and are naturally dynamic [28–30]. In contrast, NPCs are arranged in a donut-like shape (Fig 1), giving rise to both positive

and negative membrane curvatures, and their scaffold elements form extremely stable assemblies. Furthermore, NPCs function as highly selective transport gates while there is no obvious barrier layer-like structure present in COP/clathrin coats. Therefore, it is still uncertain to which extent the assembly mechanisms and organization principles of the COP/clathrin coats can be extrapolated to NPCs. In the following parts we discuss in more detail corresponding aspects of these eukaryotic membrane-deforming systems.

The membrane layer in nuclear pore complexes and membrane coats

In yeast, the membrane layer includes four transmembrane nucleoporins: Ndc1, Pom152, Pom34, which form a biochemically stable subcomplex [3,31–33•], and Pom33 [34]. Transmembrane nucleoporins are functionally redundant [33•,35,36] and evolutionary flexible [1,2]. For example, vertebrates express three transmembrane nucleoporins vNdc1, Pom121 and gp210 but only Ndc1 appears to have a clear yeast ortholog [37–40]. In addition to these transmembrane nucleoporins, two yeast paralogs Nup53 and Nup59 can also be grouped into the membrane layer. Nup53 and Nup59 are functionally redundant with Pom152 and Pom34 and contain C-terminal amphipathic helices, which in the case of Nup53 allows for direct membrane insertion [33,35,36][41,42]. Nup53/59 is widely conserved in evolution (Nup35 in vertebrates) although it was not detected in some fungal species, like Aspergillus [1,43]

The evolutionary roots of membrane nucleoporins cannot be clearly traced. However, it is interesting that the luminal domain of gp210 shares similarity with the extracellular part of intimin-like proteins, a family of prokaryotic transmembrane proteins [2,44]. Additionally, the luminal domain of yeast Pom152 displays similarity to cadherins, transmembrane proteins located in the plasma membrane [17]. It is therefore possible that membrane layer nucleoporins originated from membrane cargos initially sorted to the cell surface by membrane coats, which would make them functionally homologous to coat cargos.

Scaffold nucleoporins and coatomers

As discussed above, six scaffold nucleoporins share similarity with elements of the Sec13/31 COPII coatomer complex. In contrast, the evolutionary origin of the remaining scaffold nucleoporins is less clear. Structures of Nup170, Nup133 and Nup120 [45–48] revealed weak similarities amongst these nucleoporins [49], but did not uncover obvious homologies to any known coat proteins except for a tandem arrangement of beta-propeller and alpha-helical domains. However, there is a very rich interaction network between these nucleoporins and the membrane layer. Yeast Nup170, its paralog Nup157 and vertebrate Nup155 all make multiple direct contacts to membrane layer nucleoporins [33•,50•,51,52•]. Moreover, yNup120, vNup133 and yNup170 contain amphipathic helices known to mediate direct membrane binding [53•,54]. The tight interaction of the beta-propeller/alpha-helical scaffold nucleoporins with the membrane layer is reminiscent to the direct binding of adaptors of COP/clathrin coats to membrane cargo suggesting that they may share functional homology. Thus, scaffold nucleoporins might be functionally divided into adaptor-like (beta-propeller/alpha-helical proteins) and outer coat-like (Sec13/31-like) elements (Fig 4A and B).

Given the parallels between NPCs and COP/clathrin coats can we safely conclude that these structures all follow the same organizational principles and that NPCs also form regular lattices via anti-parallel arrangement of their scaffold subunits [29]? Current nucleoporin structures and NPC models do not give a clear answer. For example, various, mutually exclusive NPC arrangements were proposed for the Nup84 complex, the largest and the most structurally characterized NPC subcomplex, which accounts for almost a third of the total NPC mass [14••,15••,23,24,46,55•,56,57]. Therefore, the currently available data do

not appear to be sufficient to accurately predict the three-dimensional organization of NPCs suggesting that further structural analyses of higher-order nucleoporin complexes will be needed.

The barrier layer: a unique functional feature of the nuclear pore complex?

A key role of the NPC is to function as a barrier enabling the highly selective and regulated exchange of macromolecules between the cytoplasm and the nucleus in eukaryotes. This function is brought about by a set of nucleoporins all containing phenylalanine-glycine-rich (FG)-repeats, which are part of the barrier layer. The mechanistic role of FG nucleoporins in NPC selectivity remains somewhat controversial but specific interactions between FG nucleoporins and soluble nuclear transport receptors or karyopherins are a critical feature for NPC selectivity [58].

How did the filtering function of the NPC evolve? Given the unique role of the NPC as a selective transport channel, barrier FG nucleoporins might represent an NPC-specific invention. A structure with obvious homology to the FG barrier layer cannot be found on top of COP/clathrin coats. However, it is intriguing that the barrier function of the NPC is not only restricted to soluble molecules but also extends to transmembrane proteins [59,60], which would resemble the function of COP/clathrin coats that act as filtering devices to selectively retain and enrich for membrane cargos. Furthermore, membrane layer nucleoporins (yNup53, yNup59, vPom121) also contain FG-repeats [39,61], and there is evidence for interactions between scaffold nucleoporins and FG-domains [20•]. Finally, structural similarities between scaffold nucleoporins, coat proteins and soluble transport receptors/karyopherins were also noted [2,17].

Together, this might suggest that the evolutionary relationships with the COP/clathrin coat elements may extend beyond the NPC scaffold structure and may include the NPC barrier function or even the entire nucleocytoplasmic transport system.

Assembly mechanics of nuclear pore complexes and membrane coats

COP and clathrin coats form *de novo* and assemble from a soluble pool of coatomers initiated by the membrane recruitment of adaptor proteins (Fig 4C left). One mechanism of adaptor recruitment involves Arf1-like GTPases, which in their GTP-bound state bind to membranes and subsequently recruit adaptor proteins [28]. GTPase-independent mechanisms involve recruitment of adaptors via membrane cargos or direct membrane interactions [62]. The initial membrane recruitment is followed by a deposition of outer coat proteins, which induces polymerization of a regular lattice structure, membrane deformation and ultimately the production of a transport vesicle (Fig 4C left). NPCs also assemble *de novo* in interphase cells [63,64•] but the mechanism of this process is only beginning to emerge. As discussed above, multiple physical links between the membrane and scaffold layer nucleoporins exist and components of this interaction network play an important role in early steps of interphase pore assembly [33•,35,36,50•,52•,53•,65]. Additional studies will be necessary to dissect early steps of NPC biogenesis, but the role of interactions between membrane and scaffold nucleoporins may mimic adaptor-cargo interactions in early steps of COP/clathrin coat assembly (Fig 4C).

While formation of a COP/clathrin coat ultimately results in production of a transport vesicle, NPC assembly gives rise to a fusion pore between the inner and outer NE membranes (Fig 4C). Thus both processes rely on a membrane fusion event albeit with rather different topological outcomes. The scission of coated vesicles is complex and relies on the coat itself and, in addition, can depend on various activities including Sar1, dynamin or the actin cytoskeleton [66]. The mechanism of nuclear pore fusion is not understood and

it is not known whether intrinsic NPC components are sufficient or whether non-nucleoporin co-factors are needed to complete the fusion step. Interestingly, several studies point towards a critical role for the lipid composition or the membrane properties during early steps of NPC assembly prior to the incorporation of barrier nucleoporins [67•,68,69].

Another unresolved question is, how NPC assembly is specifically targeted to the NE. The GTPase Ran is the only well-established regulatory factor linked to the NPC [70]. Similar to Arf1-like GTPases, Ran belongs to the superfamily of small GTP-binding proteins, which act as regulators of molecular interactions by switching between GDP and GTP bound states often at specific subcellular locations. Unlike Arf1, however, Ran is not membrane-associated and regulates protein-protein interactions by triggering associations and dissociation between nucleo-cytoplasmic transport receptors and their cargos [70,71]. However, there is evidence that the Ran GTPase cycle is necessary for NPC biogenesis [72] and a role in NPC assembly was reported for the major Ran effector importin-beta, [63,73]. Furthermore, the membrane layer nucleoporins, yNup53 and vPom121, contain NLS sequences, which interact with transport receptors and are necessary for NPC incorporation [53•,74•,75]. The Ran-pathway might function in NPC biogenesis by affecting the interactions or localization of membrane layer nucleoporins. In addition to Ran-pathway components, the DNA-binding protein Mel28/ELYS has been implicated in targeting NPC assembly to the NE. However, its function might be restricted to post-mitotic NPC reassembly in higher eukaryotes. [53•,76–78].

Conclusions

In the last several years, considerable progress has been made in our understanding of the biochemical and structural composition of NPCs. Key questions, which remain to be answered are related to mechanistic aspects of NPC assembly and to principles of the three-dimensional organization of NPCs. It has become clear that NPCs and other membrane coating systems have at least in part a common evolutionary origin that dates back to the last eukaryotic common ancestor. The evolutionary ties between components of the membrane coating systems in eukaryotes may extend far beyond the structural resemblance of their key components. This may help us not only to get insight into aspects of NPC function but also to better understand the origins of modern eukaryotic complexity.

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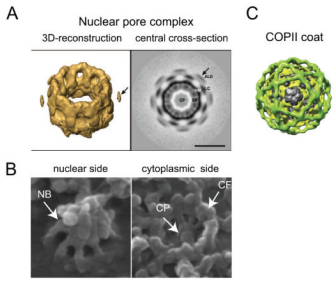

Figure 1.

Structural highlights of NPCs and COP/clathrin coats. (A) A 3D-reconstruction (left) or a central cross-section (right) of the NPC structure acquired by cryo-EM tomography using *Xenopus* NPCs. Note the characteristic 8-fold symmetry of the NPC. The image is adapted from [10•]. CP, central plug; SR, spoke ring complex; NM, nuclear membrane; LC, luminal connection; ALD, additional luminal density. (B) Surface views of the nuclear (left) and the cytoplasmic sides (right) of *Xenopus* NPC acquired using high-resolution scanning EM. The images highlight the nuclear basket (NB), cytoplasmic filaments (CF) and the central plug (CP). These structures are not clearly visible with cryo-EM tomography (Fig. 1A) due to their dynamic nature. Images were kindly provided by Dr. Elena Kiseleva. (C) Architecture of the COPII coat visualized by EM single particle reconstruction. Note the spherical architecture and highly symmetrical arrangement of coatomers within the coat structure (adapted from [30]).

Figure 2.
NPC composition in yeast and vertebrates. (A) Panel displays the distribution and approximate location of yeast nucleoporins (left) and corresponding vertebrate nucleoporins (right) within the NPC. Based on their functional properties and localization nucleoporins can be classified into 3 functional layers: the membrane layer (brown) consisting of transmembrane and membrane-associated nucleoporins, the scaffold layer (blue) composed of core structural nucleoporins, and the barrier layer (green) containing nucleoporins involved in the selective NPC permeability and other functions. (B) The correspondence between yeast (y) and vertebrate (v) nucleoporins. Colored boxes depict biochemically stable nucleoporin subcomplexes.

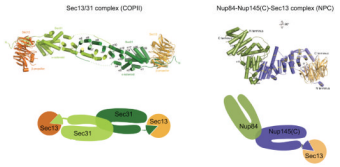

Figure 3.

Structural similarities between COPII coat elements and scaffold nucleoporins as exemplified by the Nup84-Nup145(C)-Sec13 complex structure. The top panels show structures of the Sec13/31 COPII coatomer complex (left) and the Nup84-Nup145(C)-Sec13 nucleoporin complex (right). The bottom panels display schematic representations of these complexes highlighting (a) the characteristic U-shaped organization of Nup84 and Nup145(C), which is similar to the arrangement of the Sec31 alpha-helical domain, (b) the anti-parallel mode of interaction between Nup84 and Nup145(C) and two Sec31 molecules, and (c) the in-trans blade insertions of Nup145(C) or Sec31 into the Sec13 beta-propeller. For a detailed description of these similarities see [19••,23] and main text. The structures were adapted from [23,26].

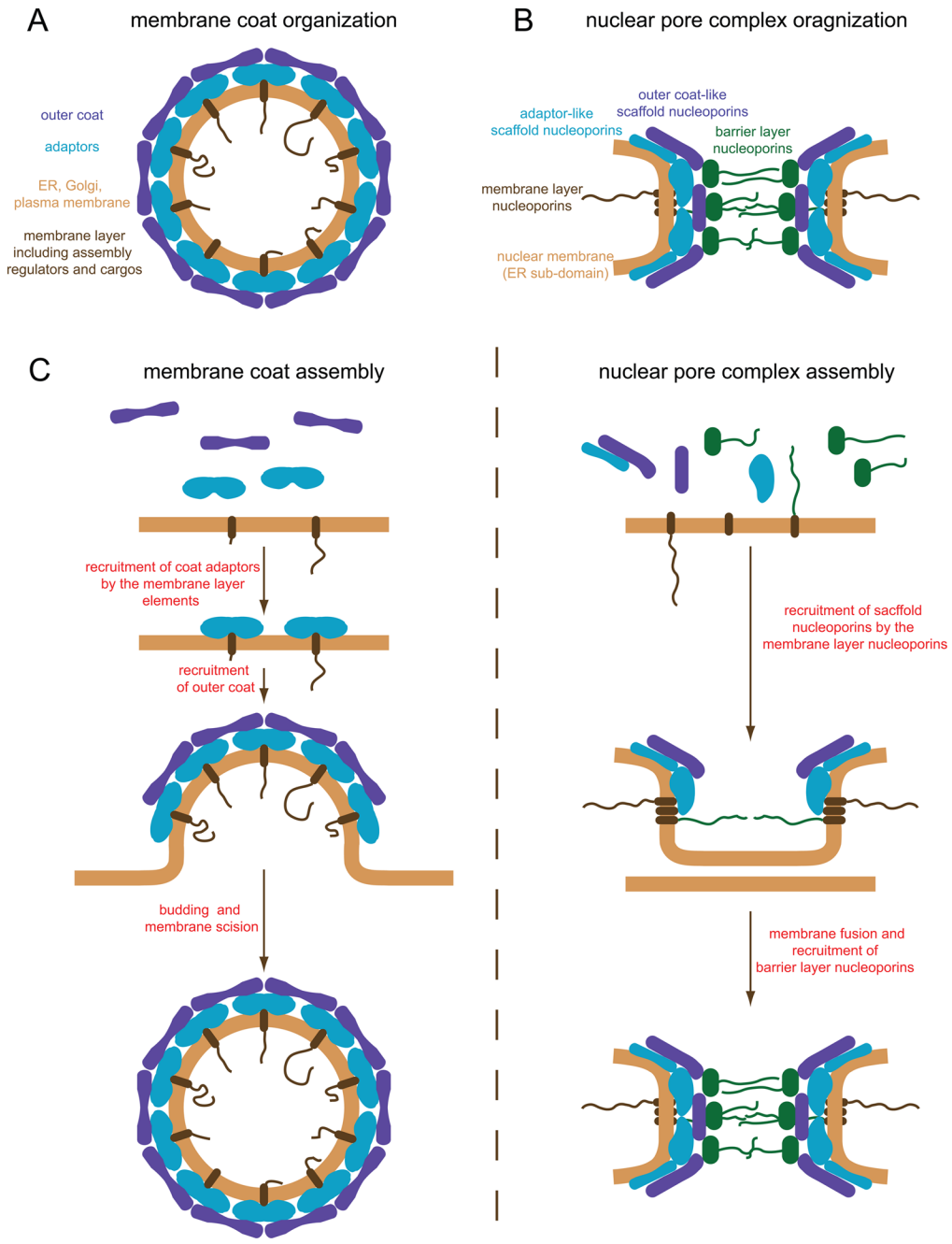

Figure 4.

(A and B) Models of the membrane coat (A) and NPC organization (B). Functionally similar parts of NPCs and membrane coats are shown in the same color. (C) Corresponding steps in the assembly of membrane coats (left) and NPCs (right).