Three-Dimensional Architecture of the Isolated Yeast Nuclear Pore Complex: Functional and Evolutionary Implications

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Summary

We have calculated a three-dimensional map of the yeast nuclear pore complex (yNPC) from frozen-hydrated specimens, thereby providing a direct comparison with the vertebrate NPC. Overall, the smaller yNPC is comprised of an octagonal inner spoke ring that is anchored within the nuclear envelope by a novel membrane-interacting ring. In addition, a cylindrical transporter is located centrally within the spokes and exhibits a variable radial expansion in projection that may reflect gating. The inner spoke ring, a transmembrane spoke domain, and the transporter are conserved between yeast and vertebrates; hence, they are required to form a functional NPC. However, significant alterations in NPC architecture have arisen during evolution that may be correlated with differences in nuclear transport regulation or mitotic behavior.

Introduction

The nuclear pore complex (NPC) spans the nuclear envelope (NE) within a specialized pore membrane domain, formed by a fusion of the inner and outer nuclear membranes. The NPC functions as a channel for the nuclear import of proteins and snRNPs, while providing an export path for tRNAs, mRNPs, ribosomal subunits, and snRNPs reviewed in Gorlich and Mattaj, 1996; Nigg, 1997. Nuclear import can be divided into three major steps: targeting/docking, translocation, and substrate release (Newmeyer and Forbes, 1988; Richardson et al., 1988; Akey and Goldfarb, 1989; Pante and Aebi, 1996). Recent work has resulted in a paradigm for nuclear import and export, in which receptors mediate the binding of transport substrates containing a nuclear localization signal (NLS) or export sequence (NES) to repeat-containing nucleoporins (GLFG/FXFG) within the NPC. It is postulated that substrate translocation may utilize cycles of GTP hydrolysis mediated by Ran (Koepp and Silver, 1996; Nigg, 1997). In addition, the NPC is thought to provide passive diffusion channels that give the NE its characteristic permeability to small molecules (Paine et al., 1975; Akey and Radermacher, 1993; Akey, 1995; Perez-Terzic et al., 1996), while peripheral channels may facilitate the redistribution of nuclear membrane proteins (Powell and Burke, 1990; Hinshaw et al., 1992, Akey and Radermacher, 1993; Wiese and Wilson, 1993). The three-dimensional structure of the vertebrate NPC has been studied in amphibian oocytes using electron microscopy and single-particle methods on frozen-hydrated (Akey and Radermacher, 1993) and negatively stained specimens (Unwin and Milligan, 1982; Hinshaw et al., 1992). These studies have provided a low resolution model of this translocation machine (see Figure 1). The NPC is comprised of an octagonal spoke-ring complex that is embedded in the NE and that encircles a central channel complex (termed the transporter; Akey, 1989, 1990). The spoke-ring complex anchors more peripherally associated components, including the cytoplasmic filaments (Richardson et al., 1988; Jarnik and Aebi, 1991; Ris, 1991) and the nuclear basket (Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992), which play a role in the docking of import (Richardson et al., 1988; Pante and Aebi, 1996; Rutherford et al., 1997) and export substrates (Kiseleva et al., 1996), respectively.

Nucleocytoplasmic transport utilizes a central channel within the NPC for import and export (Feldherr et al., 1984; Dworetzky and Feldherr, 1988). However, the mechanism of translocation across the NPC is currently unknown. Recently, atomic force microscopy and scanning electron microscopy on Xenopus NPCs (Goldberg and Allen, 1996; Perez-Terzic et al., 1996) and Chironomus NPCs (Goldberg and Allen, 1996; Kiseleva et al., 1998) have provided further observations of the NPC transporter (Akey and Radermacher, 1993). The NPC transporter is present in manually isolated NEs in a number of radially expanded configurations in projection, suggesting that the channel is gated (Akey and Goldfarb, 1989; Akey, 1990). The physical imprint of the transporter is revealed in thin sections of Balbiani ring mRNPs and nucleoporin-gold particles caught traversing the NPC (Stevens and Swift, 1966; Feldherr et al., 1984; Mehlin et al., 1992), as these substrates are constrained within a ~200-250 Å diameter channel that extends across the NPC for about 500-600 Å.

The completion of the Saccharomyces genome sequencing project (Clayton et al., 1997 and references therein) has resulted in an accelerated identification of yeast nucleoporins (nups) (Rout and Wente, 1994; Doye and Hurt, 1997). Moreover, the recent isolation of enriched fractions of yeast NPCs and NEs (Rout and Blobel, 1993; Strambio-de-Castillia et al., 1995) has opened the door to structural studies, as isolated yNPCs have dimensions similar to those in cells. However, yeast NPCs are both smaller and less massive than vNPCs (~66 MDa versus ~125 MDa; Reichelt et al., 1990; Rout and Blobel, 1993). Hence, a detailed three-dimensional (3-D) structure of the yNPC will allow an understanding of this fundamental size difference and provide a framework in which the position of yeast nucleoporins can be mapped.

In this work, we present the 3-D structure of the yNPC.
the method of Rout and Blobel (1993) and utilized computational methods to select the fraction with the highest degree of structural preservation. A representative subset of 8-fold symmetric yNPCs within this fraction were identified by rotational power spectra analysis, and the appropriate two- and three-dimensional maps were calculated.

**Mass and Symmetry of Yeast NPCs**

We have used dark-field imaging in the scanning transmission electron microscope (STEM; Wall and Hainfeld, 1986) to estimate the mass average of the enriched and highly enriched fractions of yNPCs, isolated by the method of Rout and Blobel (1993). The data for highly enriched yNPCs is shown in Figure 2A and gives a Gaussian-fitted peak of 54.5 MDa ($\pm 10.1$). Similar mass values were obtained for enriched yNPCs and cross-linked yNPCs from the highly enriched fraction (Yang, 1997). The STEM mass data and earlier estimates obtained by light scattering and sedimentation methods (Rout and Blobel, 1993) overlap significantly and place the mass of the yNPC between 55 and 66 MDa. Previous measurements rely on bulk properties of the sample and are influenced by "larger" particles (Rout and Blobel, 1993), while the STEM measures individual particles to form a mass spectrum. Hence, the differences between the midpoint values may reflect the nonideal behavior determined by electron cryomicroscopy and image processing. The resulting maps have been compared with their vertebrate counterparts, to provide insights into the function and evolution of the NPC and its subassemblies. Overall, the yNPC is smaller than the vertebrate NPC (vNPC) and maintains a conserved inner spoke ring and central transporter. In addition, yeast and vertebrate NPCs both interact with the NE at comparable radii using similar spoke domains. However, the yNPC does not have a luminal spoke ring but rather has evolved a novel membrane-interacting ring that is comprised of 8 linear arms and 8 membrane-spanning spoke domains. In projection maps, the central yNPC transporter is visualized in 3 possible transport-related configurations and demonstrates 2 related "in transit" forms, similar to that observed in vertebrate transporters (Akey and Goldfarb, 1989; Akey, 1990). The implications of these observations for the mechanism of nucleocytoplasmic transport are discussed.

**Results**

The resolution that is attainable with supramolecular assemblies in the electron cryomicroscope is often limited by the biochemical stability of the specimen. Therefore, we have characterized yNPC fractions isolated by

![A Model of the Vertebrate Nuclear Pore Complex](image)

**Figure 1. A Consensus Model of the Vertebrate NPC**

The overall structure is comprised of a wheel-like array of eight spokes connected to the inner spoke ring (ISR), which encircles the central channel complex (transporter [T] is pink). The spoke complex is framed top and bottom by the cytoplasmic (CR) and nuclear (NR) thin rings that serve as attachment sites for cytoplasmic particles (CP) and filaments and the nuclear basket. The spokes are embedded in the NE (light blue), are interconnected within the lumen to form a luminal ring (LR), and are attached to the lamina (L). The outer and inner nuclear membranes are labeled (ONM) and (INM), respectively. A set of inner ring filaments interconnect the top and bottom of the central transporter to the thin rings and for clarity, are shown only on the cytoplasmic side (bold lines).

**Structure of the Isolated Yeast NPC and Interactions with the Nuclear Envelope**

We calculated projection maps for both detergent-solubilized (dform) and nuclear envelope-associated (mform)
yNPCs in frozen-buffer and negative stain, respectively (Figures 3A and 3B). These maps were then compared with similar published maps of the vNPC (Figures 3C and 3D; Akey, 1995) to understand the structural basis of previously observed size differences and to identify the membrane-interacting spoke domain within the yNPC.

In Figure 3A, the dform map of the yNPC has an outer diameter of ~960 Å and is comprised of 8 spokes. Each spoke has 2 radial domains (circles labeled 1 and 2), with the proximal domains forming an inner spoke ring. The inner spoke ring encircles a central blurred disk that represents a global average of the transporter. An additional linear arm of density extends radially from the inner spoke ring and is connected circumferentially to both of the neighboring spoke 2 domains, to form a
Table 1. Domain Positions and Dimensions in Yeast and Vertebrate NPCs

<table>
<thead>
<tr>
<th>Structural domains</th>
<th>Yeast NPC</th>
<th>Vertebrate NPC</th>
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<tr>
<td></td>
<td>Di (Å)</td>
<td>Dc</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Transporter</td>
<td>490</td>
<td>580</td>
</tr>
<tr>
<td>Inner spoke domain</td>
<td>730</td>
<td>840</td>
</tr>
<tr>
<td>Second spoke domain</td>
<td>780</td>
<td>820</td>
</tr>
<tr>
<td>Membrane ring</td>
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<td></td>
</tr>
<tr>
<td>Nuclear membrane pore</td>
<td>860</td>
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<td>Entire NPC</td>
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Vertebrate NPC dimensions are from Akey (1989), Akey and Radermacher (1993), and Akey (1995). Di/Dc/Do are inner, center and outer diameters.

\[ a \] Measured from Akey and Radermacher (1993).

\[ b \] Including the radial arms.

\[ c \] Including the cytoplasmic particles.

ring. The spokes display approximate 2-fold symmetry when mirrored about the arrow labeled 2 (which bisects the spokes); however, the linear arm is displaced circumferentially in a counter-clockwise direction from a "local" 2-fold axis (located between the spokes as shown in Figure 3C), to interact preferentially with an adjacent spoke. This deviation from 822 symmetry may have arisen during purification, as similar circumferential distortions are present in vNPCs (Akey and Radermacher, 1993).

A comparison of 2-D maps from yeast and vertebrate NPCs suggests a reason for the observed size differences. Vertebrate spokes have 3 radial domains in projection with the positions of the innermost 2 domains being equivalent to those in yNPCs (Figures 3A and 3C, Table 1). A second projection map of the yNPC reveals that the spokes have 2 radial domains when the nuclear membrane is present (Figure 3B, see circles). Hence, it seems unlikely that the "best" yNPCs have lost major spoke domains during solubilization and purification. We suggest that the yNPC spoke may be intrinsically smaller than its vertebrate counterpart.

Although yeast and vNPCs differ in lateral diameter and mass, some features of their interaction with the NE are conserved. In Figure 3B, the nuclear membrane is present as a strong band of circumferential density that interacts with the second spoke domain in yNPCs. This interaction occurs at a similar radius within vNPCs (Figure 3D and contoured difference map in Figure 3C; Akey, 1995). However, there are notable differences between yeast and vertebrates in the details of this interaction. For example, the yNPC has an additional linear arm that is not observed in the mform yNPC map, because it is likely buried within the membrane.

Visualization of Multiple Forms of the Central Transporter

The diameter of the globally averaged central transporter in yNPCs is similar to that observed in vNPCs (~350–380 Å; Akey, 1989, 1990). The vertebrate transporter appears to have 8-fold symmetry, as 8 internal ring filaments link the transporter to the cytoplasmic and nuclear thin rings (see Figure 1; Goldberg and Allen, 1996), and these data are consistent with previous labeling studies using MAb414 (Akey and Goldfarb, 1989).

The similar diameter of the yeast and vertebrate transporters and the shared 8-fold symmetry of the spokes would argue that the yeast transporter has 8-fold symmetry, and this property was used in the following analysis. Classification was used to eliminate yNPCs with poorly defined transporters and to sort the remaining pore complexes into transporter groups (Akey, 1990; Frank, 1990). This analysis resulted in projection maps of three transporter classes shown in Figures 4A-4C, and a global average (see Figure 4D and averaged power spectrum in Figure 2E).

There are two prominent classes in which the transporter ring encircles central material, that may represent transport substrates trapped within the channel during spheroplast lysis (Figures 4B and 4C). The diameter of the transporter in Figure 4C is ~13% larger than in Figure 4B, and this radial expansion was correlated with an increase in central substrate density (after scaling within the spokes). Indeed, the intensity of the central substrate disk was computationally truncated to provide an equivalent display of the spokes in Figures 4B and 4C. Transporters with a similar "in transit" morphology were identified previously in vNPCs (Figure 4E; Akey and Goldfarb, 1989; Akey, 1990). In Figure 4A, the central transporter ring surrounds an apparently empty central channel. However, a comparison of the channel density with background levels suggests that these transporters may contain some residual material, as this class may have originated from the two "in transit" classes by the loss of trapped substrates during isolation. Finally, the central placement of the transporter within the inner spoke ring creates a set of 8 low density features that may form diffusion pores in both "species" (open circles in Figures 4A and 4E). These data suggest that transporters are general features of all NPCs and reinforce the concept that the transporter represents a gated channel involved in nucleocytoplasmic transport (Akey and Goldfarb, 1989; Akey, 1990; Akey and Radermacher, 1993).

Three-Dimensional Structure of the yNPC

Three-dimensional maps were calculated of the yNPC to provide insights into the architecture of this assembly. However, we found that 3-D maps required ~500-600 yNPCs to provide an acceptable signal-to-noise ratio with these data. Hence, at this stage we computed a
Figure 4. Classification of yNPCs Reveals the Central Transporter
(A) Transporter class 1 contains a central ring-like transporter encircling a central channel. One of eight diffusion channels is labeled with an open circle.
(B) Transporter class 2 contains a transporter ring that encircles a central bright density that, by analogy with vNPCs, may be composed of substrates caught in transit (see Akey, 1990).
(C) Transporter class 3 contains a central ring with a greater radial expansion than observed in class 2.
(D) Global average of yNPC transporters. The spoke domains ([1] and [2]), the transporter ring (T), and the putative substrate disk (S) are labeled.
(E) A radially expanded vertebrate NPC transporter (Akey, 1990). Note the similar “in transit” morphology in both vertebrate and yeast transporters. Scale bar = 300 Å.

3-D map of the global transporter class (GBT class). In addition, we used classification to obtain the most homogeneous spoke class (SC), irrespective of the quality of the associated transporter, and computed the SC map.

Central cross-sections 100 Å thick and oriented parallel to the original position of the NE are shown in Figures 5A and 5C, for the GBT and SC 3-D maps. In general, the spokes are similar in both maps, but as expected, the central transporter is rather disordered in the SC map. In this view, the spokes form an inner spoke ring, comprised of spoke domain 1 and the proximal end of the linear arms (inner closed circle, Figure 5C). In addition, the second spoke domains are interconnected with the distal end of the linear arms (outer closed circle, bright density that may reflect substrates trapped within the cylindrical walls of the channel assembly (TW)). Over-all, the isolated yNPC is a disk with dimensions of 960 Å × 350–380 Å (Table 1). A strongly thresholded surface view of the spokes without the central transporter reveals the connectivity of the spoke domains (Figure 5D).

The second radial spoke domain extends from the inner spoke ring with its attendant bridging density, as filaments were observed radiating from the yNPCs in dark-field STEM images (not shown) and peripheral filament proteins such as Nup159p are retained in these preparations (Kraemer et al., 1995). The transporter in the GBT 3-D map (Figure 5B) contains a central bright density that may reflect substrates trapped within the nuclear membrane, while the most distal parts of the ring may protrude into the lumen. Hence, we have named this novel feature the “membrane ring.”

Additional evidence for this membrane ring was obtained after detergent or heparin extraction of NEs or yNPCs, respectively (Strambio-de-Castillia et al., 1995). Extracted NEs show a filamentous meshwork in which ring structures are embedded, while strongly heparinized yNPCs give rings with a diameter commensurate with the size of the membrane ring.

Prominent spoke features were revealed by calculating an angular projection that covered an arc of ~22.5°, centered on the spoke (Figure 5B). In this view, the spoke is composed of an inner spoke domain that spans the entire yNPC and a second domain located at the spoke midplane (dashed lines). These domains are joined at the top and bottom of the inner spoke ring by a bridging density that forms an angle of ~50° relative to the central plane (closed circle in Figure 5B). Weak density present at the top and bottom of the spokes (open arrows, Figure 5B) may represent disordered filaments and nuclear baskets, as filaments were observed extending from the yNPCs in dark-field STEM images (not shown) and peripheral filament proteins such as Nup159p are retained in these preparations (Kraemer et al., 1995). The transporter in the GBT 3-D map (Figure 5B) contains a central bright density that may reflect substrates trapped within the cylindrical walls of the channel assembly (TW).

Overall, the isolated yNPC is a disk with dimensions of 960 Å × 350–380 Å (Table 1). A strongly thresholded surface view of the spokes without the central transporter reveals the connectivity of the spoke domains (Figure 5D). The second radial spoke domain extends from the inner spoke ring with its attendant bridging density, and the linear arm is located between adjacent spokes.

The relationship between the central transporter, the inner spoke ring, and the membrane ring is shown in surface views of the GBT 3-D map (Figures 6A–6D). In Figures 6A and 6B, the spoke–transporter assembly is viewed obliquely from the bottom and top of the 3-D volume, respectively. Face-on and side-on views of the...
In vertebrate NPCs, the inner and outer nuclear membranes are in close apposition to the thin nuclear and cytoplasmic layers. This arrangement allows for the transport of molecules between the cytosol and nucleus. These internal channels may play a role in the passive diffusion of small molecules between the cytosol and nucleus.

**Discussion**

**Rationale for Comparing Yeast and Vertebrate NPCs**

Yeast and vertebrate cells represent different aspects of the basic eukaryotic cell design. Generally, yeast cells are smaller, undergo rapid cell division with a closed mitosis, and have less complex cytoskeletons due to the external support of a cell wall. Vertebrate cells possess elaborations not found in yeast: they have a lamina and larger nuclei, and their NEs disassemble at mitosis. In addition, they differentiate into many cell types and undergo apoptosis. Given the evolutionary distance between yeast and vertebrates, it is not unexpected that the structure of their NPCs would differ. However, both yeast and vNPCs must contain the minimal components required to build a functional NPC.

As a prelude to this study, it was necessary to prepare enriched fractions of yNPCs. However, isolated yNPCs display a range of physical deformations and mass. We therefore used rotational power spectra to select the best yNPCs for two- and three-dimensional maps, accounting for ~2%–5% of the yNPCs. We suggest that the maps in this study are representative of the core yNPC at the current resolution of ~80 Å in 2-D and ~120–150 Å in 3-D, excluding the putative peripheral filaments and baskets that are disordered. First, 16 yeast nups coenrich quantitatively with isolated NEs and yNPCs (Rout and Blobel, 1993; M. P. R., unpublished data). Second, all known nups (~30) have been identified in the yNPC preparation using mass spectrometry (M. P. R. et al., unpublished data). Third, filaments were observed radiating from yNPCs in dark-field STEM images. As filament components such as NuB159p are present in these preparations (Kraemer et al., 1995), the data indicate that peripherally associated nups are present in the isolated yNPCs. Thus, the best yNPCs are likely to contain a full (or nearly full) complement of nups.

All existing evidence suggests that yNPCs are fundamentally smaller than vNPCs. For example, yNPCs have a simple disk-like appearance in thin sections of whole cells, nuclei, and NEs but do not contain the distinctive features at the surface of the NE that correspond to the thin coaxial rings in vertebrate NPCs (Franke and Scheer, 1974; Rout and Blobel, 1993; Strambio-de-Cas-tilla et al., 1995). The overall dimensions of our structure are in excellent agreement with measurements from thin sections of yeast nucleii (Rout and Blobel, 1993), and the morphology is similar. Interestingly, the thickness of the NE lumen is markedly different in these two eukaryotes: in yeast, the NE is ~250–300 Å thick (31 measurements from thin sections of 6 yeast nuclei in cells), while in vertebrates, the NE is 500–600 Å wide (e.g., see Goldberg and Allen, 1996; Pante and Aebi, 1996). In vertebrate NPCs, the inner and outer nuclear membranes are in close apposition to the thin nuclear and cytoplasmic rings and their connecting spoke domains.
Figure 6. Surface Views of a 3-D Map of the Yeast NPC with a Globally Averaged Transporter

(A and B) Oblique views of the yNPC revealing the bottom and top surfaces of the yNPC. The membrane ring (MR) interconnects adjacent spokes, making contact with the linear arm (*). The cylindrical transporter resides within the inner spoke ring (ISR) and contains putative central substrates (S).

(C) An en face view of the yNPC reveals details of spoke domain architecture ([1] and [2]) and suggests that diffusion channels are located between the transporter and ISR (open circle). The membrane ring (MR), transporter (T), and putative central substrate (S) are labeled.

(D) Side view of the dform yNPC indicates that the membrane ring interconnects adjacent spokes and the intervening linear arm in a sinusoidal manner (see closed circles). Scale bar = 300 Å.

(Franke and Scheer, 1974; Jarnik and Aebi, 1991; Akey and Rademaker, 1993; Goldberg and Allen, 1996). However, the thin coaxial rings are not present in the yNPC 3-D map (see next section). We suggest that major differences in the thickness of the NE lumen are correlated with the vertical size of the spokes and their mode(s) of interaction with the nuclear membrane. Hence, this observation signals a fundamental change in the architectural scale of the NPC in the two distantly related “species.”

Additional evidence for the smaller yNPC is as follows. There is no lamin homolog in yeast; thus, domains of the nuclear thin ring and spoke surface that interact with the lamina in vertebrates (Jarnik and Aebi, 1991; Goldberg and Allen, 1996) are not required in yNPCs. Abundant transmembrane nups in vNPCs (e.g., gp210 and Pom121) are not present in yeast; hence, the corresponding lumenal spoke domains are absent in yNPCs. In addition, initial solubilization studies using both nuclei and NEs revealed yNPCs with a size and morphology similar to that found in the enriched preparation (Rout and Blobel, 1993). Finally, projection maps of both dform and mform yNPCs show a similar two-domain spoke morphology; yet these preparations are made very differently, and the nuclear membrane is expected to stabilize the spokes. When taken together, all of the morphological and biochemical data indicate that yNPCs are fundamentally smaller than vNPCs; hence, our 3-D map of the isolated yNPC is likely to represent the basic architecture of the spoke-transporter assembly.

Functional Implications of Differences in Yeast and Vertebrate NPC Structure

Surface maps of yeast and vNPCs are presented to scale in Figures 7A/7B and 7C/7D. The yNPC is smaller in diameter (960 Å versus 1450 Å) and height (~350 Å versus ~800 Å) than the vNPC, and this difference reflects the mass of the respective NPCs (~60 MDa versus ~125 MDa). However, vNPC domains located at high radius (the coaxial thin rings, cytoplasmic particles, and lumenal ring) form a more open structure, and this results in an ~5-fold volume increase relative to the yNPC. When viewed from the side, the vNPC is comprised of four major ring systems surrounding the central transporter, including the inner spoke ring, the cytoplasmic and nuclear thin rings, and the lumenal ring (Figures 1, 7C, and 7D). The isolated yNPC is simpler and is comprised of an inner spoke ring that encircles the central transporter and an outer membrane interacting ring. We did not detect structural equivalents of the thin cytoplasmic and nuclear rings, cytoplasmic particles, or the lumenal spoke domains in the yNPC (Figures 7A and 7B); however, the dimensions of our 3-D structure are in good agreement with measurements of yNPCs from thin sections of cells.

Why is the vNPC larger than the yeast pore complex? Yeast and vertebrate NPCs have undergone divergent evolution from a common ancestral pore complex; hence, significant changes in morphology have resulted from alterations in surface loop size within nup homologs. Thus, vertebrate nups are often larger than their yeast counterparts (e.g., vNup107p versus yNup84p). However, each NPC may also have a complement of nucleoporins that comprise domains not present in the other.

A more useful comparison of these distantly related NPCs can be made from diagrammatic cross-sections of the structures (Figures 8A and 8B; Akey, 1995). Importantly, the equivalence of the inner spoke ring and position of the NE was used to align vertebrate and yNPCs
Figure 7. A Comparison between 3-D Structures of Yeast and Vertebrate NPCs

(A) The yeast NPC as viewed from the putative cytoplasmic surface. The transporter (T) and putative substrates are marked (S).

(B) Side view of the yeast NPC showing the marked difference in height relative to the vNPC and the lack of thin rings on either surface.

(C) The dform vNPC as viewed from the cytoplasmic surface. The transporter (T) is partly obscured by a ring of collapsed cytoplasmic filaments (CF) that emanate from the cytoplasmic particles (CP). Akey and Radermacher, 1993). The radial arms are labeled (RA).

(D) Side view of the vNPC reveals the cytoplasmic and nuclear thin rings (CR/NR) that are integral to the spoke complex. A lumenal ring (LR) is formed by the lumenal spoke domain and adjacent radial arms (see closed circles). Scale bar = 300 Å.

as shown in Figure 8A and inset. In this alignment, the inner and central spoke domains of vNPCs have equivalent domains in yNPCs, with the central or second spoke domains making a similar transmembrane insertion. In addition, the radial and vertical dimensions of these two domains are similar (Table 1). As cytoplasmic and nuclear thin rings are not present in yNPCs, the spoke domains that support the thin rings are missing. These include the inner and outer vertical supports that form a part of the respective surfaces of the vertebrate spoke. As suggested, the thin coaxial rings and lumenal ring may stabilize the larger vertebrate spokes and function in transport or its regulation (Hinshaw et al., 1992; Akey and Radermacher, 1993; Akey, 1995).

Cytoplasmic filaments and the nuclear basket provide transport substrate docking sites (Richardson et al., 1988; Kiseleva et al., 1996; Pante and Aebi, 1996) and are attached to the cytoplasmic and nuclear thin rings in vNPCs (Jarnik and Aebi, 1991; Ris, 1991; Goldberg and Allen, 1992). Biochemical and morphological data suggest that yNPCs also have these peripheral assemblies (Rout and Blobel, 1993; Kraemer et al., 1995; M. P. R., unpublished data). Presumably, the primary attachment sites for these assemblies have been conserved, but may be augmented by the thin rings in vertebrates. For example, Nup88p has been implicated in the attachment of the cytoplasmic filament protein CAN/Nup214p to the vNPC (Bastos et al., 1997; Fornerod et al., 1997). However, there is no yeast protein with significant sequence similarity to Nup88p, although Nup159p is a yeast homolog of CAN/Nup214p; hence, Nup88p may represent a vertebrate-specific protein of the cytoplasmic rings or particles.

Yeast and vNPCs differ in their interactions with the NE, although they share conserved transmembrane spoke domains. Notably, yNPCs have a novel linear arm located between adjacent spokes that, together with spoke domain 2, forms a membrane ring (Figure 8B, left). The membrane ring may function to anchor the inner spoke ring of yNPCs into the NE and provide circumferential stabilization. Recently, Pom152p has been immunolocalized near the outer surface of the yNPC (Wozniak et al., 1994); hence, we suggest that Pom152p may comprise part of the membrane ring (Strambio-de-Castillia et al., 1995). Moreover, many nup mutants display an altered NE morphology (Doye and Hurt, 1997) that may reflect the close proximity of the spoke domains to the nuclear membrane in the smaller yNPC.

A feature unique to the vNPC is a ring present within the NE lumen, comprised of the luminal spoke domains and adjacent radial arms (Figure 8B, right). Gp210 is a major membrane protein of the vNPC and is thought to be a component of the lumenal ring (Greber et al., 1992; Wozniak and Blobel, 1992; Akey and Radermacher, 1993). Our data are consistent with the lumenal position of gp210, as yNPCs do not have a luminal ring and there is no gp210 homolog in the yeast. During open mitosis, vNPCs disassemble into soluble subcomplexes in response to phosphorylation (Macaulay et al., 1995; Favreau et al., 1996). As gp210 is specifically phosphorylated in mitosis (Favreau et al., 1996), this nup may play a role in NPC disassembly. Hence, the lack of gp210 and a luminal ring may reflect the absence of NPC disassembly during closed mitosis in yeast.

Recently, it was postulated that spoke conformational plasticity may mediate the down-regulation of both active transport and the diffusion of small molecules, in response to calcium depletion from the endoplasmic
Architecture of the Yeast Nuclear Pore Complex

Figure 8. A Comparison between Models of Vertebrate and Yeast NPCs Suggests that Evolutionary Divergence Has Resulted in Dramatic Changes in the Architectural Scale of This Organelle

(A) An idealized vertical section of the yeast (left) and vertebrate (right) NPCs, cut through the middle of the spokes. The yNPC is comprised of two radial spoke domains [1] and [2] with a bridging density between them; domain 1 encircles the cylindrical transporter. Putative cytoplasmic filaments and a nuclear basket are shown on the yNPC. Components of the vNPC are: cytoplasmic filaments (CF) and particles (CP), and the inner ring filaments (IRF) that connect the transporter with a central channel (CC) to the cytoplasmic (CR) and nuclear (NR) thin rings. Inset: Aligned spoke and transporter cross-sections from yeast and vertebrates. The yeast spoke is missing the vertebrate domains highlighted in pink, which include the vertical inner and outer domains (Vi/Vo) that support the cytoplasmic and nuclear thin rings (CR/NR) and frame the luminal spoke domain (LS). The inner spoke ring and central spoke domains are conserved in both species. The outer and inner nuclear membranes (ONM/INM) make similar contacts with the C or second spoke domains; however, the overall thickness of the NE lumen mimics the vertical dimension of the NPC. The yeast transporter appears to be missing a central cylinder (pink) that gives the vertebrate transporter its hourglass shape. The yeast transporter may retain two possible gating assemblies (shown in gray).

(B) An idealized cross-section of the yeast (left) and vertebrate (right) NPCs cut within the central plane, parallel to the NE. The NPCs show conserved and divergent interactions with the nuclear envelope (NE). Yeast NPC domains labeled as in (A) with the addition of the linear arm (LA). Vertebrate NPC: inner (IS), central (CS), and luminal spoke (LS) domains, radial arms (RA), transporter (T), and central channel (CC).

The Functions of Conserved Domains in NPCs

Many nup homologs are shared between yeast and vNPCs (e.g., yeast and human Nic96p; yNup170p/yNup157p and vNup155p; yNup84p and vNup107p; Doye and Hurt, 1997), and the transport factors are conserved (Koepp and Silver, 1996). Therefore, yeast and vNPCs are expected to share a common structure that mediates nuclear transport. A comparison between yeast and vNPCs reveals a conserved inner spoke ring (Figure 8), which may function as the central organizing framework of the pore complex. The inner spoke ring is attached to similar second/central spoke domains that penetrate the nuclear membrane. In addition, the proximal region of the linear arm in the yNPC (see Figures 5A and 5C) either forms part of the inner spoke ring or is attached to an ISR component. The inner spoke ring provides a conserved framework in which the central transporter is suspended (Figures 1 and 8). Moreover, these conserved spoke domains may play a role in seeding NPC assembly.

How are substrates translocated through the NPC and peripheral assemblies? Structural data suggest that nuclear transport may span two different environments. First, substrate complexes that are bound to the cytoplasmic filaments or nuclear basket must move to the transporter (Richardson et al., 1988; Kiseleva et al., 1996; Pante and Aebi, 1996; Rutherford et al., 1997). In a second step, substrates traverse a central channel within the NPC (Feldherr et al., 1984; Akey and Goldfarb, 1989;
Akey, 1990). In our studies, both yeast and vertebrate transporters demonstrate a similar ring-like morphology and an intrinsic ability to expand radially, when viewed in projection. This conserved feature may reflect an inherent gating property of the NPC, as macromolecules without NLSs or NESs must be excluded from the central channel.

The yeast transporter is a shortened cylinder, while the vertebrate transporter is more elongated with a tripartite hourglass shape (Akey and Radermacher, 1993). Recent STEM images from Chironomus NPCs (Goldberg and Allen, 1996; Kiseleva et al., 1998) are consistent with a tripartite transporter and suggest that there are two gate assemblies localized at the cytoplasmic and nuclear faces of the transporter, which may restrict access to the central channel (Akey, 1990; Akey and Radermacher, 1993). As the translocation mechanism is probably similar in yeast and vNPCs, we suggest that the yeast transporter is formed from two opposite-facing gate assemblies, without the central extension that gives the vertebrate transporter its hourglass shape (Figure 8A, inset). The reasons for the central extension in the vertebrate transporter are unclear, but may reflect adaptations to allow transport regulation, a more efficient translocation of large substrates, or compensation for the wider NE lumen. Finally, it has been suggested that vaults and transporters may be related, given their shared 8-fold symmetry (Rome et al., 1991). However, vaults have not been reported in yeast, and a search for the complete genome does not detect vault protein homologs, indicating that transporters are distinct from vaults.

The Evolution of NPCs

Our data suggest that NPCs have undergone a fundamental change in their architectural scale between yeast and vertebrates. Furthermore, a comparison of the two NPCs enforces new boundaries on our understanding of what is required to build a functional NPC. What sort of NPC was directly ancestral to both yeast and vNPCs? What is required to build a functional NPC? What are the expected mass of 60 MDa, similar to thresholds used for other low resolution maps (Akey and Radermacher, 1993).

Experimental Procedures

Specimen Preparation and Electron Microscopy

NPCs were isolated from Saccharomyces cerevisiae (NCYC74) in detergent-extracted and membrane-associated forms. The fractions (crude, enriched, and highly enriched) were isolated by the protocol of Rout and Blobel (1993). To minimize damage, the final sample used for 3-D analysis was frozen only twice. Sample preparation: 50-100 μL of enriched yNPCs were dialyzed against 100–200 ml of 10 mM Bis-Tris (pH 6.5)/0.1 mM MgCl2/20% DMSO/0.2% TX-100/1:1000 solution P (2 mg of pepstatin, 90 mg of PMSF in absolute ethanol) at 4°C for 8–12 hr; highly enriched yNPCs used 0.01% Tween 20. To prepare frozen specimen, 10 μL of sample was incubated for 40 min on carbon-coated and air–glow–discharged copper grids. Grids were manually double-blotted and plunged into liquid ethane in a humidity-controlled chamber (Dubochet et al., 1988). Cryogrids were loaded into a Gatan cryoholder (model 626-DH) and recorded at −176°C at 13,000× on a Philips CM12 with low dose kit and specimen relocation hardware. The first zero of the contrast transfer function was at 0.70 Å, and 50° tilt image pairs were collected for random conical tilt 3-D reconstruction (Radermacher, 1988).

Yeast NPCs associated with NEs were prepared by a modification of the method in Strambio-de-Castilla et al. (1995; M. P. R., unpublished data). Bound ribosomes were stripped from the NEs by 0.5 M KCl and 1.2 × 10−6 M puromycin treatment in 1.75 M sucrose/10 mM Bis-Tris (pH 6.3)/0.1 mM MgCl2/20% DMSO for 10 min at 20°C, followed by dialysis for 4 hr at 4°C in buffer without KCl/puromycin. Stripped NEs were spun onto air glow–discharged grids and negatively stained with 2% uranyl acetate. Low dose images were recorded at 13,000× with an appropriate defocus.

Image Processing

Tilt and zero micrograph pairs (n = 42) were densitometered on an Elkonix with an image step size of 20 Å and yNPCs chosen with the WEB particles option (Frank et al., 1996), based on the circular morphology of the zero tilt particles. Initially, ~10%–15% of the possible yNPCs were chosen (n = 3264), and rotational power spectra were calculated to allow a nonbiased selection of particles (Crowther and Amos, 1971; Kocsis et al., 1995). The ratio of the 8-fold harmonic for each particle relative to the best particle was used to create a percentile distribution, which indicated that 1565 yNPCs had detectable 8-fold signal. Image processing was carried out on VMS/Alpha workstations using SPIDER (Frank et al., 1996). Alignment of untilted yNPCs utilized a multireference approach, in which the 10 best particles were aligned separately against the top 866 particles to generate new references; subsequently, a final best reference was calculated and used to realign the dataset. Resolution was calculated using the differential phase residual for projections from half-datasets. Classification of the yNPCs into transporter and spoke classes used dynamic clouds and hierarchical ascendant classification (Frank, 1990; Akey, 1995), and final classes were merged using the dendrogram and visual inspection. The 3-D maps were calculated using the R-weighted back projection method (Radermacher, 1988) and refined using projection onto convex sets (Akey and Radermacher, 1993), to correct for the missing cone. The 3-D maps in the text are comprised of the global transporter class (GBT, n = 844) that contains all yNPCs with well-defined transporters, and the best spoke class (SC, n = 577). Final 3-D maps were Fourier-filtered to 120 Å resolution, and surface models were thresholded to give a reasonable connectivity. The calculated volume of the GBT 3-D map used in the surface views corresponds to ~150% of the expected mass of 60 MDa, similar to thresholds used for other low resolution maps (Akey and Radermacher, 1993).

Mass Analysis of yNPCs

The dform yNPCs were subjected to mass analysis by scanning transmission electron microscopy using a wet film sample preparation method (Wall and Hairfield, 1986), with TMV as a mass standard. In some cases, fixation with 0.1% formaldehyde/0.2% glutaraldehyde in sample buffer was done for 25 min. Images were recorded on the STEM1 at Brookhaven National Laboratory at 40 kV with a 2.5 Å diameter beam. The intensity of yNPCs was integrated after background correction and multiplied by the TMV scale factor to obtain the correct mass. Programs were provided by Dr. G. Sosinsky.
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