Architectural nucleoporins Nup157/170 and Nup133 are structurally related and descend from a second ancestral element

James R. R. Whittle and Thomas U. Schwartz

From the Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Running Title: Structures of Nup133 and Nup157/170

The nuclear pore complex (NPC) constitutes one of the largest protein assemblies in the eukaryotic cell and forms the exclusive gateway to the nucleus. The stable, ~15-20 MDa scaffold ring of the NPC is built from two multiprotein complexes arranged around a central eight-fold axis. Here we present crystal structures of two large architectural units, yNup170\textsubscript{979-1502} and hNup107\textsubscript{658-925}•hNup133\textsubscript{517-1156}, each a constituent of one of the two multiprotein complexes. Conservation of domain arrangement and of tertiary structure suggests that Nup157/170 and Nup133 derived from a common ancestor. Together with the previously established ancestral coatamer element (ACE1), these two elements constitute the major \(\alpha\)-helical building blocks of the NPC scaffold and define its branched, lattice-like architecture, similar to vesicle coats like COPII. We hypothesize that the extant NPC evolved early during eukaryotic evolution from a rudimentary structure composed of several identical copies of a few ancestral elements, later diversified and specified by gene duplication.

The membrane-enveloped nucleus is the hallmark of the eukaryotic cell. Physical separation of nucleoplasm and cytoplasm necessitates sites for molecular exchange (1-3). Nuclear pore complexes (NPCs), plugged into circular openings where inner and outer nuclear membranes fuse, perforate the nuclear envelope and form the sole gateway. The NPC is at ~50 MDa one of the largest protein assemblies in the quiescent cell. It is modular, comprises ~30 different proteins, termed nucleoporins (nups), and forms an eight-fold symmetric ring embedded in the nuclear envelope (4). In accord with the symmetry of the complex, each nucleoporin is present in \(8*n\) copies per NPC.

The architecture of the NPC is roughly conserved among eukaryotes, measuring about 100 nm in outer diameter, with a central transport gate about 40 nm wide (5-8). The NPC is a highly dynamic assembly. Some nucleoporins are stably attached while others are more dynamic (9-11). The main scaffold ring is composed of ~15 architectural nucleoporins that anchor to the inner pore wall. A second set of nucleoporins (FG-nups) is characterized by long, phenylalanine-glycine (FG) rich filamentous extensions. These FG-fibers emanate into the central cavity of the NPC and define the main transport barrier (12-14). Ions, metabolites, and macromolecules less than 20-40 kDa diffuse, for the most part, freely through the transport gate. Larger molecules pass only when bound to dedicated nuclear transport receptors, termed karyopherins, which directly interact with FG-nups (15-17). The small GTPase Ran regulates the interaction of protein cargo with import or export karyopherins, conferring directionality to these transport processes. This regulation depends on Ran being GTP-bound in the nucleus and GDP-bound in the cytoplasm, a gradient established by the action of cytoplasmic GTPase-activating protein (RanGAP) and nuclear GTP exchange factor (RanGEF).

To better understand the myriad of functions attributed to the NPC, which go far beyond transporting molecules across the NE (18,19), we are interested in the structural characterization of the NPC, which begins with the stable scaffold structure. The ~15 architectural nucleoporins are organized in two large multiprotein complexes – the well-studied Nup84-complex and the more enigmatic Nic96-complex. The components of each are known (Table 1). The Nup84-complex contains seven universally conserved nucleoporins and adopts a characteristically branched Y-shape (20-22). In this work, the Nup84-complex is referred to as the Y-complex. Nup120 and Nup85•Seh1 form the two short arms, whereas Nup145C•Sec13, Nup84, and Nup133 build the long, kinked stalk. The Nic96-complex likely contains five distinct nucleoporins, two of them duplicated in yeast (23-27). It connects to the NE (28) as well as the FG-network (29). These two scaffold complexes likely form ring-like assemblies. Whether these rings are stacked or
concentric, or arranged some other way, is controversial (30-33). This structural framework is important to the assembly and function of the NPC. Severe defects occur when scaffold nucleoporins are deleted or depleted, including failure to recruit other nucleoporins and diminished transport of protein or RNA across the nuclear membrane (34-41).

Superficially, the architectural nucleoporins are classified by computational methods as β-propeller domains, α-helical repeat domains, or tandem combinations thereof (Table 1) (4,42,43). Experimental structural characterization, however, has revealed that this simplistic description does not adequately reflect the reality. For example, Sec13 and Seh1 are predicted as 6-bladed β-propellers, but turn out to be 7-bladed, with the final blade provided in trans by their respective binding partners (31,32,44,45). The four ACE1 nucleoporins are built around a ~65 kDa α-helical domain. They are distantly related to one another, and, strikingly also to Sec31, the main structural component of the outer coat of the COPII vesicle. This ACE1 domain is a tripartite fold-back structure of ~28 α-helices, distinct from the regular α-solenoid domains found in HEAT-, TPR-, or PPR-repeat proteins (46,47), among others. The structural similarity between these ACE1 proteins provided the proof that the NPC and COPII coat derive from a common ancestor (32), as hypothesized previously (42,43).

The ACE1 nucleoporin Nup84 binds Nup133. The structure of a fragment of the human Nup84 ortholog, hNup107 658-925, has been solved in complex with hNup133 934-1156 (48), the C terminus of the protein. This structure showed that the C terminus of Nup133 consists of two α-helical blocks. A rigid block of four α-helices, residues 934-1008, forms an interface bundle that binds Nup84(hNup107). A moderately flexible hinge connects this interface bundle to a second α-helical unit that forms a distinct lobe at the C terminus of the protein. The N terminus of Nup133 is a β-propeller, whose structure is also known (49). hNup133 934-1156 suggested that Nup133 is not an ACE1 protein.

Here we present crystallographic analysis of two architectural units, yNup170 979-1502 and hNup107 658-925•hNup133 517-1156, components of both major scaffold complexes of the NPC. Nup170, its ortholog Nup157, and Nup133 each consist of an N-terminal β-propeller followed by an ~80 kDa C-terminal α-helical domain. The structures reveal a common α-helical architecture for Nup157/170 and Nup133 that is distinct from all other known nucleoporins. This α-helical architecture is, with ACE1, another ancestral element of the NPC. We conclude that the basic NPC framework is built from a small set of recognizable structural elements that were already present in multiple copies in the last common ancestor of extant eukaryotes. During the course of evolution, gene duplications occurred and diversified these core elements, generating the complex, multi-functional machine that is the NPC.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—Nup170 from S. cerevisiae was cloned into a pET-Duet vector (Novagen) encoding an N-terminal, human rhinovirus 3C (HR3C)-cleavable His6-tag. N-terminal truncations were generated by PCR methods. Nup133 (residues 517-1156) and Nup107 (residues 658-925) from H. sapiens were cloned into a bicistronic pET-Duet vector, modified to encode N-terminal, thrombin-cleavable His6-tags. Proteins were expressed in E. coli strain BL21 (DE3)-RIL (Stratagene) in LB medium, induced with 200 μM isopropyl-β-D-1-thiogalactopyranoside at 18°C.

Cells expressing yNup170 979-1502 or yNup170 1253-1502 were homogenized at 4 °C in 50 mM Tris-HCl pH 8.5, 400 mM NaCl, 40 mM imidazol, 5 mM β-mercaptoethanol (β-ME). The protein was bound to Ni-affinity resin and eluted with 250 mM imidazole, dialyzed against 20 mM Tris-HCl pH 8.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM DTT, and purified, after affinity tags were removed, on a Superdex S75 column (GE Healthcare), equilibrated in 10 mM Tris-HCl pH 8.5, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT. SeMet-substituted protein was expressed as described (32).

hNup107 658-925•hNup133 517-1156 were bound to Ni-affinity resin in lysis buffer, 20 mM Tris-HCl, 5 mM potassium phosphate (KP) pH 8.5, 250 mM NaCl, 10 mM imidazole, 5 mM β-ME, then eluted with 250 mM imidazole, and purified, after the
affinity tags were removed, on a HiTrap FF and then a Superdex 200 column, equilibrated in 5 mM KP pH 7, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT.

Protein Crystallization—yNup170_{979-1502} was concentrated to 4-8 mg ml\(^{-1}\). An initial crystallization condition was found by vapor diffusion using commercial screens. Crystallization was greatly improved by addition of TCEP. Crystals were grown at 4 °C, in 1 µl hanging drops over 0.2 M NH\(_4\)Ac, 0.1 M Tris-HCl pH 7.9, 5-10 % polyethylene glycol (PEG) 3,350, 5 mM TCEP. Rods, \(\sim 80-100 \mu m \times \sim 80-100 \mu m \times 300-400 \mu m\), with isosceles triangular bases, formed within 3 days. They were flash-frozen in reservoir supplemented with 24% PEG 200, 24% ethylene glycol, or 25 % glycerol.

yNup170_{1253-1502} crystallized at 90 mg ml\(^{-1}\) in 1µl hanging drops over 0.1 M Tris-HCl pH 8.5, 0.2 M Li\(_2\)SO\(_4\), 50 mM NaCl, 22-24% PEG 3,350 within 3-5 days at 18 °C. Plates, 50 µm x 300 µm x 300 µm, were cryoprotected in reservoir solution supplemented with 12% glycerol and flash-frozen.

hNup107_{658-925}•hNup133_{517-1156} was concentrated to 9 mg ml\(^{-1}\) and 2% PEG 3,350 added. An initial crystallization condition was found using commercial screens. After optimization, crystals were grown in drops of 1 µl protein, supplemented with 2% PEG 3,3350, + 1 µl reservoir of 0.8-1.0 M sodium/potassium phosphate pH 7.8, 15% glycerol at 18 °C, streak-seeded after 12 hours with microcrystals. In most drops, phase separation rather than crystallization was observed. Occasionally, thin needles with dimensions of 30 µm x 30 µm x 150 µm grew within 2 days. Crystals were retrieved on MicroMounts (Mitegen), and flash-frozen in liquid nitrogen.

Data Collection, Structure Solution and Refinement—Data for yNup170_{979-1502} were collected at 100 K at microfocus beamline 24-IDE at the Advanced Photon Source (Argonne, IL). The crystals that diffracted best, to \(\sim 2.5 \AA\), were perfectly merohedrally twinned. Untwinned data were obtained to 3.2 Å and used for further analysis. Data were collected from SeMet-labeled crystals and processed with the HKL2000 package (50). Phases were determined by Se-SAD. 9 of 10 possible Se sites were identified with SOLVE (51). Se positions were refined with SOLVE-REVER (52), which also revealed the additional Se site, and experimental phases calculated. The resulting solvent-flattened electron density map was used to build a model with Coot (53).

To improve model quality and attempt to refine against the twinned data diffracting to higher resolution, the C-terminal subdomain yNup170_{1253-1502} was crystallized and 2.2 Å data collected at beamline 24-IDC at the Advanced Photon Source from a large crystal formed from several caked layers. The strongest of the observed diffraction patterns was indexed and integrated. Out of many specimen tested, morphologically indistinguishable, only this crystal diffracted strongly and belonged to space group I222 (Table 2). All others belonged to space group P6\(_3\)22, diffracted to 3.5 Å, and were not further analyzed.

A molecular replacement solution for yNup170_{1253-1502} was found using a partial model from the initially obtained 3.2 Å structure of yNup170_{979-1502}. A complete model for yNup170_{1253-1502} was built automatically, with minor intervention, using PHENIX (54). yNup170_{979-1502} was rebuilt incorporating this partial model, improving refinement parameters. We note, however, that the model of the yNup170 C-terminal subdomain did not help process the twinned 2.5Å data (not shown).

Data for hNup107_{658-925}•hNup133_{517-1156} were collected at 100 K at 24-IDE. Due to significant radiation damage, partial data sets were collected and merged from several crystals grown in the same crystallization drop, each exposed at 3-10 spots. The structure was phased by molecular replacement using the minimal 55 kDa hNup107_{658-925}•hNup133_{344-1156} interaction complex (48) as a search model in Phaser (55) in the CCP4 suite (56). The additional 45 kDa domain was built and refined with Coot (53) and PHENIX (54). Data to 3.5 Å were included, despite low I/σI, as recommended for low-resolution crystallography (57). Anisotropic diffraction was corrected by elliptical resolution truncation and anisotropic B-factor correction using the Diffraction Anisotropy Server (www.doe-mbi.ucla.edu/~sawaya/anisoscale/) (58). The obtained electron density maps allowed positioning of the secondary structure elements, which are essentially all \(\alpha\)-helical. Connections between helices were mostly visible, allowing tracing of the molecule from N to C terminus. Observed chain topology and variation in the
length of helices allowed us to assign each modeled helix unambiguously to the secondary structure as predicted by the PredictProtein server (59). In the absence of detailed positional markers, the assigned sequence in the deposited data is approximate, but is likely erroneous only in a few places and shifted by not more than 3-4 residues, i.e. one α-helical turn. Several non-helical loops could be traced confidently, including loops that are disordered in the partial structure hNup107658-925•hNup133304-1156 (48).

**Structure Analysis**—Nup170 homologs were retrieved from the NCBI website database (http://www.ncbi.nlm.nih.gov/) and a multiple sequence alignment calculated by the MUSCLE algorithm (60). An Average Distance Tree was used to select representative, divergent sequences. Residues were scored for conservation by the AMAS method in JALVIEW (61). PDB2PQR (62) and APBS (63) were used to calculate surface charge, and the PISA server to calculate accessible surface area (64). MODELLER (65) was used to build a complete model of yNup157900-1391. Pymol (http://www.pymol.org) was used to generate figures.

**RESULTS**

**Structure of the α-helical domain of Nup170**—Nup170 is predicted to contain two structural domains, an N-terminal β-propeller (residues 180-650) and a C-terminal α-helical domain (Fig. 1A) (4,42). By expressing a series of N-terminal truncations of the protein and by limited proteolysis, we defined a stable core of the predicted α-helical region, comprising residues 979 to 1502 of Nup170 from *S. cerevisiae* (data not shown). The presumed N-terminal β-propeller domain interacts weakly with the α-helical domain when separated, indicating flexible attachment (66). yNup170979-1502 was expressed recombinantly in *E. coli*, purified to homogeneity, and crystallized. The structure was solved by single-wavelength anomalous dispersion (SAD), using selenomethionine-labeled protein. The asymmetric unit contains one molecule. The experimental SAD electron density allowed for building residues 1020-1460, revealing a continuous, but bipartite stacked α-helical domain (Fig. 1). Due to the lack of strong crystal contacts, the C-terminal half of the domain is flexibly positioned. Thus, to aid structure determination, this C-terminal 29 kDa subdomain (residues 1253-1502) was separately expressed and crystallized. Data to 2.2 Å resolution were collected and phased by molecular replacement with the relevant portion of the larger protein as initially modeled. The complete, refined model of the C-terminal subdomain (*R*\(_{free}/R = 27.6/23.3\%\)) was then used to build the structure of yNup170979-1502 at 3.2 Å resolution (*R*\(_{free}/R = 32.4/30.6\%\)). Representative electron density for the 3.2 Å resolution structure is shown in Fig. S1. The crystal packing of the 2.2 Å resolution structure is shown in Movie S1. Data collection and refinement statistics are summarized in Table 2.

yNup170979-1502 adopts an irregular α-helical stack composed of 26 α-helices and overall dimensions of 12 nm x 4 nm x 4 nm (Fig. 1). We label these helices α1-26. The domain begins with helices α1/2, α3/4 and α6/7 forming three consecutive pairs of helices, of various lengths, stacked antiparallel, without superhelical twist. Helix α5 resides in a loop and does not pair to other helices. Helices α8-13 form an extended zig-zag pattern that is rotated by about 90° against the α1-7 stack. This zig-zag can be likened to a stack of three α-helical pairs that has been stretched by pulling on its ends. As a result, helices α8-13 extend over ~38 Å, reflecting a ~50% stretch compared to a tightly packed 6-helix stack, which would span only ~26 Å. The hydrophobic core of this extended zig-zag is poorly packed. Few residues are fully buried. Helix α14 is about twice as long as its direct neighbors and connects the two α-helical subdomains. The C-terminal subdomain forms a crescent only loosely definable as a stack. It starts with α15, unexpectedly positioned below, not above, helix α14. This helix abuts end-on-end to α12 of the N-terminal subdomain. The strictly conserved arginine 1232 is sandwiched between the negatively polarized C-termini of α12 and α15, presumably for charge-compensation. Nup170 then continues with helices α16-26 forming a compact hydrophobic core, implying rigidity.

To compare the structure of Nup170 with those of other proteins, we performed structure-based searches with VAST and DALI (67,68). Neither returns significant alignments. No protein aligns to Nup170 over more than 6 consecutive helices. We
conclude that Nup170 has only remote structural similarity to known proteins. 

**Nup133 adopts a quadripartite domain**—Nup133 and Nup170 are predicted to have a similar overall topology. Both comprise an N-terminal β-propeller domain linked to a C-terminal α-helical domain (Fig. 1A and 2A). To compare directly the α-helical domains of Nup133 and Nup170, we solved the structure of the complete α-helical domain of hNup133 in complex with hNup107. hNup107658-925 and hNup133517-1156 were co-expressed recombinantly in *E. coli*, purified, and crystallized. The structure was solved by molecular replacement using the 57 kDa Nup107658-925•Nup133934-1156 interaction complex (PDB 3CQC) (48). The asymmetric unit contains one heterodimer. Crystallographic analysis was challenging, because crystals were small and difficult to grow, suffered severe radiation damage, and diffracted anisotropically. Based on the resulting electron density map, we were able to assign all the helices of Nup133 and unambiguously determine the overall topology. Most connecting loops are also visible in the electron density. The 105 kDa complex was refined to R<sub>free</sub>/R = 37.0/31.2% (Table 2). The model and electron density for the novel portion is shown in Fig. S2.

hNup133517-1156 forms an elongated structure composed of 28 helices (Fig. 2). Because the N-terminal β-propeller domain of hNup133 has three helices inserted into it (PDB 1XKS) (49), we number hNup133517-1156 beginning at helix α4. The domain can be described as quadripartite. From the N-terminus, it starts with a block of 12 long helices, which form a wide and flat plane. These helices are arranged pairwise and antiparallel, except helices α9-10, which form an overhand turn. The following six helices (residues 854-944) are all short (2-3 turns) and zig-zag upward, covering a distance of 44 Å. Helices α21-24 make up the interface with Nup107 and form a α-helical bundle as described previously (48). Finally, helices α25-31 fold into a compact C-terminal subdomain.

**Comparison to minimal interacting complex**—The Nup107 moiety in the complex solved here is identical to the minimal interaction fragment previously reported. The portion of Nup133 solved here includes the entire helical portion solved previously and the 418 amino acids that connect it to the N-terminal domain. The C-terminal subdomain of Nup133 forms a crystal contact in the current structure. This subdomain is therefore more stable than in the minimal interacting complex. Several loops not modeled previously are apparent. The metazoan-specific finger helix of Nup107 (labeled α6' in Fig. 2B) also forms a crystal contact causing it to bend more than in the previous structure. Otherwise no noteworthy rearrangements occur.

**Structural comparison of Nup170 and Nup133**—Sequence- and structure-based alignments suggest remote homology between Nup170 and Nup133. Due to the multi-partite nature of the two proteins and the apparent hinges connecting the subdomains, an overall superimposition is not very informative. However, if the separate subdomains are superposed individually, commonalities become apparent (Fig. 3). The most striking similarity exists between the middle segments of Nup170 and Nup133, where the helices are short and form a characteristically extended zig-zag structure. An elastic network model suggests that both molecules may flex about this central zig-zag (Movies S2 and S3). Further, the Nup133 interface bundle and the connection to the C-terminal lobe are particularly conserved. The C-terminal lobe of Nup170 is larger than that of Nup133 (34 kDa versus 27 kDa). N-terminally attached to the zig-zag are helices that form tightly packed bundles, however in Nup133 they form a flat and extended plane, while in Nup170 we observe three tightly stacked α-helical pairs. This remote, but distinctive structural homology is matched by homology observed in the amino acid sequence. A PSI-BLAST search with hNup133517-1156 predominantly returns homologs of Nup133 and Nup170. Detected similarity spans the entire region that is structurally similar (Nup133 residues 830-1120, Nup170 residues 1150-1380). This search result suggests that Nup170 and Nup133 are more closely related to each other than to other proteins.

**yNup170979-1502 has two conserved surface features**—Nup170 is integrated into the structural framework of the NPC and must interact with other nucleoporins to exert its function, however the direct interaction partners are not yet firmly
established. Interaction sites can often be identified by conservation of neighboring surface-exposed residues. We generated a maximally diverse alignment of Nup170 sequences across all eukaryotes (Fig. S3). Surface representation of Nup170 colored by conservation suggests two conserved surfaces (Fig. 4). Helices α11-14 contribute to the first surface, which is mildly conserved and negatively charged. According to the structural alignment, Nup170 helices α11-14 correspond to the Nup133 interface bundle, the group of helices by which Nup133 binds Nup107. This alignment is shown in detail in Fig. S4. The conserved surface on Nup170 corresponds to the Nup133-Nup107 interface. In Nup133, this surface is hydrophobic. The double mutant L973E L976E prevents interaction with Nup107 by placing charged sidechains in the interface (48). The corresponding residues in Nup170, E1234 and R1238, are charged. If Nup170 binds another protein by this interface, the interaction is not hydrophobic, and likely weaker.

The second surface, built by helices α16-18, is more strongly conserved and forms a hydrophobic groove. In the sequence alignment (Fig. 4F), the exposed neighboring residues are conserved: S1305 as serine or threonine, F1308 as phenylalanine or tyrosine, F1325 as a large hydrophobic residue. Therefore, this region has the characteristics of a typical protein-protein interface. Interestingly, in the 2.2 Å crystal structure of the short yNup1701253-1502, the N-terminal helix, α14, is unwound, and the first 10 residues wrap around the surface of the protein and align in the hydrophobic groove (Fig. 4C). One may speculate whether this rearrangement and interaction has physiological relevance. With the full α-helical domain present, this motion of helix α14 would reorient the N-terminal portion dramatically, causing it to extend opposite the direction observed in this structure. Alternatively, we can consider the intramolecular interaction of these 10 residues as a serendipitous crystal artifact, which may mimic the interaction with the natural binding partner. In the later case, this structure would suggest the physical mechanism by which such interaction occurs.

Comparison of Nup170 to Nup157—In S. cerevisiae, Nup170 has a paralog, Nup157, not present in metazoa (26), likely a consequence of the whole genome duplication that occurred in the ancestor of Saccharomyces (69). We modeled the homologous α-helical domain of Nup157, which can be done confidently (sequence identity is 41% for the 60 kDa segment in question). We note that the exposed residues at the second conserved surface of Nup170 are identical in Nup157, except residue 1328 is serine instead of alanine. If this hydrophobic groove is a protein interface, we predict Nup170 and Nup157 bind to the same molecule. The most apparent difference between the α-helical domains of Nup170 and Nup157 are three insertions in the very N-terminal portion of the α-helical domain of Nup170, not part of this structure. These Nup170 extensions are predicted as disordered loops. Thus, it is likely that the structures of the two proteins have a very similar topology across the entire α-helical region.

**DISCUSSION**

The size, complexity and heterogeneity of the NPC make this complex a formidable challenge to structural biology. However, since the NPC is assembled from subcomplexes in modular fashion, the high resolution structure can be approached by a divide-and-conquer strategy (4). Two multiprotein complexes compose the principal scaffold architecture – the Y-complex and the Nic96-complex (30). We report here one structure from each: hNup107658-925-hNup133517-1156 of the Y-complex and yNup170979-1502 of the Nic96-complex. Structural similarities between Nup133 and Nup170 indicate that these nucleoporins descend from a common ancestor. Since ACE1 proteins are also found in both major structural complexes of the NPC we conclude that both complexes employ similar structural principles. The extant NPC likely derived from duplication and diversification of a few ancestral genes.

The structure of Nup133 provides a significant portion for a now nearly complete high resolution model of the heptameric core of the Y-complex, as cartooned in Fig. 5. This complex has been studied extensively by crystallography and electron microscopy (20,31,32,45,48,49,70). A central hub connects two short arms to a long and kinked stalk (20,21). The nucleoporins that build this Y connect via binary interactions with strong affinities (32,48). Crystal structures of most elements are now known. Seh1•Nup85 and Nup120 form the short arms (32,70).
Sec13•Nup145C is the proximal segment of the kinked stalk (31); Nup84 is the chain-link between Nup145C and the distal Nup133.

Electron micrographs of the Y-complex suggest that some segments can articulate with respect to one another (20,71). Our hNup107•hNup133 reveals one molecular determinant of that flexibility. The extended zig-zig at the center of the Nup133 α-helical domain provides an accordion-like transition that allows the flat, N-terminal α-helical plane of Nup133 to flex against Nup84(hNup107). This motion can be simulated using an elastic network model (72). In addition, there is a second hinge between the Nup84(hNup107) binding interface and the compact, C-terminal, all α-helical domain (49).

The Nic96-complex is less well studied than the Y-complex. At high resolution, the ACE1 domain of Nic96 is known (73,74), as well as the 15 kDa homodimerization domain of Nup53(hNup35) (75). Both structures are uncomplexed and the direct interaction partners within the Nic96-complex are not known with certainty. Nup157/170 is another member of this complex. Here we report the structure of a major portion of the α-helical stack domain of Nup170. Like Nup133, Nup170 contains a flexibly tethered N-terminal β-propeller, followed by a large, ~70 kDa, α-helical domain. The α-helical domains of Nup133 and Nup170 both are divided into rigid segments connected via flexible hinges. Pairwise superposition of these segments accentuates the structural resemblance between the two proteins, particularly in the central zig-zag and in the interface bundle (Fig. 4).

The Nic96-complex is apparently not as stably associated as the Y-complex. The many different interactions that have been reported for the Nic96-complex indicate that it has a role as a connector. The interaction with the FG-Nup-Nsp1 complex (29) on the one hand and Ndc1 on the other (76) indicates that it spans the width of the NPC scaffold.

The crystal structures solved here solidify the notion that the major scaffold complexes of the NPC are structurally related. On that basis, we suggest that the Nic96-complex adopts a branched structure and that its components are joined through binary interactions. In Nup133, the interaction with Nup84 tethers the protein to the NPC (48). It is known that, as in Nup133, the α-helical domain of Nup170 is necessary and sufficient to target the protein to the NPC (66). We show here that the surface by which Nup133 binds Nup84 is also conserved in Nup157/170, but is not as hydrophobic, arguing for perhaps a more dynamic interaction.

Weaker interactions and protein-peptide interactions will play auxiliary roles in the assembly of both scaffold complexes, and in their function. For example, the N-terminal 29 residues of the mRNA export factor Gle1 tether it via hNup155(yNup157/170) to the NPC (77). An interaction between Nup120 and Nup157/170 may join the two complexes (78).

A comparison of a variety of scaffold nucleoporins is now possible. One class shares the common ACE1 domain (32), also found in the COPII coat protein Sec31 (44). This class includes Nup85, Nup145C, Nic96 and Nup84. ACE1 consists of three α-helical modules, termed crown, trunk and tail. These fold back onto themselves to form a U-turn within the crown module. The trunk is composed of two α-helical units running in opposite directions, capped by the tail module. The tail of Nup84(hNup107) is shown here in complex with Nup133, and the tails of other ACE1 domains also support protein-protein interfaces (32,73). The ACE1 trunk domain, with multiple helices embedded in the hydrophobic core, confers greater rigidity than structures built of stacked α-helical pairs, which are more flexible. For example, all nuclear transport receptors are constructed from repeated α-helical pairs or triples, and the functional significance of flexibility is well documented (79). In Nup133 and Nup157/170, the helices do not fold back on one another as in the ACE1 trunk. Instead they typically pair, albeit without a recognizable repeat pattern. Consequently, they are more flexible than ACE1 proteins. Further, Nup133 and Nup157/170 show greater variation than the ACE1 – while a central core has much the same accordion-like structure in both, flanking this core there is substantial variation.

As both Nup133 and Nup157/170 have a β-propeller/α-helical stack tandem arrangement, one can speculate whether this alone indicates a common ancestry. However, Nup120, another nucleoporin with this domain arrangement, resembles neither Nup133 nor Nup170, nor ACE1 (70).
The overall topologies of each class of scaffold nucleoporin are shown in Fig 6. The α-helical domains of Nup133 and the ACE1 Nic96 are equally long (Fig. 6A,B), however the N-terminus of the ACE1 is at the middle of the domain rather than at one end. Nup120 has a more convoluted architecture and is shorter (Fig. 6C). Its α-helical domain is interrupted by a blade of the N-terminal β-propeller, with which it integrates to form one compact entity. Taken together, these three domain classes are not really structurally related, other than all being α-helical.

The assembly principles of vesicle and nuclear pore membrane coats are related and some of their components evolved from common ancestors. The structural similarity between the ACE1 proteins of the NPC and of the COPII vesicle coat established the common ancestry (32), as hypothesized previously (42,43). Both coat assemblies also incorporate the bifunctional protein Sec13. We expect that the scaffold structure of the NPC is as open and lattice-like as the COPII coat and has similar connectivities in parts. However, Nup133 and Nup170 have no known structural homologs outside the NPC and their specific integration into the NPC scaffold needs to be further analyzed. In addition, the NPC core scaffold contains two other large α-helical proteins, Nup188 and Nup192 (80,81), whose structures may reveal new surprises.

To build a rudimentary NPC scaffold, one might need only a few different structural building blocks. In an early eukaryote, we speculate, multiple copies of a small number of distinguishable elements formed a complete NPC scaffold, Gene duplications then created families of related nucleoporins. The members of each family evolved divergently into the distinct, non-redundant structural elements of extant NPCs. This theory for the origin of the NPC explains the perplexing observation that many structural nucleoporins are not essential in yeast. Under stressed conditions, these genes can still partially complement one another. Given that the same structural elements can be detected in the NPCs of all extant eukaryotes, this process of diversification must have occurred in the early eukaryote. No extant eukaryote has a scaffold significantly simpler than the yeast or human NPC (82). The NPC likely evolved early in eukaryotic evolution, by multiplication of a few structural elements. Today, these multiplications are still evident, though the amino acid sequences of these ancestral elements have diverged greatly.

With a growing inventory of nucleoporin structures, the next task will be to determine the higher-order assembly of the NPC. The anatomy of the NPC has been delineated by cryo-electron tomography (6-8) and a computational analysis produced a draft of the NPC (30), but there are still various ways in which these nucleoporins might arrange to form the entire assembly. The lattice-like structure of the NPC provides few spatial restraints, and interactions between complexes are largely still unknown. However, the structures of the architectural nucleoporins – those presented here and those already available – narrow the speculations about the NPC assembly. These structures now allow us to probe the NPC by genetic manipulation of specific structural elements. Some models already can be ruled out. Altogether, from a combination of structural, computational, and cell biology research, the structure of the NPC is fast emerging.

REFERENCES

1. Tran, E. J., and Wente, S. R. (2006) Cell 125, 1041-1053
2. Weis, K. (2003) Cell 112, 441-451
3. D'Angelo, M. A., and Hetzer, M. W. (2008) Trends Cell Biol 18, 456-466
4. Schwartz, T. U. (2005) Curr Opin Struct Biol 15, 221-226
5. Pante, N., and Kann, M. (2002) Mol Biol Cell 13, 425-434
6. Beck, M., Lucic, V., Forster, F., Baumeister, W., and Medalia, O. (2007) Nature 449, 611-615
7. Stoffler, D., Feja, B., Fahrenkrog, B., Walz, J., Typke, D., and Aebl, U. (2003) J Mol Biol 328, 119-130
8. Beck, M., Forster, F., Ecke, M., Plitzko, J. M., Melchior, F., Gerisch, G., Baumeister, W., and Medalia, O. (2004) Science 306, 1387-1390
9. Rabut, G., Doye, V., and Ellenberg, J. (2004) Nat Cell Biol 6, 1114-1121
10. Rabut, G., Lenart, P., and Ellenberg, J. (2004) Curr Opin Cell Biol 16, 314-321
11. Dultz, E., Zanin, E., Wurzenberger, C., Braun, M., Rabut, G., Sironi, L., and Ellenberg, J. (2008) J Cell Biol 180, 857-865
12. Lim, R. Y., Fahrenkrog, B., Koser, J., Schwarz-Herion, K., Deng, J., and Aebi, U. (2007) Science 318, 640-643
13. Frey, S., and Gorlich, D. (2007) Cell 130, 512-523
14. Jovanovic-Talisman, T., Tetenbaum-Novatt, J., McKenney, A. S., Zilman, A., Peters, R., Rout, M. P., and Chait, B. T. (2009) Nature 457, 1023-1027
15. Cook, A., Bono, F., Jinek, M., and Conti, E. (2007) Nat Rev Mol Cell Biol 8, 195-208
16. Zabel, U., Doye, V., Tekotte, H., Wepf, R., Grandi, P., and Hurt, E. (1996) J Cell Biol 133, 1141-1152
17. Marelli, M., Aitchison, J. D., and Wozniak, R. W. (1998) EMBO J 17, 6449-6464
18. Miller, B. R., Powers, M., Park, M., Fischer, W., and Forbes, D. J. (2000) Mol Biol Cell 11, 3381-3396
19. Zabel, U., Doye, V., Tekotte, H., Wepf, R., Grandi, P., and Hurt, E. C. (1996) J Cell Biol 133, 1141-1152
20. Aitchison, J. D., Rout, M. P., Marelli, M., Blobel, G., and Wozniak, R. W. (1995) J Cell Biol 131, 339-354
21. Alber, F., Dokudovskaya, S., Veenhoff, L. M., Zhang, W., Kipper, J., Devos, D., Suprapto, A., Karni-Schmidt, O., Williams, R., Chait, B. T., Sali, A., and Rout, M. P. (2007) Nature 450, 695-701
22. Hsia, K. C., Stavropoulos, P., Blobel, G., and Hoelz, A. (2007) Cell 131, 1313-1326
23. Brohawn, S. G., Leksa, N. C., Spear, E. D., Rajashankar, K. R., and Schwartz, T. U. (2008) Cell 131, 339-354
24. Gao, H., Sumanaweera, N., Bailar, S. M., and Stochaj, U. (2003) J Biol Chem 278, 25331-25340
40. Bai, S. W., Rouquette, J., Umeda, M., Faigle, W., Loew, D., Sazer, S., and Doye, V. (2004) *Mol Cell Biol* **24**, 6379-6392
41. Shulga, N., Mosammaparast, N., Wozniak, R., and Goldfarb, D. S. (2000) *J Cell Biol* **149**, 1027-1038
42. Devos, D., Dokudovskaya, S., Williams, R., Alber, F., Eswar, N., Chait, B. T., Rout, M. P., and Sali, A. (2006) *Proc Natl Acad Sci U S A* **103**, 2172-2177
43. Devos, D., Dokudovskaya, S., Alber, F., Williams, R., Chait, B. T., Sali, A., and Rout, M. P. (2004) *PLoS Biol* **2**, e380
44. Fath, S., Mancias, J. D., Bi, X., and Goldberg, J. (2007) *Cell* **129**, 1325-1336
45. Andrade, M. A., Petosa, C., O'Donoghue, S. I., Muller, C. W., and Bork, P. (2004) *J Mol Biol* **309**, 1-18
46. Bohmer, T., Jeudy, S., Berke, I. C., and Schwartz, T. U. (2008) *Mol Cell** **30**, 721-731
47. Otwinowski, Z., and Minor, W. (1997) *Macromolecular Crystallography, Pt A* **276**, 307-326
48. Sheldrick, G. M. (2008) *Acta Crystallogr A* **64**, 112-122
49. Imperato, M., and Bolognesi, P. (2004) *J Mol Biol* **309**, 1-18
50. Adams, P. D., Grosse-Kunstleve, R. W., Ioerger, T. R., McCoy, A. J., Moriarty, N. W., Read, R. J. (2002) *Acta Crystallogr D Biol Crystallogr* **58**, 1948-1954
51. Smith, D. L., and Bork, P. (2004) *Proc Natl Acad Sci U S A* **101**, 1-6
52. Kleyweg, T., and Bork, P. (2004) *PLoS Biol* **2**, e380
53. Fath, S., Mancias, J. D., Bi, X., and Goldberg, J. (2007) *Cell* **129**, 1325-1336
54. Devos, D., Dokudovskaya, S., Alber, F., Williams, R., Chait, B. T., Sali, A., and Rout, M. P. (2004) *PLoS Biol* **2**, e380
55. Imperato, M., and Bolognesi, P. (2004) *J Mol Biol* **309**, 1-18
56. Andrade, M. A., Perez-Iratxeta, C., and Ponting, C. P. (2001) *J Struct Biol* **134**, 117-131
57. Andrade, M. A., Petosa, C., O'Donoghue, S. I., Muller, C. W., and Bork, P. (2001) *J Mol Biol* **309**, 1-18
58. Berke, I. C., Boehmer, T., Blobel, G., and Schwartz, T. U. (2004) *J Cell Biol* **167**, 591-597
59. Otwinowski, Z., and Minor, W. (1997) *Macromolecular Crystallography, Pt A* **276**, 307-326
60. Sheldrick, G. M. (2008) *Acta Crystallogr A* **64**, 112-122
61. Imperato, M., and Bolognesi, P. (2004) *J Mol Biol* **309**, 1-18
62. Adams, P. D., Grosse-Kunstleve, R. W., Ioerger, T. R., McCoy, A. J., Moriarty, N. W., Read, R. J. (2002) *Acta Crystallogr D Biol Crystallogr* **58**, 1948-1954
63. Smith, D. L., and Bork, P. (2004) *Proc Natl Acad Sci U S A* **101**, 1-6
64. Kleyweg, T., and Bork, P. (2004) *PLoS Biol* **2**, e380
65. Imperato, M., and Bolognesi, P. (2004) *J Mol Biol* **309**, 1-18
66. Andrade, M. A., Perez-Iratxeta, C., and Ponting, C. P. (2001) *J Struct Biol* **134**, 117-131
67. Andrade, M. A., Petosa, C., O'Donoghue, S. I., Muller, C. W., and Bork, P. (2001) *J Mol Biol* **309**, 1-18
68. Berke, I. C., Boehmer, T., Blobel, G., and Schwartz, T. U. (2004) *J Cell Biol* **167**, 591-597
69. Otwinowski, Z., and Minor, W. (1997) *Macromolecular Crystallography, Pt A* **276**, 307-326
70. Sheldrick, G. M. (2008) *Acta Crystallogr A* **64**, 112-122
71. Imperato, M., and Bolognesi, P. (2004) *J Mol Biol* **309**, 1-18
74. Schrader, N., Stelter, P., Flemming, D., Kunze, R., Hurt, E., and Vetter, I. R. (2008) *Mol Cell* **29**, 46-55
75. Handa, N., Kukimoto-Niino, M., Akasaka, R., Kishishita, S., Murayama, K., Terada, T., Inoue, M., Kigawa, T., Kose, S., Imamoto, N., Tanaka, A., Hayashizaki, Y., Shirouzu, M., and Yokoyama, S. (2006) *J Mol Biol* **363**, 114-124
76. Hawryluk-Gara, L. A., Platani, M., Santarella, R., Wozniak, R. W., and Mattaj, I. W. (2004) *Mol Cell Proteomics* **3**, 145-155
77. Rayala, H. J., Kendirgi, F., Barry, D. M., Majerus, P. W., and Wente, S. R. (2004) *Mol Cell* **19**, 1753-1762
78. Lutzmann, M., Kunze, R., Stangl, K., Stelter, P., Toth, K. F., Bottcher, B., and Hurt, E. (2005) *J Biol Chem* **280**, 18442-18451
79. Conti, E., Muller, C. W., and Stewart, M. (2006) *Curr Opin Struct Biol* **16**, 237-244
80. Nehr bass, U., Rout, M. P., Maguire, S., Blobel, G., and Wozniak, R. W. (1996) *Journal of Cell Biology* **133**, 1153-1162
81. Kosova, B., Pante, N., Rollenhagen, C., and Hurt, E. (1999) *Journal of Biological Chemistry* **274**, 22646-22651
82. Mans, B. J., Anantharaman, V., Aravind, L., and Koonin, E. V. (2004) *Cell Cycle* **3**, 1612-1637

**FOOTNOTES**

The atomic coordinates and structure factors (codes 3I4R, 3I5P, 3I5Q) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/)

1 To whom correspondence should be addressed: Massachusetts Institute of Technology, 77 Massachusetts Avenue, Rm. 68-480, Cambridge, MA 02139. Tel.: 617-452-3851; Fax: 617-258-6553; E-mail: tus@mit.edu.
2 The abbreviations used are: NPC, nuclear pore complex; NE, nuclear envelope; PEG, polyethylene glycol; SeMet, selenomethionine; SAD, single-anomalous dispersion.

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**FIGURE LEGENDS**

Figure 1. Nup170 forms a bipartite, irregular α-helical stack

(A) The predicted domain structure of Nup170 is diagramed, with β-propeller domain, residues 180-650, and α-helical domains, residues 760-1502 drawn as ovals. Indentation at residue 1270 reflects the division into two parts. The portion of the structure solved is colored as in B and C.

(B) Cartoon representation of the 3.2 Å structure of the α-helical C-terminal domain of Nup170 with its two subdomains shown in green and aquamarine, respectively. Secondary structure elements and termini are labeled. Disordered loops connecting structural elements are drawn as dotted lines. A central, long helix, α14, spans and connects the two subdomains. The C-terminal subdomain, helices α14-26 was also expressed as a separate protein fragment and solved at 2.2 Å resolution.

(C) Structure rotated by 90° compared to B.
Figure 2. Nup133<sub>17-1156</sub> in complex with Nup84(hNup107<sub>658-925</sub>)

(A) The domain structures of Nup107 and Nup133 are diagramed. Nup107 consists of a single α-helical domain homologous to other ACE1 proteins. Nup133 has a β-propeller domain, residues 76-478, solved previously, and an α-helical domain as shown in B and C. The portions of each molecule solved here are colored. Indentation at residue 1008 of Nup133 reflects the hinge between helices α24 and α25.

(B) The α-helical domain of Nup133 (orange) in complex with Nup107<sub>658-925</sub> (blue). Loops evident in the electron density, but not modeled for the purpose of refinement, are grey. Secondary structure elements and termini of Nup133 are labeled, beginning at the N-terminus of the domain, helix α4.

(C) Structure rotated by 90° compared to B. Nup107 helix α6', a metazoan-specific structural element, is labeled.

Figure 3. The topology of Nup170 and Nup133 α-helical domains are conserved

Nup170 in green and Nup133 in orange were compared by structural alignment of segmental subdomains. Nup170 N-terminal (E), central (C), and C-terminal (A), subdomains were structural aligned to the related segments (F, D, B) in Nup133. Secondary structure elements are labeled. Unmodelled loops and connections between domains are shown as dotted lines. The N-terminal remainder of each molecule not solved here extends where marked N. The C-terminus of each molecule is marked C.

Figure 4. Surface conservation of Nup170 suggests two protein-protein interfaces

(A) Amino acid sequence conservation among Nup170 genes from maximally diverse eukaryotes was mapped on the protein, gradient-colored from white (not conserved) to orange (strongly conserved), orientated as in Fig. 1B. A conserved groove is boxed.

(B) Structure rotated 180° compared to A, with conserved surface patch boxed.

(C) Surface groove boxed in A shown magnified. Structure of C-terminal subdomain at 2.2 Å resolution is superposed and shown in aquamarine as a cartoon. Helix α14 as white cartoon, extending down and left, in the conformation seen in the full domain, as well as in aquamarine as the well-ordered, extended peptide seen in the 2.2 Å structure of the isolated C-terminal subdomain. Key residues are labeled.

(D) Surface patch boxed in B is magnified and shown as a cartoon with exposed residues labeled. Partially transparent surface representation is colored by calculated surface charge, in a gradient from negative (red) to neutral (white) to positive (blue).

(E) Homologous section of Nup133 colored and labeled as in D with extent of interface to Nup107 delimited by a solid black line.

(F) Sequence alignment of maximally diverse selection of eukaryotic Nup170 sequences, colored by conservation as in A, B, and C. Helical segments are shown as red cylinders and labeled. Bar graph shows accessible surface area (Å<sup>2</sup>) for each residue. Yellow circles mark: conserved, buried arginine 1232; two hydrophilic surface residues, glutamine 1234 and arginine 1238, that would be buried were this surface, shown in D, indeed a protein-protein interface, as in Nup133, shown in E; and residues serine 1305, phenylalanine 1308 and phenylalanine 1325, lining the groove shown in C.

Figure 5. Schematic representation of the heptameric Nup84 subcomplex.

The Nup84 subcomplex is composed of three ACE1 proteins (blues), Nup133 (orange), Nup120 (green), and β-propeller proteins Seh1 and Sec13 (grey). Nup85 and Nup145C each contribute in trans one blade of the Seh1 and Sec13 β-propellers. The portions of Nup133 and Nup107 solved here, as shown in Fig. 2, are outlined in bold. The N-terminal β-propeller of Nup133 and all portions of the Y outlined by solid lines have been solved at atomic resolution previously. Dashed lines denote portions of the Nup84 subcomplex for which no atomic resolution structures have yet been published.
Figure 6. Representative nucleoporin α-helical stack domains.
(A) The overall topology of human Nup133 (residues 75-1156) is shown. The structure is gradient-colored from orange to white from the N terminus of the helical domain to the C terminus. The β-propeller domain is colored grey.
(B) The overall topology of yeast Nie96 (residues 200-835), an ACE1, is shown. The structure is gradient colored from red to white from N to C terminus.
(C) The overall topology of yeast Nup120 (residues 1-730 of 1037) is shown. The structure is gradient-colored from magenta to white from N to C terminus. Residue 1-381 are colored grey and form β-propeller blades β1-β6 and strand β7D, which is contributed by the N terminus. Note that strands A-C of blade β7 are contributed by the portion of the α-helical domain that links helix α4 to helix α5.

TABLE 1. Proteins of the NPC core structural scaffold
Structures of the underlined proteins have been published. Gene names and PDB codes from this work are in bold.

TABLE 2. Data collection and refinement statistics
The highest resolution shell is shown in parentheses.
Figure 1.

A

Nup170

\[ \text{\( \beta \)-Propeller Domain} \]

\[ \text{\( \alpha \)-Helical Domain} \]

~180 ~650 ~760 979 1270 1502

B

C

\[ \text{\( \alpha \)}_{1} \]

\[ \text{\( \alpha \)}_{2} \]

\[ \text{\( \alpha \)}_{3} \]

\[ \text{\( \alpha \)}_{4} \]

\[ \text{\( \alpha \)}_{5} \]

\[ \text{\( \alpha \)}_{6} \]

\[ \text{\( \alpha \)}_{7} \]

\[ \text{\( \alpha \)}_{8} \]

\[ \text{\( \alpha \)}_{9} \]

\[ \text{\( \alpha \)}_{10} \]

\[ \text{\( \alpha \)}_{11} \]

\[ \text{\( \alpha \)}_{12} \]

\[ \text{\( \alpha \)}_{13} \]

\[ \text{\( \alpha \)}_{14} \]

\[ \text{\( \alpha \)}_{15} \]

\[ \text{\( \alpha \)}_{16} \]

\[ \text{\( \alpha \)}_{17} \]

\[ \text{\( \alpha \)}_{18} \]

\[ \text{\( \alpha \)}_{19} \]

\[ \text{\( \alpha \)}_{20} \]

\[ \text{\( \alpha \)}_{21} \]

\[ \text{\( \alpha \)}_{22} \]

\[ \text{\( \alpha \)}_{23} \]

\[ \text{\( \alpha \)}_{24} \]

\[ \text{\( \alpha \)}_{25} \]

\[ \text{\( \alpha \)}_{26} \]

90°
Figure 2.

A

Nup107

Nup133 binding

α-Helical Domain (ACE1)

-140 658 925

B

Nup133

β-Propeller PDB:1XKS

α-Helical Domain

76 478 516 1008 1156

C

90°
Figure 3.
Figure 5.
Figure 6.

A  
Nup133

B  
Nic96

C  
Nup120
TABLE 1
Proteins of the NPC Core Structural Scaffold
Structures of the underlined proteins have been published. Gene names and PDB codes from this work are in bold.

| Gene Name | PDB codes | Domain Architecture |
|-----------|-----------|---------------------|
| **Nip6**  | 2QX5, 2RFO|                     |
| Nip157    |           |                     |
| Nip192    |           |                     |
| Nip188    |           |                     |
| Nip53     | 1WHH      |                     |
| Nip59     |           |                     |

| **Nup170**| 3J5P, 3J5Q|                     |
| Nup157    |           |                     |
| Nup192    |           |                     |
| Nup188    |           |                     |
| Nup53     |           |                     |
| Nup59     |           |                     |

| **Nup107**| 3CQC, 3I4R|                     |
| Nup133    | 1XKS, 3CQC, 3I4R|             |
| Nup120    | 3HXR      |                     |
| Nup85     | 3EWE, 3F3F|                     |
| Sch1      | 3EWE, 3F3F|                     |
| Nup145C   | 3BG1      |                     |
| Sec13     | 3BG1      |                     |

Legend:
- α-helical domain
- α-helical domain (ACE1)
- β-propeller domain
- β-propeller insertion blade
- coiled-coil
- α/β domain
- loop
### TABLE 2
**Data Collection and Refinement Statistics**
The highest resolution shell is shown in parentheses.

|                     | hNup107<sub>658-925</sub> | hNup133<sub>917-1356</sub> | yNup170<sub>973-1582</sub> | yNup170<sub>1235-1582</sub> |
|---------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **PDB Code**        | 3I4R                        | 3I5P                        | 3I5Q                        | I222                        |
| **Data Collection** |                             |                             |                             |                             |
| Spacegroup          | P2₁2₁2₁                     | H32                         | H32                         | I222                        |
| Cell Dimensions     |                             |                             |                             |                             |
| a, b, c (Å)         | 115.6, 133.0, 176.3         | 121.4, 121.4, 258.1         | 121.4, 121.4, 256.7         | 78.4, 113.0, 150.9          |
| α, β, γ (°)         | 90, 90, 90                  | 90, 90, 120                 | 90, 90, 120                 | 90, 90, 90                  |
| Resolution range (Å)| 37.6-3.5                    | 30-3.5                      | 30-3.2                      | 30-2.2                      |
| (3.63-3.5)           |                             |                             |                             |                             |
| No. of unique reflections | 29866                      | 9123                       | 12125                       | 33936                       |
| Completeness (%)    | 85.7 (40)                   | 96.8 (96.4)                 | 98.6 (90.0)                 | 99.7 (99.2)                 |
| Redundancy          | 5.4 (2.6)                   | 7.9 (7.2)                   | 3.4 (3.1)                   | 5.3 (4.1)                   |
| R<sub>mea</sub>(I) (%) | 15.7 (-)                  | 9.8 (37.5)                  | 5.6 (25.4)                  | 12.8 (54.4)                 |
| I/σ (I)             | 11.6 (0.59)                 | 26.7 (4.5)                  | 25.9 (2.6)                  | 19.4 (2.3)                  |
| Elliptical Truncation Radii (Å) | 4.3, 3.5, 4.2 |                             |                             |                             |
| Anisotropic Scale Factors (Å<sup>2</sup>) | 39.9, 8.50, -48.4 |                             |                             |                             |
| No. of unique reflection remaining | 23455                      |                             |                             |                             |
| **Refinement**      |                             |                             |                             |                             |
| Resolution range (Å)| 37.6-3.5                    | 30-3.2                      | 30-2.2                      |                             |
| R<sub>work</sub> (%) | 31.2                       | 30.6                        | 23.2                        | 27.2                        |
| R<sub>free</sub> (%) | 37                         | 32.4                        | 27.2                        | 36.8                        |
| Number of reflections | 23455                      | 11975                       | 32541                       |                             |
| Number of atoms     |                             |                             |                             |                             |
| Total               | 5485                       | 3552                        | 4057                        |                             |
| Waters              | 0                          | 0                           | 368                         |                             |
| Average B-factor (Å<sup>2</sup>) | 185                        | 148                         | 43.6                        |                             |
| Ramachandran analysis (%) |                             |                             |                             |                             |
| Favored             | 90.9                       | 90.4                        | 97.4                        |                             |
| Allowed             | 7.9                        | 8.9                         | 2.6                         |                             |
| Disallowed          | 1.2                        | 0.7                         | 0.0                         |                             |
Architectural nucleoporins Nup157/170 and Nup133 are structurally related and descend from a second ancestral element
James R. R. Whittle and Thomas U. Schwartz

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