Atomic structure of the Y complex of the nuclear pore

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The nuclear pore complex (NPC) is the principal gateway for transport into and out of the nucleus. Selectivity is achieved through the hydrogel-like core of the NPC. The structural integrity of the NPC depends on ~15 architectural proteins, which are organized in distinct subcomplexes to form the ~40-MDa ring-like structure. Here we present the 4.1-Å crystal structure of a heterotetrameric core element (‘hub’) of the Y complex, the essential NPC building block, from *Myceliophthora thermophila*. Using the hub structure together with known Y-complex fragments, we built the entire ~0.5-MDa Y complex. Our data reveal that the conserved core of the Y complex has six rather than seven members. Evolutionarily distant Y-complex assemblies share a conserved core that is very similar in shape and dimension, thus suggesting that there are closely related architectural codes for constructing the NPC in all eukaryotes.

In all eukaryotic cells, transcription and translation are physically separated between the nucleus and cytoplasm. This allows for distinct gene expression-control mechanisms, for example in cell differentiation and development, which are unavailable to prokaryotes. NPCs, which perforate the nuclear envelope and act as the main transport gates, therefore have a fundamental role in cellular homeostasis1,2. The NPC is a modular, donut-shaped assembly of ~30 different proteins (nucleoporins or nups), arranged in multiples of eight around a central axis that is aligned with the main transport channel3. Nups can be classified as (i) architectural nups, which form the stable scaffold of the NPC; (ii) peripheral nups, with various degrees of mobility; and (iii) nups with characteristic phenylalanine-glycine (FG)-repeat elements in disordered extensions that form the permeability barrier.

To gain mechanistic insight into NPC function, considerable effort has been undertaken to determine the NPC structure at high resolution. Owing to its enormous size of ~40–120 MDa (refs. 4–6), this will ultimately be possible only with a combination of different visualization techniques, notably X-ray crystallography and cryo-EM.

One of the main architectural elements of the NPC is the Y-shaped complex, which is essential for NPC formation7,8. In *Saccharomyces cerevisiae*, it is a seven-membered 575-kDa complex composed of Nup84, Nup85, Nup120, Nup133, Nup145C, Sec13 and Seh1 (ref. 9). Homologs for these seven members are also found in humans, thus suggesting conservation within all opisthokonts10–12. It is widely believed that the Y complex, together with the heteromeric Nic96 complex, forms the principal NPC scaffold13–15. Thus, the atomic structures of the assembled Y and Nic96 complexes are important milestones toward understanding the NPC at the highest resolution.

All seven members of the Y complex have been studied crystallographically, though only individually or as heterodimeric or trimeric complexes, typically in truncated forms16–27. The branch point or hub of the Y complex, where the two short arms and the long stalk meet, is structurally the least understood even though it is arguably the most important element (Fig. 1a).

Here we now report the structure of the Y-complex hub, which enables us to combine all the additional fragmentary structures into a highly accurate assembled structure of the Y complex. We show that the Y-complex structure is widely conserved among all eukaryotes. Species-specific additions to the Y complex decorate but do not principally alter the overall structure.

**RESULTS**

**Structure of the Y-complex hub**

We generated a series of structure-based expression constructs containing the elements of Nup120, Nup145C and Nup85, which are known to directly interact with each other at the Y-complex hub18,20,22,28. In addition, we designed these constructs to overlap at least partially with the already structurally characterized Y-complex fragments. We succeeded in obtaining crystals of a heterotetrameric construct containing Nup85257–1181, Nup120952–1241, Nup145C233–791 and Sec13 from the thermophilic fungus *M. thermophila* (mt), which diffracted to 4.1-Å resolution (Table 1). We solved the structure with a combination of molecular replacement and single anomalous dispersion (SAD), using selenomethionine-derivatized protein (Online Methods). The crystals have a high solvent content (68%), and the structure exhibits substantial positional disorder (Wilson B factor of 144 Å2). Despite the high Wilson B factor, we were able to properly assign the sequence to all four proteins within the assembled complex on the basis of model building guided by selenomethionine positions, homology models and phylogenetic considerations (Supplementary Table 1).

The overall structure of the heterotetramer is roughly V shaped, composed of three helical units (Nup85, Nup120 and Nup145C) and a laterally attached β-propeller (Sec13) (Fig. 1b and Supplementary Fig. 1). Nup85 and Nup145C form the long sides of the V, whereas...
Nup120 is sandwiched between the two sides and acts as the main connector. Sec13 is bound to Nup145C as previously described in the Nup145C–Sec13–Nup84 structure from S. cerevisiae (sc)19,25, namely by the insertion of a seventh blade into its open six-bladed β-propeller. When viewed from the side, it is noticeable that the heterotetramer is substantially bent rather than flat (Fig. 1c). The overall dimensions of the complex are ~125 Å × ~140 Å × ~50 Å.

Nup85 and Nup145C belong to the ancestral coatomer element1 of ACE1 class of proteins. These proteins are characterized by an ~65-kDa tripartite helical segment composed of a crown, a trunk and a tail element, which adopts a characteristic fold-back structure involving ~30 α-helices3,18. ACE1 proteins are found exclusively in the NPC scaffold and the COPII vesicle coat29. The scNup85 and scNup145C fragments solved previously both lack the tail elements, which are present in this structure. These tail elements are in direct contact with Nup120. Even though the identity between the M. thermophila and S. cerevisiae sequences is low (14% for Nup85, 20% for Nup145C) (Supplementary Table 2 and Supplementary Notes 1–4), the structures superpose well (Supplementary Fig. 2). Therefore, we assigned secondary-structure elements in the M. thermophila proteins in accord with the published S. cerevisiae fragments18,19. Nup145C has 27 helices in total. Helices α1–α3 and α12–α20 form the trunk, α4–α11 the crown and α21–α27 the tail. The entire helical stack of Nup145C has a crescent shape. To stabilize the Sec13 interaction, we fused Sec13 N terminally to 145C, similarly to what was previously done for scSec13-Nup145C19. For this reason, our Nup145C construct is lacking 232 N-terminal residues that are predicted to be disordered.

Nup85 has 33 helices in total. Helices α1–α3 and α12–α20 form the trunk, α4–α11 the crown and α21–α30 the tail. As compared to that of mtNup145C, the mtNup85 tail domain is longer and contains four additional helices. In S. cerevisiae, Nup85 binds Seh1 very similarly to Nup145C binding Sec13, i.e., via an insertion blade that closes the open six-bladed β-propeller. Although Sordariomycota, including M. thermophila and Chaetomium thermophilum, also contain a recognizable Seh1 homolog, this homolog does not bind to Nup85 (ref. 30). The structure now reveals that mtNup85 lacks the essential Seh1-binding site, i.e., the insertion blade. Instead, it contains an additional N-terminal helix α0 (Fig. 1 and Supplementary Fig. 3), which is incompatible with Seh1 binding. In light of this observation, we suggest that Seh1 is not a member of the conserved core of the Y complex. This core is a heterohexamer rather than a heterotetramer. The presence of an Seh1 homolog in Sordariomycota is probably due to its function in the GATOR or SEA complex31,32.

The C-terminal fragment of Nup120 contains ten helices (α2–α29, α29α, α29β and α30), eight of which form a regular stack. This stack superimposes very well (r.m.s. deviation of 2.2 Å over 135 Cα positions) with the C terminus of Nup120 from Schizosaccharomyces pombe32. The C-terminal mtNup120 element is wedged between the tail domains of Nup85 and Nup145C, and it forms the core of the hub. The interface between Nup120 and Nup85 buries an ~940 Å²

| Data collection and refinement statistics | Nup85(SeMet)257–1181, Nup120902–1241, Sec13-Nup145C233–791 | Nup85(SeMet)257–1181, Nup120(SeMet)202–1241, Sec13-Nup145C233–791 |
|-----------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Cell dimensions                        | a, b, c (Å)                                      | α, β, γ (°)                                      |
|                                        | 104.98, 212.02, 170.64                           | 90.107, 90.72                                  |
| Resolution (Å)                         | 163–4.10 (4.25–4.10)                            | 157–4.00 (4.14–4.00)                           |
| Rwork / Rfree                          | 0.19 (1.00)                                      | 0.21 (0.97)                                    |
| Completeness (%)                       | 98.2 (93.5)                                      | 90.3 (81.3)                                    |
| Redundancy                             | 6.1 (4.7)                                        | 3.6 (2.9)                                      |

Supplementary Table 2

Nup85(SeMet)257–1181, Nup120(SeMet)202–1241, Sec13-Nup145C233–791
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ARTICLES

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Figure 2  Fitness analysis of hub interactions. (a) Growth curves of NUP85Δ strains carrying NUP85::URA3 and either empty pRS315 (negative control), Nup85 wild type or Nup85 Δα30 grown in the presence of 5-fluoro-orotic acid (5-FOA). The positive control is the NUP85Δ strain carrying NUP85::URA3 and empty pRS315 grown in the absence of 5-FOA. OD, optical density. (b) Growth curves of NUP145Δ strains carrying NUP145::URA3 and either empty pRS315 (negative control), Nup145C wild type or Nup145C Δα27 grown in the presence of 5-FOA. The positive control is the NUP145Δ strain carrying NUP145::URA3 and empty pRS315 grown in the absence of 5-FOA. (c) Growth curves of NUP120Δa strains carrying YClac33 empty vector (negative control), Nup120 wild type or Nup120 Δα30 grown in yeast, peptone, dextrose (YPD) medium. Four technical replicates (OD measurements) for each of three biological replicates from separate colonies, were performed at 30 °C for all experiments. Error bars, s.e.m. of the three biological replicates.

Fitness analysis

To evaluate the importance of the hub interface in vivo, we used a fitness test in S. cerevisiae. We designed C-terminal truncations of the last helix of each of Nup85, Nup120 and Nup145C to selectively disrupt the mapped interfaces between the three helical proteins. The Nup85Δα30 mutant had the most severe phenotype and showed drastically reduced growth (Fig. 2). Nup145CΔα27 and Nup120Δα30 had progressively milder phenotypes. Nup85Δα30 almost recapitulated the phenotype of the lethal Nup85 knockout33, thus suggesting that the Nup85-Nup120 interaction is critical for NPC assembly. For Nup120 and Nup145C, it is likely that interface elements N terminal to the truncations are still sufficient to rescue interaction or that they are not the exclusive elements that integrate these proteins into the NPC, and that additional contacts exist. The N-terminal extension of Nup145C, which is past the Sec13 insertion blade and is not part of our structure, is likely to play a part in this. However, contacts to other scaffold nucleoporins need to be considered as well. Additionally, although we did not formally quantify the protein levels or test the fold retention of the individual truncated proteins, on the basis of previous in vivo28 and in vitro18,20,22,34 experiments, we can assume that our specific truncations are folded correctly and are expressed at wild-type levels.

Composite high-resolution structure of the Y complex

With the heterotetrameric hub assembly in hand, we set out to build a complete high-resolution, composite structure of the entire Y complex (Supplementary Data Sets 1 and 2 for Homo sapiens and S. cerevisiae, respectively). The structures of full-length Nup120 (ref. 22) and Nup84–Nup145C–Sec13 (ref. 19) contain overlapping elements with the hub structure, of 19 kDa and 85 kDa, respectively, and superposed with high confidence (Supplementary Fig. 4). This generated a heteropentameric complex, in which Nup84 is the preliminary terminal fragment of the long stalk of the Y. Nup84 is an ACE1 protein, but the published structure lacks the tail domain (residues 443–724) that interacts with Nup133. However, the structure of most of the tail domain of Nup107, the human Nup84 homolog, in complex with Nup133 is known17. Therefore, we were able to model full-length Nup84 on the basis of the experimentally known N- and C-terminal fragments, and the homology-modeled structure of the intervening 84-residue segment, by using other ACE1 domains (Online Methods). After Nup84 was positioned, Nup133 could be docked on the basis of the structure of the Nup107–Nup133 complex21. Finally, the last element on the long stalk of the Y complex is the N-terminal Nup133 β-propeller, which is loosely tethered to the C-terminal α-helical stalk element16,35. The resulting composite structure constitutes the conserved heterohexameric core of the Y complex (Fig. 3a).
Because the composite model is built from structural elements of four different organisms, we examined the extent to which this might affect the overall structure. Therefore, we also built models for the heteroheptameric Y complex in S. cerevisiae, including Seh1, as well as the heterodecameric Y complex in H. sapiens, including Seh1 and Nup37 (Fig. 3b,c). The Seh1 position can be deduced from the structure of the Seh1–Nup85 complex\(^\text{18,24}\), whereas the Nup37 position is known from the structure of the Nup37–Nup120 complex\(^\text{22,36}\). The positions of Nup43 and ELYS within the human complex are not well understood and were therefore excluded from our model. By comparison, we observed that the conserved, heterohexameric core changes only in local areas between the three models and that the overall shape and dimensions of the Y complex appear to be conserved in all opisthokonts. Organism-specific proteins decorate the Y core but do not substantially influence its overall structure otherwise.

Our composite structure reveals that the Y complex, when viewed from the front, measures about 20 nm wide and 40 nm high (Fig. 3). This is in good agreement with published EM structures and computational models\(^\text{15,28,37}\). However, the principal angles between the three extensions from the hub deviate substantially between our X-ray–based structure and the EM structures, as evident upon superimposing the different sets of data (Fig. 4). Strikingly, when viewed from the side or the top, the composite crystal structure reveals a distinct three-dimensional (3D) shape (Figs. 3e and 4b,f), whereas the previously reported structures were essentially flat (Fig. 4d,h). At the hub, the three extensions, namely the two arms and the stalk, exhibit strong curvature and form a dome-shaped structure. As a result, we measure a thickness of ~8 nm for the Y complex (Fig. 3e), in comparison to ~4 nm reported in previous EM analyses. Theoretically, this difference could be the result of flexibility within the Y complex, which is well documented. In our composite structure, we can now specify the main hinge regions and flexible areas (Fig. 5). These flexible regions are mapped on the basis of general considerations regarding
protein structure, domain boundaries established by limited proteolysis and flexibility observed in previous crystallographic and EM studies\textsuperscript{15–17,21,22,37}. The hub itself is rather rigid because four proteins engage in a tightly coordinated interface. To flatten the Y complex, the helical Nup120 C-terminal domain or the Nup85 ACE1 element would have to bend by nearly 90° with respect to each other. Helical stacks have the propensity to bend, as best exemplified by various nuclear transport receptors\textsuperscript{38,39}, but the direction of bending is determined by the helical orientation. For the flattening of the Y, the elements predicted to be bendable are, however, oriented in an unfavorable way. This means that the necessary distortions in Nup120 or Nup85 would be energetically costly because of the disruption to the hydrophobic core that they would generate; hence, we consider this scenario to be rather unlikely. Therefore, the simplest explanation for the discrepancy between our composite crystal structure and previous random-conical-tilt negative-stain EM structures is that the latter were artificially flattened in the direction normal to the EM grid, a well-known phenomenon.

Implications for NPC assembly models

Next, we tested whether our composite human Y complex could be positioned into the recently published 3.2-nm cryo-ET density map\textsuperscript{15} of the human NPC, which predicted a staggered two-ring, head-to-tail orientation of Y complexes, symmetrically positioned on the nucleoplasmic and the cytoplasmic faces of the NPC. We were able to recapitulate the published results of docking the flat Y-complex structure determined by random-conical-tilt negative-stain EM (EMD-\textsuperscript{2443})\textsuperscript{15} into the cryo-electron tomography (cryo-ET) map\textsuperscript{15}. We then tried the same procedure with our highly curved Y-complex structure. We searched with the human Y-complex model, omitting Nup133, presumably the most flexible Y-complex element. Nup133 has an N-terminal \(\beta\)-propeller flexibly connected to a C-terminal helical-stack domain\textsuperscript{16}. The helical domain of Nup133 is tripartite, with hinges connecting the three helical segments\textsuperscript{21}. Using this stubbed Y complex, we found three top numerical solutions (Online Methods). Two solutions roughly coincided with the outer Y-complex ring postulated by Bui \textit{et al.}\textsuperscript{15} (Fig. 6), and the third solution coincided with the inner ring of that study (Supplementary Fig. 5). While our work was in review, Stuwe \textit{et al.} reported the docking of a similarly stubbed Y complex from...
S. cerevisiae, from a 7.4-Å crystal structure showing a curved topology generally consistent with our structure, and arrived at a similar solution34. However, when we added the Nup107 tail and Nup133 structures back to the docked Y-complex model of our third solution in topologically reasonable ways, we observed extensive steric clashes with the neighboring Y complex that seem implausible (Supplementary Fig. 5). Thus, we did not consider this solution further. Regarding the two top solutions, they are rotated around the hub by approximately 20° relative to each other. In each solution, the long stalk could be fitted reasonably well to two different regions in the EM density. Both solutions result in a seemingly closed ring when Nup133 is added, although the head-to-tail contact would be different in each case. To fit each solution, the long stalk would need to adopt different conformations, largely by adjusting Nup133; this would seem realistic because of the expected flexibility around distinct hinge points (Fig. 6c). Obviously, both solutions cannot coexist, owing to excessive steric clashes. Therefore, the easiest way to explain our docking results is to suggest that the Y-complex ring is a single rather than a double ring but that it can adopt at least two conformations. We argue that because of subtomogram averaging, we might see an overlay of the two equally and most populated states of the Y-complex ring in the cryo-ET density.

DISCUSSION

As reasonable as our docking attempts may appear, we would like to express caution about the interpretation of these results. First, the available cryo-ET map (EMD-2444)15 is calculated on the basis of assumptions that we still do not know to be necessarily correct. For example, a strict eight-fold rotational symmetry is assumed, which may be appropriate at nanometer resolution but possibly not at atomic resolution. If this symmetry is not true on the atomic level, the calculated map could be intrinsically flawed. Because of the similarity of various scaffold nups on a nanometer scale, this is particularly troublesome. Second, docking at ∼3-nm resolution is at best tentative and is reasonable to attempt only because of the distinct and large size of the Y complex. It is possible that an entire portion of the Y complex is so flexible in the NPC assembly that it could simply be averaged out in the cryo-ET study. This could in principle be true for the long stalk as well as most of the Nup120 arm, which can bend perpendicular to the long axis of its C-terminal helical stack domain22. Many additional docking solutions would need to be considered if such possibilities were entertained.

One way of independently confirming a specific assembly model initially derived from docking is to map the assumed contacts between neighboring complexes. Bui et al. attempted this by cross-linking experiments15. Surprisingly, only two such cross-links (Nup107-Nup133 and Nup43-Nup96) were found. However, these interactions could not be confirmed by additional experiments and therefore are insufficient to distinguish between the models. A two-ring model in particular would generate many inter-Y contact sites; thus, the paucity of detected cross-links is unexpected. Taking additional studies into account does not resolve the discrepancy between the reticulated two-ring model and a flexible one-ring model. For example, the radial distribution of fluorescently labeled scaffold nucleoporins was determined by super-resolution microscopy40. However, the raw localization precision of 20–30 nm attained in that study is insufficient to distinguish between the different ring models. Stoichiometric considerations, which have major model implications, are also not definitive. For example, a recent study concluded that there are 32 copies of Y complexes per NPC in HeLa cells41, a number supporting a two-ring model, whereas earlier studies argued for 16 copies6,42, which would favor a one-ring model. Recent advancements in super-resolution microscopy should allow for the direct counting of individual nucleoporins within an NPC, putting this controversy to rest soon43. Finally, mass estimates for the NPC range from 40 MDa to 120 MDa, depending on the species and technique used44–6. Again, it is premature to use this information to definitively validate a specific model. In conclusion, to confirm any model, additional experimental data that have an appropriate resolution are needed. Interaction data that would confirm contacts between neighboring subcomplexes, or cryo-ET maps at higher resolution, would probably be the most helpful tools. Regardless, the Y-complex structure presented here at least provides a benchmark that any reasonable model should be consistent with.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Coordinates and structure factors have been deposited in the Protein Data Bank under accession code 4YCZ.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

T.U.S., K.K. and K.E.K. designed the study. K.K. and K.E.K. performed the experiments. K.K., K.E.K. and T.U.S. analyzed the data. G.K. performed and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Hetzer, M.W. & Wente, S.R. Border control at the nucleus: biogenesis and organization of the nuclear membrane and pore complexes. Dev. Cell 17, 606–616 (2009).
2. Strambio-De-Castillia, C., Niepel, M. & Rout, M.P. The nuclear pore complex: bridging nuclear transport and gene regulation. Nat. Rev. Mol. Cell Biol. 11, 490–501 (2010).
3. Brohawn, S.G., Partridge, J.R., Whitlle, J.R.R. & Schwartz, T.U. The nuclear pore complex has entered the atomic age. Structure 17, 1156–1168 (2009).
4. Reichelt, R. et al. Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. J. Cell Biol. 110, 883–894 (1990).
5. Yang, Q., Rout, M.P. & Akey, C.W. Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. Mol. Cell 1, 223–234 (1998).
6. Rout, M.P. et al. The yeast nuclear pore complex: composition, architecture, and transport mechanism. J. Cell Biol. 148, 635–651 (2000).
7. Walther, T.C. et al. The conserved Nup107–160 complex is critical for nuclear pore complex assembly. Cell 113, 195–206 (2003).
8. Harel, A. et al. Removal of a single pore subcomplex results in vertebrate nuclei devoid of nuclear pores. Mol. Cell 11, 853–864 (2003).
9. Lutzmann, M., Kunze, R., Buerer, A., Aebi, U. & Hurt, E. Modular self-assembly of a Y-shaped multienzyme complex from seven nucleoporins. EMBO J. 21, 387–397 (2002).
Nagvenkar, V. et al. Structure of a trimeric nucleoporin complex reveals alternate oligomerization states. Proc. Natl. Acad. Sci. USA 106, 17693–17698 (2009).

26. Seco, H.-S. et al. Structural and functional analysis of Nup120 suggests ring formation of the Nup84 complex. Proc. Natl. Acad. Sci. USA 106, 14281–14286 (2009).

27. Sampathkumar, P. et al. Structure of the C-terminal domain of Saccharomyces cerevisiae Nup133, a component of the nuclear pore complex. Proteins 79, 1672–1677 (2011).

28. Fernandez-Martinez, J. et al. Structure-function mapping of a heptameric module in the nuclear pore complex. J. Cell Biol. 196, 419–434 (2012).

29. Whittle, J.R.R. & Schwartz, T.U. Structure of the Sec13-Sec16 edge element, a template for assembly of the COPII vesicle coat. J. Cell Biol. 190, 347–361 (2010).

30. Thierbach, K. et al. Protein interfaces of the conserved Nup84 complex from Chaetomium thermophilum shown by crosslinking mass spectrometry and electron microscopy. Structure 21, 1672–1682 (2013).

31. Bar-Peled, L. et al. A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 340, 1100–1106 (2013).

32. Alagri, R. et al. Molecular architecture and function of the SEA complex, a modulator of the TORC1 pathway. Mol. Cell. Proteomics 13, 2855–2870 (2014).

33. Siniosoglou, S. et al. A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. Cell 84, 265–275 (1996).

34. Stuewe, T. et al. Architecture of the nuclear pore complex coat. Science 347, 1148–1152 (2015).

35. Kim, S.J. et al. Integrative structure-function mapping of the nucleoporin Nup133 suggests a conserved mechanism for membrane anchoring of the nuclear pore complex. Mol. Cell. Proteomics 13, 2911–2926 (2014).

36. Liu, X. et al. Structural evolution of the membrane-coating module of the nuclear pore complex. Proc. Natl. Acad. Sci. USA 109, 16498–16503 (2012).

37. Kampmann, M. &Blobel, G. Three-dimensional structure and flexibility of a membrane-coating module of the nuclear pore complex. Nat. Struct. Mol. Biol. 16, 782–788 (2009).

38. Sjöblom, K.E. et al. Three-dimensional super-resolution microscopy via nanobodies. J. Cell Biol. 196, 342–355 (2012).

39. Cook, A. et al. Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging. Science 341, 655–658 (2013).

40. Ori, A. et al. Cell-type specific nuclear pores: a case in point for context-dependent stoichiometry of molecular machines. Mol. Syst. Biol. 9, 648 (2013).

41. Eghlidi, H. et al. Protein-membrane interaction of the mammalian nuclear pore complex. J. Cell Biol. 158, 915–927 (2002).

42. Thierbach, K. et al. Novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. Cell 84, 265–275 (1996).

43. Sampathkumar, P. et al. Structure of the C-terminal domain of Saccharomyces cerevisiae Nup133, a component of the nuclear pore complex. Proteins 79, 1672–1677 (2011).

28. Fernandez-Martinez, J. et al. Structure-function mapping of a heptameric module in the nuclear pore complex. J. Cell Biol. 196, 419–434 (2012).

29. Whittle, J.R.R. & Schwartz, T.U. Structure of the Sec13-Sec16 edge element, a template for assembly of the COPII vesicle coat. J. Cell Biol. 190, 347–361 (2010).

30. Thierbach, K. et al. Protein interfaces of the conserved Nup84 complex from Chaetomium thermophilum shown by crosslinking mass spectrometry and electron microscopy. Structure 21, 1672–1682 (2013).

31. Bar-Peled, L. et al. A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 340, 1100–1106 (2013).

32. Alagri, R. et al. Molecular architecture and function of the SEA complex, a modulator of the TORC1 pathway. Mol. Cell. Proteomics 13, 2855–2870 (2014).

33. Siniosoglou, S. et al. A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. Cell 84, 265–275 (1996).

34. Stuewe, T. et al. Architecture of the nuclear pore complex coat. Science 347, 1148–1152 (2015).

35. Kim, S.J. et al. Integrative structure-function mapping of the nucleoporin Nup133 suggests a conserved mechanism for membrane anchoring of the nuclear pore complex. Mol. Cell. Proteomics 13, 2911–2926 (2014).

36. Liu, X. et al. Structural evolution of the membrane-coating module of the nuclear pore complex. Proc. Natl. Acad. Sci. USA 109, 16498–16503 (2012).

37. Kampmann, M. &Blobel, G. Three-dimensional structure and flexibility of a membrane-coating module of the nuclear pore complex. Nat. Struct. Mol. Biol. 16, 782–788 (2009).

38. Sjöblom, K.E. et al. Three-dimensional super-resolution microscopy via nanobodies. J. Cell Biol. 196, 342–355 (2012).

39. Cook, A. et al. Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging. Science 341, 655–658 (2013).

40. Ori, A. et al. Cell-type specific nuclear pores: a case in point for context-dependent stoichiometry of molecular machines. Mol. Syst. Biol. 9, 648 (2013).

41. Eghlidi, H. et al. Protein-membrane interaction of the mammalian nuclear pore complex. J. Cell Biol. 158, 915–927 (2002).

42. Thierbach, K. et al. Novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. Cell 84, 265–275 (1996).
ONLINE METHODS

Construct generation. Nup120952–1241, Nup145C233–791, Nup85257–1181 and full-length Sec13 were cloned from M. thermophila. Nup120952–1241 was C-terminally fused with a His16 tag and cloned into a kanamycin-resistant T7 promoter–based bacterial expression vector. To increase stability, full-length Sec13 was fused C-to-N-terminally to Nup145C233–791, with a flexible four-residue linker, in analogy to a previously described S. cerevisiae construct19. The Sec13–Nup145CFusion was N-terminally tethered to a 3C protease–degradable SUMO tag and cloned into an ampicillin-resistant T7 promoter–based bacterial expression vector. Nup85257–1181 was N-terminally fused with a 3C protease–degradable His16–Arg8–SUMO tag. The tetrameric complex is referred to as Nup120–Sec13–Nup145C–Nup85 for simplicity.

Protein production and purification. Nup120 and Sec13–Nup145C vectors were cotransformed into Escherichia coli LOBSTR-RIL(DE3) (Kerafast) cells, and protein production was induced with 0.2 mM IPTG at 18 °C for 12–14 h. Nup85 was expressed separately under identical conditions. Cells were centrifuged at 6,000 g, resuspended in lysis buffer (50 mM potassium phosphate, pH 8.0, 500 mM NaCl, 30 mM imidazole, 3 mM β-mercaptoethanol (βME) and 1 mM PMSF) and lysed with a cell disruptor (Constent Systems). The lysate was cleared by centrifugation at 12,500g for 15 min. The soluble fraction was incubated with Ni-Sepharose 6 Fast Flow beads (GE Healthcare) for 30 min on ice. After washing of the beads with lysis buffer, the protein was eluted (250 mM imidazole, pH 8.0, 150 mM NaCl and 3 mM βME). The Nup85 Ni eluate was dialyzed 1:1 with 20 mM HEPES-KOH, pH 7.4, 0.1 mM EDTA and 1 mM dithiothreitol (DTT), and was subjected to cation-exchange chromatography on a HiTrap column (GE Healthcare) with a linear NaCl gradient. The Nup120–Sec13–Nup145C Ni eluate was incubated with 3C and dialyzed overnight at 4 °C (20 mM HEPES-KOH, pH 7.4, 150 mM NaCl, 0.1 mM EDTA and 1 mM DTT). Both samples were further purified separately via size-exclusion chromatography on a Superdex S200 16/60 column (GE Healthcare) equilibrated in running buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM Nic96 and 1 mM DTT). Purified His16–Arg8–SUMO-Nup85 was incubated with an excess of purified Nup120–Sec13–Nup145C, and the assembled quaternary complex was separated via cation-exchange chromatography. Nup120–Sec13–Nup145C–Nup85 complex was incubated with 3C overnight at 4 °C and subjected to a final purification by size-exclusion chromatography on Superdex S200 (GE Healthcare). Selenomethionine (SeMet)–derivated Nup85 and Nup120–Sec13–Nup145C–Nup85 was prepared as described previously21 and purified as the native version, except that the reducing-agent concentration (βME and DTT) was 5 mM in all buffers.

Crystallization. Initial crystals of Nup120–Sec13–Nup145C–Nup85 were obtained at 18 °C in 9 %d in sitting drops over a reservoir containing 50 mM Tris-HCl, pH 8.5, and 1 mM Na2SO4, 10 mM EDTA as an additive reduced nucleation and improved the shape of the crystals. Hanging drops of 1 µl protein at 3 mg/ml and 1 µl of precipitant (50 mM Tris-HCl, pH 8.23, 0.7 M (NH4)2SO4 and 20 mM βME) were supplemented on the third day of incubation with 0.2 µl seed stock to yield diffraction-quality, thin triangular plates. Selenomethionine–derivated protein crystalized under identical conditions. Crystals were cryoprotected in mother liquor supplemented with 20% (v/v) ethylene glycol.

Data collection and structure determination. Data collection was performed at the Advanced Photon Source end station 24-IDC. All data-processing steps were carried out with programs provided through SBgrid45. Data reduction was performed with HKL2000 (ref. 46). Sec13 from S. cerevisiae (PDB 3MZK, chain A)47 was used as a search model for molecular replacement (MR). One unique solution was found in a Nup85-only SeMet derivative (space group P21, one copy per asymmetric unit). Partial MR phases were then used to find 12 out of 20 possible SeMet positions (eight were in disordered regions) with Phaser within the PHENIX suite48 with the MR–single anomalous dispersion (MR-SAD) protocol. An interpretable 4.1-Å electronic density map was obtained after solvent modification with Parrot from the CCP4 suite48. The backbone of Nup133 stacked with the closest Nup homolog to Nup84 and vice versa, despite a low sequence identity (8%). The Nup133 stacked β-helical domain and its junction with Nup107 are from S. cerevisiae (PDB 3I4R)21. Four helices between the trunk and tail domains on Nup84 were modeled from Nup96 (PDB 2QK5 and 2RFO)52,53 with Phyre2 one-to-one threading54. Nic96 was chosen as the template because superposition between its trunk and tail domains and the corresponding regions on Nup84 yielded the lowest r.m.s. deviation of all solved ACE1 domain proteins. Additionally, the BackPhyre54 structure-prediction server suggests that Nic96 is the closest Nup homolog to Nup84 and vice versa, despite a low sequence identity of 8%. The Nup133 stacked β-helical domain and its junction with Nup107 are from S. cerevisiae (PDB 3I4R)21. The interface between the Nup133 β-propeller (PDB 1XKS)16 and the stacked β-helical domain is not known but is expected to be flexible, on the basis of limited proteolysis data (data not shown).

Composite structures of the heptameric S. cerevisiae and the octameric H. sapiens Y complexes were generated in Coot with SSM superposition onto the minimal composite structure of solved and modeled structure fragments generated by one-to-one threading with Phyre2. For the S. cerevisiae Y-complex composite, scNup120714–1036 was modeled with spNup120 (PDB 4FHM)22 as the template. scNup85915–741 was modeled with mtNup85. scNup145C534–712 was modeled with mtNup145C. scNup133 was modeled with hsNup133 (PDB 1XKS and 3I4R)52,53, scNup120425–726 was modeled with hsNup120 and scNic96 as described above. For the human Y-complex composite, hsNup160 was modeled with spNup120 (PDB 4FHM)22 as the template. hsNup8521, as modeled with scNup85 (PDB 3EWE and 3F3F)18,24, hsNup85415–652 was modeled with mtNup85. hsNup96617–732 was modeled with scNup145C (PDB 3IRO and 3IKO)19,25, hsNup96633–407 was modeled with mtNup145C. hsNup107167 was modeled with scNup84 and scNic96 as described above. The composite structures are in Supplementary Data Sets 1 and 2.

Fitting composite Y-complex structures into single-particle 3D-reconstruction EM maps of Y complexes. Composite H. sapiens (omitting Nup133 and Nup107)77,92 and S. cerevisiae Y-complex structures were fitted into the published EM maps for the respective species (EMD-2443 and EMD-515215) with the Fitmap tool from Chimera55. 1,000 trials were run with an apparent resolution of 33 Å for the H. sapiens and 35 Å for the S. cerevisiae composite structures. For fitting the human composite model into EMD-2443 (ref. 15), the best solution had a correlation score of 0.8235 with 116/1,000 hits (Fig. 4c). The next-best solution (not shown) had a correlation score of 0.8219 with 8/1,000 hits. For fitting the yeast composite model into EMD-5152 (ref. 15), the best solution had a correlation score of 0.6992 with 18/1,000 hits (Fig. 4g). The next-best solution (not shown) had a correlation score of 0.6374 with 19/1,000 hits.

Fitting composite H. sapiens and S. cerevisiae Y-complex structures into the 3D-reconstruction EM tomography map of the entire NPC. A procedure similar to the one outlined in Bui et al.13 was followed to fit both human and yeast composite Y-complex structures into the published EM map of the human NPC
Yeast plasmid construction. The entire NUP145, NUP85 and NUP120 genes with their endogenous promoters and terminators (~1,000 nucleotides before the start codon and ~400 after the stop codon) were separately cloned into the multiple cloning site of the centromeric YCplac33 shuttle vector. Additionally, the entire NUP145 and NUP85 genes with their endogenous promoters and terminators were separately cloned into the multiple cloning site of the centromeric prs315 vector. All cloning was performed according to the standard Gibson assembly method. The constructed wild-type vectors used in this study were named as follows: GKYp01 (NUP120, URA3, CEN), GKYp02 (NUP85, URA3, CEN), GKYp03 (NUP145, URA3, CEN), GKYp04 (NUP85, LEU2, CEN) and GKYp05 (NUP145, LEU2, CEN).

Vectors in which the last C-terminal α-helix of Nup120 (Δα30, Δα106–1037), Nup85 (Δα30, Δα19–744) and Nup145C (Δα27, Δα689–712) had been deleted were also generated with GKYp01, GKYp04 and GKYp05, respectively. The deletion vectors were created by introducing an early stop codon with the QuikChange method. The constructed deletion vectors used in this study were named as follows: GKYp06 (NUP120Δα30, URA3, CEN), GKYp07 (NUP85Δα30, LEU2, CEN), and GKYp08 (NUP145Δα27, LEU2, CEN).

Yeast strain construction. Yeast strains used in this study are listed in Supplementary Table 3. The ΔNUP120 haploid strain was obtained from the S. cerevisiae deletion consortium (background BY4742, no. 14906), transformed with the YCplac33 empty vector (negative control), GKYp01 (positive control) or GKYp06 (truncation), and selected on plates lacking uracil. The diploid ΔNUP85 and ΔNUP145 strains were also obtained from the S. cerevisiae deletion consortium (background BY4743, nos. 26840 and 24459, respectively), transformed with either GKYp02 or GKYp03, respectively, and selected on uracil negative plates. The transformed diploids were then sporulated and subjected to tetrad dissection. The resulting ΔNUP85 haploids containing GKYp02 were transformed with prs315 empty vector (negative control), GKYp04 (positive control) or GKYp07 (truncation), whereas the ΔNUP145 haploids containing GKYp03 were transformed with prs315 empty vector (negative control), GKYp05 (positive control) or GKYp08 (truncation). The ΔNUP85 and ΔNUP145 haploid transformations were selected on plates lacking leucine.

Cell growth analysis. The ΔNUP120 strains containing YCplac33, GKYp01 or GKYp06 were grown as liquid cultures in YPD overnight, with shaking, at 30 °C. The cultures were then diluted in YPD to an OD600 of 0.1, and aliquots were deposited into a 96-well plate (100 μl of culture/well). The plate was placed into a Synergy 2 multimode microplate reader (BioTek), and the OD600 of all the wells containing a culture was monitored every 15 min for 24 h. The plate was shaken continuously and kept at 30 °C.

The ΔNUP85 haploid strains containing GKYp02 and prs315, GKY04 or GKY07, and the ΔNUP145 haploid strains containing GKYp03 and prs315, GKY05 or GKY08 were grown as liquid cultures in YPD overnight, with shaking, at 30 °C. The cultures were then diluted in either SC medium (containing all 20 amino acids, uracil and 2% glucose) or SC medium containing 5-fluoro-orotic acid (5-FOA) at 1 mg/ml. The plate was placed into a Synergy 2 multimode microplate reader, and the OD600 of all the wells containing a culture was monitored every 15 min for 24 h. The plate was shaken continuously and kept at 30 °C.

The growth of three separate clones of each strain was tested in quintuplicates. The data were graphed in Prism (GraphPad Software). All error bars represent s.e.m.