

Piecing together nuclear pore complex assembly during interphase

Michael Rexach

Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064

All nucleocytoplasmic traffic of macromolecules occurs through nuclear pore complexes (NPCs), which function as stents in the nuclear envelope to keep nuclear pores open but gated. Three studies in this issue (Flemming, D., P. Sarges, P. Stelter, A. Hellwig, B. Böttcher, and E. Hurt. 2009. *J. Cell Biol.* 185:387–395; Makio, T., L.H. Stanton, C.-C. Lin, D.S. Goldfarb, K. Weis, and R.W. Wozniak. 2009. *J. Cell Biol.* 185:459–473; Onishchenko, E., L.H. Stanton, A.S. Madrid, T. Kieselbach, and K. Weis. 2009. *J. Cell Biol.* 185:475–491) further our understanding of the NPC assembly process by reporting what happens when the supply lines of key proteins that provide a foundation for building these marvelous supramolecular structures are disrupted.

NPCs assemble at the nuclear envelope in two different ways. The first occurs during reformation of the nuclear envelope at the end of mitosis and involves the recruitment of NPC components (nucleoporins or Nups) and membrane vesicles to chromatin. The second occurs during interphase and involves synthesis of new NPC components. Much less is known about this latter pathway, which is of particular importance in organisms such as *Saccharomyces cerevisiae* that do not undergo nuclear envelope and NPC breakdown in mitosis. Now, three studies in this issue (see Flemming et al. on p. 387, Makio et al. on p. 459, and Onishchenko et al. on p. 475) shed some light on how new pores are formed in this organism. Together, these studies show that the nucleoporins Nup170 and Nup157 help to build new NPCs by recruiting nucleoporins and candidate membrane fusogens to sites of NPC assembly in the nuclear envelope.

Budding yeast NPCs are formed by the intimate interaction of ~30 different nucleoporins in multiple copies for a total of ~450 nucleoporins per NPC (Alber et al., 2007). The complexity of assembling this 50-mD structure could be greater than the complexity of assembling the 3.2-mD yeast ribosome, which is comprised of ~80 protein and RNA components (Morgan et al., 2000). By genetically manipulating *S. cerevisiae*, Onishchenko

et al. (2009) show that the lipophilic nucleoporins Nup59/53 and the integral pore membrane nucleoporins Pom152 and Pom34 have redundant functions, i.e., to tether Nup170 and a third integral membrane nucleoporin Ndc1 to sites of new NPC assembly in the nuclear envelope. In the absence of Nup59 and Nup53 and Pom152 and Pom34, nucleoporin-rich foci accumulate throughout the cytoplasm (likely at peripheral ER sites), and the diameter of nuclear pores in the envelope increases (Onishchenko et al., 2009). This finding echoes recent work from Dawson et al. (2009), showing that a set of membrane-bending proteins, the ER reticulons Yop1 and Rtn1, which display genetic interactions with the Poms, play an essential role in the formation of new NPCs. Without reticulons, NPC-like intermediates also accumulate in the outer and inner membranes of the nuclear envelope but not at sites in nuclear pores where these membranes normally join.

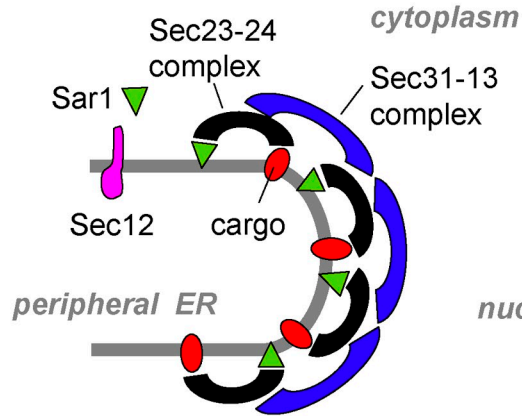
Approaching NPC assembly from a different angle, Makio et al. (2009) present evidence that depletion of Nup170 and its homologue Nup157 also causes the accumulation of NPC-like structures in the inner nuclear membrane and at cytoplasmic foci rather than properly localized to nuclear pores spanning the nuclear envelope. Likewise, Flemming et al. (2009) demonstrate that overexpression of just the Nup170 C terminus in cells lacking full-length Nup170 also causes the accumulation of NPC-like structures at peripheral ER membranes.

In all of these featured studies, the genetic defects created by the investigators led to a reduction of the total number of NPCs per nucleus and a consequent reduction of nucleocytoplasmic transport. The common phenotype was partly assembled NPC precursors accumulating at inner or outer membranes of the nuclear envelope (and the continuous peripheral ER membranes) unable to fuse across the luminal chasm to create new pores. Many of the NPC-like structures had dimensions similar to mature NPCs but represented distinct assembly intermediates given the assortment of nucleoporins detected in them. Notably, the cytoplasmic NPC-like structures were depleted of nucleoplasmic facing Nups (e.g., Nup1, Nup60, Nup2, and Mlp1), and the NPC-like structures in the envelope were depleted of cytoplasmic facing Nups (e.g., Nup82 and Nup159; Makio et al., 2009), suggesting that in the mutants, the two apposing halves

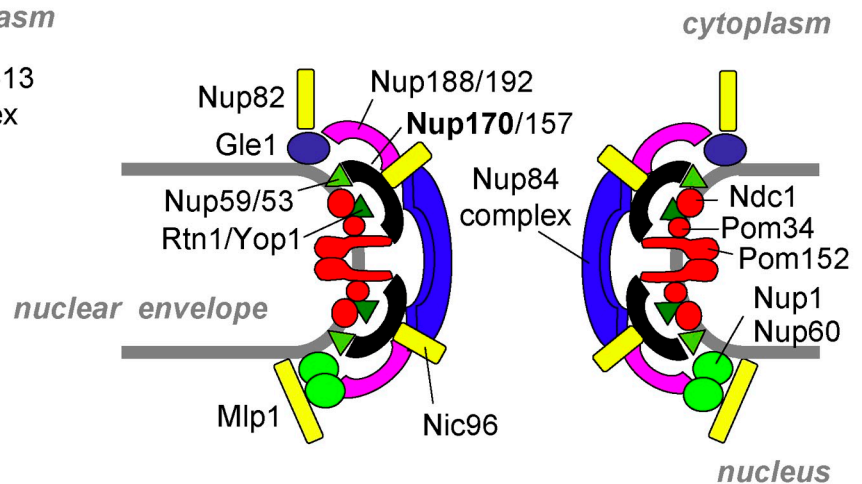
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Correspondence to Michael Rexach: rexach@biology.ucsc.edu

A COPII membrane coat



B Nuclear pore membrane coat



C

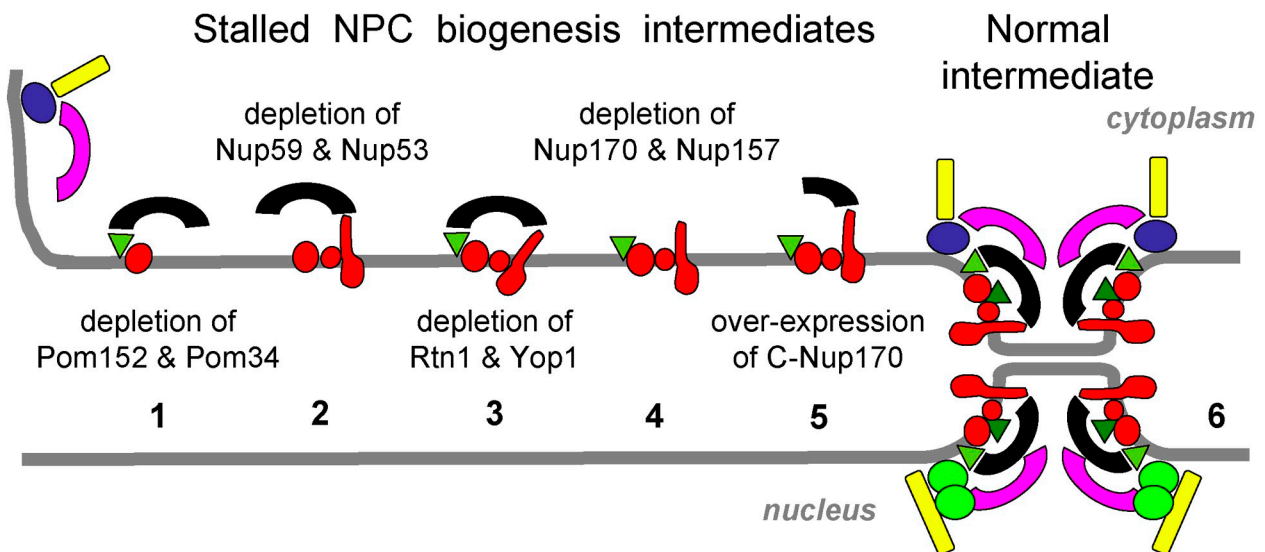


Figure 1. Similarities between COPII coats and nuclear pore coats. (A) Structure of a COPII coat driving vesicle budding from the ER membrane. The integral membrane protein (Sec12) marks the site of budding and recruits Sar1 to the membrane. Sar1 then inserts itself in the outer leaflet of the membrane via an amphipathic α -helix and imparts curvature to the membrane (Lee et al., 2005). Sar1 then recruits the Sec23–Sec24 coat complex, which traps membrane protein cargos using a large concave surface (Fromme et al., 2008). Another set of coat proteins (Sec13–Sec31) docks to Sec23–Sec24 and oligomerizes to create an outer cage structure (Fath et al., 2007) that helps Sar1 drive membrane fusion and vesicle scission (Lee et al., 2005). (B) Structure of the proposed nuclear pore membrane coat formed by Poms, Nups, and reticulons. See the main text for a detailed description. The topology of the Nup84 complex (as shown) is different than suggested in previous models (Alber et al., 2007; Hsia et al., 2007) but is consistent with the data used to generate them. In this alternate configuration, 16 concave-shaped Nup84 heptamers (each ~ 8 nm wide \times 40 nm long; Hsia et al., 2007) would stack laterally in an antiparallel arrangement (joined at the midbody) with their concave sides facing outwards toward the pore membrane. This would create an hourglass scaffold structure with a minimum inner diameter of 40 nm, which is consistent with the size limit for facilitated transport across NPC. Interestingly, this layout could physically isolate the pore membrane region from the central conduit region. (C) Diagram depicting what happens to cells when supply lines of Poms and Nups that provide the foundation for building new NPCs are disrupted. Without Pom152 and Pom34 (scenario 1) or Nup53 and Nup59 (scenario 2), Ndc1 and cytoplasmic facing nucleoporins (e.g., Nup82 and associated Nups; Alber et al., 2007) are not secured at new sites of NPC assembly and can wander off path to peripheral ER membranes (Onishchenko et al., 2009). Without the reticulons Rtn1 and Yop1 (scenario 3), the curved membranes at sites of new NPC assembly would be unstable, leading to the accumulation of NPC-like intermediates at the nuclear envelope (Dawson et al., 2009). Without the Nup170/Nup157 homologues (scenario 4; Makio et al., 2009) or in cells depleted of Nup170 but overexpressing its C terminus (scenario 5; Flemming et al., 2009), the Nup53–Ndc1–Pom34–Pom152 complex is unstable and unable to serve as a foundation for building new NPCs. A normal intermediate of NPC assembly depicting a later stage is shown for comparison (scenario 6). A more mature NPC is shown in B.

of the otherwise symmetric NPC fail to join at nuclear pores during biogenesis. Perhaps most importantly, Makio et al. (2009) and Onishchenko et al. (2009) demonstrate, using a photoconvertible nucleoporin–Dendra approach to distinguish old from new NPCs, that the stalled Nup complexes that accumulate in the cytoplasmic foci remain active as assembly

intermediates and can be recruited promptly to new NPCs upon reversal of the genetic block.

To understand some of the earliest events during NPC biogenesis, including those that drive the fusion between the inner and outer nuclear membranes, and to bring the results of the featured reports into focus, one can draw a functional parallel

between the cellular machinery used in the formation of COPII-coated transport vesicles (Fig. 1 A; Fromme et al., 2008) and the nucleoporins that coat nuclear pores (Fig. 1 B). This comparison is justified because the peripheral ER membrane is continuous with the nuclear envelope, and several nucleoporins, including Nup170/Nup157, Nup188/192, and the heptameric Nup84 complex, are predicted to resemble vesicle coat proteins at the structural level (Devos et al., 2006; Hsia et al., 2007; Boehmer et al., 2008). In fact, the COPII coat protein Sec13 is also a component of the Nup84 complex. Based on the events that occur for COPII budding (marking a site of assembly, recruitment of proteins that promote membrane bending and curvature, followed by a membrane fusion event), one can envision the following scenario (among others) for NPC formation.

During new NPC assembly, the complex between the three membrane-spanning nucleoporins Pom152, Pom34, and Ndc1 could mark the sites of new NPC assembly at inner and outer nuclear envelope membranes, which is consistent with the observation that NPC assembly proceeds from both sides of the envelope (D'Angelo et al., 2006). This membrane complex then recruits Nup53/59 and the reticulons Yop1/Dbp1 to the membrane via the direct interactions (Dawson et al., 2009; Onishchenko et al., 2009). These recruits insert themselves in the outer leaflet of the membrane using monotopic membrane insertion domains, imparting local curvature to the membrane. Nup53/59 and Pom152 then cooperate to recruit the crescent moon-shaped Nup170/Nup157 coat protein, which further stabilizes the pore membrane complex and the curved membrane. Nup170/157 then recruits a second putative coat protein Nup188/192, which serves to tether peripheral Nup structures (those containing strictly cytoplasmic or nucleoplasmic facing Nups) and the linker nucleoporins Nup82 and Nic96, to the pore membrane. These linkers then recruit natively unfolded nucleoporins (and other Nups not shown; Alber et al., 2007) to seal and gate the aqueous conduit that will form. In a final act, the luminal domains of Pom152 molecules in apposing membranes zipper up together and oligomerize with Pom34 to form the ring complex isolated by Alber et al. (2007). This ring and the other membrane-proximal proteins would drive the membrane fusion event and could set the diameter of the pore membrane (Onishchenko et al., 2009). Finally, 16 concave-shaped Nup84 heptamers (two per spoke, as shown in Fig. 1 B) would clamp the cytoplasmic and nucleoplasmic halves of the NPC together. These heptamers would need to stack laterally in an antiparallel fashion joined at the midbody (Hsia et al., 2007) with their concave surface facing outwards toward the pore membrane to create an hourglass scaffold structure capable of stabilizing curved membranes all around the periphery of a nuclear pore. If the Pom ring complex and the membrane-inserted proteins were to drive membrane fusion without help from coat proteins, as suggested by a morphological study (Goldberg et al., 1997), then the membrane coats would assemble promptly at nascent pores to stabilize the curved membrane. Ultimately, any defect in the assembly process could cause the accumulation of Nup complexes and/or NPC-like structures that end up diffusing along nuclear envelope and peripheral ER membranes as observed in the featured studies (Fig. 1 C).

In the end, should we care about which assembly method is used to build NPCs (i.e., from new or old components)? Apparently we should, as new NPCs are recruited to daughter cells preferentially (Shchebrova et al., 2008). Perhaps this asymmetrical distribution mechanism occurs because every cell knows that old NPCs can become leaky, and any leaky pore can breach the nucleocytoplasmic permeability barrier to cause problems related to aging (D'Angelo et al., 2009). Thus, when looking for a fountain of youth to slow down aging or just for a good puzzle to solve, one needs to look no further than the process of new NPC biogenesis.

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