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Nuclear envelope insertion of spindle pole bodies and nuclear pore complexes

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Abbreviations: SPB, spindle pole body; NPC, nuclear pore complex; NE, nuclear envelope; INM, inner nuclear membrane; ONM, outer nuclear membrane; MTOC, microtubule organizing center; ALPS, ArfGAP1 lipid packing sensor; SUN, Sad1-UNC-84 homology domain; PNS, perinuclear space; KASH, klarsicht ANC-1 syne homology; Pom, pore membrane protein; SIF, shared insertion factor

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The defining feature of eukaryotic cells is the double lipid bilayer of the nuclear envelope (NE) that serves as a physical barrier separating the genome from the cytosol. Nuclear pore complexes (NPCs) are embedded in the NE to facilitate transport of proteins and other macromolecules into and out of the nucleus. In fungi and early embryos where the NE does not completely breakdown during mitosis, microtubule-organizing centers such as the spindle pole body (SPB) must also be inserted into the NE to facilitate organization of the mitotic spindle. Several recent papers have shed light on the mechanism by which SPB complexes are inserted into the NE. An unexpected link between the SPB and NPCs suggests that assembly of these NE complexes is tightly coordinated. We review the findings of these reports in light of our current knowledge of SPB, NPC and NE structure, assembly and function.

The Nuclear Envelope and Nuclear Envelope Proteins

The nuclear envelope (NE) is a double lipid bilayer (**Fig. 1A**). The outer nuclear membrane (ONM) is contiguous with the endoplasmic reticulum (ER) and shares a number of integral membrane components. This includes many lipid biosynthetic enzymes. In contrast, the inner nuclear membrane (INM) contains a distinct set of proteins and lipids from either the ONM or the ER. Proteomic analysis of the INM indicates that it is composed of over 100 distinct proteins, most of which are uncharacterized.[1] In metazoans, the NE is also associated with the lamin intermediate filament network. Nuclear lamins along with lamin-associated proteins provide structural support for the NE and contribute to the intranuclear arrangement of chromosomes. Cells lacking lamins or lamin-associated proteins have an altered nuclear morphology, changes in chromosome organization and aberrant gene expression, resulting in a broad spectrum of human diseases ranging from tissue- specific diseases of muscle, bone and fat cells to multi-system diseases, such as the premature aging syndrome progeria and cancer.[2,3] What nuclear processes are altered and why different cell types are differentially affected is not well understood. A recent analysis of lamin B knockout mice showed a requirement for this major component of the nuclear lamina in organogenesis, but surprisingly, embryonic stem cells derived from these animals do not exhibit changes in NE morphology or gene expression.[4] This result strongly suggests that additional proteins function in parallel to the lamins in the maintenance of nuclear structure.

The Sad1-UNC-84 (SUN) homology domain proteins are leading candidates to function in lamin-independent control of nuclear architecture because of their role in chromosome attachment at the nuclear periphery, in nuclear pore complex (NPC) assembly and in duplication and tethering of microtubule-organizing centers (MTOCs) to the NE.[5-7] In higher eukaryotes, localization of certain SUN proteins requires lamins,[8-11] indicating at least an indirect connection between these two classes of NE organizers.

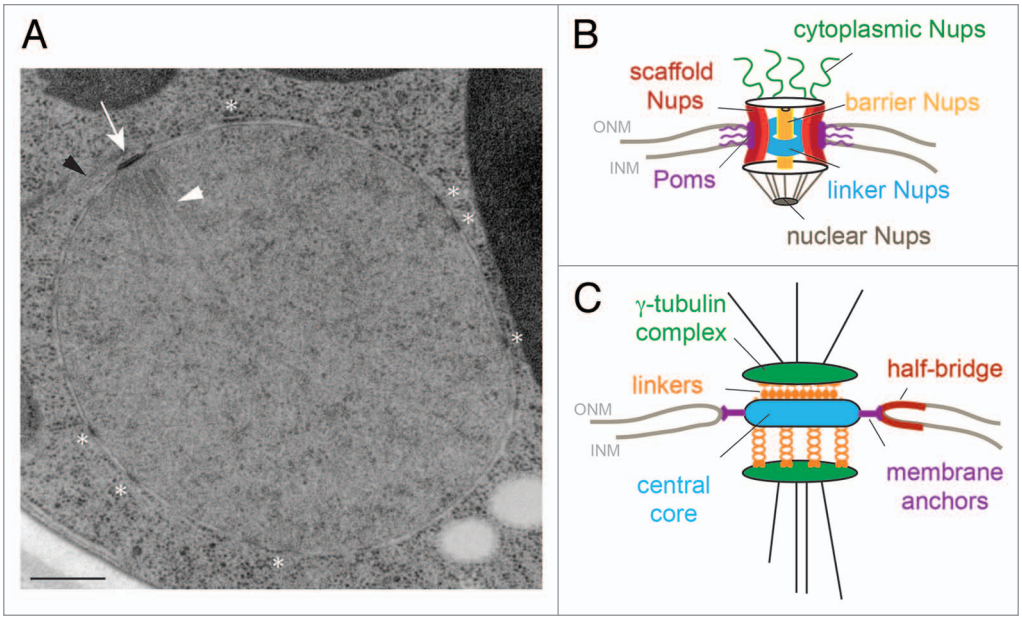

Figure 1. Nuclear envelope protein complexes. (A) Thin-section electron micrograph of a budding yeast nucleus. Embedded in the nuclear envelope are the SPB (white arrow) and multiple NPCs (asterisks). The positions of nuclear (white arrowhead) and cytoplasmic (black arrowhead) microtubules are also indicated. Bar, 0.2 μm. (B) Schematic of the NPC showing major subcomplexes: the cytoplasmic filaments, the nuclear basket, the central core composed of many FG-Nups, the scaffold and linker Nups and the membrane-associated Poms that tether the NPC in the NE. (C) Schematic of the SPB showing the organization of SPB components into five sub-complexes: the γ-tubulin complex that nucleates microtubules, the linker proteins that connect the γ-tubulin complex to the cytoplasmic and nuclear face of the core SPB, the soluble core SPB/satellite components that form the foundation of the SPB and SPB precursor, the membrane anchors that tether the core SPB in the NE and the half-bridge components that are important for SPB assembly.

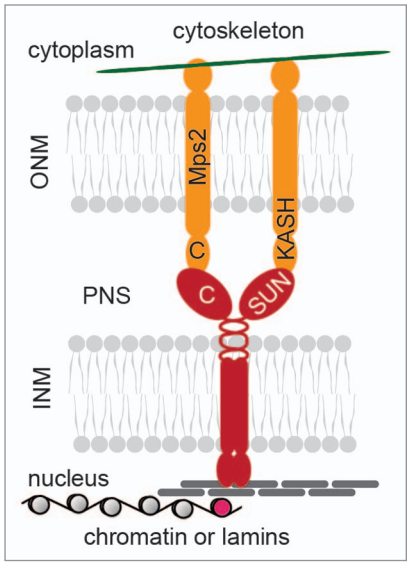

Figure 2. SUN proteins. The INM SUN proteins interact with ONM proteins, such as Mps2 or KASH-domain proteins, in the PNS to link the nucleus to the cytoskeleton.

However, because SUN proteins are present in plants and lower eukaryotes such as fungi that lack lamins, their function in chromosome organization, insertion of MTOCs into the NE and NE morphology must be lamin-independent. Originally identified in *Caenorhabditis elegans* and *Schizosaccharomyces pombe*, the SUN domain-containing proteins are widely conserved components of the INM of all eukaryotes. At least one gene encoding a SUN protein has been found in every eukaryote sequenced to date.[5,12,13] Many eukaryotes, such as mammals, have multiple genes encoding SUN proteins; of the five mammalian SUN genes, two are ubiquitously expressed, while three appear to be expressed primarily in the male germline. Budding yeast was originally not predicted to contain a SUN protein,[14] however more advanced sequence alignment tools facilitated identification of a SUN domain in the spindle pole body (SPB) component Mps3.[12]

Localized to the INM, the integral membrane SUN proteins contain an N-terminal domain that is located in the nucleoplasm where it is thought to interact with chromatin and/or the nuclear lamina (**Fig. 2**). The larger C-terminal domain generally contains at least one coiled-coil motif as well as the conserved SUN domain. Although the exact function of the ~175 amino acid SUN domain is not known, several lines of evidence from multiple species point to a role in binding and localization of ONM proteins, particularly those that have a small C-terminal domain.[5-7] A weak degree of homology has been observed in the tails of these ONM proteins (a KASH motif, for Klarsicht ANC-1 Syne homology), although there is evidence that SUN proteins may interact with non-KASH domain-containing ONM proteins, as illustrated by the interaction between Mps3 and its binding partner Mps2.[12] The most notable feature of SUN binding proteins is their connection via an N-terminal domain to the actin, microtubule or intermediate cytoskeleton. In this way, the SUN proteins form a linker complex, coupling the cytoplasmic cytoskeleton with chromosomes in the nucleus, even in the presence of an intact NE (**Fig. 2**). This linker complex is important for nuclear migration and for chromosome movement, particularly during meiotic prophase.[6] The role of SUN proteins in nuclear processes has been the

topic of several recent reviews in references 5–7. We will focus on recent studies linking SUN proteins to insertion of NPCs and MTOCs into the bilayers of the NE and propose a model linking the assembly of these complexes.

NPC Assembly

NPCs are large protein complexes approximately 40–70 MDa found in the NE of all eukaryotes.[13] Each NPC is composed of roughly 30 proteins present in multiple copies. The NPC controls the transport of proteins greater than approximately 40 kDa and macromolecules such as RNAs into and out of the nucleus. Regulation of nuclear-cytoplasmic trafficking can have dramatic effects on transcription, chromosome integrity, nuclear organization and many other cellular processes.[15,16] Control of NPC insertion is one way that cells can regulate the bidirectional transport of cargos. An increase in the number of NPCs must be coupled to the cell cycle and nuclear division to ensure that the cell has an adequate capacity for nuclear-cytoplasmic transport as the nucleus expands during cell growth and contracts/divides following cytokinesis.[17-19]

NPCs are embedded in the NE; the INM and ONM are contiguous at the NPC, forming a highly curved membrane known as the pore membrane (**Fig. 1A and B**). In metazoans, NPCs are assembled into the NE during its reassembly around the chromosomes following anaphase. This pathway is coupled with changes in NE structure that occur during mitosis, including disassembly and reassembly of the nuclear lamina.[20] However, NPCs are also inserted into the NE by a de novo assembly pathway that is active during interphase.[21] In organisms such as *Saccharomyces cerevisiae* that undergo a closed mitosis where the NE remains intact, de novo insertion of NPCs is the sole assembly mechanism.[19] Genetic and cytological analysis of de novo NPC assembly suggests that it occurs in a stepwise manner and does not involve division or splitting of pre-existing NPCs.[15] Instead, changes in membrane organization and the sequential recruitment of NPC subcomplexes are thought to be necessary for de novo NPC assembly (**Fig. 3**).

EM analysis of NPC assembly suggests that a change in NE organization is essential for NPC insertion (**Fig. 3**). Based on their observations that the spacing between INM and ONM decreased prior to the detection of NPC complexes, Goldberg and colleagues suggested that interaction of INM and ONM proteins within the perinuclear space (PNS) drives formation of the pore membrane early in NPC assembly.[22] Molecular studies have suggested that the lumenal domains of the integral membrane proteins of the NPC (known as Poms, for pore membrane proteins) are probably involved in this early step, although other membrane proteins including the SUN proteins may also play a role (**Fig. 4A**).[23-27] Depletion or mutation of reticulons, membrane-bending proteins of the ER, results in defects in NPC assembly, suggesting their involvement in the generation of membrane curvature during de novo NPC assembly.[28] Presumably, these proteins would act on the outer leaflets of the NE (**Figs. 3 and 4A**). What corresponding or compensatory changes occur on the inner membrane leaflets is not known. Also, since the reticulons are not stably associated with intact NPCs, additional factors must stabilize the curved membrane generated by the reticulons. Several NPC subunits (for example, Nup133, Nup120, Nup85, Nup170 and Nup188 in yeast) contain an ALPS motif (for ArfGAP1 lipid packing sensor). ALPS domain-containing proteins contain an amphipathic α-helix with a hydrophobic patch, which allows for preferential hydrophobic interactions with lipid tails.[29,30] Because lipid tails are only accessible on curved membranes (in a tightly packed lipid bilayer the lipid tails are shielded), ALPS-domain containing Nups presumably bind to and stabilize the highly curved pore membranes. The formation of a coat complex on the NE by these proteins facilitates NPC insertion by generation of a membrane structure where INM and ONM are fused (**Fig. 4A**). Once the pore membrane site has been formed, additional soluble NPC subunits can assemble, often as partially assembled sub-complexes that are preassembled in the cytoplasm or the nucleoplasm (**Fig. 3**). This completes NPC assembly, and a new functional NPC is embedded in the NE.

Despite the fact that many of the players involved in NPC assembly have been identified and their temporal order of function and structural organization has been carefully studied in both yeast and metazoan systems, many questions regarding the process of de novo NPC duplication remain. How is the initial site of NPC assembly marked? Is NPC assembly controlled by the availability of one or more of the Pom proteins? How do the Pom proteins drive changes in membrane organization and why are they so poorly conserved? What types of membrane changes are needed to form a pore membrane? What is the function of the ALPS domain in vivo?

In vertebrate cells, Sun1 is associated with NPCs and is involved in their assembly and distribution in the NE.[23,25] However, in lower eukaryotes, neither Mps3 nor Sad1, the budding and fission yeast SUN proteins, respectively, co-localize with NPCs or are required for NPC assembly.[31,32] Instead, analysis of SUN proteins in yeast has revealed a role in NE insertion of the SPB through a mechanism that probably requires membrane-remodeling events similar to those involved in de novo NPC assembly.[33-35] Furthermore, these studies on the mechanism of SPB duplication have demonstrated an unexpected connection between the NPC and SPB. Characterization of SPB duplication has added to our understanding of the molecular events required for NE fusion, including a role for SUN proteins that may also be important for de novo NPC assembly.

The Spindle Pole Body

The NE has evolved as a barrier to organize and protect the genome.[13] However, the NE presents a unique challenge to cells in terms of accessing the chromosomes within the nucleus with soluble cytoplasmic components of the cytoskeleton. This is particularly an issue in organisms such as budding and fission yeast that undergo a closed mitosis. How can cytoplasmic microtubules mediate chromosome segregation in the nucleus? The solution employed by *S. cerevisiae* is to embed the SPB in the NE throughout the yeast lifecycle (**Fig. 1A**).[36] Therefore,

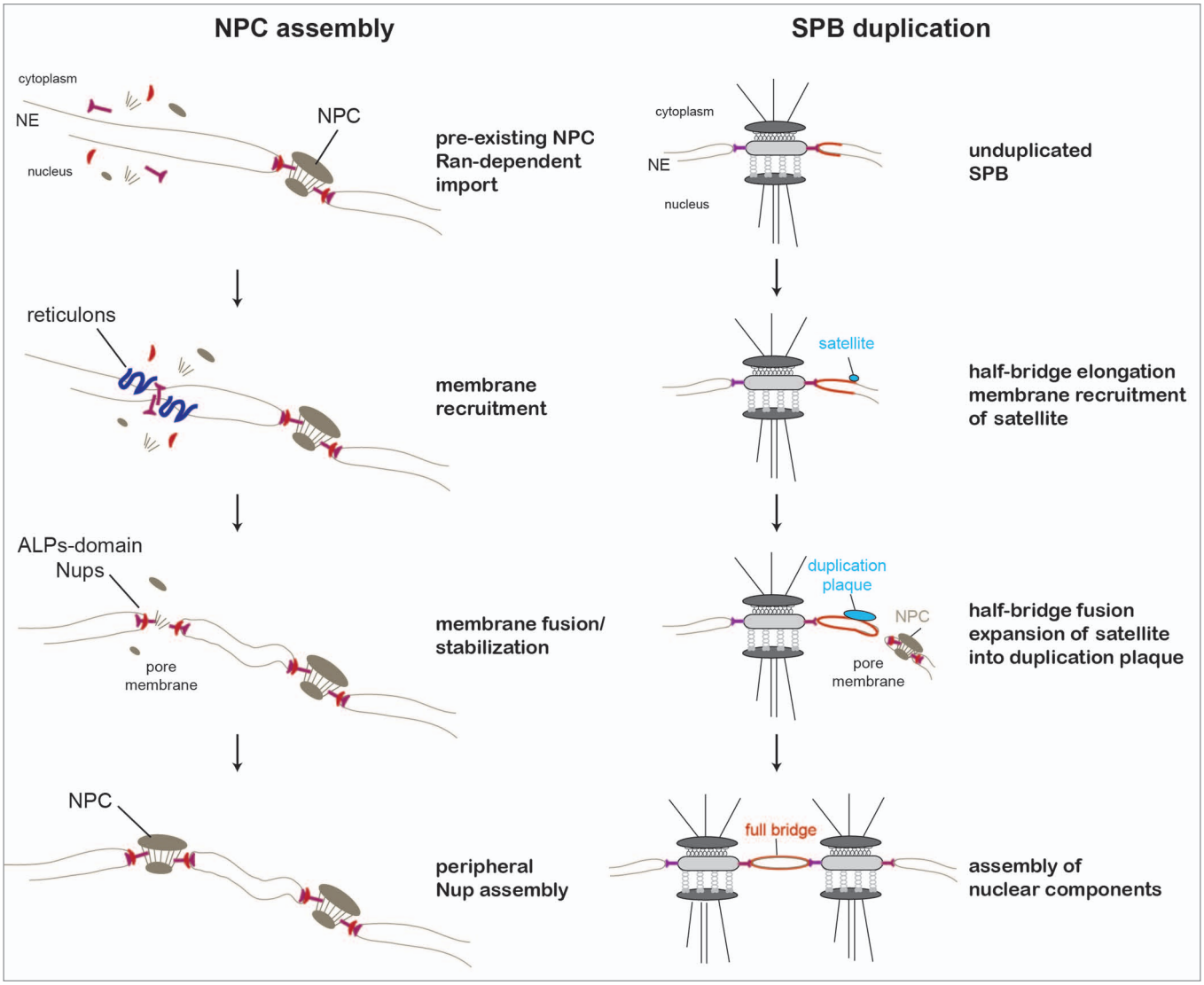

Figure 3. Duplication of the NPC and SPB requires the formation of a NE pore. Genetic analysis of NPC assembly in yeast and cytological studies of de novo NPC insertion in mammals indicates a series of discrete steps are involved: following Ran-dependent import of NPC subunits and other proteins through pre-existing NPCs, lumenal interactions of Pom proteins occurs early during assembly, presumably leading to dimpling of the NE; further NE remodeling occurs through the recruitment of the reticulons; this is followed by association of Nups, including those containing an ALPS domain to bind and stabilize the curved pore membrane; assembly of additional Nups from both the cytoplasmic and nucleoplasmic side of the NE leads to the formation of a functional NPC. Similarly, cytological analysis of SPB intermediates in wild type and mutant yeast cells suggests that SPB duplication can be divided into three steps: elongation of the half-bridge and formation of the satellite, which contains soluble precursors to the SPB; expansion of the satellite into a duplication plaque and fenestration of the NE; and insertion into the NE and assembly of nuclear components to create duplicated side-by-side SPBs.

the SPB simultaneously nucleates nuclear microtubules involved in spindle assembly and cytoplasmic microtubules required to position the nucleus (**Fig. 1C**). In *S. pombe*, the SPB is tethered to the NE throughout the cell cycle, but it is inserted into the NE prior to entry into mitosis and is extruded from the NE upon mitotic exit.[37]

SPB duplication in both yeasts has been extensively characterized at the level of electron microscopy, and many SPB components have been identified using genetic and biochemical methods.[38-40] At the mechanistic and molecular level, duplication of the budding yeast SPB has been extensively studied so we will focus primarily on its assembly into the NE. As depicted in **Figure 1C**, the 18 *S. cerevisiae* SPB components can be arranged into five sub-complexes. Based on cytological analysis of SPB intermediates in wild-type and mutant yeast cells, it is thought that a SPB precursor, known as the satellite, forms on the distal tip of the half-bridge early in SPB assembly. Continued expansion of the satellite by addition of soluble precursors and expansion of the half-bridge leads to the formation of a duplication plaque and fenestration of the NE. SPB duplication is completed by insertion of the duplication plaque into the NE and assembly of nuclear components to create duplicated side-by-side SPBs (**Fig. 3**). Unlike NPC

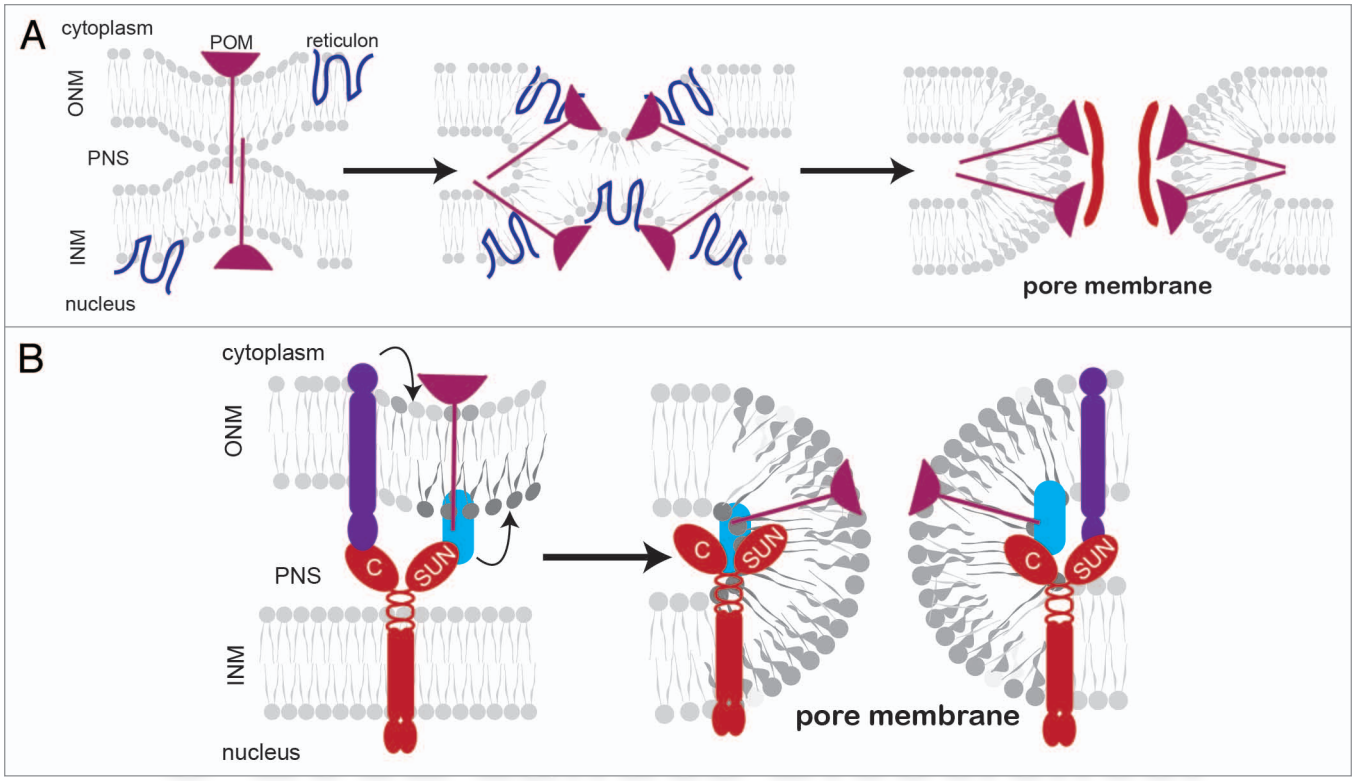

Figure 4. Proposed role of SUN proteins in NE structure. (A) Remodeling of the NE that is thought to occur during NPC insertion. Lumenal interactions between Poms (magenta) decrease the distance between INM and ONM. In addition, insertion of reticulons (blue) into the outer leaflets of both INM and ONM helps to generate curvature. Other membrane remodeling events, such as addition of very long chain fatty acids and incorporation of unsaturated polar lipids and sterols, is also likely involved in the formation of the pore membrane, and the curved membrane is stabilized by binding of ALPS domain-containing Nups (red) (B). We propose that the C-terminus of SUN proteins associates with proteins in the PNS to decrease the distance between INM and ONM as well as proteins involved in membrane remodeling (purple and cyan). This could drive changes in the physical properties of the NE (including altering lipid composition, which is depicted by lipids with different degrees of saturation and chain length in lighter and darker gray) required for the formation of a pore membrane found at the NPC and SPB. In addition, SUN proteins might also interact with proteins that form and stabilize the pore membrane (magenta).

assembly, SPB duplication is spatially and temporally restricted. The new SPB is assembled during late G_1 phase at the distal tip of the half-bridge, approximately 100 nm from the pre-existing SPB.[36] Therefore, SPB duplication serves as an excellent model to study how the NE is reorganized to allow for protein complex insertion.

As the sole site of microtubule nucleation in budding yeast, the SPB must be assembled and inserted into the NE once every cell cycle. Although the exact mechanism of SPB insertion is unknown, its insertion into the NE requires the formation of a pore membrane similar to that found at the NPC (**Fig. 3**). Modification of the NE leaflets, fusion of INM and ONM, binding of proteins that stabilize a curved membrane and assembly of soluble proteins are thought to be required during SPB assembly. Genetic analysis of SPB assembly has shown that the membrane anchors and half-bridge components such as Mps3 play a role in SPB insertion.[34,35,41-46] One of the SPB membrane anchors is Ndc1, a conserved Pom, which is also required for NPC assembly.[47] Unlike factors involved in NPC assembly, there is little redundancy in SPB insertion factors. All four membrane anchors and Mps3 are encoded by essential genes; yeast cells harboring deletions in any of these genes are inviable, and cells containing specific mutated versions of each have a defect in SPB insertion.

Therefore, we were surprised to discover that the function of Mps3 in SPB assembly could be bypassed if specific subunits of the NPC were also deleted.[35] Similar results were also reported for the membrane protein Mps2 and its binding partner Bbp1[46] and for Ndc1.[47] It is difficult to envision how the SPB could duplicate in the absence of a structural protein such as Mps3 and or how it could be tethered in the NE without the membrane anchors Mps2, Bbp1 or Ndc1. At least two possible models could account for these observations and are supported by additional evidence in the literature. One focuses on a limiting protein that modulates the insertion of large protein complexes such as the NPC and SPB into the membrane (Model A) and one focuses on changes in the NE in terms of lipids to facilitate SPB insertion (Model B) (**Fig. 5**). Either model could account for the Mps3-independent SPB duplication that has been observed.[35]

Model A: the shared insertion factor model. Because NPCs and SPBs must both insert into the NE and because many of the same molecular events are likely required for both (**Figs. 3 and 4**), NPCs and SPBs may compete for a shared

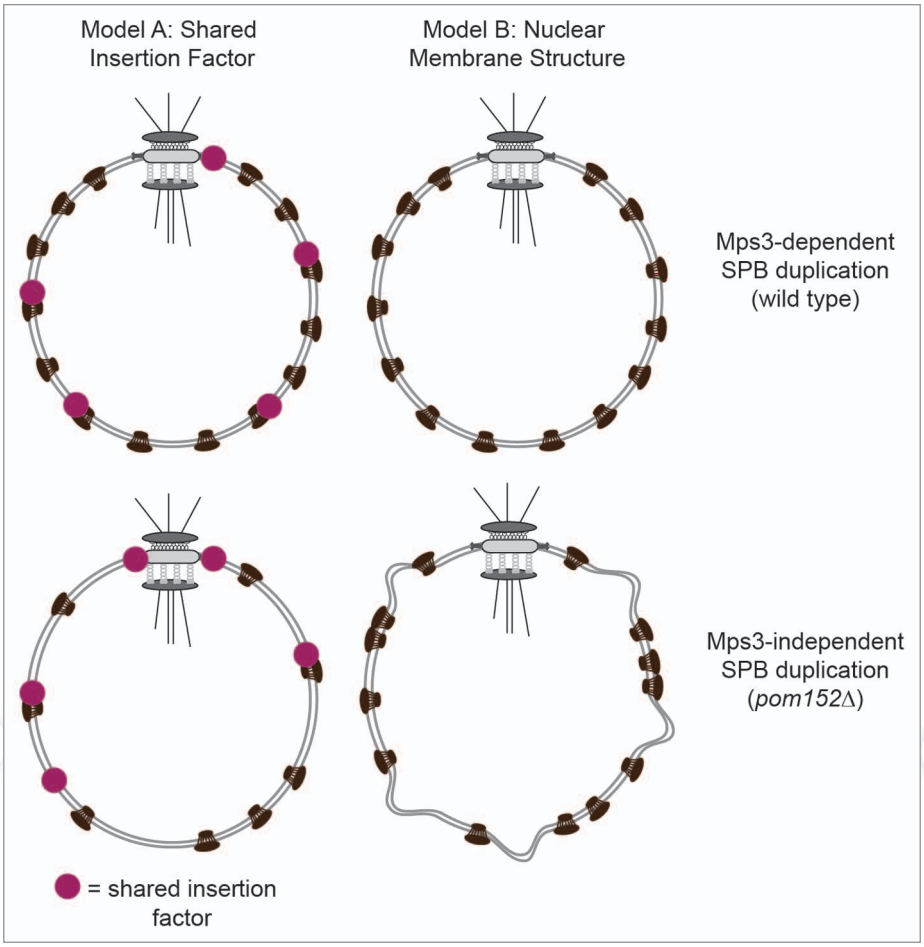

Figure 5. Two possible models to account for Mps3-independent SPB assembly. Model A, the shared insertion factor model, is based on the hypothesis that SPBs and NPCs compete for a shared insertion factor, such as Ndc1. Model B, the nuclear membrane structure model, is based on the hypothesis that NPCs impart structural rigidity to the NE. See text for additional details.

insertion factor (SIF) (**Fig. 5**). By reducing NPC insertion through deletion of specific Poms or Nups, more of the SIF is available to facilitate SPB insertion, and this eliminates the need for certain SPB components. This model is attractive for a number of reasons. First, the SIF model accounts for genetic interactions observed between mutants in genes encoding components of the SPB and NPC.[35,46-48] Second, this model provides a potential explanation for why many SPB mutants spontaneously diploidize at the permissive temperature (for examples review refs. 12, 41–45, 47 and 49). Although a failure in SPB duplication and a monopolar mitosis could account for the initial increase-in-ploidy associated with these mutants, it is not clear why they remain stable diploids and do not undergo additional monopolar mitotic events. The diploid state may be stable due to an adequate amount of the SIF. Although diploids have twice the chromosome content of haploids and have a 2-fold increase in cell and nuclear volume, the nuclear surface area only increases 1.6-fold from haploids to diploids,[50-52] and thus the effective concentration of the SIF could be higher in diploids than in haploids-perhaps sufficiently high to make diploidy the most stable state. Third, a protein-mediated, membrane insertion-based mechanism controlling SPB assembly could coordinate SPB duplication with cell growth and/or the cell cycle and explain the nuclear autonomous coordination of SPB duplication in the contiguous cytoplasm of *Ashbya gossypii* hyphae, which is an ancient ancestor of budding yeast that contains a similar plaque-like SPB.[53] It is likely that levels of the SIF increase in G_1 since most genes involved in SPB assembly are controlled by cell cycle specific transcriptional elements (MCB elements). Nuclear volume is small in G_1 as the result of nuclear division in the preceding mitosis, thus the effective concentration of the SIF in the NE is high, permitting SPB duplication and insertion in late G_1 phase. Continued expansion of the nucleus during S and G_2/M in the absence of ongoing synthesis of the SIF would be non-permissive for SPB assembly. Lastly, the SIF model could account for the observation that deletion of several Nups, such as *NUP170*, delay in metaphase of the cell cycle and account for genetic interactions between Nups and the spindle assembly checkpoint machinery, which detects defects in the mitotic spindle.[54,55] In the absence of the Nup, more of the SIF is titrated away from the SPB to rescue the assembly defect of the ailing NPCs. Loss of the SIF from the SPB results in a mild SPB insertion defect, which triggers the checkpoint and causes a delay in metaphase.

A major feature of this model is the identity of the proposed SIF. The NE protein Ndc1 is an excellent candidate to be the SIF. Perhaps because it is present at both NPCs and SPBs, yeast cells are highly sensitive to Ndc1 levels.[47,56] In addition, Ndc1 is involved in generating the pore membrane at both the NPC and SPB.[26,27,42,47,57] Unlike other membrane anchors, its function cannot be bypassed by deletion of other Poms or by other genetic methods, although some alleles of *NDC1* are suppressed by *pom152*Δ or *pom34*Δ.[35,46,47] At the NPC, the C-terminal tail of Ndc1 directly interacts with the N-terminus of Pom152 and probably other Poms and Nups.[26,58,59] Nbp1 is the only known Ndc1 binding partner at the SPB, although *NDC1* genetically interacts with other membrane anchors and half-bridge proteins.[12,44,60] A simple way for Ndc1 to modulate insertion of SPBs and NPCs is to bind to NPC and SPB components through the same domain. In this way, the cell could partition Ndc1 to either the SPB or NPC depending on conditions. A more thorough understanding of Ndc1 binding partners at both the NPC and SPB and determining how the protein is distributed between the two NE complexes will help test this model.

Model B: the membrane structure model. This model proposes that proteins such as Mps3 function to facilitate membrane insertion events by locally altering the lipid concentration of the NE (**Fig. 5**). Because there are many NPCs in the NE (between 65 and 182 in the nuclei of haploid yeast vs. a single SPB),[19] it is likely that they impart structural rigidity to the NE that makes SPB insertion difficult without half-bridge components or membrane anchors. If we imagine that this "rigidity" is a block to insertion of a newly duplicated SPB, then we can understand the observation that NPC mutants can suppress certain SPB mutants in terms of SPB assembly as follows: by blocking NPC insertion, the composition or structure of the membrane is altered in a manner that accommodates SPB assembly in the absence of Mps3 or other SPB components. There is precedent for this type of a model at the plasma membrane, where high concentrations of receptors and other membrane proteins result in the formation of membrane rafts that are rich in sterols and sphingolipids.[61,62]

The recent finding that a dominant allele of *MPS3* displays defects in SPB insertion and NE structure and the observation that deletion of *MPS3* restores the balance of certain types of lipids to wild-type levels in cells lacking nucleoporins is most consistent with this hypothesis.[34] Perhaps due to a change in nuclear transport, deletion of *POM152* resulted in decreased levels of total sterol ester levels and diacylglycerol compared with wild-type cells. Cells lacking both *POM152* and *MPS3* have wild-type levels of both classes of lipids, raising the intriguing question of why the transport defect might be ameliorated when the SUN protein is eliminated.

Perhaps the most compelling evidence that changes in NE composition must accompany SPB insertion comes from studies in *S. pombe*. The fission yeast SPB is associated with the NE throughout interphase, sitting in a small NE fenestra.[37,40,63] Prior to mitotic entry, the SPB duplicates and the two SPBs are inserted into pores in the NE. This process, commonly referred to as polar fenestration, enables assembly of nuclear microtubules. Following completion of mitosis, the SPBs are extruded from the nucleus and the pore is sealed. In a screen for mutants defective in polar fenestration, the Hagan lab identified a mutant in *brr6*$^{+}$, a gene thought to be required for NPC assembly in budding yeast. They went on to show that Brr6 and its associated protein Apq12 transiently associate with the SPB during polar fenestration but are not localized to the SPB during the remainder of the cell cycle, suggesting that they may be required for NE remodeling associated with SPB insertion.[32] Interestingly, Brr6 is not found in the genomes of metazoans or plants; it is only found in the genomes of organisms that undergo polar fenestration,[64] raising the question as to whether the primary function of Brr6, its paralog Brl1 and Apq12 is NE remodeling during SPB duplication (see below). In fission yeast, no NPC assembly defects were observed in cells containing mutations in *brr6* or *apq12*.Δ.[32]

SUN proteins could directly affect NE structure to facilitate membrane organization and complex insertion by tethering proteins involved in lipid modification in the NE (**Fig. 4B**). Ndc1, Mps3 and most other membrane and half-bridge components of SPB lack any structural motifs that provide insight into the mechanism of SPB insertion. The membrane at the site of SPB insertion is highly curved, and certain physical properties are thought to be required for this type of membrane structure and for fusion of the INM and ONM, including very long-chain fatty acids, membrane bending by reticulons and stabilization of curved membranes by ALPS-domain containing proteins.[15,29,30,65,66] Recent work from the Scheibel lab demonstrated that the Ndc1 binding protein Nbp1 contains an ALPS domain, however, Nbp1 shows no preference for binding to curved membranes in vitro, implicating additional targeting or curvature factors.[45] Further evidence that additional factors involved in membrane remodeling are involved in SPB assembly comes from the observation that *NBP1* is non-essential under certain conditions (Jaspersen S.L., unpublished). Therefore, it seems reasonable to propose that SUN proteins might selectively tether enzymes and other proteins involved in membrane remodeling in the ONM or lumenal space (**Fig. 4B**).

What proteins might these be? In budding yeast, *MPS3* genetically interacts with *SPO7*, a gene involved in the regulation of phospholipid biosynthesis and the maintenance of nuclear morphology.[35,67-69] The aberrant nuclear membranes formed in cells lacking Spo7, its binding partner Nem1 or their target, lipin (Pah1 in yeast), contain NPCs, suggesting that assembly of NPCs is not affected.[68-70] Therefore, the genetic interaction between *spo7*Δ and certain *MPS3* mutants is most easily explained by a change in NE composition, and it is possible that Mps3 tethers Spo7 in the NE, although this has never been experimentally tested. Several proteins involved in lipid synthesis and vesicle formation, including acetyl coA carboxylase (Cut6), the reticulon interacting protein Sey1, the AP-1 coat protein Apm1, the t-SNARE Ufe1 and the nucleoporin Nup40 (orthologous to Nup53 in budding yeast) were identified as putative Sad1 binding partners in a yeast two-hybrid

screen.[71] Although interactions between these proteins and the SUN protein may be indirect, deletion or mutation of many of these Sad1 interacting factors exacerbates the SPB insertion defect associated with a mutation in the SUN protein, suggesting a link between the membrane-based processes and SPB duplication. In metazoans, mutations in UNC-84, but not its two KASH partners, have been reported to result in decreased fat levels in *C. elegans*,[72] so it is plausible that UNC-84 also tethers unknown proteins involved in lipid metabolism in the NE/ONM. Recent reports have shown that mammalian SUN proteins localize, at least at low levels, to ER and Golgi membranes, which are major sites of lipid synthesis.[73-75] Therefore, tethering enzymes involved in membrane remodeling may be a conserved function of SUN proteins throughout eukaryotes.

Model A and B: the hybrid model? It is probable that aspects of both the SIF model and the membrane architecture model contribute to SPB assembly. EM examination of duplicating SPBs has often revealed adjacent NPCs even in mutants where NPCs are clustered in distal regions of the NE.[47,76] One explanation for this phenomenon is that the NPC, or at least a subset of NPC proteins such as the Poms, Nup157/Nup170 and the Nup84 complex, is required for SPB insertion. Many of these Nups and Poms genetically interact with SPB mutants and are found in small amounts in preparations of SPB components.[26,35,46-48] Interestingly, several of these Nups and Poms are involved in generating and stabilizing the highly curved membranes at the site of NPC assembly, raising the possibility that they may be required to perform similar functions during SPB insertion.[15,16] Because none are essential, we presume that their function at the SPB is redundant much as it is at the NPC.

One attractive feature of the hybrid model is that it could account for some of the perplexing phenotypes associated with the NE proteins Apq12, Brr6 and Brl1 in budding yeast. Brr6 and Brl1 are essential integral membrane proteins required for nuclear transport; Apq12 is an integral membrane protein required for growth and nuclear transport at low temperatures.[77-79] Mutations in *BRR6* lead to mis-localization of at least a subset of Nups, abnormal NPC distribution and NE morphology defects.[80] Similar, although not completely identical, phenotypes are observed in cells containing mutant versions of *BRL1* or a deletion of *APQ12*.[78,81] Analysis of NPC assembly using cycloheximide showed that new NPC insertion was specifically blocked in cells lacking Apq12 function.[81] At lower temperatures, cells respond to environmental change by altering the lipid composition of their membranes.[82-84] The observation that cells lacking *APQ12* are unable to grow at lower temperatures, combined with the fact that NPC assembly defects are partially rescued by changing membrane properties, has pointed to a role for Apq12 in membrane remodeling. Based on the fact that overexpression of *BRR6* rescues many defects associated with *apq12*Δ and that *brr6-1* mutants exhibit a broad range of genetic interactions with NPC and lipid biosynthesis genes, Brr6 and its paralog Brl1 are thought to act together with Apq12 in NPC assembly.[64,80] Interestingly, *apq12*Δ and *brr6-1* mutants have aberrant lipid profiles similar to that seen in cells lacking *MPS3* function.[80] Moreover, neutral lipids were not efficiently partitioned into lipid droplets in these mutant cells, resulting in accumulation of large amounts of neutral lipids in the ER and probably PNS. It is this change in membrane composition that is proposed to affect nuclear morphology and NPC insertion.

Although *brr6* and *brl1* mutants were originally identified based on their nuclear export defects,[77-79] an additional study in budding yeast found that *apq12*Δ was synthetically lethal or displayed diminished growth when combined with kinetochore mutants.[85] The kinetochore is a specialized protein structure required to attach microtubules to the centromeric DNA of each chromosome. Why would a protein involved in NPC insertion and NE remodeling genetically interact with kinetochore mutant? One explanation is that the *apq12*Δ mutant actually has a SPB duplication defect, and like several SPB mutants, displays genetic interactions with genes encoding kinetochore proteins and spindle checkpoint components.[34,48,86-90] The cold-sensitivity of *apq12*Δ could also be interpreted in the context of a SPB defect since microtubules depolymerize into α and β tubulin dimers at low temperatures.[91-93] Affecting both SPB insertion and microtubule dynamics could result in lethality. If we assume that Apq12 is required for membrane remodeling at both the NPC and SPB, the inability to insert the SPB results in the growth arrest observed in the mutant, and in the absence of Apq12, the SPB is unable to insert, so more of the SIF is titrated away from the NPC, resulting in severe defects in NPC assembly and nuclear transport defects. A similar competition between NPCs and SPBs may also occur in *brr6*^{ts} and *brl1*^{ts} mutants. By coordination of membrane remodeling and the distribution of a SIF, the cell can spatially and temporally regulate NPC and SPB insertion.

Beyond the SPB and the NPC

Is the connection between the SPB and the NPC a specific strategy evolved in fungi and other eukaryotes that undergo a closed mitosis to regulate NE composition and control assembly of NE complexes? It is clear that metazoan MTOCs, known as centrosomes, are structurally distinct from the SPBs found in fungi.[39,94] Despite this morphological diversity, many components are shared between the two organelles. Although centrosomes are not embedded in the NE, they are tethered to the NE by SUN-KASH proteins in certain cell types.[5-7,38] Based on the observation that cells derived from patients with Emery-Dreifuss muscular dystrophy (one example of a "laminopathy") fail to tether centrosomes to the NE, it is thought that failure to attach the centrosome to the NE contributes to disease pathology, possibly by affecting nuclear positioning and/or chromosome integrity.[3,95,96] Therefore, it is important to understand at a molecular level how centrosomes are connected to the NE. Interestingly, a recent report implicated the NPC in NE linkage of the centrosome, suggesting that a functional and/or structural connection between the MTOC and NPC may extend throughout eukaryotes.[97] The connection between NE membrane complexes such as the NPC and the MTOC may have an ancient origin derived from

the roles these proteins played in genome partitioning in the last eukaryotic common ancestor.[13]

Through genetic analysis of SPB duplication in a model organism, an unexpected connection between two protein complexes of the NE was discovered. Both SPBs and NPCs must insert into the NE, and this requires changes in the NE, both in terms of lipids and integral membrane proteins. In comparison to other membranes, little is known about NE composition.[61,62,98] Although NE proteins have been identified by mass spectroscopic analysis, many have not been verified in vivo and their function is unknown.[1] Furthermore, the lipid composition of the nuclear envelope in normal proliferating cells is poorly characterized. A major challenge to analysis of NE composition is the tight connection between the NE and the ER; it is virtually impossible to isolate "pure" NE membranes by biochemical methods so subtractive approaches have been used to infer both its protein and lipid composition.[1,61,99-101] Given the link between INM-ONM spacing, NE shape and the control of NE protein distribution and human disease, the use of classical genetics and sophisticated live cell imaging methods will be important in solving questions of NE structure and will be critical to advancing our understanding of MTOC insertion/tethering at the NE and NPC assembly.

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