Nuclear compartmentalization is achieved through the enclosure of the genome by the nuclear envelope; the nuclear envelope is perforated by nuclear pore complexes (NPCs), which form portals that control molecular exchange between the nucleus and cytoplasm. The number of NPCs per nucleus establishes a limit to the flux of molecules across the nuclear envelope and might directly impact genome organization and gene expression in a cell type specific manner. Mechanisms that control NPC number remain ill defined. Our recent study implicates a cytoplasmic pool of the nucleoporin Nsp1 as a factor that controls NPC number during the asymmetric division of budding yeast; Nsp1 acts to ensure that daughters inherit NPCs. We place our data within an emerging model of NPC inheritance in yeast and consider potential analogous mechanisms in multicellular eukaryotes, including the functional conservation of a cytoplasmic pool of Nsp1.

Introduction

Nuclear pore complexes (NPCs) form gateways in the nuclear envelope that control the exchange of molecules between the nucleus and cytoplasm in all eukaryotes. They are constructed from ~30 subunits termed nucleoporins or nups that form a ring-like scaffold and central transport channel. The number of NPCs within the nuclear envelope establishes a limit to the total flux of molecules that can permeate the nucleus at a given time. Further, the growing functional and physical connections between NPCs and chromatin support the concept that NPC number could directly influence gene expression, perhaps in a cell type specific fashion. Consistent with this idea, NPC number and distribution has been shown to vary between different cell types and has even been correlated with cancer cell multidrug resistance. The inputs and pathways that regulate NPC number remain to be fully uncovered.

Since there is no evidence suggesting that NPCs can be removed from the nuclear envelope, NPC number is likely controlled either by up or downregulation of the de novo NPC assembly pathway, or, as our recent work in yeast suggests, by ensuring inheritance of NPCs by daughter cells during cell division. There has been considerable interest in understanding NPC inheritance in Saccharomyces cerevisiae beyond mechanisms of NPC number control; there is also a potential relationship between NPCs and aging. In part, this is due to the remarkable lifetime—up to several years—of some of the NPC scaffold nups and the propensity for long lived proteins to accumulate damage with age. This posits that cells like yeast, which often restrict the passage of damaged or misfolded proteins to daughters in order to ensure their lifespan, might have evolved mechanisms to restrict the transmission of NPCs as well. Since NPCs remain intact during mitosis in budding yeast, it provides a facile experimental system to investigate mechanisms that promote or restrict NPC transmission to daughters.

Our recent study helps to define an emerging consensus by which NPCs are...
transmitted to daughter cells through a mechanism that depends on four factors: (1) the dynamics of individual NPCs imposed by the asymmetric distribution of NPC binding partners; (2) a cytoplasmic pool of the nup, Nsp1; (3) a myosin motor (Myo2) and intact actin cytoskeleton; and (4) the modulation of a bud neck barrier (Fig. 1). In the following, we will interpret our data in the context of several additional studies that have investigated NPC inheritance and discuss the broader themes applicable to organisms that undergo an open mitosis in which NPCs break down during mitosis.

**Potential Models of NPC Transmission to Daughter Cells**

There are at least three plausible mechanisms that could contribute to the transmission of NPCs to daughter cells during the asymmetric division of budding yeast: first, NPCs could freely diffuse along the nuclear envelope with NPC inheritance being a stochastic process largely dependent on the nuclear surface area inherited after nuclear division. Second, NPCs could be tethered to cytoplasmic or nuclear structures. In this scenario, NPC inheritance would be secondary to mechanisms that either retain or segregate the tethering factor. Third, in analogy to how several organelles are transported to daughters, NPCs could be moved by direct connections between motor proteins and NPCs. Importantly, any of the above mechanisms would be influenced by a barrier or restriction at the bud neck that could physically impede NPC transmission to the daughter (Fig. 1).

**NPC diffusion**

While individual NPCs may freely diffuse along the nuclear envelope, such a mechanism cannot explain the observation that there is an increased density of NPCs and a bias of “old” nups in daughters after anaphase.\(^8,11\) While an increased NPC density in daughters could also suggest enhanced daughter-specific NPC assembly, our data argue that this is not the case, as inhibiting NPC assembly does not influence NPC transmission. We also failed to detect a bias in the accumulation of newly synthesized NPC protomers in daughter cells. Perhaps most importantly, we are able to decouple nuclear envelope inheritance from NPC transmission by inhibiting the nup, Nsp1, or the myosin-V motor, Myo2.\(^8\) Cumulatively, these results support that diffusion alone cannot explain NPC transmission and another mechanism is required to enrich NPCs in daughter cells.

**Figure 1.** Mechanism of NPC inheritance in budding yeast. NPC dynamics and their segregation between mother and daughter cells are influenced by binding to cytoplasmic and nuclear structures including SPBs (1), and chromatin and/or chromatin binding partners like the LEM proteins (2). At least a subset of NPCs are likely attached to either a microtubule or actin-based cytoskeleton (3). There is a barrier at the bud neck (4) that impedes the passage of NPCs and other organelles, which likely responds to changes in cellular physiology. Under wild type conditions, Nsp1\(_{\text{CYT}}\) is translocated into the bud through a mechanism that requires an actin cytoskeleton and Myo2. Nsp1\(_{\text{CYT}}\) moves with ER tubules that extend from the mother nuclear envelope and contact the bud cortex; this ER might be connected to the cortex through the exocyst complex. The passage of Nsp1\(_{\text{CYT}}\) licenses NPC passage by contributing to the dissolution of the barrier (arrow). Under conditions in which there are disruptions to bud physiology and/or fitness, we propose that Nsp1\(_{\text{CYT}}\) function is inhibited, the barrier remains intact and NPCs are not transmitted.
NPC dynamics mediated by binding to cellular structures

NPCs in budding yeast are mobile within the nuclear envelope suggesting that, as a population, they are not tethered to a stationary network analogous to the mammalian nuclear lamina. However, individual NPCs interact with several cellular factors, both in the cytoplasm and nucleus that would directly influence their dynamics (Fig. 1). Should one of these factors be retained in mothers or segregated to daughters, this could impact the ultimate distribution of associated NPCs. A potential candidate for driving such asymmetry is the yeast centrosome (spindle pole body, SPB); NPCs have been observed clustered around SPBs, potentially through an interaction with the nuclear basket protein, Mlp2. Centrosome interactions might also contribute to the polarization of NPCs at the nuclear envelope observed in several organisms including *Plasmodium* and *Ochlamydomonas*.

NPCs also interact with chromatin binding factors including the integral inner nuclear membrane proteins of the Lap2-emerin-MAN1 (LEM) family (Heh1 and Heh2), and others reviewed in references 26, 27. Interestingly, in *Schizosaccharomyces japonicus* where there is a partial nuclear envelope break down during mitosis, the LEM proteins play a role in equally partitioning the nucleus and NPCs between daughters, perhaps by directly connecting NPCs to segregating chromosomes. More insight into how interactions between NPCs and these binding factors are controlled and whether they are asymmetrically segregated between mother and daughter cells is required to fully understand their contribution to NPC transmission.

Motor-driven movement of NPCs

Several motor proteins have been shown to interact with NPCs in yeast and in multicellular eukaryotes. Consistent with the idea that NPCs can be coupled directly to the cytoskeleton through motor proteins, ATP-dependent movement of NPCs has been visualized in several yeasts including *S. cerevisiae*, where this movement also requires an intact actin cytoskeleton. This dependence on actin might help to explain our observation that the myosin motor Myo2 is uniquely required for NPC density in daughter cells, although a direct connection between Myo2 and NPCs has not been established. The directional movement of Nup2 foci between mother and daughter nuclei observed in budding yeast is also consistent with an active motor-directed movement of NPCs, although since Nup2 is mobile these results could benefit from visualizing a more stable component of the NPC. To clearly establish whether motors are required in NPC transmission will require the use of several nup markers and likely super-resolution approaches to resolve individual NPCs and monitor their movement during anaphase.

A Bud Neck Barrier Controls NPC Inheritance

We envision that under different internal or external conditions, the contribution of any of the above mechanisms to NPC transmission could be altered to either promote or inhibit the numbers of NPCs inherited by daughters (Fig. 1). Furthermore, at least three studies, including our own, support the existence of a physical barrier at the bud neck that acts to prevent NPC transmission to daughters. Controversy over its existence is in large part due to an inability to reach a consensus as to the biochemical composition of the barrier, and the physiological conditions in which the barrier might be regulated. Indeed, as described above, we observe that NPCs are able to access daughter cells during anaphase suggesting that in a permissive laboratory setting and in vegetatively growing cells, the barrier is not overly active toward NPCs. In contrast, when Nsp1 is inhibited, we observe a striking reduction of the inheritance of NPCs. While in principle, the inhibition of Nsp1 could influence any one of the three putative mechanisms of NPC transmission outlined above, by, for example, coupling NPCs to either cellular structures or motor proteins, our data support that it is a cytoplasmic pool of Nsp1 and not the NPC pool that impacts NPC inheritance. Therefore, the most plausible interpretation of our data is that Nsp1 acts by influencing the function of the bud neck barrier (Fig. 1).

Functional Conservation of Nsp1<sub>CYT</sub>

Perhaps one of the more surprising and interesting findings of our work is that a pool of Nsp1 that exists outside of NPCs, which we term “Nsp1<sub>CYT</sub>” is responsible for ensuring NPC inheritance. To show this, we used a conditional “anchor-away” approach for inhibiting nup function, which specifically inactivated cytoplasmic nups and not those bound to NPCs. The notion that Nsp1 can function outside of the NPC is not without precedent. Indeed, a quantitative proteomic analysis of fission yeast supports that Nsp1 is by far the most abundant nup. Whereas most nups were found in -2000 to -7000 copies per cell, Nsp1 was found in -40 000 copies, an order of magnitude more. Since there is no evidence that there is 10-fold more Nsp1 in NPCs compared with other nups, this excess Nsp1 likely exists outside of the NPC. Moreover, in HeLa cells, the Nsp1 ortholog Nup62 localizes to the leading edge of migrating cells and contributes to cell migration; this function is mediated by an interaction with the exocyst—a protein complex best known for its role in tethering secretory vesicles to the plasma membrane during exocytosis. Further, Nup62 interacts with the endoplasmic reticulum (ER)-resident oxysterol binding protein, ORP8, which might compete for binding with the exocyst subunit, Exo70. Consistent with this, downregulation of ORP8 leads to the localization of Nup62 to the cell’s leading edge and a concomitant increase in cell migration. Lastly, Nup62 has been found at the mid-body ring prior to cell abscission, an interaction that might be mediated by the actin-cap binding protein CapG. Cumulatively, these studies point to a role for a cytoplasmic pool of Nup62 at the interface of membrane and/or actin-mediated processes at both the cell edge and mid-body ring. Interestingly, we observe analogous dynamics and interactions for Nsp1<sub>CYT</sub> in budding yeast including interactions with ER and the exocyst complex.
In analogy to the polarized delivery of Nup62 to the leading edge of migrating cells, Nsp1\textsubscript{CYT} is observed as a focus that moves from the mother cell to the bud tip directly preceding anaphase. During anaphase, Nsp1\textsubscript{CYT} is directed back toward the bud neck and the elongating anaphase nucleus, where it integrates into the nuclear envelope.\textsuperscript{a} The dynamics of this movement are reminiscent of a battery of growth factors influenced by the polarization of the actin cytoskeleton, which is apically polarized during bud growth and then redirected to the bud neck during anaphase.\textsuperscript{43} Indeed, actin depolymerization affects the localization of Nsp1\textsubscript{CYT} and the most abundant Nsp1\textsubscript{CYT} foci are observed in the context of extreme apical growth after overstimulation of the Wsc1 kinase. Further, we observe a relationship between bud size and the abundance and bud-bias of the Nsp1\textsubscript{CYT} foci. Together, these data are suggestive of a link between Nsp1\textsubscript{CYT} function and actin-mediated processes that oversee bud growth and physiology.

**Bud Fitness Modulates the Bud Neck Barrier**

We hypothesize that Nsp1\textsubscript{CYT} is part of a network that couples bud physiology and/or fitness to modulation of the bud neck barrier in order to license NPC transmission (Fig. 1). Consistent with the sensitivity of the bud neck barrier to inputs from cellular physiology, it has been recently shown that ER stress is signaled through the MAP kinase Slt2, which regulates a bud neck barrier dependent on septin function.\textsuperscript{44} It is possible that Nsp1\textsubscript{CYT} functions within this or an analogous pathway to either directly (or through another factor[s]) promote barrier dissolution. While a better understanding of Nsp1\textsubscript{CYT}’s direct binding partners is a necessity to evaluate this model, we are able to show that it interacts with ER, either through direct membrane binding\textsuperscript{45} or a yet to be identified ER protein.

Nsp1\textsubscript{CYT} associates with ER tubules that extend from the mother nuclear envelope and contact the bud cortex (Fig. 1). We suspect that these tubules are identical to those seen being drawn into the bud from the mother nuclear envelope in tomographic reconstructions from electron microscopy of the yeast ER\textsuperscript{46} and visualized by light microscopy by several groups.\textsuperscript{47-49} There is evidence that these tubules might be linked to the cortex through the exocyst.\textsuperscript{47} Interestingly, acting through these ER-bud cortex connections, the exocyst plays a role in maintaining nuclear positioning at the bud neck prior to anaphase. This is achieved through the generation of forces that transiently pull the mother nuclear envelope into the bud and form so-called “nucleopodia.”\textsuperscript{47} The dynamics of nucleopodia resemble “tugging”, suggesting that there is a dynamic fluctuation in the forces applied to the mother nuclear envelope. Since we show that nucleopodia are often directed toward Nsp1\textsubscript{CYT} foci, a key to unraveling the function of Nsp1\textsubscript{CYT} may be in understanding the nature of these forces and how they might translate information from the bud cortex to the mother nuclear envelope. We speculate that these forces might directly contribute to barrier dissolution, but testing this hypothesis awaits a more molecular understanding of this pathway.

**Outlook to Multicellular Eukaryotes**

The asymmetric division of budding yeast can serve as a model for mitoses in multicellular eukaryotes that result in daughter cells with distinct cell fates. While NPCs are thought to break down during mitosis into a small number of discrete subcomplexes, it seems feasible that the segregation of these building blocks might be biased to one daughter by diffusion barriers or the attachment to a polarity and/or motor-driven machinery, or to centrosomes. We suggest that this might be necessary because it is likely more efficient to reassemble NPCs from a small subset of preformed building blocks rather than from producing and assembling ~30 individual subunits. Moreover, the observation that interphase de novo NPC assembly is arrested in post-mitotic cells might place an additional burden on the inheritance of nups at the last mitosis before these cells enter quiescence.\textsuperscript{14} How Nsp1/Nup62 might contribute to these putative mechanisms awaits further investigation and will require the capacity to visualize “new” and “old” nups through cell division and the completion of differentiation programs. The convergence of gene-editing technologies where fluorescent protein tags can be engineered into endogenous genes with super-resolution microscopy capable of visualizing the NPC substructure, promises to open the door for these experiments.

**Disclosure of Potential Conflicts of Interest**

No potential conflict of interest was disclosed.

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