An auxiliary, membrane-based mechanism for nuclear migration in budding yeast

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ABSTRACT
How nuclear shape correlates with nuclear movements during the cell cycle is poorly understood. We investigated changes in nuclear morphology during nuclear migration in budding yeast. In preanaphase cells, nuclear protrusions (nucleopodia [NP]) extend into the bud, preceding insertion of chromosomes into the bud neck. Surprisingly, formation of nucleopodia did not depend on the established nuclear migration pathways. We show that generation and maintenance of NP requires nuclear membrane expansion, actin, and the exocyst complex. Exocyst mutations cause nuclear positioning defects and display genetic interactions with mutations that deactivate astral microtubule-dependent nuclear migration. Cells that cannot perform DNA replication also fail to form nucleopodia. We propose that nuclear membrane expansion, DNA replication, and exocyst-dependent anchoring of the nuclear envelope to the bud affect nuclear morphology and facilitate correct positioning of nucleus and chromosomes relative to the cleavage apparatus.

INTRODUCTION
Spatial coordination of cell cleavage with chromosome segregation ensures that the cleavage apparatus bisects the partitioned chromosomes and the nucleus after anaphase. To achieve this in Saccharomyces cerevisiae, the nucleus, the intranuclear mitotic spindle, and the chromosomes migrate toward the bud and insert into the bud neck, the future site of cytokinesis. Two conserved microtubule-dependent pathways facilitate nuclear/spindle migration in budding yeast (Miller et al., 1998; Moore and Cooper, 2010). The Kar9 pathway mediates migration of the nucleus–spindle–chromosome system toward the bud neck and orients the mitotic spindle with the old spindle pole facing the bud (Pereira et al., 2001; Liakopoulos et al., 2003). Insertion of the nucleus and the spindle into the bud neck occurs with the help of dynein-mediated spindle oscillations, during which the entire spindle traverses the bud neck (Saunders et al., 1995; Yeh et al., 1995).

During spindle elongation in anaphase, the anaphase spindle stretches and partitions the nuclear envelope between mother and bud, resulting in generation of two nuclei at the end of mitosis. Nuclear duplication requires that both the nuclear membrane and the perinuclear endoplasmic reticulum (ER) increase in mass during the cell cycle (Jorgensen et al., 2007). Growth of the nuclear membrane depends on the coordinated action of the diacylglycerol kinase Dgk1 and the mammalian lipin homologue, the phosphatidate phosphatase Pah1 (Siniossoglou, 2009). These enzymes regulate the production of phosphatidic acid, which acts as a positive signal for nuclear membrane growth (Loewen et al., 2004). Nuclear membrane expansion is induced upon phosphorylation and deactivation of Pah1 by multiple kinases, including Cdk1/cyclin B, whereas dephosphorylation of the enzyme by the heterodimeric phosphatase Sp07-Nem1 activates Pah1 and inhibits growth of the nuclear membrane (Santos-Rosa et al., 2005; O’Hara et al., 2006; Karanasios et al., 2010; Choi et al., 2012; Su et al., 2012).

Of interest, nuclear membrane growth and nuclear shape are linked (Zhang and Oliferenko, 2012). Nuclear membrane overexpansion leads to deformation of the nuclear envelope (Han et al., 2008), and membrane/vesicle trafficking maintains nuclear shape during proliferation of the nuclear membrane (Webster et al., 2010).
The DNA in the nucleus seems to also participate in the regulation of nuclear shape. Expansion of the nuclear envelope takes place at the area of the nuclear envelope next to the nucleolus (Campbell et al., 2006; Witkin et al., 2012). In contrast, the nuclear envelope associated with the DNA mass seems to resist nuclear membrane expansion. Thus association of chromosomes with the nuclear envelope may contribute to preservation of spherical nuclear shape.

Nuclear migration into the bud is accompanied by dramatic changes in nuclear morphology. In preanaphase, the nuclear envelope loses its spherical shape and forms protrusions that extend into the bud and lack DNA (Fehrenbacher et al., 2002). These poorly characterized structures exhibit sweeping motions and contact the cell cortex. Of importance, these nuclear protrusions form before anaphase and extend into the bud while the metaphase spindle is still in the mother cell. It is not clear what causes the nucleus to migrate into the bud ahead of the mitotic spindle and how the nuclear protrusions are formed and maintained.

RESULTS

Nuclear migration into the bud can occur independently of Kar9 and dynein

In preanaphase, the nucleus of budding yeast obtains a dumbbell morphology (Palmer et al., 1989). This occurs because the mitotic spindle and the nucleus are pulled by astral microtubules into the bud neck. The bud neck constricts the nucleus, making it appear like a dumbbell (Figure 1A). Surprisingly, we and others observed that some preanaphase cells generated dynamic nuclear protrusions that grew into the bud ahead of the mitotic spindle, before dumbbell formation (Figure 1B and Supplemental Videos S1 and S2; Fehrenbacher et al., 2002). We named these protrusions nucleopodia (NP). Nucleopodia have been suggested to contribute to spindle alignment and nuclear migration (Fehrenbacher et al., 2002). We verified that NP contained little, if any, DNA (Figure 1B) and that they were indeed protrusions of the nucleus, since they contained nucleoporins (Nup1-green fluorescent protein [GFP]), nucleoplasm (visualized with tetR-NLS-GFP), and the nuclear protein Nop1-cyan fluorescent protein [CFP] (Figures 1, B and D, and 2A, and Supplemental Videos S1 and S2). Nucleopodia formed in full or selective media at 30, 34, and 37°C.

We investigated NP formation during the cell cycle more closely. After release of Nurp1-GFP, mCherry-Tub1-expressing cells from G1, 10% of the cells formed NP, and anaphase followed immediately after (Supplemental Figure S1A and Supplemental Video S3; Tub1 is the yeast α-tubulin). To conveniently observe NP formation, we prevented cells from undergoing anaphase after release from G1. For this we placed the chromosomal copy of the CDC20 gene under the control of the glucose-repressible GAL1-10 promoter (P<sub>GAL</sub>CD20 cells). Addition of glucose in the medium results in G2/M arrest of the P<sub>GAL</sub>CD20 cells due to depletion of Cdcd20, an activator of the anaphase-promoting complex. When we released P<sub>GAL</sub>CD20 cells expressing Nurp1-GFP and mCherry-Tub1 from G1 (α-factor) in glucose-containing medium, nearly half of the cell population formed NP (Figure 1, C and D; for this and all following quantifications, N > 100 cells/time point were counted, N > 500 cells/time course, and three to five independent experiments were performed). Time-lapse microscopy showed that NP form after one pole of the mitotic spindle is pulled transiently through the bud neck (Figure 1D), in agreement with Fehrenbacher et al. (2002). Subsequently the spindle pole is pulled back into the mother cell, leaving part of the nuclear membrane inside the bud. There the nuclear membrane forms dynamic protrusions (see also Supplemental Video S3).

Eventually, the mitotic spindle and the associated DNA mass are pulled into the bud neck, forming a dumbbell nucleus.

Spindle insertion into the bud and oscillating spindle movements depend on the activity of cytoplasmic dynein, Dyn1 (Yeh et al., 1995). Surprisingly, dyn1Δ CD20 cells did not display significant defects in NP formation; Figure 2, A and B, and Supplemental Figure S1B, whereas insertion of the spindle into the bud (dumbbell nuclei) was severely reduced, as expected. Formation of NP was largely unaffected in kar9Δ cells (Figure 2B and Supplemental Figure S1B). We also occasionally observed NP in dyn1Δ kar9Δ cells that lack both pathways and display an extremely slow growth phenotype (Figure 2C; Miller et al., 1998). Depolymerization of the microtubule cytoskeleton inhibited NP generation (Figure 3A). However, disruption of microtubules did not affect already formed NP, indicating that the microtubule cytoskeleton is not essential for maintenance of NP (Figure 3B). These results suggest that dynein-dependent nuclear oscillations may facilitate NP formation but are not essential for it. Thus the nucleus is able to protrude into the bud by a dynein- and Kar9-independent mechanism.

Protrusion of the nucleus into the bud requires nuclear membrane growth

Nuclear membrane expansion relies on phospholipid biosynthesis and is up-regulated by mitotic Cdk1 complexes (Santos-Rosa et al., 2005). We reasoned that NP might form after entry into the mitotic cell cycle by growth of the nuclear membrane into the bud. To investigate this possibility, we generated cells that had a large bud but had not entered mitosis. CDC34 encodes a ubiquitin-conjugating enzyme required for the progression from G1 to the S phase of the cell cycle. Shift of temperature-sensitive cdc34-2 cells to the restrictive temperature prevents cells from entering S phase but does not affect bud growth, which occurs normally in late G1. G1-arrested cdc34-2 cells did not form NP, even after growing very large buds (Figure 4A). Nuclear volume was reduced in these cells compared with preanaphase-arrested P<sub>GAL</sub>CD20 cells at the same temperature (cdc34-2: 6 ± 1.6 vs. 11 ± 0.7 μm<sup>3</sup>), suggesting that the nuclear membrane expands only after cells enter mitosis.

We next investigated whether inhibition of nuclear membrane growth would abrogate NP formation. Growth of the nuclear membrane depends on fatty acid synthesis (Schneiter et al., 1996) and requires the activity of fatty acid synthase. First, we used cerulenin, an inhibitor of fatty acid synthase that has been shown to inhibit growth of the nuclear membrane in Schizosaccharomyces pombe and Schizosaccharomyces japonicus (Yam et al., 2011). Treatment of cells with 10 μM cerulenin abrogated NP formation (Figure 4B) without affecting cell cycle progression, as cerulenin-treated cells exhibited bud sizes and spindle lengths comparable to those of solvent-treated cells (Supplemental Figure S1, C and E). Moreover, the effect was not due to cell death because cell viability was not severely reduced during the course of the treatment (Supplemental Figure S1D). We also blocked lipid synthesis by inhibiting the yeast acetyl-CoA-carboxylase Acc1. For this we used the specific inhibitor of Acc1, soraphen A (Vahlensieck et al., 1994). Similar to cerulenin, soraphen A inhibited formation of NP without severely affecting cell growth (Figure 4B and Supplemental Figure S1E). Finally, we used genetic means to inhibit nuclear membrane growth and overexpressed the Nem1-Spo7 phosphatase (Santos-Rosa et al., 2005). Formation of nucleopodia was severely reduced in Nem1-Spo7-overexpressing cells (Figure 4C). Again, cell cycle progression to G2/M was not affected in Nem1-Spo7-overexpressing cells, since spindle length was similar to control cells (Supplemental Figure S1F). Taken together, these data indicate that protrusion of the
We depolymerized actin in cells that had not yet formed any NP (Figure 5A) by treating cells with latrunculin A (latA) 60 min after release from G1 in glucose medium. We did not observe any further increase in the percentage of cells with NP at later time points. When latA was added after cells had formed NP, the latter shrunk, became rounded, and lost their contact to the bud cortex (Figure 5, B and C). We concluded that both NP formation and maintenance require an intact actin cytoskeleton.

Nucleopodia formation and maintenance depend on the actin cytoskeleton and the exocyst complex

We next asked whether disruption of the actin cytoskeleton would affect NP formation. In this experiment we used $P_{\text{GAL}}\text{CDC20}^{\Delta}$ cells to avoid dumbbell formation and observe only nucleopodia.

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The cortical ER depends on the actin cytoskeleton for proper inheritance and cortical anchoring (Prinz et al., 2000; Fehrenbacher et al., 2002; Wiederkehr et al., 2003). Therefore we reasoned that defects in generation of NP upon actin depolymerization could reflect defects caused to cortical ER anchoring, to which the nuclear envelope is connected (Preuss et al., 1991; Fehrenbacher et al., 2002; West et al., 2011). To test this idea, we examined generation of NP in a series of $P_{GAL}CDC20$ strains harboring mutations that impair ER inheritance (Du et al., 2004). Neither myo4Δ cells nor swa2Δ cells (Du et al., 2001; Estrada et al., 2003) showed any severe defect in NP formation (Supplemental Figure S2A). In contrast, 105 min after release from G1, NPs were almost absent when we shifted exocyst mutant sec3-2 cells to the restrictive temperature, whereas >50% of wild-type cells with comparable bud size had formed NP at this temperature (Figure 6, A and B, and Supplemental Figure S2B). Cells with a temperature-sensitive mutation in Sec4, a GTPase that mediates...
A high percentage of binucleated cells, which increased when the sec3-2 and sec4-8 mutations were combined with kar9Δ and dyn1Δ deletions (Figure 7B; 16.8% binucleated sec3-2 kar9Δ and 16.5% binucleated sec3-2 dyn1Δ cells vs. 10% for sec3-2, 3.7% for kar9Δ, and 5.6% for dyn1Δ single mutants). In fact, sec4-8 kar9Δ, and sec4-8 dyn1Δ cells had increased numbers of binucleated cells even at the permissive temperature. We did not observe any mislocalization of Kar9 or dynein in exocyst mutants (Supplemental Figure S2C). Finally, we found that the distance between the spindle and the bud neck increased in sec3-2 cells at the restrictive temperature, indicating that spindles are mispositioned in this mutant (Figure 7C).

To directly examine whether NP facilitate spindle positioning, we measured the position of the spindle in cells with or without NP. To eliminate any contribution of dynein-dependent insertion of the nuclear envelope into the bud, we performed this measurement in dyn1Δ cells. Time-lapse imaging showed that NP retracted after 20 min at the restrictive temperature (Figure 6E). Of importance, after retraction of the NP, the nucleus lost its position close to the bud neck and fell back into the mother cell. These data suggested that anchoring of NP could be important for maintaining the position of the nucleus and the spindle close to the bud.

Exocyst mutants display nuclear- and spindle-positioning defects

If NP facilitate nuclear positioning, mutations defective in NP formation or maintenance should exacerbate the nuclear- and spindle-positioning defects of kar9Δ and dyn1Δ cells and lead to increased numbers of binucleated cells. Indeed, all tested mutant alleles encoding different exocyst subunits (sec3-2, sec4-8, sec6-4, and sec10-1) displayed synthetic growth defects upon deletion of DYN1 or KAR9 (Figure 7A). Moreover, sec3-2 and sec4-8 cells displayed a high percentage of binucleated cells, which increased when the sec3-2 and sec4-8 mutations were combined with kar9Δ and dyn1Δ deletions (Figure 7B; 16.8% binucleated sec3-2 kar9Δ and 16.5% binucleated sec3-2 dyn1Δ cells vs. 10% for sec3-2, 3.7% for kar9Δ, and 5.6% for dyn1Δ single mutants). In fact, sec4-8 kar9Δ, and sec4-8 dyn1Δ cells had increased numbers of binucleated cells even at the permissive temperature. We did not observe any mislocalization of Kar9 or dynein in exocyst mutants (Supplemental Figure S2C). Finally, we found that the distance between the spindle and the bud neck increased in sec3-2 cells at the restrictive temperature, indicating that spindles are mispositioned in this mutant (Figure 7C). To directly examine whether NP facilitate spindle positioning, we measured the position of the spindle in cells with or without NP. To eliminate any contribution of dynein-dependent insertion of the nuclear envelope into the bud, we performed this measurement in dyn1Δ cells. We found that the spindle resided closer to the bud neck in cells with NP (Figure 7C). Therefore NP seem to stabilize the position of the nucleus close to the bud and facilitate spindle positioning.

We sought evidence to determine whether the exocyst mediates a physical connection between NP and the cortical ER. In P_GAL CDC20 cells expressing Sec61-GFP (the major subunit of the ER translocon), Sec3-CFP, and mCherry-Tub1, we detected ER tubules
Nuclear protrusions in nuclear migration

Connecting NP to the cortical ER at sites occupied by the exocyst subunit Sec3 (Figure 7D and Supplemental Videos S4–S6). Therefore, consistent with the role of NP and the exocyst in nuclear migration, NP may contribute to stabilization of the nuclear/spindle position by anchoring the nucleus to the cortical ER in the bud.

DNA replication is required for nucleopodia formation

Previous studies showed that nuclear envelope proliferation does not take place around the bulk of the DNA, which seems to restrict membrane expansion (Campbell et al., 2006; Witkin et al., 2012). DNA replication was shown to disrupt anchoring of telomeres to the nuclear envelope (Taddei et al., 2004; Ebrahimi and Donaldson, 2008). We thus wondered whether, besides nuclear membrane expansion, untethering of chromosomes from the nuclear envelope due to DNA replication would be required for generation of NP.

To test this, we examined cdc7-1 cells that are unable to replicate their DNA and arrest due to activation of the spindle assembly checkpoint (Biggins and Murray, 2001) with high mitotic Cdk1/Cdc28 activity, a mitotic spindle, and inactivated APC.Cdk20. In contrast to Cdc20-depleted cells, cdc7-1 cells were defective in NP formation (Figure 7E). Of importance, the inability of replication-defective cdc7-1 cells to form NP was not due to failure to expand their nuclear membrane. The nuclear volume of G2/M arrested cdc7-1 cells (10.8 ± 1 μm³) did not differ significantly from the nuclear volume of arrested PgalCDC20 cells (11.3 ± 0.7 μm³). We attempted to restore NP formation in cdc7-1 cells by deactivating factors shown to tether telomeric regions to the nuclear envelope. However, we did not observe any increase in NP formation after combining the cdc7-1 mutation with esc1Δ, yku70Δ (Taddei et al., 2004; Ferreira et al., 2011), mps3Δpom152Δ (the pom152Δ mutation was used here to suppress the inviability of mps3Δ; Friederichs et al., 2011), or siz1Δsiz2Δ (Taddei et al., 2004; Ferreira et al., 2011). Nevertheless, these data suggest that DNA replication is required for NP formation and efficient nuclear migration into the bud.

**DISCUSSION**

In this article we analyzed the changes in nuclear morphology accompanying positioning of the nucleus relative to the cleavage apparatus at the bud neck. We showed that the nuclear membrane can migrate ahead of the mitotic spindle into the bud by generating protrusions, which depend on nuclear membrane growth. Clearly, NP have been previously described and can be observed in any cycling yeast culture (Prinz et al., 2000; Fehrenbacher et al., 2002). However, to unambiguously distinguish NP from dumbbell or anaphase nuclei, the mitotic spindle (or the DNA) and the nuclear envelope must be simultaneously visualized. Nucleopodia are most easily observed whenever cells (also cycling cells) display a G2/M delay. We do not think that generation of NP is a checkpoint-specific process; rather, the G2/M cell cycle delay simply reveals the formed NP and its connection to the bud, which are obscured in fast-cycling cells.

Our data indicate that NP generation depends on phospholipid biosynthesis, actin, and the exocyst complex. Biosynthesis of phospholipids takes place in the ER, whereas actin is required for the delivery of cortical ER into the daughter cells (Estrada et al., 2003). Nucleopodia could form when ER tubules connect the nuclear envelope with the cortical ER in the bud as the nucleus migrates close to the bud neck (Figure 8). Because the bud ER may be biosynthetically more active, incorporation of new lipids causes the nuclear envelope to proliferate in the direction of the bud. It was recently suggested that vesicle trafficking might regulate the availability of the nuclear membrane and the shape of the nuclear envelope (Webster et al., 2010). We were not able to test mutants defective in vesicular trafficking.
in generation of NP: indeed, myo2 mutants are defective in NP formation (Supplemental Figure S2E). However, myo2 mutants also affect actin polarity (Lillie and Brown, 1994), and therefore their defect in NP formation is not clear.

Why is DNA replication required for NP formation? Previous reports demonstrated that the DNA mass restricts nuclear membrane expansion, which instead takes place at the nuclear membrane adjacent to the nucleolus (Campbell et al., 2006; Witkin et al., 2012). Consistent with this, we find that NP in cells grown at 28°C. Therefore chromosomal regions other than telomeres may interact with the nuclear envelope to restrain nuclear membrane expansion.

Formation of NP seems to act in parallel to the KAR9 and DYN1 pathways to facilitate nuclear/spindle migration. There are several examples for the role of cortical ER in spindle positioning.

To our surprise, we found that NP formation does not depend on dynein-mediated spindle oscillations. This raises the question of whether the opposite could be the case: nuclear membrane expansion may be required for dynein-dependent oscillations to occur. It would be interesting to study spindle oscillations when nuclear membrane growth is inhibited (i.e., upon Nem1-Spo7 overexpression).

The exocyst complex seems to be a docking/attachment site for ER tubules and growing NP. One could also speculate that the exocyst may play an active role in formation of NP, since exocyst homologues are involved in generation of membrane nanotubes (Hase et al., 2009). In this respect, the Myo2 motor, which directly interacts with exocyst components Sec15 and Sec4, could be involved in generation of NP: indeed, myo2 mutants are defective in NP formation (Supplemental Figure S2E). However, myo2 mutants also affect actin polarity (Lillie and Brown, 1994), and therefore their defect in NP formation is not clear.

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Formation of NP seems to act in parallel to the KAR9 and DYN1 pathways to facilitate nuclear/spindle migration. There are several examples for the role of cortical ER in spindle positioning. During ascidian asymmetric divisions, the cortical centrosome attracting body, a mass of ER containing asymmetrically segregated mRNAs and the cortical PAR-3/PAR-6/aPKC complex, keeps one centrosome

**FIGURE 6:** The exocyst is required for nucleopodia formation and maintenance. (A) Generation of NP depends on a functional exocyst complex. Quantification of NP formation during preanaphase arrest of PGAL CDC20 sec3-2 mutants at permissive (28°C) and restrictive temperature (34°C). Control quantification at 34°C and bud sizes are shown in Supplemental Figure S2, A and B, respectively. (B) Images showing lack of NP in arrested PGAL CDC20 sec3-2 cells at the restrictive temperature. Arrows show NP in cells grown at 28°C. (C) Quantification of NP formation during preanaphase arrest of PGAL CDC20 sec4-8 cells. (D) The exocyst is required for maintenance of NP. The quantification shows reduction of the number of cells with NP after shift of arrested PGAL CDC20 sec3-2 cells to the restrictive temperature. (E) Shrinkage of NP (arrow) after shift of a PGAL CDC20 sec3-2 cell to the restrictive temperature. Note that the spindle–nucleus falls back into the mother cell after NP shrinkage. The gray dashed line is drawn for comparison of the spindle position. Bars, SEM; **p < 10⁻² in a Student’s t-test.
NP formation and exocyst-dependent connections to the cortical ER may stabilize the position of the nucleus and the mitotic spindle, facilitating chromosome segregation in anaphase. It will be interesting to explore whether similar connections contribute to stabilization of the nuclear position in higher eukaryotic cells.

FIGURE 7: The exocyst and DNA replication facilitate nuclear migration. (A) Exocyst mutations display synthetic growth defects when combined with dyn1Δ or kar9Δ deletion. Shown are serial dilutions of cells. (B) Exocyst mutants display nuclear migration defects, resulting in a high percentage of binucleated cells. The defects are additive when combined with dyn1Δ or kar9Δ mutations, already at permissive temperatures. No semipermissive temperature could be identified for sec4-8 cells. (C) Left, exocyst mutants display spindle-positioning defects. Shown is the distance of the middle of the mitotic spindle to the middle of the bud neck. Right, the presence of NP correlates with improved position of the spindle. The spindle is closer to the bud neck in dyn1Δ cells that have NP compared with cells that lack NP. (D) Selected deconvolved image slices from 3D image stacks showing tubular ER connections (yellow arrow) between NP (white arrow), the cortical ER, and Sec3. Each image is a different z-slice; distance between slices is 0.2 μm. (E) Cells that do not perform DNA replication do not form NP. Deconvolved image of an arrested cdc7-1 cell (left) at the restrictive temperature (37°C) and quantification of NP formation during preanaphase arrest. Compared are two time points at which cells of the two strains have the same bud sizes. The nuclear DNA is associated with the spindle and surrounded by the nuclear membrane. DAPI staining outside the nucleus is due to mitochondrial DNA.

in the proximity of the cortex by regulating microtubule–cortical interactions (Munro, 2007). Spindle positioning through anchoring of one centrosome to the spectrosome, an ER-rich structure of Drosophila germline stem cells, is another example (Lin et al., 1994; Yamashita et al., 2003). In a variation of this mechanism in budding yeast, NP formation and exocyst-dependent connections to the cortical ER may stabilize the position of the nucleus and the mitotic spindle, facilitating chromosome segregation in anaphase. It will be interesting to explore whether similar connections contribute to stabilization of the nuclear position in higher eukaryotic cells.
bles were calculated by Imaris 5.7.2 (Bitplane, Zurich, Switzerland).

nuclear membrane. Deconvolved 3D images were thresholded and nuclear volume was determined using Essential 3.4 (Scientific Volume Imaging, Hilversum, Netherlands) and statistical analysis was performed with Excel (Microsoft, Redmond, WA).

neither Kar9, dynein, nor NP formation is absolutely essential for nuclear migration. However, all three pathways likely cooperate to guarantee robust inheritance of nucleus and chromosomes.

FIGURE 8: Model showing events during nuclear migration/spindle positioning in budding yeast. Early in the cell cycle the Kar9 pathway moves the nucleus close to the bud neck. After entry into mitosis, replication starts and the nuclear membrane expands. Dynein-dependent spindle oscillations pull part of the nuclear envelope into the bud, which creates NP, as it continues to proliferate. This nuclear subdomain is connected to the cortical ER (dashed line) by the exocyst, stabilizing the position of the nucleus and the spindle close to the bud. Eventually, dynein pulls the spindle and the DNA mass into the bud neck, creating a dumbbell nucleus. Why DNA replication is required for NP formation is not understood; it may un tether chromosomes from the nuclear envelope, enabling the nuclear membrane to move through the bud neck. Neither Kar9, dynein, nor NP formation is absolutely essential for nuclear migration. However, all three pathways likely cooperate to guarantee robust inheritance of nucleus and chromosomes.

MATERIALS AND METHODS

Yeast strains, growth conditions, and drug concentrations

For strains see Supplemental Table S1. All strains were routinely grown in standard yeast extract/peptone/dextrose (YPD) or selective medium. Strains expressing CDC20 under the control of the GAL1-10 promoter were grown in YPSSG (YP supplemented with 2% sucrose + 2% galactose). Endogenously tagged fusions were created by standard yeast techniques. For α-factor arrest, cells were incubated with 5 μg/ml α-factor for 3 h. Latrunculin A was used at 100 μM, nocodazole at 10 μg/ml, cerulenin at 10 μM, and soraphen A at 0.25 μg/ml final concentration. After release from α-factor arrest, cells were incubated for 30 min at 28°C to allow the formation of a small bud before addition of drugs. For 4′,6-diamidino-2-phenylindole (DAPI) staining of living cells, cells from logarithmically growing cultures were briefly incubated in 200 μl of phosphate-buffered saline (PBS), containing 2 μl of 1 μg/ml DAPI in PBS. Cells were washed twice to remove the DAPI and resuspended in 2.5 μl of nonfluorescent medium (Waddle et al., 1996) for microscopy.

Fluorescence microscopy and image analysis

For GFP and mCherry visualization, cells were grown overnight in YPSG liquid cultures containing additional adenine, tryptophan, and uracil. Yeast cells expressing mCherry-Tub1 and tagged Nup1-GFP were analyzed by time-lapse fluorescence microscopy using an Olympus IX81 microscope (Tokyo, Japan) equipped with the CellIR imaging system. Images were acquired with an APO 100x objective as z-series of five focal planes separated by 0.5 μm and projected into a single plane. Images were analyzed using ImageJ (National Institutes of Health, Bethesda, MD), and statistical analysis was performed with Excel (Microsoft, Redmond, WA).

For the three-dimensional (3D) reconstruction of yeast nuclei and deconvolution, a z-series of 41–81 z-stacks with 0.1-μm z distance were background subtracted and deconvolved using Huygens Essential 3.4 (Scientific Volume Imaging, Hilversum, Netherlands) with a point spread function experimentally obtained for the specific lens. Deconvolved 3D images were thresholded and nuclear volumes were calculated by Imaris 5.7.2 (Bitplane, Zurich, Switzerland).

Thresholding was arbitrary, but only nuclei that generated a closed surface were chosen for quantification of nuclear volumes. Recalculation of the volume of the same nucleus resulted in a value that differed by <5% from the first calculated value.

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