

# Dynein-dependent nuclear dynamics affect morphogenesis in *Candida albicans* by means of the Bub2p spindle checkpoint

Kenneth R. Finley<sup>1</sup>, Kelly J. Bouchonville<sup>1</sup>, Aaron Quick<sup>1</sup> and Judith Berman<sup>1,2,\*</sup>

Departments of Genetics, Cell Biology, and Development<sup>1</sup> and Microbiology<sup>2</sup>, University of Minnesota, Minneapolis, Minnesota, 6-160 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, USA

\*Author for correspondence (e-mail: jberman@umn.edu)

Accepted 15 November 2007

Journal of Cell Science 121, 466-476 Published by The Company of Biologists 2008  
doi:10.1242/jcs.015172

## Summary

*Candida albicans*, the most prevalent fungal pathogen of humans, grows with multiple morphologies. The dynamics of nuclear movement are similar in wild-type yeast and pseudohyphae: nuclei divide across the bud neck. By contrast, in hyphae, nuclei migrate 10-20  $\mu\text{m}$  into the growing germ tube before dividing. We analyzed the role of the dynein-dynactin complex in hyphal and yeast cells using time-lapse fluorescence microscopy. Cells lacking the heavy chain of cytoplasmic dynein or the p150<sup>Glued</sup> subunit of dynactin were defective in the position and orientation of the spindle. Hyphal cells often failed to deliver a nucleus to the daughter cell, resulting in defects in morphogenesis. Under yeast growth conditions, cultures included a mixture of yeast and pseudohyphal-like cells that exhibited distinctive defects in nuclear dynamics: in yeast, nuclei divided within the mother cell, and the spindle position checkpoint protein Bub2p ensured the delivery of the

daughter nucleus to the daughter cell before cytokinesis; in pseudohyphal-like cells, pre-mitotic nuclei migrated into the daughter and no checkpoint ensured return of a nucleus to the mother cell before cytokinesis. Analysis of double mutants indicated that Bub2p also mediated the pre-anaphase arrest and polarization of pseudohyphal-like cells. Thus, Bub2p has two distinct roles in *C. albicans* cells lacking dynein: it mediates pre-anaphase arrest and it coordinates spindle disassembly with mitotic exit.

Supplementary material available online at  
<http://jcs.biologists.org/cgi/content/full/121/4/466/DC1>

Key words: *Candida albicans*, Cell cycle, Cytoplasmic dynein, Morphogenesis, Spindle checkpoints

## Introduction

*Candida albicans* is a multimorphic human pathogen that alters its morphology in vivo and in vitro. Most *C. albicans* vegetative cultures are a mixture of yeast, pseudohyphae and true hyphae. Yeast and pseudohyphae grow by budding: initial polarized growth is followed by a switch to isotropic growth that produces round cells with a constriction at the bud neck, the site where buds emerge and where septa eventually form. Pseudohyphae are more elongated than yeast cells and wider than true hyphae (Merson-Davies and Odds, 1989; Sudbery et al., 2004). By contrast, true hyphae grow continuously in a polarized manner and never switch to isotropic growth, resulting in long, narrow cells with parallel cell walls. The hyphal septum forms within the hyphal tube ~10-20  $\mu\text{m}$  from the mother cell. Importantly, the site of nuclear division and septation is different in budding and hyphal cells. In yeast and pseudohyphae, nuclear division occurs across the bud neck; in hyphae, nuclei move out of the basal cell and divide at the presumptive site of septation that forms 10-20  $\mu\text{m}$  into the growing germ tube (Finley and Berman, 2005).

Nuclear movements and chromosome segregation are mediated by microtubules (MTs) and the mitotic spindle. In filamentous fungi such as *Aspergillus nidulans*, *Ashbya gossypii* and *Neurospora crassa*, apical nuclei migrate behind the elongating hyphal tip and trailing nuclei are positioned periodically within the hypha (reviewed in Xiang and Fischer, 2004). In *A. gossypii*,

asynchronous nuclear divisions are coordinated with septin rings that localize to hyphal tips and branch sites (Helfer and Gladfelter, 2006). By contrast, in *C. albicans* hyphae, a single unreplicated nucleus remains in the basal cell until after the site of septation is specified, as indicated by the appearance of the septin rings 10-15  $\mu\text{m}$  into the hyphal tube (Finley and Berman, 2005).

Spindle pole bodies (SPBs), which are the fungal equivalent of mammalian centrosomes, anchor and nucleate microtubules from their minus-ends, while the plus-ends are free in the cytoplasm. In fungi, dynamic instability (sudden transitions between MT growth and shrinkage) is exclusive to plus-ends; there is no evidence for subunit addition at the minus-ends of SPB-bound MTs. As replication begins, SPBs duplicate. Spindle assembly begins when the duplicated SPBs separate to form a bipolar spindle. In *C. albicans* hyphae, the bipolar spindle then migrates into the growing germ tube (Finley and Berman, 2005). MTs associate laterally with and appear to slide along the cell cortex, suggesting that bipolar spindle movement into and within the germ tube is mediated by a minus-end-directed motor protein (Finley and Berman, 2005). Anaphase occurs across the future site of septation (presumptum) and then the mother nucleus is propelled towards the mother cell, initially as a consequence of mitotic spindle elongation (Finley and Berman, 2005).

Cytoplasmic dynein, the minus-end-directed microtubule motor protein, and its regulatory complex, dynactin, play important roles

in regulating nuclear position with respect to morphology in all fungi where it has been examined. While nuclei fail to migrate into the hyphae of *A. nidulans* dynein mutants (Xiang et al., 1994), nuclei cluster within the hyphae of *N. crassa* and *Nectria haematococca* dynein mutants (Inoue et al., 1998; Plamann et al., 1994). Strikingly, nuclei collect near the hyphal tip in *A. gossypii* dynein mutants, suggesting that cytoplasmic dynein opposes another force that moves nuclei towards hyphal tips (Alberti-Segui et al., 2001). Thus, it appears that there are other mechanisms that can move nuclei in the absence of cytoplasmic dynein, but these mechanisms are masked by the presence of dynein activity in wild-type cells. Furthermore, nuclei in dynein-deficient cells occasionally move limited distances into hyphae (Plamann et al., 1994; Xiang et al., 1994).

In the budding yeast *Saccharomyces cerevisiae*, cells lacking dynein function have pre-anaphase spindles that are further from the bud neck and that fail to orient parallel to the mother-bud axis (Yeh et al., 1995). As a result, the mitotic spindle elongates within the mother cell, generating binucleate mother cells. Elegant microscopic work identified the transport of MT plus-ends along the cortex (Hwang et al., 2003) and the shrinkage of MT plus-ends at the cortex (Gupta et al., 2006) as mechanisms that were previously masked by dynein activity.

Cells must position nuclei with respect to cell shape to ensure the faithful segregation of genetic material to daughter cells. Checkpoints monitor the cell cycle to ensure that crucial events occur in the appropriate order and delay cell cycle progression when they do not occur properly (Hartwell and Weinert, 1989). In *S. cerevisiae*, the Bub2p-dependent spindle position checkpoint monitors the position and orientation of the mitotic spindle relative to the bud neck (reviewed in Lew and Burke, 2003). In the absence of dynein-dynactin, Bub2p inhibits spindle disassembly until the daughter nucleus resides within the daughter cell. The morphogenesis checkpoint protein Swe1p inhibits nuclear division when the actin cytoskeleton or the septin ring is perturbed (reviewed in Keaton and Lew, 2006). Similarly, in *A. gossypii*, AgSwe1p coordinates nuclear division near hyphal branch points, where septin rings form (Helfer and Gladfelter, 2006). Thus, fungi employ checkpoints to ensure that nuclei move appropriately with respect to morphology. Unlike *S. cerevisiae*, activation of the DNA damage (Bedell et al., 1980; Shi et al., 2007) or spindle checkpoints (Bachewich et al., 2003; Bai et al., 2002) does not arrest bud growth in *C. albicans*; the result is the formation of elongated pseudohyphal-like buds (Berman, 2006). Interestingly, nuclei migrate into these elongated buds before mitosis, with dynamics reminiscent of the migration of nuclei into hyphal germ tubes.

To understand the mechanisms that underlie nuclear dynamics in *C. albicans* hyphal and budding cells, we analyzed strains lacking genes encoding either the cytoplasmic dynein heavy chain (*DYN1*) or the p150<sup>Glued</sup> subunit of dynactin (*NIP100*). Surprisingly, under yeast growth conditions, cells lacking dynein or dynactin grew as a mixed population of yeast and pseudohyphal-like cells. Yeast cells lacking dynein exhibited spindle position and orientation defects, but a checkpoint ensured that each daughter cell eventually received a nucleus. In pseudohyphal-like cells, nuclei migrated into the daughter cell, divided there, and no checkpoint ensured that each mother received a nucleus; this resulted in a high proportion of anucleate mother and binucleate daughter cells. Under hyphal growth conditions, ~33% of nuclei migrated into the germ tube, albeit with extremely slow kinetics. In contrast to growth in yeast conditions, growth under hyphal

conditions did not alter cell morphology until after nuclear migration defects occurred. Analysis of double mutants revealed that the spindle position checkpoint (Bub2p), but neither the spindle assembly checkpoint (Mad2p) nor the morphogenesis checkpoint (Swe1p), monitored at least two distinct steps of cell cycle progression in cells lacking dynein. Bub2p inhibited spindle disassembly in yeast cells and also inhibited onset of anaphase in pseudohyphal-like cells. Furthermore, Bub2p mediated the polarized growth of *dyn1Δ/Δ* pseudohyphal-like cells.

## Results

### Nuclear migration occurs in a subset of *dyn1Δ/Δ* hyphal cells

To understand better the mechanisms by which nuclei migrate long distances in *C. albicans* hyphae, we generated strain YJB8973, which lacks both alleles of *DYN1* and expresses Tub2-GFP for the visualization of MTs, SPBs and spindle morphology during time-lapse microscopy. In a wild-type hypha, the bipolar spindle migrates out of the basal (mother) cell, into the germ tube and to the presumptum, where the spindle elongates and subsequently disassembles just before the septation event that yields two separate cells (Fig. 1A) (Finley and Berman, 2005). Surprisingly, in 33% (18 of 54) of *dyn1Δ/Δ* hyphal cells, the bipolar spindles did migrate into the germ tubes (Fig. 1B): during spindle elongation, the daughter SPBs (dSPBs) moved across the presumptum before spindle disassembly and septation ensued in all 18 of these cells, thereby recapitulating the chain of events of the wild type. Following spindle disassembly, the growth of the daughter hyphal cell was normal, at least until the next septum was evident. The migration of undivided nuclei into elongating *dyn1Δ/Δ* germ tubes was surprising because a previous report indicated that Dyn1p was essential for nuclear migration into hyphae (Martin et al., 2004) and, second, because it resembles the dynamics of nuclei in wild-type hyphae (Finley and Berman, 2005).

In the majority (66%) of *dyn1Δ/Δ* hyphae, nuclei failed to migrate into the germ tube before anaphase; in these cells, any movement of the daughter nucleus into the germ tube was a result of spindle elongation (Fig. 1C). In all of these *dyn1Δ/Δ* hyphae, germ tube morphology remained normal until the time of septation, when growth arrested abruptly (Fig. 1C, 167 minutes). These results are consistent with the idea that, in *dyn1Δ/Δ* cells, hyphal morphology is perturbed as a secondary effect of the failure to deliver a nucleus into the apical cell. That is, irregular hyphal morphology is not a direct effect of the lack of dynein or slower nuclear dynamics on morphogenesis.

### Dynein mediates MT release from the spindle pole body and masks at least one other mechanism of nuclear migration

In addition to dynein motor activity, several MT-based processes, such as shrinkage of a MT attached to the cortex (Gupta, Jr et al., 2006; Varga et al., 2006) or the translocation of a MT plus-end along the cortex (Hwang et al., 2003), can mediate nuclear migration. To ask how nuclear movement is accomplished in the absence of the dynein-dynactin complex, we compared the MT dynamics of wild-type, *dyn1Δ/Δ* and *nip100Δ/Δ* strains using Tub2-GFP and time-lapse microscopy. MT dynamics (MT growth and shrinkage) were generally similar between wild-type and dynein-dynactin mutants (Fig. 2 and Table 1) when measured with 3-second time-lapse intervals. MTs were nucleated from SPBs at similar rates, grew long within the hypha and interacted with the hyphal cortex along the way (Fig. 2A,B). In wild-type cells, it was not possible to distinguish MT plus-end transport or MT shrinkage

from MT sliding events. In *dyn1* $\Delta/\Delta$  cells analyzed at 3-second intervals, spindle displacements were too small (<200 nm) to be measured accurately and photobleaching and phototoxicity affected the ability to detect appreciable spindle movements. Using 15-second time-lapse intervals, migratory spindles were observed, although the magnitude of their movement remained very small (Fig. 2C). Therefore, individual spindle displacements could not be associated with specific MT-cortex interactions (e.g. MT depolymerization or plus-end transport).

In *C. albicans*, wild-type spindles exhibit highly dynamic oscillatory movements that result in net migration in one direction (Finley and Berman, 2005). By contrast, in the *dyn1* $\Delta/\Delta$  hyphae, bipolar spindle displacements were much less dynamic in the *dyn1* $\Delta/\Delta$  hyphae (Fig. 1). If spindle movements in the *dyn1* $\Delta/\Delta$  hyphae are generally linear rather than oscillatory, then the rates of spindle movement determined using different time-lapse intervals are expected to be similar. To ask whether this was the case, we compared the rates of bipolar spindle position over time for *dyn1* $\Delta/\Delta$  hyphae imaged at 15-second (Fig. 2C) and at 5-minute intervals (e.g. Fig. 1B). Calculation of the respective rates using linear regression analysis revealed that the average rate of spindle movement in 8 cells imaged at 15-second intervals and in 12 cells imaged at 5-minute intervals was not significantly different ( $0.25\pm 0.04$  and  $0.29\pm 0.03$   $\mu\text{m}/\text{min}$ , respectively,  $P>0.1$ ). Thus, dynein-independent spindle migration is dramatically slower than wild-type MT spindle translocation ( $9.2\pm 0.7$   $\mu\text{m}/\text{min}$ ) and it occurs in a linear manner that does not involve major oscillations. This

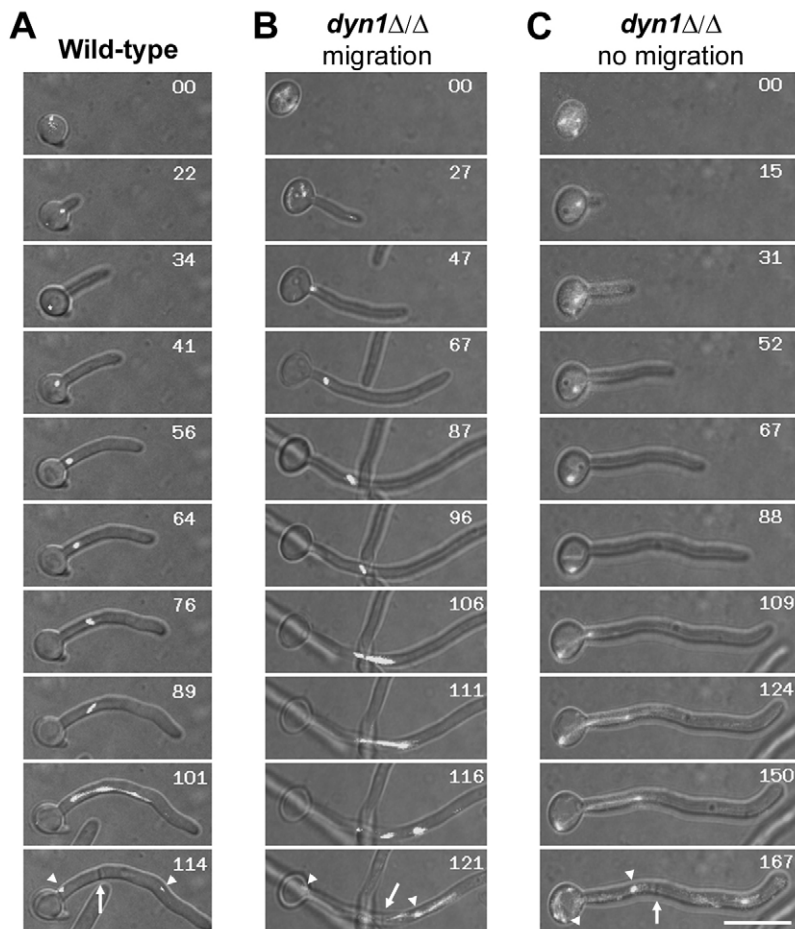
implies that dynein-dynactin moves nuclei using the oscillatory dynamics that rapidly deliver the nucleus into the germ tube.

The loss of dynein-dynactin affected only one major aspect of MT dynamics: *dyn1* $\Delta/\Delta$  and *nip100* $\Delta/\Delta$  cells exhibited dramatically lower rates of MT release from the SPB (reduced by a factor of 7.3 and 3.4 relative to wild-type, respectively, Table 1). Because MT release from the SPB terminates MT growth, a reduction in MT release rates increases the amount of time that MTs remain attached to the SPB and thus are subject to other growth-terminating events. Consistent with this, the proportion of other MT growth-terminating events (catastrophe and breakage) were higher in *dyn1* $\Delta/\Delta$  and *nip100* $\Delta/\Delta$  cells relative to wild-type cells (Table 1). Thus, loss of dynein-dynactin decreased the frequency of MT release and, as a consequence, also increased the rates of MT catastrophe and breakage events. These results support the idea that dynein-dynactin facilitates bipolar spindle movements by pulling on astral MTs from the cortex, implying that the force generated by cytoplasmic dynein is sufficient to either pull MTs out of the SPB or cause them to break near the SPB.

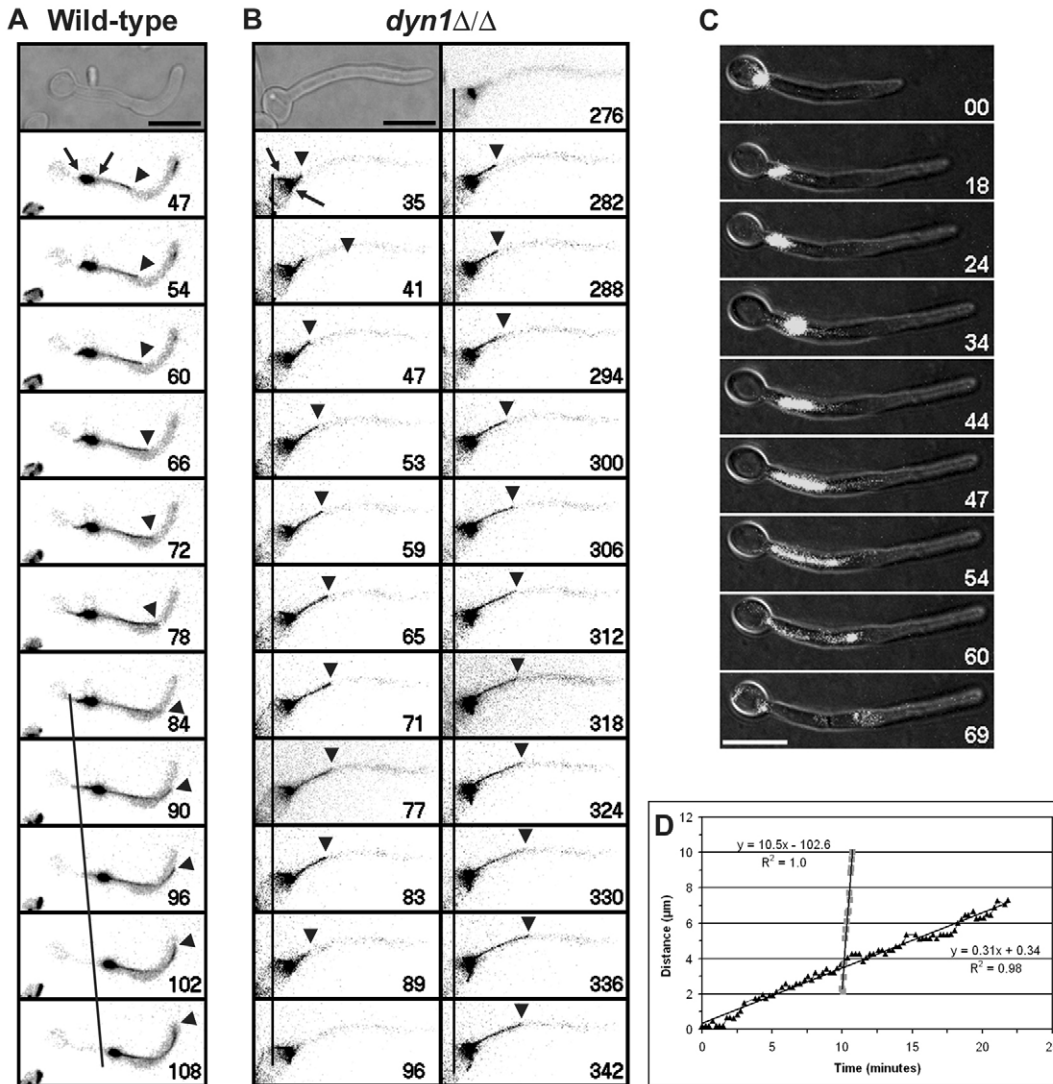
#### Pre-mitotic nuclear migration in hydroxyurea-treated cells is partially dependent on dynein-dynactin

Cells treated with hydroxyurea (HU, an inhibitor of ribonucleotide reductase that slows DNA replication by limiting nucleotide pools) become elongated and the undivided nucleus moves into the elongating bud. This phenotype is reminiscent of the situation in *A. gossypii* dynein mutants, where nuclei move along behind the

hyphal tip (Alberti-Segui et al., 2001). To ask whether dynein-dynactin is required for nuclear migration in HU-arrested cells, we treated wild-type and *dyn1* $\Delta/\Delta$  cells with 100 mM HU for 6 hours, fixed them with ethanol and stained them with DAPI to detect the nuclei. Because *dyn1* $\Delta/\Delta$  cells are variable in morphology and nuclear number (see below), we restricted our analysis to *dyn1* $\Delta/\Delta$  cells with only one bud and one nucleus (Fig. 3A). We found that HU-treated *dyn1* $\Delta/\Delta$  cells were generally more elongated than HU-treated wild-type cells. This increased length relative to WT cells could be a function of HU treatment, checkpoint-related cell elongation (below) and/or the presence of long cells in the *dyn1* $\Delta/\Delta$  culture before HU treatment. To compare the nuclear movement in *dyn1* $\Delta/\Delta$  and wild-type cells, we measured the distance between the nucleus and the bud tip. In wild-type cells, a plot of the relationship



**Fig. 1.** Nuclear dynamics in *dyn1* $\Delta/\Delta$  hyphae. Time-lapse microscopy was performed as described in Materials and Methods. Images were acquired at 5-minute intervals. Time, minutes. (A) Spindle dynamics in wild-type hyphae. The bipolar spindle moves into the germ tube (56 minutes) before anaphase (101 minutes). The septum becomes evident after spindle disassembly (arrow, 114 minutes). (B) In a *dyn1* $\Delta/\Delta$  hypha, a bipolar spindle moves into the germ tube (47 minutes) and reaches the presumptum (96 minutes) before anaphase (106 minutes). The septum is evident after spindle disassembly (arrow, 121 minutes). (C) Spindle migration fails in a *dyn1* $\Delta/\Delta$  hypha. Anaphase begins with the bipolar spindle in the basal cell (109 minutes). The dSPB does not cross the presumptum before septation (167 minutes). After septation, the hyphal tip swelled slightly before elongation arrested. Arrowheads, SPBs after septation. Bar, 10  $\mu\text{m}$ .



**Fig. 2.** MT dynamics during spindle movement in *dyn1*Δ/Δ hyphae. (A) A bipolar spindle in a wild-type hypha migrates into the germ tube (angled line) even as the dSPB-bound MT grows longer (arrowheads). Time, seconds. (B) A bipolar spindle in a *dyn1*Δ/Δ hypha remains at the bud neck (straight line) despite repeated MT growth, shrinkage and interaction with the cortex. Arrows, SPBs; arrowheads, MT plus-ends; time, seconds.

(C) Bipolar spindle movement captured in a *dyn1*Δ/Δ hypha at 15-second intervals. Time, minutes. Bar, 10 μm. (D) An example of spindle position (y-axis) plotted against time (x-axis) used to indirectly calculate rates of spindle movement in *dyn1*Δ/Δ hyphae (black triangles). An example of spindle position versus time in a wild-type hypha is also shown for comparison (grey squares).

between bud length and the distance of the nucleus from the bud tip was linear, and the distribution of cells on this plot remained fairly narrow, suggesting that this relationship was tightly regulated (Fig. 3B). In the *dyn1*Δ/Δ cells, the distance between the nucleus and the bud tip also increased with increasing bud length; however, the relationship between bud length and nuclear position was less tightly maintained. This suggests that, in the absence of dynein, a mechanism exists that moves the nucleus into the bud, but this movement is not coordinated with bud length. Thus, the movement of an undivided nucleus into a bud that is elongating in response to HU treatment is mediated by both dynein-dependent and dynein-independent mechanisms; furthermore, dynein appears to mediate movement that is tightly coordinated with bud length.

Loss of dynein affects morphogenesis in some, but not all, yeast cells

To analyze further the effects of losing cytoplasmic dynein-dynactin in *C. albicans* under yeast growth conditions, we constructed strains lacking both copies of *DYNI* (YJB7812) or *NIP100* (YJB9107). On YPD-agar plates at 30°C, where wild-type and *dyn1*Δ/*DYNI* cells formed smooth colonies (Fig. 4A and data not shown), the *dyn1*Δ/Δ and *nip100*Δ/Δ strains grew more slowly,

forming small colonies with filaments at the colony edges (Fig. 4B,C). In liquid cultures grown under yeast conditions (30°C YPD medium, Fig. 4D-F), wild-type cultures consisted of only yeast cells (Fig. 4D). By contrast, the *dyn1*Δ/Δ and *nip100*Δ/Δ strains produced a mixture of yeast (Fig. 4E,F, arrowheads) (45% and 38%, respectively) and pseudohyphal (55% and 62%, respectively) cells. Some of the pseudohyphal cells had a single nucleus (28% in *dyn1*Δ/Δ and 38% in *nip100*Δ/Δ), some were binucleate (Fig. 4E,F, arrows) (33% in *dyn1*Δ/Δ and 27% in *nip100*Δ/Δ) or multinucleate (2% in *dyn1*Δ/Δ and 3% in *nip100*Δ/Δ), and others were anucleate (30% in *dyn1*Δ/Δ and 41% in *nip100*Δ/Δ) (Fig. 4E,F, carets). Thus, the loss of cytoplasmic dynein-dynactin affected cell morphology and nuclear status in some, but not all, *C. albicans* cells growing under yeast growth conditions.

Nuclear dynamics are defective, albeit in different ways, in *dyn1*Δ/Δ yeast and pseudohyphae

Dynein affects nuclear dynamics and cell cycle progression in several organisms (May and Hardwick, 2006). In *C. albicans*, perturbing cell cycle progression stimulates polarized growth (Berman, 2006). To test the hypothesis that *dyn1*Δ/Δ pseudohyphal cells had more severe defects in spindle position, orientation or

**Table 1. G2 microtubule dynamics in wild-type, *dyn1Δ/Δ* and *nip100Δ/Δ* hyphae**

	Rate of MT:			Frequency of MT:		% of MT growth terminated by:		
	Growth	Shrinkage	SPB displacement	Catastrophe	Rescue	Catastrophe	Breakage	Release
	$\mu\text{m min}^{-1} \pm \text{s.e.m. (n)}$			Events $\text{min}^{-1}$				
WT*	6.4±0.3 (31)	26.6±1.9 (27)	9.2±0.7 (17)	0.34	0.02	54.8%	22.6%	22.6%
<i>dyn1</i> <sup>‡</sup>	7.1±0.3 (44)	31.2±1.7 (27)	0.25-0.29 <sup>§</sup>	0.45	0.02	69.2%	26.2%	3.1%
<i>nip100</i> <sup>¶</sup>	7.1±0.3 (21)	24.4±2.6 (14)	ND	ND	ND	60.7%	38.2%	6.7%

\*19 cells; 36 life histories; 58 minutes; WT, wild type (for details, see Finley and Berman, 2005).  
<sup>‡</sup>12 cells; 75 life histories; 110 minutes.  
<sup>§</sup>Indirectly determined, as described in the text.  
<sup>¶</sup>Five cells; 25 life histories; 28 minutes; ND, not determined.

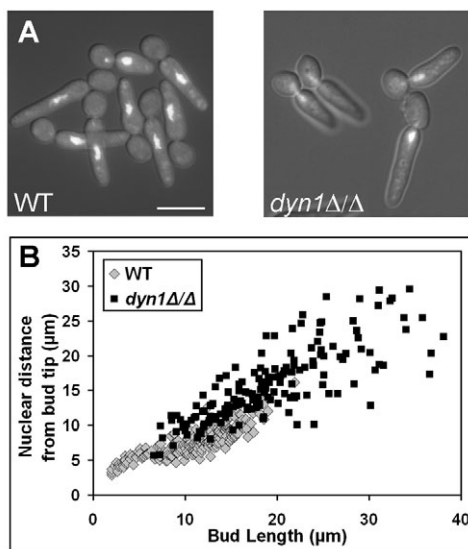
migration than *dyn1Δ/Δ* yeast cells, we performed time-lapse microscopy on wild-type and *dyn1Δ/Δ* yeast and pseudohyphal cells that expressed either Nop1-YFP, a marker of nuclear dynamics (Finley and Berman, 2005), or Tub2-GFP, a marker of spindle dynamics.

In wild-type yeast at 30°C (Fig. 5A) and 35°C (data not shown) as well as in wild-type pseudohyphae at 35°C (Fig. 5B), the daughter nucleus reached the bud upon chromosome separation (Fig. 5A,B) because the bipolar spindle moved close to the bud neck [average distance 1.1±0.3  $\mu\text{m}$  ( $n=30$ )] and was oriented parallel to the mother-bud axis (Fig. 5A, Tub2, 56 minutes). The daughter pole of the mitotic spindle moved into the bud within 5 minutes of onset of anaphase (Fig. 5A, Tub2, 61 minutes).

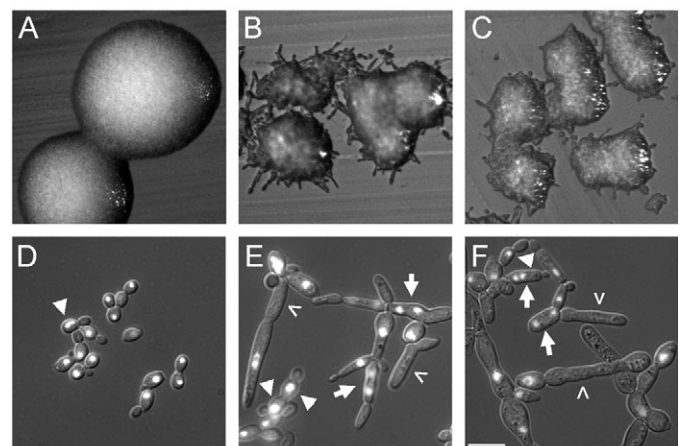
By contrast, bipolar spindles were positioned farther from the bud neck in *dyn1Δ/Δ* yeast cells (2.7±0.3  $\mu\text{m}$ ,  $n=28$ ,  $P<0.01$ ). Furthermore, spindles often were not parallel to the mother-bud axis (Fig. 5C, Tub2, 40 minutes). As a result, spindle elongation occurred within mother cells of all 30 *dyn1Δ/Δ* yeast cells (Fig. 5C, Tub2, 45 minutes). Thus, defects in spindle position and orientation were evident in all *dyn1Δ/Δ* yeast cells. Despite the

defects in spindle position and orientation in *dyn1Δ/Δ* yeast cells, all the daughter nuclei eventually segregated into the buds before spindle disassembly; binucleate mother cells and anucleate buds existed only transiently while the spindle re-oriented along the mother-bud axis. A previous study of *C. albicans* dynein mutants used GFP-labeled histones and concluded that delayed segregation of nuclei occurred post-mitosis (Martin et al., 2004). By contrast, our analysis of spindle dynamics using Tub2-GFP-labeled *dyn1Δ/Δ* yeast cells indicated that mitotic spindles remained intact until the dSPB moved into the bud (Fig. 5C, Tub2, cf. 60 minutes and 80 minutes). This indicates that mitotic exit in *C. albicans* and *S. cerevisiae* is similar: cells remain in mitosis, with delayed spindle disassembly and septation, until a daughter nucleus is delivered to the bud. Thus, nuclear segregation in *dyn1Δ/Δ* yeast cells occurs during a protracted anaphase, implying that a checkpoint mediates this anaphase delay until proper nuclear segregation occurs.

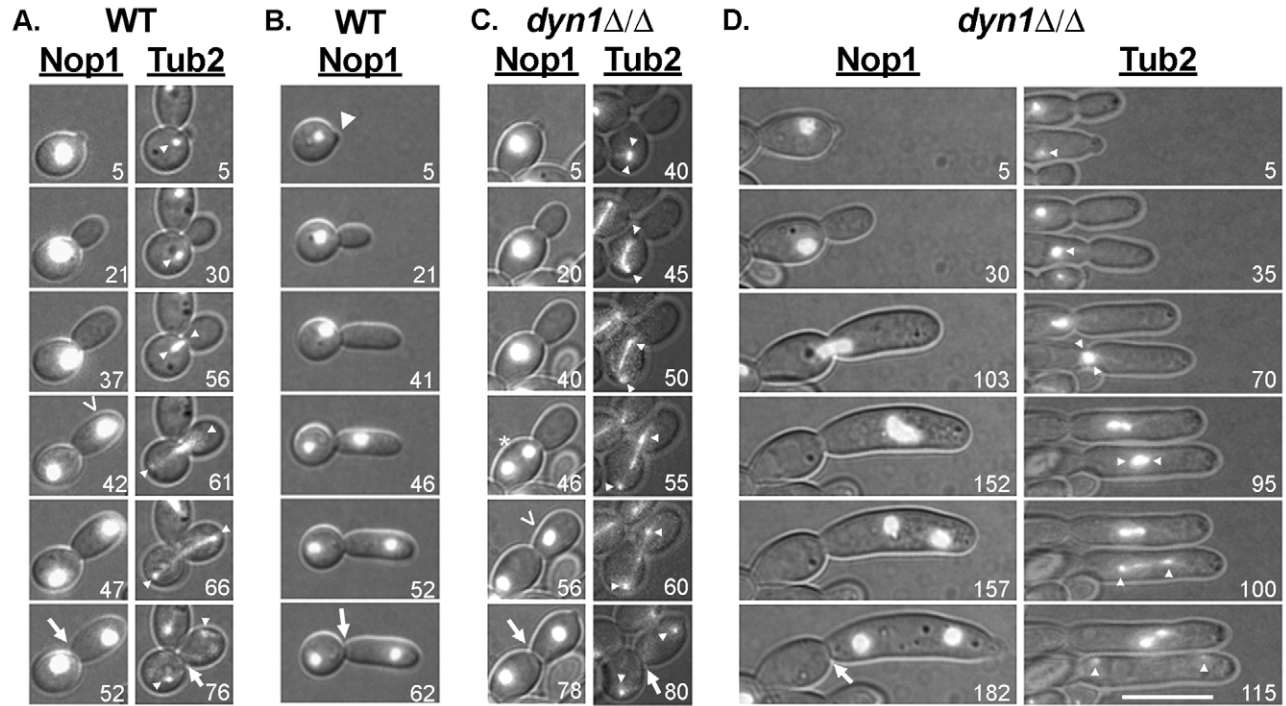
The *dyn1Δ/Δ* pseudohyphal-like cells, like *dyn1Δ/Δ* yeast cells, also exhibited defects in spindle position and orientation early in the cell cycle. In addition, the short bipolar spindle moved into the elongating bud before the onset of anaphase (Fig. 5D, Tub2, 95 minutes). As the bipolar spindle moved through the neck, it became oriented parallel to the mother-bud axis. However, neither this



**Fig. 3.** Dynein contributes to nuclear migration during cell cycle arrest. (A) Wild-type (YJB6284) and *dyn1Δ/Δ* (YJB7812) strains were grown to stationary phase overnight in YPD at 30°C. They were then diluted 1:100 into fresh YPD containing 100 mM HU and grown at 30°C for 6 hours before being fixed with ethanol and stained with DAPI for imaging. Bar, 10  $\mu\text{m}$ . (B) Scatter plot of nuclear position with respect to the bud tip (y-axis) plotted against bud length (x-axis). Strains: wild-type, BWP17; *dyn1Δ/Δ*, YJB7812.



**Fig. 4.** Colony and cellular morphology of dynein-dynactin mutants. (A-C) Colony morphology of wild-type (A), *dyn1Δ/Δ* (B) and *nip100Δ/Δ* (C) colonies after 2 days of growth on YPD-agar at 30°C. (D-F) Cellular morphology of wild-type (D), *dyn1Δ/Δ* (E) and *nip100Δ/Δ* (F) cells diluted to  $\text{OD}_{600}=1.0$  in YPD liquid medium and grown at 30°C for 4 hours. Cells were collected, fixed with ethanol and stained with DAPI for simultaneous DIC-DAPI microscopy. Arrowheads, yeast cells with a single nucleus; arrows, binucleate cells; carets, anucleate cells. Bar, 10  $\mu\text{m}$ .

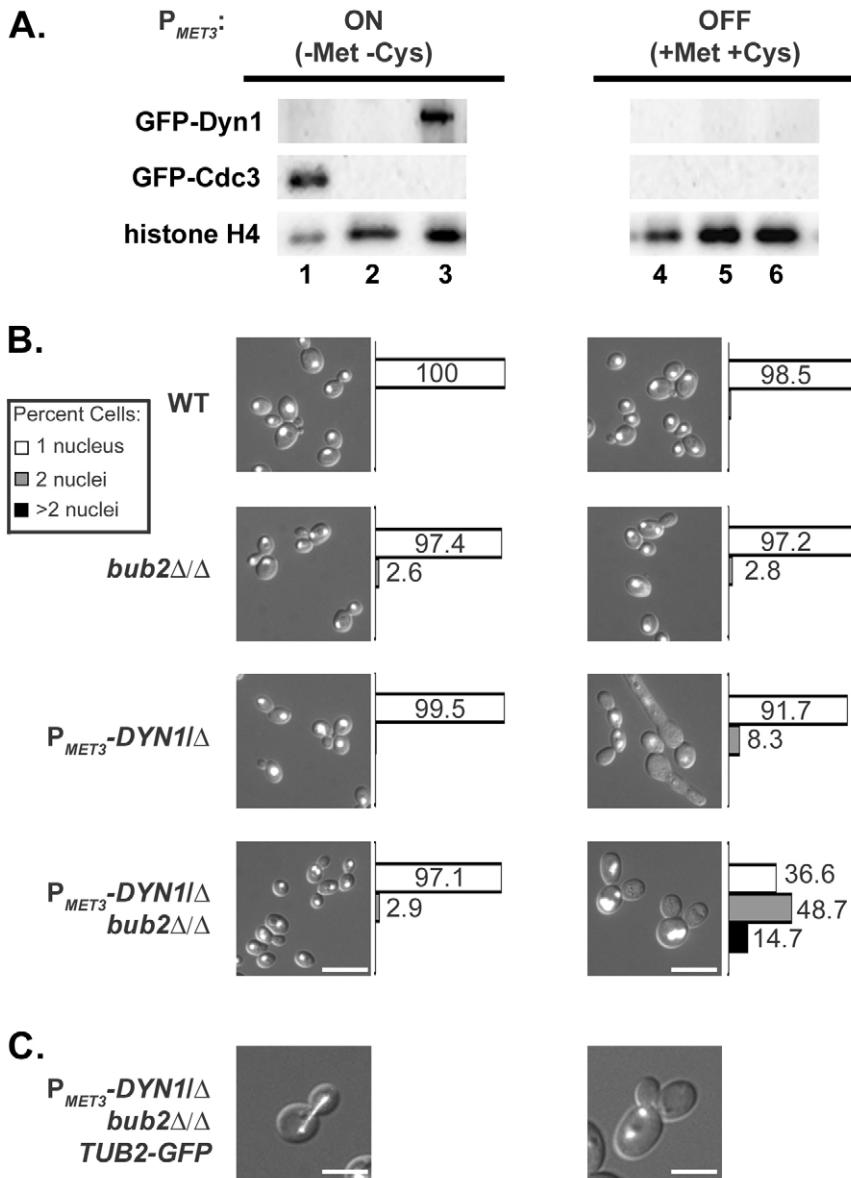


**Fig. 5.** Nuclear dynamics in wild-type and *dyn1* $\Delta/\Delta$  yeast and pseudohyphae. Time-lapse microscopy images were acquired at  $\sim$ 5-minute intervals. Time, minutes. (A) In wild-type yeast cells, segregation of the daughter nucleus (Nop1) occurs within 5 minutes of nuclear segregation (caret, 42 minutes). During anaphase, the spindle is oriented parallel to the mother-bud axis (Tub2, 56–66 minutes). The dSPB penetrates the bud within 5 minutes of onset of anaphase (61 minutes). Septation, which is detected as a darkening of the bud neck (arrows), occurs after nuclear segregation (Nop1, 52 minutes) and spindle disassembly (Tub2, 76 minutes). (B) In wild-type pseudohyphae grown at 35°C, nuclear dynamics observed with Nop1-YFP are similar to those of yeast cells. Tub2-GFP is not shown because of a temperature-sensitive phenotype at 35°C. (C) In *dyn1* $\Delta/\Delta$  yeast cells, mother cells are transiently binucleate (Nop1, 46 minutes, asterisk). Bipolar spindles are positioned away from the bud neck (Tub2, 40 minutes) and oriented oblique to the mother-bud axis during anaphase (45–50 minutes). The spindle remains intact until the dSPB penetrates the bud (60 minutes). Septation occurs after nuclear segregation (Nop1 arrow, 78 minutes) and spindle disassembly (Tub2, 80 minutes, arrow). (D) In *dyn1* $\Delta/\Delta$  pseudohyphal cells, undivided nuclei (Nop1, 103 minutes) and bipolar spindles (Tub2, 95 minutes) migrate into the elongating bud. Nuclei separate (Nop1, 157 minutes) as the spindle elongates entirely within the bud (Tub2, 100 minutes). The spindle disassembles without the mSPB returning to the mother cell (Tub2, 115 minutes). Septation occurs before the mother nucleus returns to the mother cell (Nop1, 182 minutes). Arrowheads, SPBs; bar, 10  $\mu$ m. Strains: (A) wild-type Nop1-YFP, YJB7062; Tub2-GFP YJB8953; (B) wild-type Nop1-YFP, YJB7062; (C,D) *dyn1* $\Delta/\Delta$  Nop1-YFP, YJB9430; *dyn1* $\Delta/\Delta$  Tub2-GFP, YJB8973.

correction of spindle position/orientation nor the fact that the dSPB moved into the bud was sufficient to stimulate anaphase. Instead, anaphase occurred much later, and it occurred within the elongated bud. In 6/21 cells, elongation of the mitotic spindle within the bud was sufficient to return the nucleus to the mother cell; in these cells, spindle disassembly was followed by cytokinesis and two uninucleate cells resulted (data not shown). In the other 15 cells, spindle elongation within the bud moved the mother SPB (mSPB) towards, but not into, the mother cell before spindle disassembly. Importantly, septation occurred despite the failure to deliver a nucleus back into the mother cell (Fig. 5D). Thus, these pseudohyphal-like cells lost the relationship between nuclear position and the onset of mitotic exit (spindle disassembly and septation). In support of this conclusion, the time elapsed between spindle disassembly and septation was not significantly different in wild-type yeast ( $6.6 \pm 0.8$ ,  $n=30$ ), *dyn1* $\Delta/\Delta$  yeast ( $5.6 \pm 0.8$  minutes,  $n=27$ ) and *dyn1* $\Delta/\Delta$  pseudohyphal-like cells ( $5.4 \pm 0.7$  minutes,  $n=20$ ,  $P>0.1$ ). Together, these results indicate that, although both yeast and pseudohyphal-like *dyn1* $\Delta/\Delta$  cells have spindle defects, the defects are distinct. In *dyn1* $\Delta/\Delta$  yeast, spindle elongation always occurs within the mother cell and a checkpoint delays mitotic exit until a nucleus is delivered to the bud. In *dyn1* $\Delta/\Delta$  pseudohyphal-like cells, the pre-anaphase nucleus moves

into the bud, anaphase occurs within the bud and there is no apparent checkpoint mechanism to ensure that the mother nucleus returns to the mother cell.

In *S. cerevisiae*, Dyn1p localizes asymmetrically to MTs associated with the dSPB early in anaphase (Grava et al., 2006; Shaw et al., 1997; Sheeman et al., 2003). Later in anaphase, Dyn1p localizes symmetrically to both poles of the spindle (Grava et al., 2006; Shaw et al., 1997). To ask whether *C. albicans* Dyn1p exhibited similar properties and whether Dyn1p mislocalization could be the mechanistic basis for the filamentation phenotype, we followed the localization of Dyn1-YFP, integrated as the only *DYNI* allele, into a strain expressing *TUB2-CFP*. The Dyn1-YFP localized preferentially to MTs that extend towards and into the bud during G2-M and showed enrichment towards the MT plus-ends (supplementary material Fig. S1A). After the dSPB had moved into the bud, Dyn1-YFP localized to MTs associated with both SPBs (supplementary material Fig. S1A). Thus, Dyn1p localizes asymmetrically to daughter-bound MTs in G2-M and becomes symmetrically distributed later in anaphase. Importantly, in *nip100* $\Delta/\Delta$  cells, both yeast and pseudohyphal-like cells lost Dyn1-YFP asymmetry (supplementary material Fig. S1B). Thus, the loss of symmetrical Dyn1p localization in the mutant cannot explain the



**Fig. 6.** Bub2p affects cell cycle progression and morphogenesis in dynein-depleted yeast cells. (A) Immunoblots of  $P_{MET3}$ -GFP-Dyn1p expression (left) or repression (right). Strains YJB6755 ( $P_{MET3}$ -GFP-*CDC3/CDC3*, lanes 1 and 4), YJB7944 (*DYN1-GFP/DYN1*, lanes 2 and 5) and YJB8046 ( $P_{MET3}$ -GFP-*DYN1/dyn1Δ*, lanes 3 and 6) were grown overnight in  $P_{MET3}$  ON conditions, diluted to  $OD_{600}=0.01$  in fresh SDC medium containing 10 mM methionine and 2 mM cysteine ( $P_{MET3}$  OFF) or lacking methionine and cysteine ( $P_{MET3}$  ON) and incubated at 30°C for 14 hours. Immunoblots were probed with antibodies against GFP or histone H4, as described in Materials and Methods. Dyn1-GFP in YJB7944 was not detectable under these conditions, presumably owing to low expression from the native promoter; it was detectable by microscopy (supplementary material Fig. S1). (B) Bub2p monitors two steps of anaphase in dynein-depleted cells. The strains indicated were grown as in A. The number of DAPI-stained nuclei per cell is plotted as a histogram at the side of each micrograph. Strains: wild-type, BWP17; *bub2Δ/Δ*, CB432;  $P_{MET3}$ -*DYN1/dyn1Δ*, YJB8039;  $P_{MET3}$ -*DYN1/dyn1Δ* *bub2Δ/Δ*, YJB9993. (C) Bub2p inhibits disassembly of the mitotic spindle in dynein-deficient cells. Strain YJB10232 ( $P_{MET3}$ -*DYN1Δ* *bub2Δ/Δ* *TUB2-GFP:NAT1*) was cultured as in A. When  $P_{MET3}$ -*DYN1* was expressed (left), mitotic spindles remained intact until after the dSPB penetrated the bud; when  $P_{MET3}$ -*DYN1* was repressed (right), mitotic spindles disassembled prematurely. DIC and fluorescent images were merged in B and C. Bars, 5 μm.

difference between the yeast and pseudohyphal morphologies of *C. albicans* dynein-dynactin mutants.

#### The Bub2p checkpoint protein mediates polarized growth in *dyn1Δ/Δ* yeast cells

In *S. cerevisiae*, Bub2p inhibits spindle disassembly and mitotic exit until a nucleus has been delivered to the bud (Bardin et al., 2000; Bloecher et al., 2000; Pereira et al., 2000). We asked whether Bub2p has an analogous role in *C. albicans* *dyn1Δ/Δ* cells. For these experiments, we repressed expression of the only *DYN1* allele using the regulatable *MET3* promoter (Care et al., 1999) in *BUB2* (YJB10155) and in *bub2Δ/Δ* (YJB9993) strains. Approximately 14 hours after transfer of the strains to  $P_{MET3}$ -repressing medium, GFP-Dyn1p was depleted (Fig. 6A) and *dyn1Δ/Δ* phenotypes were evident in the dynein-depleted wild-type *BUB2* strain: the culture contained pseudohyphal-like cells with undivided nuclei as well as yeast cells with two nuclei (Fig. 6B). By contrast, depletion of Dyn1p in YJB9993 ( $P_{MET3}$ -*DYN1/dyn1Δ* *bub2Δ/Δ*) resulted in three phenotypes: first, many binucleate and multinucleate,

multibudded cells appeared (48.7% and 14.7%, respectively; Fig. 6B); second, the pseudohyphal-like phenotype was rarely seen. These changes suggest at least two defects: failure of nuclear segregation and failure to activate the polarized growth seen in *dyn1Δ/Δ* cells.

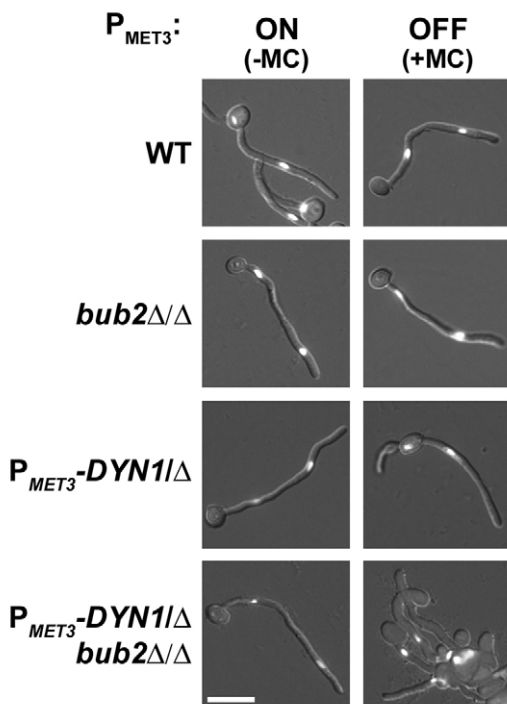
We integrated *TUB2-GFP* into YJB9993 and followed spindle dynamics in the resulting strain (YJB10232). When  $P_{MET3}$ -*DYN1* was expressed, mitotic spindles were intact and stretched across the neck of cells that had a single bud. By contrast, when  $P_{MET3}$ -*DYN1* was repressed in the strain, cells with multiple buds and multiple SPBs, but no mitotic spindles, appeared (Fig. 6C). This phenotype indicates that nuclei failed to reach the bud, yet the spindles had disassembled.

We repeated these experiments by depleting dynein in strains lacking either the spindle assembly checkpoint protein Mad2p or the morphogenesis checkpoint protein Swe1p. In both cases, the phenotypes were similar to phenotypes of Dyn1p-depleted cells: pseudohyphal cells were evident in the population (K.R.F., unpublished). Thus, only the Bub2p spindle position checkpoint is

required for the pseudohyphal-like phenotype in cells lacking cytoplasmic dynein.

In Dyn1p-depleted hyphal cells the Bub2p spindle position checkpoint mediates delayed nuclear division and hyphal morphogenesis

We next asked about the role of Bub2p in hyphal cells depleted for dynein. We induced hyphal growth in the same strains detailed above. After 10 hours in *MET3*-repressing medium and two hours in hypha-inducing conditions, wild-type and *bub2Δ/Δ* hyphae contained nuclei that had migrated and divided (Fig. 7). Under the same conditions, *MET3-DYN1/Δ* hyphae had morphologies and nuclear migration phenotypes like those of *dyn1Δ/Δ* cells induced for hyphal growth (cf. Fig. 7 and Fig. 1). By contrast, under these depletion conditions, *MET3-DYN1/Δ bub2Δ/Δ* cells exhibited severe defects in morphology: the cells were elongated but with wide, pseudohyphal-like morphology (Fig. 7). Thus, Bub2p is required to maintain a hyphal morphology in cells lacking dynein. Importantly, many of the cells had multiple nuclei within the basal cell, indicating that mitosis had occurred before nuclear migration. This is consistent with the idea that, in the absence of dynein, Bub2p delays cell cycle progression long enough for dynein-independent activities to move the nuclei in some cells, but loss of Bub2p allows the onset of anaphase and mitotic exit to occur before, or in the absence of, nuclear movement. Thus, Bub2p coordinates nuclear migration with hyphal morphogenesis in cells that lack dynein.



**Fig. 7.** Bub2p mediates cell cycle progression and morphogenesis in dynein-depleted hyphae. Strains were grown to stationary phase overnight at 30°C in either  $P_{MET3}$  ON or  $P_{MET3}$  OFF conditions and then diluted 1:20 into the same medium containing 10% serum and prewarmed to 37°C. They were grown at 37°C for 2 hours, fixed with ethanol and stained with DAPI. Strains: wild-type, YJB6284 (Bensen et al., 2002),  $P_{MET3}$ -*DYN1/dyn1Δ*, YJB10155, *bub2Δ/Δ*, CB432 (Bachewich et al., 2005),  $P_{MET3}$ -*DYN1/dyn1Δ bub2Δ/Δ*, YJB9993.

## Discussion

Nuclear dynamics are dramatically different in wild-type *C. albicans* true hyphae and budding cells. The *C. albicans* genome contains single alleles for six kinesin-like proteins and single alleles of cytoplasmic dynein and dynactin; no orthologs of conventional kinesin are present (K.R.F., data not shown). Therefore, dynein-dynactin is likely to be the major minus-end-directed microtubule motor protein in *C. albicans*. Indeed, in the absence of dynein or dynactin, nuclear dynamics are slowed dramatically and nuclear migration into daughter cells is accomplished by spindle elongation in all yeast and in the majority of hyphal cells. In addition, dynein-independent mechanisms move nuclei forward in three different cell types. In pseudohyphal-like *dyn1Δ/Δ* cells, HU-arrested *dyn1Δ/Δ* cells and some *dyn1Δ/Δ* hyphal cells, nuclei migrate into the daughter cells, albeit very slowly, and there does not appear to be a mechanism to ensure that a nucleus returns to the mother cell. By contrast, the Bub2p checkpoint protein monitors two different stages of mitosis: onset of anaphase and disassembly of the spindle/initiation of mitotic exit. Thus, dynein-dynactin is the major activity that moves nuclei in wild-type cells.

### MT dynamics in the presence and absence of dynein

Loss of dynein-dynactin did not affect the rates of MT growth and shrinkage in *C. albicans* (Table 1). This is different from *S. cerevisiae*, where the loss of dynein (Dyn1) decreases rates of MT growth and shrinkage (Carminati and Stearns, 1997), whereas the loss of dynactin (Arp1) increases the rates of MT growth and shrinkage (Adames and Cooper, 2000). It also differs from the situation in *A. nidulans*, where the loss of dynein decreases the rate of MT shrinkage but not the rate of MT growth (Han et al., 2001). Because the loss of dynein-dynactin affects MT dynamics differently in different organisms, dynein-dynactin most likely regulates MT dynamics indirectly rather than directly.

The reduced frequency of MT release from the SPB seen in *C. albicans* dynein dynamics mutants has not been reported in other fungi. This suggests that dynein mediates the frequent detachment of MTs from the SPB seen in wild-type cells (Finley and Berman, 2005) and is consistent with the idea that dynein facilitates spindle positioning by pulling SPB-bound MTs towards their minus-ends.

Dynein makes a large contribution to nuclear migration in *C. albicans* hyphae; dynein-independent activities are far less (by a factor of ~34) efficient at moving nuclei out of the mother cell and into the bud. In *A. gossypii*, the dynein-independent activity that moves nuclei to hyphal tips is strong and pulls in the opposite direction relative to dynein (Alberti-Segui et al., 2001). Our work indicates that, in *C. albicans*, the weaker dynein-independent activities appear to be cooperative with dynein, moving nuclei in the same direction relative to dynein. This makes it difficult to detect these weak activities when dynein is present.

Dynein-independent nuclear movements also have been seen in *A. nidulans* (Xiang et al., 1995) and *S. cerevisiae* (Eshel et al., 1993; Li et al., 2005). In *S. cerevisiae*, two dynein-independent, yet microtubule-dependent, mechanisms of nuclear migration operate during interphase: microtubule plus-end transport and microtubule shrinkage. In microtubule plus-end transport, the plus-ends of SPB-bound microtubules (MTs) are translocated along the cortex by means of a type V myosin-dependent connection to the cortical actin cytoskeleton (reviewed in Pearson and Bloom, 2004), which can deliver MT ends to cortical sites such as the bud neck or bud tip (Miller and Rose, 1998). Microtubule shrinkage involves active

depolymerization of MT plus-ends that remain attached to the cortex by microtubule depolymerases, such as the kinesin-8 Kip3p (Gupta et al., 2006; Varga et al., 2006). Mutations in the kinesin-8 orthologs in both *S. cerevisiae* (Kip3p) (Miller et al., 1998) and *A. nidulans* (KipB) (Rischitor et al., 2004) perturb spindle positioning, suggesting that the role of kinesin-8 in nuclear movement might be conserved among fungi.

#### Morphogenesis defects in *C. albicans* cells lacking dynein-dynactin

In *C. albicans*, as in other filamentous fungi (Straube et al., 2001), a lack of dynein-dynactin results in morphogenesis defects. While some of the morphogenetic defects in *C. albicans* *dyn1Δ/Δ* have been reported previously (Martin et al., 2004), the incomplete penetrance of the morphogenesis phenotype and the distinctive defects in nuclear dynamics in cells with different morphologies had not been appreciated. Our study supports the idea that the timing of cell cycle events ultimately modulates yeast cell morphogenesis. The mechanism(s) by which morphogenesis is affected by the loss of dynein-dynactin must differ from that in *S. cerevisiae*, where lack of dynein does not cause a defect in morphogenesis.

Approximately half of the cells in a population growing logarithmically at 30°C resemble pseudohyphae. In pseudohyphal-like cells, defects in spindle position and orientation are eventually overcome during an extended G2 phase that is maintained by an active checkpoint. Together, these results indicate that defective spindle position and orientation per se cannot account for the difference in cell morphologies between *dyn1Δ/Δ* yeast and pseudohyphal-like cells. Furthermore, asymmetric dynein localization cannot cause cells to grow with yeast versus pseudohyphal-like dynein-dynactin morphologies as *nip100Δ/Δ* mutants also formed both yeast and pseudohyphal-cells at 30°C, yet dynein localization was symmetric in both cell types.

#### Role of Bub2p in monitoring cell cycle progression

Checkpoints ensure that cell cycle events happen in a specific, crucial order. For example, mitotic exit events must not occur until both the dSPB moves into the daughter cell and the mother and daughter chromosomes separate. Execution of only one of these events is not sufficient to trigger mitotic exit in the absence of dynein: in yeast cells, anaphase separation of chromosomes does not trigger mitotic exit until the dSPB moves into the daughter cell; in pseudohyphal-like cells, the migration of the dSPB on the bipolar spindle into the daughter cell does not trigger mitotic exit until anaphase ensues. Thus, in *C. albicans*, both yeast and pseudohyphal-like cells appear to delay mitotic exit until both events (anaphase and movement of a SPB into the daughter) have occurred, even though they occur in a different order in pseudohyphal-like cells relative to yeast cells. It is interesting that nuclear dynamics in the pseudohyphal-like cells and in HU-treated cells is reminiscent of the nuclear dynamics in true hyphae.

The requirements for mitotic exit in hyphae lacking dynein appear to be different; the septum forms 10–15 μm into the germ tube and the anaphase spindle often is not long enough to deliver the dSPB past the site of septation. Nonetheless, spindle disassembly eventually occurs, resulting in binucleate basal cells and anucleate tip cells. Defects in hyphal morphology occur after these late defects in cell cycle progression, suggesting that morphogenetic defects are a secondary effect of defects in cell cycle progression, rather than a direct effect of the loss of dynein.

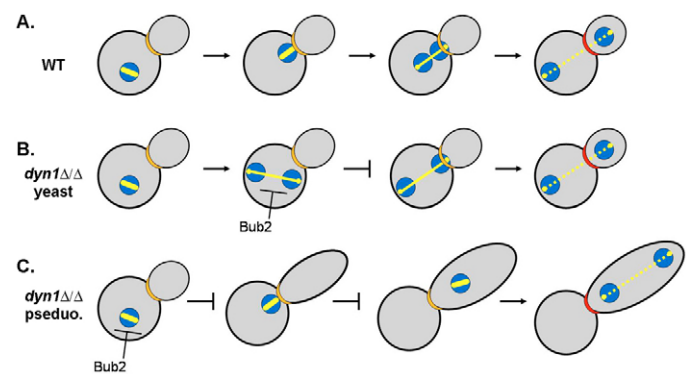
Thus, in hyphal cells, the onset of anaphase is delayed, but not blocked, if the spindle does not migrate near the presumptive site of septation.

The morphology of elongated *dyn1Δ/Δ* cells appears to depend upon the Bub2p checkpoint protein. Specifically, in *dyn1Δ/Δ* pseudohyphal-like cells, Bub2p inhibits the onset of anaphase; polarized growth occurs primarily during the long delay in G2 phase (K.R.F. and J.B., unpublished) that precedes nuclear migration to the bud. In the absence of Bub2p, cell elongation is not observed; virtually all of the cells are round. While we cannot exclude a direct role for Bub2p in morphogenesis, this result is consistent with the idea that cell cycle delays mediate polarized growth (Berman, 2006).

In *dyn1Δ/Δ* hyphae, dynein-independent mechanisms can move nuclei to the germ tube, and then spindle elongation can push the daughter nucleus into the daughter hyphal compartment. In cells lacking both dynein and Bub2p, neither onset of anaphase nor disassembly of the spindle is delayed long enough for nuclei to move into the germ tube and to reach the daughter cell. This results in anucleate daughter cells with highly unusual morphologies. As with budding cells, the abnormal morphologies appear to be a secondary effect of defects in cell cycle progression (lack of a nucleus) rather than a direct effect of dynein on hyphal morphogenesis.

#### Bub2p monitors two dynein-dependent steps of anaphase

Interestingly, we found that *C. albicans* Bub2p responds to two dynein-dependent defects in cell cycle progression (Fig. 8). First, Bub2p delays the onset of anaphase, maintaining spindle integrity when pre-anaphase nuclear migration fails. Second, Bub2p ensures that cells do not initiate exit from mitosis until nuclei have undergone anaphase. The first mechanism is seen in *C. albicans* and not in *S. cerevisiae*. Bub2p mediates the G2 arrest and



**Fig. 8.** Model for the role of *C. albicans* Bub2p in regulating nuclear position. (A) In unperturbed wild-type cells, the bipolar spindle (yellow bar) migrates to the bud neck (orange) and elongates across the bud neck. After the dSPB/daughter nucleus (blue) resides in the bud, the spindle disassembles (dashed yellow line) and septation ensues (red). (B) Yeast cells lacking dynein-dynactin function undergo anaphase within the mother cell. Bub2p inhibits spindle disassembly and septation until the dSPB/daughter nucleus has moved into the bud. (C) Bub2p inhibits onset of anaphase and stimulates polarized growth in *dyn1Δ/Δ* cells. In pseudohyphal-like cells lacking dynein, the bipolar spindle migrates into the elongating bud in a dynein-independent, bud length-dependent, fashion that might be mediated by interactions between the spindle and the bud tip. Spindle elongation eventually ensues. Spindle disassembly is not inhibited because the dSPB is already within the bud. Septation can occur before the mSPB/mother nucleus returns to the mother cell because no checkpoint is activated.

associated polarized growth response of *dyn1Δ/Δ* cells (Fig. 8C). At present, it is not clear why some *dyn1Δ/Δ BUB2/BUB2* cells proceed into anaphase despite defective spindle position and why other cells maintain a G2 arrest even while the bipolar spindle migrates through the neck.

One mechanism resembles that in *S. cerevisiae*: Bub2p blocks mitotic exit when the anaphase spindle elongates in the mother cell (Fig. 8B), resulting in the appearance of multiply budded cells containing multiple nuclei and disassembled spindles. These phenotypes are hallmarks of loss of a cell cycle checkpoint (Hoyt et al., 1991) and indicate that Bub2p is required to ensure that the dSPB traverses the bud neck before the onset of exit from mitosis.

Cell cycle inhibition by Bub2p is stimulated by different mechanisms in *S. cerevisiae* (Hu and Elledge, 2002; Hu et al., 2001; Kim and Song, 2006), where Bub2p is a GTPase-activating protein that is thought to keep the Tem1p GTPase in the inactive, GDP-bound state. However, Tem1p has high intrinsic GTPase activity (Geymonat et al., 2002) and thus might be capable of stimulating exit from mitosis during prolonged cell cycle delay. It is tempting to speculate that the different requirements for the initiation of exit from mitosis in *C. albicans* hyphae and yeast are due to an effect of cell length and the length of time that Bub2p can inhibit Tem1p. Clearly, in *C. albicans*, dynein has a partial role in regulating at least two different aspects of cell cycle progression (onset of anaphase and exit from mitosis) and Bub2p monitors both of them.

## Materials and Methods

### Strain construction

The strains used in this study (supplementary material Table 2) were constructed in *C. albicans* strain background BWP17 and transformed as described previously (Wilson et al., 1999) using SDC medium (Rose, 1987) lacking the appropriate amino acids or uridine as necessary for selection. Unless stated otherwise, uridine was always included at 80 μg/ml and cells were always grown at 30°C. Open reading frames were disrupted using pGEM-HIS1, pRS-Arg4ΔSpeI (Wilson et al., 1999) or pDDB57 (Wilson et al., 2000) as templates with disruption primers (supplementary material Table 2). Strains were made prototrophic by transforming with plasmids pRS-Arg4ΔSpeI, pRS-ARG-URA-BN (Davis et al., 2000) or pGEM-HIS1 linearized with *EcoRI*, *NotI* or *NruI*, respectively. To select for cells that excised *URA3* from *dyn1Δ::URA3-dpl200* (YJB7753), YJB7709 was passaged twice for single colonies on SDC plates containing 0.5g/L 5'fluoroorotic acid (U.S. Biologicals, Swampscott, MA). *URA3* loss was verified by PCR and lack of growth on SDC plates lacking uridine. C-terminal fluorescent fusions to *NOP1* and *TUB2* were constructed as described previously (Finley and Berman, 2005; Gerami-Nejad et al., 2001). The expression or repression of *DYN1* from the *MET3* promoter (Care et al., 1999) was controlled by growth in SDC medium lacking methionine and cysteine or containing 10 mM methionine and 2 mM cysteine, respectively. To minimize the possibility of allele-specific phenotypes, independent null strains were constructed from two independently derived heterozygotes. The colony and cellular morphology of *DYN1/dyn1Δ* and *NIP100/nip100Δ* strains was no different from wild-type strains (data not shown).

### DAPI staining

Cells were harvested by centrifugation at the desired time after reinoculation into fresh medium, resuspended in 1 ml of 70% ethanol, incubated at room temperature for 5-10 minutes, pelleted, resuspended in 1 ml of 1× PBS (130 mM NaCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.2) containing 1 μg/ml DAPI (4'-6-diamidino-2-phenylindole, Sigma Chemical Co., St Louis, MO) and incubated at room temperature for 5-10 minutes or overnight at 4°C. Microscopy and image analysis were performed as described previously (Finley and Berman, 2005). Simultaneous differential interference contrast (DIC)-DAPI microscopy was performed using low-level transmitted light and full-intensity fluorescent light in combination with manually adjusted exposure times.

### Culturing strains for time-lapse microscopy

The strains used in the time-lapse microscopy experiments were cultured overnight in liquid YPD, diluted to OD<sub>600</sub>=1.0 in fresh YPD liquid and incubated at 30°C with shaking for 1.5-2 hours. 200 μl of this culture was spread onto prewarmed (30°C) YPD-agar plates. A cover slip was then placed over the cells and a single differential interference contrast (DIC) image and four YFP images were collected with 1-μm

z-axis intervals, as described previously (Finley and Berman, 2005), except that the objective heater was set to 30°C or 37°C and four different stage positions were used at each time-point. For fluorescent images, YFP illumination was used regardless of whether the fluorescent fusion protein was Nop1-YFP or Tub2-GFP.

### Immunoblot analysis

Protein gels were prepared, electrophoresed and transferred to Hybond-P (Amersham Biosciences) membrane (Bensen et al., 2005). GFP was detected with monoclonal mouse antibody against GFP (Roche Applied Science, Indianapolis, IN) and polyclonal HRP-conjugated goat antibody against GFP (Abcam, Cambridge, MA), followed by a HRP-anti-mouse secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and histone H4 (loading control) was detected with a rabbit antibody against *S. cerevisiae* H4 (Glowczewski et al., 2004), which cross-reacts with *C. albicans* histone H4, followed by a HRP-anti-rabbit secondary antibody (Santa Cruz Biotechnology).

### Data analysis

Data from the time-lapse experiments were collected in Metamorph, v6.2 (Universal Imaging Corp., Westchester, PA) or NIH Image J v.1.34s (Rasband, 1997-2006) and exported to Microsoft Excel (Microsoft Corp., Redmond, WA) for analysis. Error bars represent the s.e.m. Student's *t*-test was used to determine statistical significance.

We thank Carrie Ketel, Cheryl Gale, Duncan Clarke, Kerry Bloom and Pete Sudbery for critical reading of the manuscript. We thank Maryam Gerami-Nejad for technical assistance and Catherine Bachewich and Malcolm Whiteway for strain CB432. This work was supported by NIH grant AI/DE 14666 to J.B. K.J.B. was supported, in part, by the Graduate School 3M Fellowship in Science and Technology. K.R.F. was supported, in part, by NIH Biotechnology Training Grant GM08347.

## References

- Adames, N. R. and Cooper, J. A. (2000). Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae*. *J. Cell Biol.* **149**, 863-874.
- Alberti-Segui, C., Dietrich, F., Altmann-Johl, R., Hoepfner, D. and Philippsen, P. (2001). Cytoplasmic dynein is required to oppose the force that moves nuclei towards the hyphal tip in the filamentous ascomycete *Ashbya gossypii*. *J. Cell Sci.* **114**, 975-986.
- Bachewich, C., Thomas, D. Y. and Whiteway, M. (2003). Depletion of a polo-like kinase in *Candida albicans* activates cyclase-dependent Hyphal-like growth. *Mol. Biol. Cell* **14**, 2163-2180.
- Bachewich, C., Nantel, A. and Whiteway, M. (2005). Cell cycle arrest during S or M phase generates polarized growth via distinct signals in *Candida albicans*. *Mol. Microbiol.* **57**, 942-959.
- Bai, C., Ramanan, N., Wang, Y. M. and Wang, Y. (2002). Spindle assembly checkpoint component CaMad2p is indispensable for *Candida albicans* survival and virulence in mice. *Mol. Microbiol.* **45**, 31-44.
- Bardin, A. J., Visintin, R. and Amon, A. (2000). A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell* **102**, 21-31.
- Bedell, G. W., Werth, A. and Soll, D. R. (1980). The regulation of nuclear migration and division during synchronous bud formation in released stationary phase cultures of the yeast *Candida albicans*. *Exp. Cell Res.* **127**, 103-113.
- Bensen, E. S., Filler, S. G. and Berman, J. (2002). A forkhead transcription factor is important for true hyphal as well as yeast morphogenesis in *Candida albicans*. *Eukaryotic Cell* **1**, 787-798.
- Bensen, E. S., Clemente-Blanco, A., Finley, K. R., Correa-Bordes, J. and Berman, J. (2005). The mitotic cyclins Clb2p and Clb4p affect morphogenesis in *Candida albicans*. *Mol. Biol. Cell* **16**, 3387-3400.
- Berman, J. (2006). Morphogenesis and cell cycle progression in *Candida albicans*. *Curr. Opin. Microbiol.* **9**, 595-601.
- Blocher, A., Venturi, G. M. and Tatchell, K. (2000). Anaphase spindle position is monitored by the BUB2 checkpoint. *Nat. Cell Biol.* **2**, 556-558.
- Care, R. S., Trevethick, J., Binley, K. M. and Sudbery, P. E. (1999). The *MET3* promoter: a new tool for *Candida albicans* molecular genetics. *Mol. Microbiol.* **34**, 792-798.
- Carminati, J. L. and Stearns, T. (1997). Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. *J. Cell Biol.* **138**, 629-641.
- Davis, D., Edwards, J. E., Jr, Mitchell, A. P. and Ibrahim, A. S. (2000). *Candida albicans* RIM101 pH response pathway is required for host-pathogen interactions. *Infect. Immun.* **68**, 5953-5959.
- Eshel, D., Urrestarazu, L., Vissers, S., Jauniaux, J., van Vliet-Reedijk, J., Planta, R. and Gibbons, I. (1993). Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Natl. Acad. Sci. USA* **90**, 11172-11176.
- Finley, K. R. and Berman, J. (2005). Microtubules in *Candida albicans* hyphae drive nuclear dynamics and connect cell cycle progression to morphogenesis. *Eukaryotic Cell* **4**, 1697-1711.
- Gerami-Nejad, M., Berman, J. and Gale, C. A. (2001). Cassettes for PCR-mediated construction of green, yellow, and cyan fluorescent protein fusions in *Candida albicans*. *Yeast* **18**, 859-864.

- Geymonat, M., Spanos, A., Smith, S. J., Wheatley, E., Rittinger, K., Johnston, L. H. and Sedgwick, S. G. (2002). Control of mitotic exit in budding yeast. In vitro regulation of Tem1 GTPase by Bub2 and Bfa1. *J. Biol. Chem.* **277**, 28439-28445.
- Glowczewski, L., Waterborg, J. H. and Berman, J. G. (2004). Yeast chromatin assembly complex 1 protein excludes nonacetylatable forms of histone H4 from chromatin and the nucleus. *Mol. Cell Biol.* **24**, 10180-10192.
- Grava, S., Schaerer, F., Faty, M., Philippesen, P. and Barral, Y. (2006). Asymmetric recruitment of dynein to spindle poles and microtubules promotes proper spindle orientation in yeast. *Dev. Cell* **10**, 425-439.
- Gupta, M. L., Jr, Carvalho, P., Roof, D. M. and Pellman, D. (2006). Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. *Nat. Cell Biol.* **8**, 913-923.
- Han, G., Liu, B., Zhang, J., Zuo, W., Morris, N. R. and Xiang, X. (2001). The *Aspergillus* cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics. *Curr. Biol.* **11**, 719-724.
- Hartwell, L. H. and Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629-634.
- Helper, H. and Gladfelter, A. S. (2006). AgSwe1p regulates mitosis in response to morphogenesis and nutrients in multinucleated *Ashbya gossypii* cells. *Mol. Biol. Cell* **17**, 4494-4512.
- Hoyt, M. A., Totis, L. and Roberts, B. T. (1991). *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**, 507-517.
- Hu, F. and Elledge, S. J. (2002). Bub2 is a cell cycle regulated phospho-protein controlled by multiple checkpoints. *Cell Cycle* **1**, 351-355.
- Hu, F., Wang, Y., Liu, D., Li, Y., Qin, J. and Elledge, S. J. (2001). Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell* **107**, 655-665.
- Hwang, E., Kusch, J., Barral, Y. and Huffaker, T. C. (2003). Spindle orientation in *Saccharomyces cerevisiae* depends on the transport of microtubule ends along polarized actin cables. *J. Cell Biol.* **161**, 483-488.
- Inoue, S., Turgeon, B., Yoder, O. and Aist, J. (1998). Role of fungal dynein in hyphal growth, microtubule organization, spindle pole body motility and nuclear migration. *J. Cell Sci.* **111**, 1555-1566.
- Keaton, M. A. and Lew, D. J. (2006). Eavesdropping on the cytoskeleton: progress and controversy in the yeast morphogenesis checkpoint. *Curr. Opin. Microbiol.* **9**, 540-546.
- Kim, J. and Song, K. (2006). The study of Bfa1p(E438K) suggests that Bfa1 control the mitotic exit network in different mechanisms depending on different checkpoint-activating signals. *Mol. Cells* **21**, 251-260.
- Lew, D. J. and Burke, D. J. (2003). The spindle assembly and spindle position checkpoints. *Annu. Rev. Genet.* **37**, 251-282.
- Li, C. R., Wang, Y. M., De Zheng, X., Liang, H. Y., Tang, J. C. and Wang, Y. (2005). The formin family protein CaBn1p has a role in cell polarity control during both yeast and hyphal growth in *Candida albicans*. *J. Cell Sci.* **118**, 2637-2648.
- Martin, R., Walther, A. and Wendland, J. (2004). Deletion of the dynein heavy-chain gene *DYN1* leads to aberrant nuclear positioning and defective hyphal development in *Candida albicans*. *Eukaryotic Cell* **3**, 1574-1588.
- May, K. M. and Hardwick, K. G. (2006). The spindle checkpoint. *J. Cell Sci.* **119**, 4139-4142.
- Merson-Davies, L. A. and Odds, F. C. (1989). A morphology index for characterization of cell shape in *Candida albicans*. *J. Gen. Microbiol.* **135**, 3143-3152.
- Miller, R. K. and Rose, M. D. (1998). Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. *J. Cell Biol.* **140**, 377-390.
- Miller, R. K., Heller, K. K., Frisen, L., Wallack, D. L., Loayza, D., Gammie, A. E. and Rose, M. D. (1998). The kinesin-related proteins, Kip2p and Kip3p, function differently in nuclear migration in yeast. *Mol. Biol. Cell* **9**, 2051-2068.
- Pearson, C. G. and Bloom, K. (2004). Dynamic microtubules lead the way for spindle positioning. *Nat. Rev. Mol. Cell Biol.* **5**, 481-492.
- Pereira, G., Hofken, T., Grindlay, J., Manson, C. and Schiebel, E. (2000). The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol. Cell* **6**, 1-10.
- Plamann, M., Mink, P. F., Tinsley, J. H. and Bruno, K. S. (1994). Cytoplasmic dynein and actin-related protein Arp1 are required for normal nuclear distribution in filamentous fungi. *J. Cell Biol.* **127**, 139-149.
- Rasband, W. (1997-2006). ImageJ. Bethesda, MD: US National Institutes of Health, <http://rsb.info.nih.gov/ij/>.
- Rischitor, P. E., Konzack, S. and Fischer, R. (2004). The Kip3-like kinesin KipB moves along microtubules and determines spindle position during synchronized mitoses in *Aspergillus nidulans* hyphae. *Eukaryotic Cell* **3**, 632-645.
- Rose, M. D. (1987). Isolation of genes by complementation in yeast. *Meth. Enzymol.* **152**, 481-504.
- Shaw, S. L., Yeh, E., Maddox, P., Salmon, E. D. and Bloom, K. (1997). Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. *J. Cell Biol.* **139**, 985-994.
- Sheeman, B., Carvalho, P., Sagot, I., Geiser, J., Kho, D., Hoyt, M. A. and Pellman, D. (2003). Determinants of *S. cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. *Curr. Biol.* **13**, 364-372.
- Shi, Q. M., Wang, Y. M., Zheng, X. D., Teck Ho Lee, R. and Wang, Y. (2007). Critical role of DNA checkpoints in mediating genotoxic-stress-induced filamentous growth in *Candida albicans*. *Mol. Biol. Cell* **18**, 815-826.
- Straube, A., Enard, W., Berner, A., Wedlich-Soldner, R., Kahmann, R. and Steinberg, G. (2001). A split motor domain in a cytoplasmic dynein. *EMBO J.* **20**, 5091-5100.
- Sudbery, P., Gow, N. and Berman, J. (2004). The distinct morphogenic states of *Candida albicans*. *Trends Microbiol.* **12**, 317-324.
- Varga, V., Helenius, J., Tanaka, K., Hyman, A. A., Tanaka, T. U. and Howard, J. (2006). Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nat. Cell Biol.* **8**, 957-962.
- Wilson, R. B., Davis, D. and Mitchell, A. P. (1999). Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J. Bacteriol.* **181**, 1868-1874.
- Wilson, R. B., Davis, D., Enloe, B. M. and Mitchell, A. P. (2000). A recyclable *Candida albicans* *URA3* cassette for PCR product-directed gene disruptions. *Yeast* **16**, 65-70.
- Xiang, X. and Fischer, R. (2004). Nuclear migration and positioning in filamentous fungi. *Fungal Genet. Biol.* **41**, 411-419.
- Xiang, X., Beckwith, S. and Morris, N. (1994). Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* **91**, 2100-2104.
- Xiang, X., Roghi, C. and Morris, N. (1995). Characterization and localization of the cytoplasmic dynein heavy chain in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* **92**, 9890-9894.
- Yeh, E., Skibbens, R., Cheng, J., Salmon, E. and Bloom, K. (1995). Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* **130**, 687-700.