Cytoplasmic dynein’s mitotic-spindle-pole localization requires a functional
anaphase-promoting complex, γ-tubulin and NUDF/LIS1 in *Aspergillus nidulans*

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Running title: Dynein’s mitotic-spindle-pole targeting

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Abstract:

In *Aspergillus nidulans*, cytoplasmic dynein and NUDF/LIS1 are found at the spindle poles during mitosis, but they appear to be targeted to this location via different mechanisms. The spindle-pole localization of cytoplasmic dynein requires the function of the anaphase-promoting complex (APC), while that of NUDF does not. Moreover, while NUDF’s localization to the spindle poles does not require a fully functional dynein motor, the function of NUDF is important for cytoplasmic dynein's targeting to the spindle poles. Interestingly, a γ-tubulin mutation, *mipAR63*, nearly eliminates the localization of cytoplasmic dynein to the spindle poles, but has no apparent effect on NUDF’s spindle-pole localization. Live cell analysis of the *mipAR63* mutant revealed a defect in chromosome separation accompanied by unscheduled spindle elongation before the completion of anaphase A, suggesting that γ-tubulin may recruit regulatory proteins to the spindle poles for mitotic progression. In *A. nidulans*, dynein is not apparently required for mitotic progression. In the presence of a low amount of benomyl, a microtubule depolymerizing agent, however, a dynein mutant diploid strain exhibits a more pronounced chromosome-loss phenotype than the control, indicating that cytoplasmic dynein plays a role in chromosome segregation.
Introduction:

The minus-end-directed microtubule motor cytoplasmic dynein has been implicated in a variety of cellular functions including mitosis, transport of vesicles and distribution of nuclei and other organelles (Karki and Holzbaur, 1999). Cytoplasmic dynein is a multisubunit complex with heavy chains, intermediate chains, light intermediate chains and light chains (King, 2000; Tynan et al., 2000). The heavy chains contain the motor activity, while the other components have been implicated in targeting the motor to various cargoes. The functions of cytoplasmic dynein require dynactin, another multisubunit complex that may link the motor with membranous cargos, and, in addition, enhance the processivity of the dynein motor on microtubules (Holleran et al., 1998; King and Schroer 2000; Schroer 2004). Besides the components of the cytoplasmic dynein and dynactin complexes, additional regulators of cytoplasmic dynein have been discovered. One of them is LIS1, the product of the causal gene for type I lissencephaly, a human disease characterized by brain malformation due to a defect in neuronal migration (Gupta et al., 2002; Reiner 2000). Studies in fungi and in higher eukaryotic systems have indicated that LIS1 functions in the cytoplasmic dynein pathway (Morris 2000; Vallee et al., 2001; Gupta et al., 2002; Xiang et al., 1995a; Geiser et al., 1997; Cockell et al., 2004; Rehberg et al., 2005). LIS1 physically interacts with the heavy- and intermediate chains of cytoplasmic dynein, and the dynamitin subunit of the dynactin complex (Faulkner et al., 2000; Niethammer et al., 2000; Sasaki et al., 2000). Two regions of the dynein heavy chain: the first AAA repeat and an N-terminal site implicated in cargo binding, directly interact with LIS1 (Tai et al., 2002). The mechanism by which LIS1 regulates dynein remains unclear.
Consistent with the multiple roles of cytoplasmic dynein in the cell, cytoplasmic dynein, dynactin and LIS1 are found at various cellular sites. Cytoplasmic dynein and dynactin have been found on organelle membranes (Roghi and Allan, 1999; Habermann et al., 2001), consistent with their roles in organizing and transporting organelles. Cytoplasmic dynein, dynactin and LIS1 have also been localized to microtubule plus ends in both lower and higher eukaryotic cells (Vaughan et al., 1999; 2002; Han et al., 2001; Coquelle et al., 2002; Lee et al., 2003; Sheeman et al., 2003; Zhang et al., 2002, 2003; Carvalho et al., 2003). The microtubule plus ends may represent dynein’s cargo-loading sites (Vaughan et al., 2002). In addition, plus-end dynein may be involved in regulating microtubule dynamics and/or microtubule-cortex interaction (Carminati and Stearns, 1997; Shaw et al., 1997; Han et al., 2001); Plus-end dynein may also be delivered to the cortex where dynein may play a role as a minus-end-directed motor to walk along astral microtubules for spindle positioning during mitosis (Lee, et al., 2003; 2005; Sheeman et al., 2003; Adames and Cooper 2000; Heil-Chapdelaine 2000). Dynein at the cell cortex or adhesion junctions may be required for pulling or tethering microtubules for centrosome positioning during interphase and/or mediating microtubule-actin interactions (Ligon et al., 2001; Dujardin and Vallee, 2002; Dujardin et al., 2003; Koonce et al., 1999). Cytoplasmic dynein is also located at the kinetochore where it may interact with and transport checkpoint proteins away from the kinetochore, thereby allowing spindle checkpoint inactivation and entry into anaphase (Pfarr et al., 1990; Steuer et al., 1990; Echeverri et al., 1996; Wojcik et al., 2001; Howell et al., 2001).

Cytoplasmic dynein and dynactin are also found at centrosomes and at mitotic spindle poles in higher eukaryotic cells, where they may focus microtubule minus ends at spindle
poles during mitosis and organize cytoplasmic microtubules during interphase (Merdes et al., 1996; Heald et al., 1997; Compton, 2000; Heald, 2000; Quintyne et al., 1999; Quintyne and Schroer, 2002). In addition, cytoplasmic dynein anchored at the nuclear membrane may walk toward the centrosome, and this has been implicated in nuclear membrane breakdown during mitosis (Salina et al., 2002), separation of the daughter centrosomes (Gonczy et al., 1999), and coupling of the centrosome to the nucleus (Robinson et al., 1999; Malone et al., 2003). The perinuclear and centrosomal localizations of cytoplasmic dynein and LIS1 have also been implicated in centrosome-nuclear coupling during neuronal cell migration (Tanaka et al., 2004; Shu et al., 2004; Aumais et al., 2001). Although the multiple functions of dynein require it to be targeted to different subcellular locations, exactly how dynein is targeted to these sites remains to be investigated.

In fungi, the centrosome-equivalent organelle is the spindle pole body (SPB), which is embedded in the nuclear membrane. With its bound γ-tubulin, the SPB serves as a microtubule-organizing center for both the spindle microtubules and cytoplasmic microtubules (Oakley et al., 1990; Job et al., 2003). In the budding yeast S. cerevisiae and the fission yeast S. pombe, cytoplasmic dynein has been found at the SPB during both interphase and mitosis (Yeh et al., 1995; Sheeman et al., 2003; Miki et al., 2002; Yamamoto et al., 1999). In the filamentous fungus Aspergillus nidulans, cytoplasmic dynein has not been detected at the SPB in interphase cells. In this study, we have investigated the localization of cytoplasmic dynein and NUDF (A. nidulans LIS1) during mitosis and have found that they both localize to the mitotic spindle poles. They localize to the spindle poles by different mechanisms, however. While NUDF is located at the
poles of spindles of various lengths, dynein only appears at the poles of longer spindles, and this localization of dynein is dependent upon the function of the anaphase-promoting complex (APC). In addition, while NUDF’s spindle-pole localization did not require the full function of dynein, the spindle-pole localization of dynein is positively regulated by NUDF. Transient benomyl treatment of mitotic cells that destroyed both astral and spindle microtubules did not eliminate the localization of dynein and NUDF at the spindle poles. Thus, dynein and NUDF are likely to bind to the SPB rather than just accumulate at the minus ends of microtubules. Importantly, a previously reported $\gamma$ tubulin mutation, $mipAR63$, nearly eliminated dynein’s localization to the spindle poles. By contrast, the same mutation did not have an obvious effect on NUDF’s spindle-pole localization. The $mipAR63$ mutant exhibits abnormally elongated spindles accompanied by an obvious inhibition in anaphase A. We suggest that a fully functional $\gamma$-tubulin may be required for the association of regulatory proteins to the spindle poles to facilitate anaphase A progression.

Results:

1. Cytoplasmic dynein localizes to the mitotic spindle poles in A. nidulans and this localization depends on the function of the anaphase-promoting complex (APC).

In this study, we have generally used the yellow fluorescence protein (YFP)-tagged NUDI (cytoplasmic dynein intermediate chain) to report the location of the dynein complex. In a previous study, we have shown that the functional GFP-NUDI and GFP-NUDA (cytoplasmic dynein heavy chain) fusions both localize to the plus ends of cytoplasmic microtubules, and they depend on each other for this localization (Zhang et
In addition, in the nudA2 temperature sensitive (ts) mutant grown at the restrictive temperature of 42°C for 2 days, the GFP-NUDI protein level was drastically reduced and the GFP-NUDI signal was barely detectable on Western blots. Thus, the stability of GFP-NUDI depends on the presence of the normal NUDA heavy chain (Zhang et al., 2002). Based on these results and the fact that both YFP-NUDI and GFP-NUDI are functional, we conclude that the vast majority of the YFP-NUDI molecules should be associated with the dynein complex, and that YFP-NUDI’s localization reflects the localization of the dynein complex.

By observing living cells containing cyan fluorescence protein (CFP)-labeled microtubules and YFP-NUDI, we detected cytoplasmic dynein signals at the two poles of mitotic spindles. However, this pole localization was more obvious on longer spindles. To correlate cytoplasmic dynein’s spindle-pole localization with spindle lengths, randomly chosen spindles of various lengths were analyzed in an asynchronous cell population (Figure 1). The spindle-pole signals of YFP-NUDI were either absent or barely detectable with spindles shorter than 1.5 μm. However, YFP-NUDI was easily observed at poles of spindles that were longer than 3 μm, suggesting that more dynein accumulates at spindle poles during spindle elongation. A similar pattern of localization was also detected using a strain containing GFP-NUDA (cytoplasmic dynein heavy chain) and CFP-TUBA (Figure 1), confirming that this mode of localization faithfully reflects the localization of the dynein complex during mitosis.

We next tried to correlate spindle length with cell cycle stage by measuring spindles in a ts mutant of bimE (APC1), bimE7 (Osmani et al., 1988; Engle et al., 1990; Peters 2002; Osmani and Mirabito 2004). Studies in several other systems indicate that...
the APC, a multi-subunit E3 ubiquitin ligase, is required for anaphase entry by activating a protease called separase, which is required for the proteolytic cleavage of the cohesin complex that holds sister chromatids together after they replicate. The activation of separase involves two mechanisms (Peters, 2002 and references therein): (1) the degradation of securin, which binds and inhibits separase; (2) the partial degradation of cyclin B to reduce the activity of cdk1 that negatively regulates separase via phosphorylation. For chromosomal segregation during anaphase, the APC-mediated degradation of the kinesin-like protein Xkid is also important. At the end of mitosis, the APC is required for a complete degradation of cyclin B and other mitotic regulators to effect mitotic exit (Peters, 2002; Castro et al., 2005 and references therein).

Cells carrying the bimE7 mutation and the CFP-tubA fusion were cultured overnight at the permissive temperature of 32°C and then shifted to the restrictive temperature of 42°C for 2 or 6 hours before microscopic observation at 42°C. Under these conditions, a significant fraction of cells were blocked in mitosis as judged by the presence of CFP-labeled spindles. However, the spindles in the cell population were not uniform in length. To observe the bimE7-block directly, we crossed the bimE7 mutation into a strain carrying both CFP-histone-H2A and GFP-tubA (Su et al., 2004), and observed nuclei and spindles simultaneously in live cells. Typically, we found that most cells were blocked with spindles shorter than 4 μm at the restrictive temperature. Within this population of cells, shorter spindles were often surrounded by a chromatin mass, while relatively longer spindles had chromosomes dotted along them (Figure 2, E-H). Since a metaphase plate is not seen in A. nidulans it is not clear whether these configurations represent a problematic anaphase or a blockage of the metaphase-to-
anaphase transition. Based on the known function of the APC in anaphase entry, however, we think that the latter possibility is more likely. The cell-to-cell variability in spindle length at this block point may contribute to how easily we can observe chromosomes spread along the spindle. Occasionally, we saw cells with segregated chromosomes but containing very long spindles that failed to dissemble (Figure 2, I-K). These cells were most likely blocked at a stage before mitotic exit due to incomplete degradation of cyclin B and other mitotic regulators.

Although we could not precisely correlate the spindle lengths with the different stages of mitosis, the dependency of dynein’s spindle-pole localization on spindle length suggests a mitotic regulation of this localization. To test whether dynein’s localization to the spindle poles requires the function of the APC, we compared YFP-NUDI’s spindle-pole localization in wild type cells with that in the \( \textit{bimE7} \) mutant (Figure 3). The cells were incubated at the permissive temperature overnight and then shifted to the restrictive temperature for 6 hours. The profile of dynein’s spindle-pole localization in wild type cells was similar to that measured at the permissive temperature (data not shown). We examined about sixty spindles in the \( \textit{bimE7} \) mutant, and found that almost none of the spindles in the \( \textit{bimE7} \) mutant showed spindle-pole signals (Figure 3). Some mutant spindles showed dynein signals along the spindle, but the signals were not focused at the spindle pole as that in wild type cells. These results indicate that dynein localization to the spindle poles is abolished in the \( \textit{bimE7} \) mutant at the restrictive temperature. Importantly, not only the shorter spindles, but also the spindles that were longer than 3 \( \mu \text{m} \) (n=29) failed to show focused spindle-pole dynein signal while in wild type cells, more than 90% of the spindles longer than 3 \( \mu \text{m} \) showed dynein’s spindle-pole
localization. Thus, the function of the APC is required for targeting dynein to the spindle poles and/or for maintaining dynein at the poles during mitotic progression.

2. NUDF/LIS1 positively regulates cytoplasmic dynein’s spindle-pole localization.

LIS1, a protein required for neuronal migration during brain development, has been shown to be involved in dynein function in a variety of organisms (Morris 2000; Vallee et al., 2001; Gupta et al., 2002). Exactly how LIS1 affects dynein’s function is an important question being pursued in different experimental systems. In the budding yeast *S. cerevisiae*, loss of the Pac1/LIS1 protein abolishes the microtubule-plus-end localization of dynein (Lee et al., 2003; Sheeman et al., 2003). However, in *A. nidulans*, the comet-like structures that represent the microtubule-plus-end dynein are more prominent in the absence of NUDF/LIS1 (Zhang et al., 2003). Similarly, prominent dynein comets are observed in cells without the NUDF/LIS1 binding protein NUDE or its *Neurospora* homolog RO11 (Efimov, 2003; Minke et al., 1999). Therefore, in filamentous fungi, LIS1 may affect other aspects of dynein’s activity rather than its localization to the microtubule plus ends.

Since the spindle pole is the site associated with microtubule minus ends, we were interested in determining how loss-of-function of NUDF/LIS1 could affect cytoplasmic dynein’s spindle-pole association. However, these analyses are hard to perform in the *nudF* deletion mutant because of the clustering of spindles at the spore end, which precludes a clear view of the individual spindle poles. Moreover, the deletion mutant is severely defective in producing asexual spores at any temperature, thus, it is impossible for us to inoculate enough starting materials for the microscopic analysis that requires a
large number of cells (mitotic cells only represent about 4% of total cells under normal conditions). To circumvent this problem, we introduced the CFP-TUBA and YFP-NUDI fusions into the nudF7 mutant, which contains a temperature sensitive, loss-of-function mutation of nudF (Xiang et al., 1995a). The spores were harvested at the permissive temperature (32°C), and the cells were incubated at 32°C overnight to allow nuclei to move into the germ tube. We then shifted the cells to a restrictive temperature (42°C) for 8 hours and observed many randomly chosen spindles. Compared to a wild type control, the spindle-pole dynein signal intensity was much lower in the nudF mutant, and far fewer cells with elongated spindles showed detectable spindle-pole dynein signals (Figure 4). We also looked at GFP-NUDA/CFP-TUBA in the nudF7 background, and obtained the same conclusion (data not shown). These results suggest that functional NUDF/LIS1 is important for the localization of dynein to spindle poles.

3. NUDF/LIS1’s localization to the spindle poles does not require the full function of the APC and cytoplasmic dynein.

To determine whether NUDF/LIS1 in A. nidulans is also localized to the mitotic spindle poles, we observed the YFP-NUDF fusion in a strain that contains CFP-labeled microtubules. The YFP-NUDF fusion is functional like the previously described GFP-NUDF fusion (Han et al., 2001). Similar to cytoplasmic dynein, NUDF was found at the mitotic spindle poles (Figure 5). However, NUDF’s spindle-pole signal was detected on spindles of various lengths (Figure 5). Furthermore, NUDF’s spindle-pole localization was readily observed in the bimE7 mutant cells at the restrictive temperature (Figure 5),
indicating that unlike dynein, NUDF localization to the spindle poles is not dependent on APC.

Although NUDF’s spindle-pole signal appears earlier than that of dynein, it is still possible that NUDF may use the dynein motor for its initial targeting to the spindle pole and then becomes associated while dynein does not. To determine whether NUDF’s targeting to the spindle pole is dynein-dependent, we introduced the YFP-NUDF and CFP-TUBA fusions into the nudA mutant, which is a temperature sensitive dynein heavy chain loss-of-function mutant (Xiang et al., 1995b). We allowed the cells to grow at the permissive temperature (32°C) overnight to let the nuclei move into the germ tube. We then shifted the culture to the restrictive temperature (42°C) for 8 hours and observed the cells at 42°C. In a previous study, we have found that shifting the nudA mutant cells to 42°C for only 6 hours is sufficient to cause an obvious defect in microtubule dynamics (Han et al., 2001). In this study, we found that after shifting the nudA mutant cells to 42°C for 7 or 8 hours, the YFP-NUDI intermediate chain can no longer be observed at the spindle poles (data not shown), further indicating that dynein is not fully functional at these time points. After 8 hours at the restrictive temperature, YFP-NUDF was easily observed at the spindle poles in the nudA mutant (Figure 6). This result suggests that NUDF localization to the spindle does not require a fully functional dynein motor.

4. The association of dynein and NUDF with the spindle poles is resistant to transient microtubule depolymerization.

Since cytoplasmic dynein is a minus-end-directed motor, and the spindle poles are where the minus ends of microtubules locate in the cell, it is possible, in principle, that
the spindle-pole localization of dynein may simply be due to dynein moving along microtubules and accumulating at the minus ends. In addition, the appearance of dynein at the spindle poles may reflect dynein’s localization to the plus ends of very short astral microtubules. To rule out these possibilities, we treated the cells with benomyl (2.4 μg/ml), a microtubule-depolymerizing drug, to quickly depolymerize microtubules. We found that treatment with benomyl completely depolymerized microtubules as judged by the disappearance of all CFP-labeled microtubules. There were no CFP spots left near the spindle poles, indicating that during mitosis in *A. nidulans*, microtubules near the spindle pole body (SPB) are not well protected by the nuclear membrane from being depolymerized by the drug. Interestingly, after the disappearance of both cytoplasmic and spindle microtubules, dynein remained at the same positions (Figure 7), suggesting that dynein is associated with the SPB. The same treatment did not abolish NUDF’s spindle-pole localization either, suggesting that NUDF is also associated directly with the SPB during mitosis (Figure 8).

5. The spindle-pole association of dynein but not NUDF is abolished in the *mipA R63* γ-tubulin mutant.

Whether dynein and NUDF/LIS1 interact directly with a SPB component is not clear. In mammalian cells, a LIS1-binding protein, mNUDE, is located at the centrosome and interacts with several centrosomal components including γ-tubulin (Feng *et al*., 2000). In *A. nidulans*, γ-tubulin is located at the SPB during all cell cycle stages and plays multiple roles in mitotic progression (Oakley *et al*., 1990; Jung *et al*., 2001; Prigozhina *et al*., 2001; 2004). A previous study in tissue culture cells and *Xenopus*...
extracts suggested that γ-tubulin may be transported to the centrosome by dynein (Young et al., 2000). In this study, we constructed a strain in which γ-tubulin is tagged at the N-terminus with YFP, and we found that the YFP-γ-tubulin fusion localized to the spindle poles as expected. This localization was not affected by the nudF7 and nudA2 mutations at the restrictive temperature (Figure 9), suggesting that dynein’s function is not essential for γ-tubulin’s targeting to the SPB in A. nidulans. We next asked if γ-tubulin recruits dynein or NUDF/LIS1 to the mitotic spindle poles. Towards this goal, we crossed a γ-tubulin mutant, mipAR63, with the strains expressing YFP-NUDI/CFP-TUBA and YFP-NUDF/CFP-TUBA, and obtained the progeny that carried the γ-tubulin mutation and the YFP-NUDI (or NUDF)/CFP-TUBA fusions. The rationale for using this specific γ-tubulin mutant is that this allele exhibits a partial defect in nuclear positioning in the hyphae (Jung et al., 2001), which suggests a possible link with dynein since all dynein loss-of-function mutants exhibit a severe nuclear distribution defect in A. nidulans (Xiang et al., 1995b). Since the colony size of this γ-tubulin mutant is significantly reduced at room temperature (about 25°C), we incubated the cells at 32°C overnight and then shifted them to room temperature for 3 hours before observation. A previous study using fixed cells has suggested that this γ-tubulin mutant exhibited long mitotic spindles (Jung et al., 2001). Here we measured spindle length in live cells containing YFP-NUDI/CFP-TUBA and the mipAR63 mutation, and found that over 60% of the total mitotic spindles were longer than 3 μm (n=57), while in the wild-type background only 13.2% of the spindles were longer than 3 μm (n=53). In wild-type cells, spindle-pole signals of the YFP-NUDI dynein intermediate chain were easily observed on spindles longer than 3 μm. However, in the mipAR63 mutant, YFP-NUDI’s spindle-pole association was greatly diminished.
(Figure 10), suggesting that γ tubulin is important for dynein’s spindle-pole localization. Interestingly, YFP-NUDF was easily observed at the spindle poles in the same mutant background (Figure 10), indicating that NUDF’s spindle-pole localization does not require the full function of γ tubulin. In a few cells, GFP-NUDF fluorescent dots were seen to move along the spindle towards or away from one of the poles (data not shown), but the significance of this observation is not clear at this moment.

6. The mipAR63 mutant exhibits a severe inhibition of anaphase A.

Mitotic and microtubule defects associated with the mipAR63 mutant have been noticed in a previous study (Jung et al., 2001). Because the mipAR63 mutation affected dynein’s spindle-pole localization during mitosis, we examined in more detail the mitotic defect associated with this mutation in live cells. By observing CFP-labeled spindles in a strain that contains YFP-nudI and mipAR63, we found that many mipAR63 mutant cells exhibited long spindles (longer than 4 μm). These long spindles did not undergo further elongation or disassembly within 5 minutes while spindles in wild type cells underwent dramatic elongation and disassembly during the same time period (Table 2). Since in wild-type cells spindles longer than 4 μm were usually seen after initiation of anaphase B (Su et al., 2004), we initially speculated that the mipAR63 mutants might exhibit a mitotic exit delay. To further examine the mitotic defect in a strain in which both chromosomes and microtubules are labeled, we crossed the mipAR63 γ-tubulin mutation into a strain containing GFP-tubA/CFP-Histone-H2A and observed the behaviors of the nuclei and the microtubules simultaneously during mitosis (Figure 11). Interestingly, about 30% of the mipAR63 mutant cells (n=50) exhibited a severe defect in either the initiation or the
progression of anaphase A such that they failed to complete anaphase A within the 11 minutes time period of image acquisition (Figure 11, E-H, I-L). These cells were blocked with a mitotic configuration in which chromosomes were located near the middle portion or spread along the spindles. This configuration was also observed in the bimE7 APC1 mutant except that spindles in the mipAR63 γ-tubulin mutant were much longer (Figure 11, E-H, I-L). The rest of the cells did finish anaphase A during the 11 minutes of observation, but many of them took longer compared to wild-type cells. Some cells also took longer to disassemble spindles after anaphase A was completed (Figure 11, M-P), and a few cells exhibited abnormally curved spindles (Figure 11, Q-T). Taken together, our results suggest that the mipAR63 γ-tubulin mutation allows spindle elongation prior to chromosomal disjunction and significantly impedes anaphase A chromosomal separation.

7. *A. nidulans* dynein plays a non-essential role in chromosome segregation.

Our previous studies have suggested that *A. nidulans* dynein is not essential for nuclear division (Xiang *et al.*, 1995b). To determine if dynein plays a subtle role in chromosome segregation, we have carried out a chromosome loss assay. In *A. nidulans*, diploid nuclei are formed at a low frequency during vegetative growth, and a pure diploid strain can be maintained. The growth of a diploid colony is more sensitive to benomyl than that of a haploid colony (data not shown), suggesting that chromosome segregation in diploids is more sensitive to microtubule-depolymerizing drugs than in haploid cells. This is consistent with a higher demand for effective kinetochore-microtubule interactions with increased ploidy (Lin *et al.*, 2001). If a diploid loses one chromosome, the resulting aneuploid often rapidly loses additional chromosomes to become a haploid.
Benomyl at a concentration of 0.7 μg/ml can induce chromosome loss, leading to the formation of haploid sectors in a diploid colony. In *A. nidulans*, this so-called “parasexual cycle” of haploid formation from a diploid has been routinely used for genetic mapping of genes to specifically marked chromosomes (Clutterbuck 1992). To detect the formation of haploid sectors directly, we routinely use haploid strains with different color markers to derive a green diploid, then look for the appearance of haploid sectors with different colors such as yellow, chartreuse, white or yellow chartreuse as a function of chromosome loss. To determine if the dynein mutant diploid has an increased frequency of chromosome loss, we first constructed a *nudA1/nudA5* diploid (*nudA1* and *nudA5* are both loss-of-function ts alleles of the dynein heavy chain gene as previously described in Xiang *et al.*., 1995b). This mutant diploid exhibits a nud phenotype at a restrictive temperature of 42°C. We also constructed a *nudA+/nudA5* (or +/-*nudA5*) heterozygous diploid and a wild type diploid as controls (see table 1). The heterozygous diploid behaves similarly to the wild type diploid strain in growth and in chromosome loss assays (data not shown). Thus, we focused on the difference between the *nudA1/nudA5* and the +/-*nudA5* diploid (control diploid) strains that contain exactly the same nutritional and color markers. We first incubated the strains at the permissive temperature (32°C) for 1 day, and then shifted the plates to the restrictive temperature (42°C) for 1 day to inhibit dynein function. Since a nud mutant is defective in conidia (asexual spore) formation, we shifted the plates back to the permissive temperature (32°C) for 2 days to see the colors of the haploid sectors. In the absence of benomyl, neither the mutant diploid nor the control diploid produced enough sectors to allow a quantitative analysis. Thus, dynein loss-of-function does not
appear to cause significant chromosome loss during mitosis under normal conditions. However, in the presence of a low amount of benomyl (0.4 μg/ml), the mutant diploid produced significantly more haploid sectors than the control (Figure 13, p<0.006). This result suggests that although *A. nidulans* dynein is not essential for mitosis, it does play a role in chromosome segregation in diploids.

**Discussion:**

1. **NUDF is important for cytoplasmic dynein’s spindle-pole targeting.**

   In this study, we have found that in the filamentous fungus *A. nidulans*, cytoplasmic dynein and its regulator NUDF/LIS1 both localize to the poles of mitotic spindles. This localization is in addition to their microtubule-plus-end localization described in previous studies (Han *et al.*, 2001; Zhang *et al.*, 2002, 2003). Surprisingly, while NUDF’s spindle-pole localization is obvious on spindles of various lengths and is APC-independent, dynein’s spindle-pole localization is only obvious on longer spindles and is APC-dependent. Furthermore, while a γ-tubulin defect severely impairs dynein’s spindle-pole localization, it does not apparently affect NUDF’s localization to the same site. These results suggest that NUDF’s localization to the spindle poles is mechanistically different from dynein’s spindle-pole localization.

   LIS1 is thought to be involved in spindle assembly and mitotic progression in higher eukaryotic cells (Faulkner *et al.*, 2000; Feng *et al.*, 2004; Cockell *et al.*, 2004). We have shown here that NUDF is important for dynein’s spindle-pole localization during mitosis in *A. nidulans*. One explanation for this result is that NUDF may provide a binding site for dynein at the spindle poles. Alternatively, NUDF at the plus end of a
microtubule or along the microtubule may act as a dynein activator to facilitate its movement toward the spindle poles. Experiments in mammalian cells have also suggested a role of LIS1 as a positive regulator of dynein motor activity (Smith et al., 2000; Sasaki et al., 2000; Tai et al., 2002). In addition, it has been found that expression of a Nudel (a LIS1 binding protein) mutant protein impaired in LIS1 binding causes a defect in dynein’s movement to the spindle poles (Yan et al., 2003). Interestingly, our current study suggests that NUDF is targeted to the spindle poles before APC activation while dynein’s localization at the spindle poles occurs upon or after APC activation. How NUDF is targeted to the spindle poles and how it affects dynein’s localization to the same sites will require further study.

2. The involvement of the APC and γ-tubulin in dynein’s spindle-pole localization

The bimE7 mutation in APC1, and the mipAR63 mutation in γ-tubulin, both affect dynein’s spindle-pole localization, but the mechanisms of the effect are not clear. In A. nidulans, γ-tubulin and a protein in the APC complex, BIMA (a homolog of CDC27/APC3) are both localized to the SPB (Oakley et al., 1990; Mirabito and Morris, 1993). The APC is a multisubunit ubiquitin ligase E3 that is used for the degradation of securin and later of cyclin B and other regulators, thereby initiating the metaphase-to-anaphase transition and later mitotic exit (Harper et al., 2002; Peters 2002). The temporal-spatial regulation of APC during mitosis is still not clear, although SPB-specific degradation of cyclin B during the end of metaphase has been reported (Clute and Pines, 1999; Huang and Raff, 1999). Compared to the APC, the function of γ-tubulin during mitosis is much less well defined. The γ-tubulin complex is involved in nucleating
microtubule assembly and in minus-end capping (Gunawardane et al., 2000; Wiese and Zheng, 2000; Patel and Stearns, 2002; Job et al., 2003). Previous studies have shown that in addition to nucleating microtubule assembly, γ-tubulin is important for spindle pole separation during spindle assembly and for coordinating mitotic events in A. nidulans and the fission yeast S. pombe (Paluh et al., 2000; Prigiozhina et al., 2001; 2004; Vardy et al., 2002). In the A. nidulans mipAR63 mutant, long spindles are found before anaphase A is completed, suggesting either a premature spindle elongation or a delayed chromosomal disjunction. Besides the abnormally elongated spindles, about 30% of the cells carrying the mipAR63 γ-tubulin mutation fail to achieve anaphase A chromosome separation. This may be due to a defect of this mutant in recruiting regulatory molecules to the SPB. Alternatively, the mipAR63 γ-tubulin mutation may stabilize the plus ends of kinetochore microtubules, thereby affecting anaphase A chromosome segregation. In S. pombe, defects in the γ-tubulin complex cause the plus ends of both spindle and cytoplasmic microtubules to become more stable (Zimmerman and Chang, 2005), and a defective anaphase A has also been observed (Paluh et al., 2000). The fact that the mipAR63 mutant contains long and stable spindles is consistent with an alteration of spindle microtubule dynamics at the plus ends. How γ-tubulin mutation(s) may affect plus-end microtubule dynamics is an intriguing question that deserves to be explored further.

It is possible that the bimE7 and mipAR63 mutations affect dynein’s spindle-pole localization because they block the cell cycle at specific point(s). In addition, astral microtubules are not easily visible in the mipAR63 mutant cells (data not shown), or in the bimE7 mutant cells (data not shown, Osmani et al., 2003). The deficiency in astral microtubules may contribute to the lack of dynein at the spindle poles. However, our
observations suggest that dynein’s spindle-pole localization does not depend upon astral microtubules. In cells treated with a low amount of benomyl, astral microtubules were not visible, yet dynein’s localization at the poles was clearly visible on slowly elongating spindles that most likely had passed the metaphase-to-anaphase transition (data not shown). It is not clear whether the spindle-pole dynein comes from the kinetochore via kinetochore microtubules, from the nucleoplasm, or from the cytoplasm. In *A. nidulans*, the nuclear pore complex is partially disassembled during “closed” mitosis (De Souza et al., 2004), which may allow a big complex such as dynein to move in and out of the nucleus.

3. Possible functions of cytoplasmic dynein at the spindle poles

Cytoplasmic dynein is known to organize the spindle poles in higher eukaryotic cells (Merdes *et al.*, 1996; Heald *et al.*, 1997; Compton, 2000; Heald, 2000), however, its function at the spindle poles is not clear in most fungi where the SPB is embedded in the nuclear membrane. In another filamentous fungus, *Nectria haematococca*, cytoplasmic dynein is required for mitotic aster formation (Inoue *et al.*, 1998). However, this does not seem to be the case in *A. nidulans* since astral microtubules can be observed in dynein mutants or mutants in the dynein pathway (data not shown, and Osmani *et al.*, 1990).

In higher eukaryotic cells, cytoplasmic dynein has been implicated in transporting proteins to the spindle poles during mitosis. For example, dynein may transport microtubule-depolymerizing molecules such as a KinI kinesin to the spindle poles, which may be required for spindle microtubule flux during metaphase (Gaetz *et al.*, 2004). However, the spindle-pole-associated microtubule-minus-ends in fungi seem to be non-dynamic during anaphase and possibly also during metaphase (Maddox *et al.*, 2000;
Zimmerman and Chang, 2005; B. R. Oakley, unpublished data). In Drosophila and in cultured cells, dynein has been implicated in moving the spindle-assembly-checkpoint proteins from the kinetochore towards the spindle poles for inactivating the checkpoint, which is consistent with dynein’s role in the metaphase-to-anaphase transition in these cells (Wojcik et al., 2001; Howell et al., 2001; Goshima and Vale 2003; Yan et al., 2003). In *Tetrahymena thermophila*, gene disruption of the ubiquitous cytoplasmic dynen results in a failure of proper chromosome segregation during micronuclear mitosis (Lee et al., 1999). But it is not clear whether this defect is caused by a failure in the inactivation of the spindle-assembly checkpoint or in force generation during chromosome movement. In the budding yeast *S. cerevisiae*, cytoplasmic dynein plays a redundant role with the Cin8 and Kip1 kinesins in anaphase chromosome segregation (Saunders et al., 1995). However, the impact of dynein is most likely on spindle elongation during anaphase B when dynein-mediated interactions between astral-microtubules and the cell cortex may help to pull the spindle poles apart (Saunders et al., 1995). This function is more likely to be achieved by dynein at the plus ends of astral microtubule rather than by dynein at the SPB (Sheeman et al., 2003; Lee et al., 2003).

In *A. nidulans*, cytoplasmic dynein is not essential for mitotic progression (data not shown, and Xiang et al., 1995b), but our current study has suggested a possible role of dynein in chromosome segregation. Loss of dynein function does not cause an obvious chromosome-segregation defect under normal conditions, which is consistent with an early result from *S. cerevisiae* (Li et al., 1993). However, in the presence of a low amount of the microtubule drug benomyl, the dynein mutant diploid undergoes chromosome-loss-induced haploid formation with a higher frequency than that of the control diploid.
Chromosome loss may be caused by a defect in kinetochore-to-microtubule attachment during prometaphase, metaphase or anaphase, if such a defect is not caught by the spindle assembly checkpoint and corrected. Thus, dynein may play a role in kinetochore-microtubule interactions and this role becomes more critical if kinetochore microtubules are not in their normal dynamic state. In mammalian cells, cytoplasmic dynein locates to prometaphase kinetochores (Pfarr et al., 1990; Steuer et al., 1990; Echeverri et al., 1996). Defects in dynein function perturb chromosome alignment at the metaphase plate, and also cause problems in spindle assembly (Echeverri et al., 1996; Faulkner et al., 2000). In *A. nidulans*, mutations affecting the spindle-assembly checkpoint severely exacerbate the growth defects of the dynein mutants (Efimov and Morris, 1998), which is consistent with dynein’s role in spindle integrity. In fungi, both chromosomes and spindles are much smaller than the ones in higher eukaryotic cells and fungal dynein has not been shown to locate at the kinetochores. Similar to yeasts, *A. nidulans* kinetochores also seem to cluster in the vicinity of the SPB at the end of mitosis (Yang et al., 2004; Wigge and Kilmartin, 2001 and references therein). One possibility we can not exclude at this stage is that a low amount of dynein molecules are located at the kinetochore and remain bound till the end of mitosis. However, it is also possible that dynein may play a role at the spindle poles in maintaining spindle integrity, thereby influencing microtubule-kinetochore interactions required for anaphase A chromosome movement.

Cytoplasmic dynein may be functionally involved in the later stages of mitosis. In *S. cerevisiae*, absence of the dynein heavy chain significantly decreases the SPB signal intensity of a mitotic exit network (MEN) protein Tem1p (Molk et al., 2004). In higher eukaryotic cells, dynein regulators have been found at the midbody (Karki et al., 1998),
and are important for cytokinesis (Zhou et al., 2003; Aumais et al., 2003). In *A. nidulans*, dynein is important for specifying the position of the first septum because dynein mutants show a misplaced first septum (Liu and Morris, 2000; Liu et al., 2003). Whether septum position is related to the spindle-pole-associated dynein is an interesting question. But so far we have not observed any apparent abnormality in septum position in the *mipAR63* mutant where dynein’s spindle-pole association is nearly abolished (data not shown), suggesting that dynein at the spindle poles is unlikely to be critical for septation.

**Materials and Methods:**

**Construction of CFP-*tubA* and YFP-*nudI/YFP-nudF* strains**

Previously, we made various GFP constructs to observe microtubules, dynein and NUDF in *A. nidulans* (Xiang et al., 2000; Han et al., 2001; Zhang et al., 2002; 2003). Because of the difference in codon preference, the commercially available GFP variants did not express well in *A. nidulans*. For this reason, we mutated the GFP-*tubA* plasmid to change GFP to CFP (cyan fluorescent protein), and the GFP-*nudI/GFP-nudF* plasmids to change GFP to YFP (yellow fluorescent protein), using the Stratagene quick-change multi-sites mutagenesis kit. The change of GFP to CFP was made as described previously (Su, et al., 2004). The change of GFP to YFP required five amino acid substitutions: S65G, V68L, Q69K, S72A, and T203Y. Two oligonucleotides were used for mutagenesis: M4:

GTCACTACTTTCCGGTTATGGTCTCAAGTGCTTTGCCAGATACCCAGATC; M5:

CCATTACCTGCTCACCAATCTGCCCCTTTTC. The CFP-*tubA* plasmid was transformed into SO121 (*pyrG*89; *nicB*8). The YFP-*nudI* and YFP-*nudF* plasmid were
transformed into GR5 (pyrG89, pyroA4 and wA3). Phenotypic analysis was used to screen for strains in which YFP-nudI or YFP-nudF was integrated at its correct locus. Because the fusions were driven by the alcA promoter that can be shut off by glucose, a strain with a site-specific integration of the plasmid would produce a nud phenotype on a YUU plate (Han et al., 2001; Zhang et al., 2002). Southern blot analyses were used to confirm that the integration indeed occurred at the right site. Strains carrying CFP-tubA and YFP-nudF both contain single integrations at the correct sites, and were crossed to each other to produce a strain that carries both CFP-tubA and YFP-nudF (data not shown). The desired progeny were identified by their nud-like growth phenotype 2-3 days after being streaked on a YUU plate, and by their CFP-labeled microtubules under a fluorescence microscope. The original YFP-nudI strain carried the correct integration of the plasmid but also an extra integration, and this strain was crossed to the CFP-tubA strain. Two strains carrying both CFP-tubA and YFP-nudI were obtained (SL25 and SL26). While SL25 contains the correct integration plus the extra integration, SL26 contains only the correct integration. However, these two strains exhibited nearly identical behaviors in NUDI localization at the spindle pole, indicating that the extra integration does not interfere with this study. Since SL25 contains a nutritional marker that can be used for crosses, we crossed SL25 to various mutant strains for further analyses. We also crossed the previously published GFP-nudA strain (Xiang et al., 2000) to the CFP-tubA strain and analyzed GFP-NUDA’s spindle-pole localization.

Introducing CFP-TUBA/YFP-NUDI, CFP-TUBA/YFP-NUDF and CFP-H2A/GFP-TUBA fusions to various mutant backgrounds
To obtain CFP-TUBA/YFP-NUDI fusions in the nudF7 background, and the CFP-TUBA/YFP-NUDF fusions in the nudA2 background, standard genetic crosses were carried out. The desired progeny were identified based on the nud-like growth phenotype of the YFP-nudI or YFP-nudF carrying strain on glucose plates and the temperature sensitive nud phenotype of the nudF7 or the nudA2 mutant on glycerol plates, and the CFP-labeled microtubules under a fluorescence microscope. To obtain CFP-TUBA/YFP-NUDI and CFP-TUBA/YFP-NUDF fusions in the bimE7 background, genetic crosses were set up. The desired progeny were identified based on the nud-like growth phenotype of the YFP-nudI or YFP-nudF carrying strain on glucose plates, the temperature sensitive bimE7 mutant phenotype at 42°C, and the CFP-labeled microtubules under a fluorescence microscope. To obtain CFP-TUBA/YFP-NUDI and CFP-TUBA/YFP-NUDF fusions in the mipAR63 background, genetic crosses were set up. The desired progeny were identified based on the nud-like growth phenotype of the YFP-nudI or YFP-nudF carrying strain on glucose plates, the cold sensitive mipAR63 mutant phenotype at room temperature, and the CFP-labeled microtubules under a fluorescence microscope. To obtain CFP-H2A/GFP-TUBA fusions in the bimE7 or the mipAR63 background, the desired progeny were identified from genetic crosses based on the cold sensitive mipAR63 mutant phenotype at room temperature or the temperature sensitive bimE7 mutant phenotype at 42°C, the CFP-labeled nuclei and the GFP-labeled microtubules under a fluorescence microscope.

Construction of strains containing GFP-γ-tubulin or YFP-γ-tubulin
Two oligonucleotides, N-γ (5’-GGGCGGCCGCTGCCTAGGTATACCCTCC-3’) and C-γ (5’-GGGCGGCCGCAGAACAATGTATGGACAG-3’) (the two underlined regions represent the NotI sites), were used to amplify a 2.1 kb region of the γ-tubulin gene and the PCR product was digested with NotI followed by ligation to the NotI site of the LB01 plasmid that carries the alcA promoter and GFP (Liu and Morris, 2000). The resulting plasmid contains alcA-GFP fused with the γ-tubulin gene from the start codon (but ATG was changed to CTG to allow in-frame fusion with GFP) to the 3’ untranslated region. To make the YFP-γ-tubulin plasmid, we digested the YFP-nudF plasmid with NotI and the plasmid backbone was ligated to the 2.1 kb, NotI-digested γ-tubulin fragment. These plasmids were transformed to the A. nidulans strain GR5 separately to obtain strains containing GFP-γ-tubulin or YFP-γ-tubulin. The YFP-γ-tubulin fusion was introduced to the strain expressing CFP-TUBA by genetic crosses. The CFP-TUBA and YFP-γ-tubulin fusions were introduced to the nudA2 or the nudF7 background by genetic crosses. Progeny selection was based on the nud phenotype at 42°C and microscopic observation of the CFP and YFP fusions.

**Image acquisition and analyses**

Cells were grown in ΔTC3 culture dishes (Bioptechs) containing 1.5 ml medium. Images were captured using an Olympus IX70 inverted fluorescence microscope (with a 100X objective) linked to a PCO/Cooke corporation Sensicam QE cooled CCD camera. A Bioptechs heating stage and heated objective system was used for capturing images at 32°C or 42°C. Ludl Electronic Products dual individual excitation and emission motorized filter wheels were used for observing YFP and CFP signals in the same living
cell. Chroma 8600 filters for CFP (430 nm peak excitation with a bandwidth of 25 nm, 470 nm peak emission with a bandwidth of 30 nm) and for YFP (500 nm peak excitation with a bandwidth of 20 nm, 535 nm peak emission with a bandwidth of 30 nm) were used. Custom macros in the IPLab software were written by Jim Paladino of BioVision Technologies.
Acknowledgement:

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Figure legend:

Figure 1 YFP-NUDI (cytoplasmic dynein intermediate chain) accumulates at spindle poles during spindle elongation. YFP-NUDI’s spindle-pole signal is not present on a short spindle (A, B, C), but is present on a longer spindle (D, E, F). CFP-labeled microtubules were pseudo-colored red (A, D), YFP-labeled NUDI was pseudo-colored green (B, E), and merged to show both colors (C, F). The percentage of spindles with the spindle-pole YFP-NUDI (left) signal increases with spindle elongation (G, left). A similar profile was observed in a cell population with GFP-labeled NUDA (cytoplasmic dynein heavy chain) and CFP-labeled microtubules (G, right). Standard errors were calculated using three data collections, and in each collection we analyzed 60 or more spindles chosen randomly from cells grown at 32°C. Bar, 5 μm.

Figure 2 The mitotic phenotype of the bimE7 mutant. Cells were grown at the permissive temperature of 32°C overnight and shifted to the restrictive temperature of 42°C for 6 hours. Images were taken at 42°C. Four consecutive frames of a time lapse image set with a 60-second pause time between frames were shown for each cell (except for the last series in I-K). CFP-labeled chromosomes were pseudo-colored red, and GFP-labeled microtubules were pseudo-colored green. A-D, a wild type cell underwent mitotic progression. E-H, a bimE7 mutant blocked at the metaphase-to-anaphase transition. I-K, a bimE7 mutant cell blocked before mitotic exit. One daughter nucleus was located in a branch, and the spindle was bent.
Figure 3  The spindle-pole accumulation of YFP-NUDI (pseudo-colored green) is abolished in the *bimE7* mutant at the restrictive temperature (42°C) for 6 hours. Wild type (A) and *bimE7* mutant cells (B) grown under the same conditions are shown.

Figure 4  The spindle-pole accumulation of YFP-NUDI is significantly decreased in the *nudF7* mutant. Spindles of the *nudF7* mutant at the restrictive temperature (42°C) were compared with spindles of wild type cells grown under the same condition. YFP-NUDI’s spindle-pole signal is present on a long spindle of the wild type cell (A, B, C) but undetectable on a long spindle of the *nudF7* mutant (D, E, F). CFP-labeled microtubules were pseudo-colored red (A, D), YFP-labeled NUDI was pseudo-colored green (B, E), and merged to show both colors (C, F). For image analysis, 71 wild type spindles (left side of the two adjacent columns) and 63 mutant spindles (right side of the two adjacent columns) were chosen randomly from cells grown at 42°C with various spindle lengths (G).

Figure 5  YFP-NUDF is localized to the poles of mitotic spindles of various lengths and this localization is APC-independent. YFP-NUDF’s spindle-pole signal (pseudo-colored with green) is present on both short and long spindles (pseudo-colored with red) (A, B). The percentage of spindles with the spindle-pole YFP-NUDF signal does not change significantly with spindle elongation. Standard errors were calculated from two data collections, and in each collection we analyzed about 60 spindles chosen randomly from cells grown at 32°C (C). The spindle-pole accumulation of YFP-NUDF is not affected by
the \textit{bimE7} mutation (D, E). A wild type (D) and a \textit{bimE7} mutant cell (E) grown at the restrictive temperature (42°C) are shown.

Figure 6  The spindle-pole localization of NUDF is not affected in the \textit{nudA2} dynein heavy chain mutant. Spindles of the \textit{nudA2} mutant at the restrictive temperature (42°C) were compared with spindles of wild type cells grown under the same conditions. YFP-NUDF’s spindle-pole signal is present on a spindle of the wild type cell (A, B, C) and a spindle of the \textit{nudA2} mutant (D, E, F). CFP-labeled microtubules were pseudo-colored with red (A, D), YFP-labeled NUDI was pseudo-colored with green (B, E), and merged to show both colors (C, F). For image analysis, 83 wild type (left side of the two adjacent columns) and 68 mutant (right side of the two adjacent columns) spindles of various lengths were chosen randomly from cell grown at 42°C.

Figure 7  Live cell imaging demonstrating that the localization of cytoplasmic dynein to the mitotic spindle poles is resistant to transient microtubule depolymerization. CFP-labeled microtubules were pseudo-colored red (A, D, G), YFP-labeled cytoplasmic dynein intermediate chain (NUDI) was pseudo-colored green (B, E, H), and merged to show both colors (C, F, I). Cells were grown at 32°C for overnight and images were acquired at 32°C. Benomyl was added to the culture dish to a final concentration of 2.4 \( \mu \text{g/ml} \) and images were acquired after 15 minutes (D, E, F) and 25 minutes (G, H, I). Bar, 5 \( \mu \text{m} \).
Figure 8  Live cell imaging demonstrating that the localization of NUDF to the mitotic spindle poles is resistant to transient microtubule depolymerization. CFP-labeled microtubules were pseudo-colored red (A, D), YFP-labeled NUDF was pseudo-colored green (B, E), and merged to show both colors (C, F). Benomyl was added to the culture dish to a final concentration of 2.4 μg/ml and images were acquired after 25 minutes (D, E, F).

Figure 9  The localization of γ-tubulin to the SPB is not affected by the nudA2 or nudF7 mutation. YFP-labeled γ-tubulin was pseudo-colored green, and CFP-labeled spindles were pseudo-colored red. Wild type (A), nudA2 (B) and nudF7 (C) cells grown at the restrictive temperature (42°C) are shown.

Figure 10  The spindle-pole accumulation of YFP-NUDI but not YFP-NUDF is abolished in the mipAR63 mutant. Cells were grown at the permissive temperature (32°C) overnight and shifted to the restrictive temperature (room temperature) for 3 hours. Cells containing YFP-NUDI (pseudo-colored with green) in wild type (A) and the mipAR63 mutant background (B-D), and cells containing YFP-NUDF in wild type (E) and the mipAR63 mutant background (F) are shown.

Figure 11  The mipAR63 mutation causes defects in mitotic progression. Cells were grown at the permissive temperature 32°C overnight and shifted to room temperature for 3 hours. Four consecutive frames of a time lapse with a 60-second pause time between frames are shown for each cell. A-D, a wild type cell underwent mitotic progression. E-
H, a mipAR63 mutant containing a long spindle with chromosomes associated with the middle portion. I-L, a mipAR63 mutant containing a long spindle with chromosomes along it. M-P, a mipAR63 mutant cell with a completed anaphase A but a relatively more stable spindle compared to that in wild type cells. Q-T, a mipAR63 mutant cell that finished anaphase A with a bent spindle.

Figure 12  The dynein mutant diploid (nudA1/nudA5) forms more haploid sectors than the control diploid (+/nudA5) in the presence of 0.4 μg/ml benomyl. Upper: A picture showing 3 haploid sectors formed from the mutant diploid colony, and a control diploid colony with no sectors. KCl with a final concentration of 0.6M was present in the complete medium YUU to enhance spore formation of the nud mutants. Lower: Quantitative analyses of haploid sector formation. Standard errors were calculated using two data collections. 32 total colonies were analyzed.
References:


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Table 1 *A. nidulans* strains used in this work (All the strains have the veA1 marker)

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<td></td>
<td>`nudA1/nudA5, other markers from the parental</td>
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<tr>
<td></td>
<td>strains GG1 and XX10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterozygous diploid (DS2)</td>
<td></td>
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<tr>
<td></td>
<td>`+/nudA5, other markers from the parental</td>
<td></td>
</tr>
<tr>
<td></td>
<td>strains R21 and XX10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wild type diploid (DS1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Markers from the parental strains R21 and GR5</td>
<td></td>
</tr>
</tbody>
</table>

This work

45
Table 2 Spindle length and stability in the *mipAR63* mutant

<table>
<thead>
<tr>
<th></th>
<th>Spindles longer than 4μm before anaphase A is completed</th>
<th>Spindles not disassembled 5 minutes after their lengths reach 4 μm</th>
<th>Spindles not disassembled 5 minutes after anaphase A is completed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild type</strong></td>
<td>17.1% (n=35)</td>
<td>2.8% (n=36)</td>
<td>5.6% (n=36)</td>
</tr>
<tr>
<td><strong>mipAR63</strong></td>
<td>71.4% (n=49)</td>
<td>52.0% (n=50)</td>
<td>17.6% (n=34)</td>
</tr>
</tbody>
</table>

Legend:
For these analyses, cells were grown under the same conditions as described in Figure 11 legend.
Figure 2
CFP-H2A/GFP-TUBA

WT 0 s  60 s  120 s  180 s
A   B   C   D

bimE7 0 s  60 s  120 s  180 s
E   F   G   H

bimE7 0 s  60 s  120 s
I   J   K
Figure 3
CFP-TUBA/YFP-NUDI

WT

A

bimE7

B
Figure 5

YFP-NUDF/CFP-TUBA

(A, B) Images showing spindles.

(C) Bar chart showing percentage of spindles with spindle-pole NUDF for different spindle length categories.

(D, E) Images comparing WT and bimE7 conditions.
Figure 6

<table>
<thead>
<tr>
<th>CFP-TUBA</th>
<th>YFP-NUDF</th>
<th>Merge</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>nudA2</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

G

<table>
<thead>
<tr>
<th>Spindle length (µm)</th>
<th>Spindles with spindle-pole NUDF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.5</td>
<td>wild type: 90%</td>
</tr>
<tr>
<td>1.5-3.0</td>
<td>wild type: 80%</td>
</tr>
<tr>
<td>&gt;3.0</td>
<td>wild type: 100%</td>
</tr>
</tbody>
</table>
Figure 7

CFP-TUBA

YFP-NUDI

Merge

A  benomyl 0 min  B  C  

D  benomyl 15 min  E  F  

G  benomyl 25 min  H  I
Figure 8

CFP-TUBA  YFP-NUDF  Merge

benomyl 0 min

A  B  C

benomyl 25 min

D  E  F
Figure 9

YFP-γ-tubulin/CFP-TUBA

WT  nudA2  nudF7
Figure 10

YFP-NUDI/CFP-TUBA

A  WT  B  mipAR63  C  mipAR63  D  mipAR63

YFP-NUDF/CFP-TUBA

E  WT  F  mipAR63