The LIS1-related Protein NUDF of *Aspergillus nidulans* and Its Interaction Partner NUDE Bind Directly to Specific Subunits of Dynein and Dynactin and to α- and γ-Tubulin*

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The NUDF protein of *Aspergillus nidulans*, which is required for nuclear migration through the fungal mycelium, closely resembles the LIS1 protein required for migration of neurons to the cerebral cortex in humans. Genetic experiments suggested that NUDF influences nuclear migration by affecting cytoplasmic dynein. NUDF interacts with another protein, NUDE, which also affects nuclear migration in *A. nidulans*. Interactions among LIS1, NUDE, dynein, and γ-tubulin have been demonstrated in animal cells. In this paper we examine the interactions of the *A. nidulans* NUDF and NUDE proteins with components of dynein, dynactin, and with α- and γ-tubulin. We show that NUDF binds directly to α- and γ-tubulin and to the first P-loop of the cytoplasmic dynein heavy chain, whereas NUDE binds directly to α- and γ-tubulin, to NUDK (actin-related protein 1) and to the NUDG dynein LC8 light chain. The data suggest a direct role for NUDF in regulating dynein function and a role for NUDE in the regulation of cytoplasmic dynein and dynactin subunits via NUDE. The interactions of NUDF and NUDE with components of dynein and dynactin complexes are the main components of the nuclear distribution machinery (3–8). Three additional proteins, NUDC, NUDEF, and NUDE, whose functions are still not well understood, are also required for nuclear migration in *A. nidulans*. NUDC is a 22-kDa protein required to maintain the intracellular concentration of NUDF (9). NUDF (a homolog of yeast *Pac1*) is a homodimeric 49-kDa protein with seven WD40 repeats (10) and a short predicted N-terminal coiled-coil domain that participates in homodimer formation (11). *A. nidulans* NUDF colocalizes with the NUDA dynein heavy chain in comet-like structures at the plus ends of cytoplasmic microtubules *in vivo* (12, 13). The location of NUDA and NUDF at the ends of microtubules may have functional significance because the deletion of either protein increases microtubule stability (13). NUDE (RO11 in *Neurospora crassa*) is a 70-kDa protein that was initially shown to be required for nuclear migration in *N. crassa* (3). It was subsequently identified in *A. nidulans* as a high copy suppressor of a temperature-sensitive NUDE mutation (14). NUDE has a long, very basic N-terminal coiled-coil domain and a C-terminal domain rich in proline and glycine. In the yeast two-hybrid assay *in vitro*, NUDE via the coiled-coil domain similarly binds NUDE, like dynein and dynactin, to the plus ends of microtubules. The data indicate that NUDF and NUDE affect cytoplasmic dynein and dynactin function.

During growth of *Aspergillus* and other filamentous fungi nuclei migrate into the germ tube and distribute evenly along the cell length (1–3). Analysis of mutations that affect nuclear distribution in these fungi has shown that microtubules and the cytoplasmic dynein and dynactin complexes are the main components of the nuclear distribution machinery (3–8). Three additional proteins, NUDC, NUDEF, and NUDE, whose functions are still not well understood, are also required for nuclear migration in *A. nidulans*. NUDC is a 22-kDa protein required to maintain the intracellular concentration of NUDF (9). NUDF (a homolog of yeast *Pac1*) is a homodimeric 49-kDa protein with seven WD40 repeats (10) and a short predicted N-terminal coiled-coil domain that participates in homodimer formation (11). *A. nidulans* NUDF colocalizes with the NUDA dynein heavy chain in comet-like structures at the plus ends of cytoplasmic microtubules *in vivo* (12, 13). The location of NUDA and NUDF at the ends of microtubules may have functional significance because the deletion of either protein increases microtubule stability (13). NUDE (RO11 in *Neurospora crassa*) is a 70-kDa protein that was initially shown to be required for nuclear migration in *N. crassa* (3). It was subsequently identified in *A. nidulans* as a high copy suppressor of a temperature-sensitive NUDE mutation (14). NUDE has a long, very basic N-terminal coiled-coil domain and a C-terminal domain rich in proline and glycine. In the yeast two-hybrid assay *in vitro*, NUDE via the coiled-coil domain similarly binds NUDE, like dynein and dynactin, to the plus ends of microtubules. The data indicate that NUDF and NUDE affect cytoplasmic dynein and dynactin function.

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* The abbreviations used are: GFP, green fluorescent protein; 3-AT, 3-aminotriazole; VSV, vesicular stomatitis virus; PAGE, polyacrylamide gel electrophoresis; ARP1, actin-related protein 1.

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A. nidulans strains and expressed tagged protein versions

| Strain name/transformed strain | Construct (plasmids contain pyr4) | Genotype | Ref. |
|-------------------------------|-----------------------------------|----------|-----|
| GR5 | VSVG-NUDE | wA3; pyrG89; pyroA4; veA1 | 52 |
| XX21 × 6–17 | ANBHI | ya2 | 14 |
| ANBH1 | VSVG-NUDE | ya2; pyrG | This work |
| ANBH2/ANBH1 | FLAG-NUDG-FLAG | wA3; pyroA4; veA1; pyrG | This work |
| ANBH3/GR5 | Protein C-NUDG- protein C | wA3; pyroA4; veA1; pyrG89 | This work |
| ANBH4/GR5 | FLAG-NUDG-FLAG | ya2; pyrG | This work |
| ANBH5/ANBH1 | Protein C-NUDG- protein C | wA3; pyroA4; veA1; pyrG89 | This work |
| ANB17/ANBH1 | Protein C-NUDI | ya2; pyrG | This work |
| ANBH6/GR5 | FLAG-M1A-FLAG | wA3; pyroA4; veA1; pyrG89 | This work |
| ANBH6/GR5 | FLAG-TUBA | wA3; pyroA4; veA1; pyrG89 | This work |
| ANBH10/GR5 | FLAG-NUDE-FLAG | wA3; pyroA4; veA1; pyrG89 | This work |
| ANBH11/GR5 | FLAG-P-loopNUDA- FLAG | wA3; pyroA4; veA1; pyrG89 | This work |
| CA1[pAAFS] | NUDF-protein S | Wild-type | 11 |

and other centrosomal proteins in the yeast two-hybrid system and in communoprecipitation experiments (20, 22, 26, 28, 29, 31). The protein interaction experiments are informative; but because they were mainly done in unpurified systems, one cannot conclude that the observed interactions are direct, as they could be mediated by intervening proteins. In the present paper we have examined the interactions of the A. nidulans NUDF and NUDE proteins with each other as well as with component proteins of dynein and dynactin and with α- and γ-tubulin using yeast two-hybrid assays, communoprecipitations, and pull-down experiments. We have additionally studied the binding of purified proteins to each other to determine whether their interactions are direct or indirect. These results indicate that both NUDE and NUDF bind directly to α- and γ-tubulin, but they bind differently. NUDF binds directly to the dynein heavy chain, whereas the NUDE protein is associated with the light chain and the NUDG actin-related protein of dynactin. We also show that NUDE and NUDF can bind simultaneously to NUDE as a scaffold protein. The interactions between NUDE and NUDF and the γ-tubulin, also observed in mammalian cells (24), support the additional possibility that γ-tubulin may mediate these protein interactions.

EXPERIMENTAL PROCEDURES

A. nidulans Media—YG (5 g of yeast extract and 20 g of glucose/liter supplemented with 0.1% trace elements) was used as a complete liquid medium for A. nidulans strains (34). Minimal medium (6 g/liter NaNO₃, 0.52 g/liter KCl, 0.52 g/liter MgSO₄, 1.52 g/liter KH₂PO₄, 2% glucose, and 0.1% trace elements) was from Pontecorvo et al. (35). 2% agar was added to solidify media where appropriate. To support the growth of strains carrying pyrG mutant alleles, media were supplemented with 10 mM uridine and uracil.

Construction of Yeast Strains Carrying Gal4p Fusion Proteins—The full-length, shortened and mutated constructs of NUDE, NUDF, LIS1, and human NUDE for use in the yeast two-hybrid system were described previously (11, 14). Full-length open reading frames of A. nidulans nudK (dynactin actin-related protein), nudG (dynein light chain), nudf (dynein intermediate chain), tutA (α-tubulin), tutB (β-tubulin), benA (β-tubulin), minpA (γ-tubulin), N. crassa ro10 (putative dynactin-associated protein) (3), ro2 (putative dynactin subunit) (36), and ro3 (dynactin p150sub10) (37) were made by polymerase chain reaction. Some N. crassa dynactin subunits were used for two-hybrid analysis because N. crassa and A. nidulans are closely related ascomycetes, and the A. nidulans genes have not yet been cloned. The primers contained additional restriction sites for insertion into the yeast two-hybrid plasmids pGBKT7 and pGADT7 (Matchmaker 3 system, CLONTECH). The open reading frame of nudA (dynein heavy chain) was inserted into both plasmids as three fragments of approximately equal length. The first third of NUDA included amino acids 1–1675. The second part encoded amino acids 1676–3162, and the third contained the C-terminal coding sequence (amino acids 3163–4344).

An additional two-hybrid NUDA fragment was constructed spanning the first P-loop of the dynein heavy chain (amino acids 1676–2138) according to Sasaki et al. (20). Yeast Two-Hybrid Analysis—The yeast two-hybrid strain PJ69-4A was used containing three different reporter constructs driven by different Gal4p-dependent promoters, GAL1-HIS3, GAL2-AD2, and GAL7-lacZ (38). After plating in liquid medium for 7 days, colonies from different plates were grown on different amounts of 3-aminotriazole (3AT, 1 mg/liter) to assess the interaction between NUDE and NUDF. Cells were analyzed after a 3-day incubation.

Expression of Tagged Versions of Tubulins, NUDF and NUDE Interactions with Dynein/Dynactin and Tubulins

We also show that more than one dynein or dynactin subunit can bind simultaneously to NUDE, arguing for a possible functionality that may influence dynein function.

Expression of Tagged Proteins—For high expression of tagged proteins the strains were grown in a preinduction medium (0.5% yeast extract, 1% glycerol, 0.1% trace elements) for 12 h, harvested, washed twice with H₂O, and shifted to induction medium (0.25% yeast extract, 0.075% glycerol, 0.1% trace elements) containing 0.5% methyl ethyl ketone to induce alca promoter-driven protein expression. Strains were grown for an additional 16–20 h, harvested, and ground with a mortar and pestle in liquid nitrogen. Proteins were extracted by vortexing 5 g of mycelial powder in a 50-ml tube containing 20 ml of buffer that contained a protease inhibitor mixture for fungal extracts (Sigma). Buffers were those indicated for the isolation of the various tagged proteins according to the manufacturer’s manuals. After a 4°C centrifugation at 3,000 × g for 3 min to remove cell wall debris, supernatants...
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Fig. 1. NUDF and NUDE proteins interact with dynein/dynactin and microtubule subunits in the yeast two-hybrid system. S. cerevisiae strain PJ69-4A was transformed with pairwise combinations of plasmids expressing the indicated fusion proteins with either the Gal4p DNA binding (DBD) or Gal4p activating (AD) domains. NUDE-cc and human NUDE-cc (hNUDE-cc) express the N-terminal domain of NUDE proteins (residues 1–195). The NUDF-m construct contains seven isoleucine and leucine to alanine substitutions in the NUDF coiled-coil region. Protein names of the known mammalian homologues are shown in parentheses. RO proteins came from N. crassa. Growth was tested on four different media (from left to right at the bottom of the figure): SC/-Leu/-Trp/-His; SC/-Leu/-Trp/-His with 2 mM 3AT; SC/-Leu/-Trp-adenine. The first three media select for the expression of the LEU2 reporter gene, and the fourth medium selects for the expression of the 3AT reporter gene. Growth in the absence of histidine or adenine indicates a strong interaction and the absence of 3AT or lacking adenine and 3AT and suggests a weak interaction. For each pair of plasmids, yeast growth was tested on the indicated media and the resulting background was subtracted from the growth that resulted from interactions between the proteins encoded by the plasmids. Growth on medium SD/-Leu/-Trp/-His indicates a weak interaction between the expressed proteins. Stronger interactions correspond to growth on medium SD/-Leu/-Trp/-His with 1 mM 3AT or the absence of 3AT or lacking adenine and 3AT and are indicated by green, blue, and red dots, respectively. Note that fusions specific to the absence of any interactions. 1 mM 3AT, a competitive inhibitor of growth. Interactions were tested in both directions. Growth results were essentially identical. Two-hybrid interactions confirmed by direct protein-protein interaction tests were only performed with A. nidulans NUDE and NUDF proteins. Details of the two-hybrid system will be published elsewhere.

RESULTS

NUDF and NUDE Interact with Subunits of Dynein, Dynactin, and Tubulin in the Yeast Two-Hybrid System—We have used the yeast two-hybrid assay system to study the interactions of NUDF and NUDE with various components of cytoplasmic dynein, dynactin, and microtubules. Because of its large size we were unable to test the whole NUDA dynactin heavy chain directly in the two-hybrid system. We therefore tested constructs that encoded fragments of the dynein heavy chain, each encoding about one-third of the molecule and an additional smaller fragment containing the P-loop. Other interaction targets included the NUDF dynein intermediate chain, the NUDG light chain, the NUDK ARP1 actin-related protein of dynactin, and α-, β-, and γ-tubulins. We also tested three components of the system from the related ascomycete N. crassa: the RO3 p150 component of dynactin (37), the dynactin-associated protein RO2 (36), and RO10, whose function in nuclear migration is not known (3). Different re-
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porter constructs were tested to determine the relative strength of the interactions (8). All of the proteins were tested as both bait and activators (Fig. 1). As described previously, NUDF interacted strongly with NUDE and with the coiled-coil region of NUDE (14). It also interacted with the dynein heavy chain fragment containing the first P-loop even though no interaction was seen with the middle one-third fragment of the dynein heavy chain, which includes the P-loop. This middle third fragment, however, also failed to show a two-hybrid interaction with any of the other proteins shown in Fig. 1, suggesting that the reason for its lack of reaction with NUDF may have been because it was not correctly folded (data not shown). NUDF also exhibited a moderately strong interaction with the NUDI intermediate chain and with the TUBA and MIPA α- and γ-tubulin proteins but did not interact with the NUDG dynein LC8 light chain or with the NUDK ARP1 subunit of dynactin. To determine whether these interactions involved the coiled-coil region of NUDF, we used a mutant NUF construct in which the seven leucines involved in the coiled-coil interaction were mutated to alanines to destroy the ability of this region to undergo a coiled-coil dimerization. This mutant construct failed to interact with any of the above components of dynein or dynactin as shown previously for the failed interaction between NUDE and the mutant NUDF. This suggests either that the coiled-coil region of NUDF was directly involved in these interactions or that it was required to maintain the structure of NUDF. We attempted to test LIS1, the human homologue of NUDF, for its ability to interact with these same proteins, but the LIS1 protein by itself induced significant Gal4p-dependent transcription. LIS1 showed an interaction above this background only with α- and γ-tubulin. The full-length NUDE protein interacted strongly with itself in the two-hybrid system, as did the NUDE coiled-coil domain. NUDE also interacted with NUDF (14), with the NUDI dynein intermediate and NUDG LC8 light chains, with the ARP1 of dynactin, and with the α- and γ-tubulin proteins. The NUDE coiled-coil domain gave a somewhat different interaction pattern. Like the full-length protein it gave a strong signal with NUDE, NUDE, and the dynein LC8 and intermediate chains, but it did not interact with the NUDK (ARP1) protein of dynactin or with the tubulin proteins. A human homologue of NUDE, hNUDE, which has a similar conserved coiled-coil domain, exhibited interactions similar to those of the Aspergillus NUDE except that it failed to interact with the NUDK ARP1 protein of dynactin (20). Similarly, the coiled-coil domain of hNUDE interacted with the same proteins as the coiled-coil

FIG. 2. NUDE and NUDF interact with α-, β-, and γ-tubulins. Panel A, coprecipitation experiments with purified proteins. Proteins NUDF (NUDF-Prot.S), NUDE (NUDE-Prot.S), NUDE (NUDF-Prot.S), and NUDG (NUDG (CDLC)-Flag) were isolated by tag affinity purification from strains CA1[pAAFS], XX21 [pAAFS], and XX21 [pAAFS], respectively, and NUDF and NUDE complexes were purified by tag affinity purification. The tagged proteins are marked by a star. The proteins were transferred to a membrane, and Western blot analyses were performed (lanes a, b, and unsp., respectively). Panel B, coimmunoprecipitation experiments with purified proteins. Crude extracts containing the VSV-G-NUDE. Antibodies against the VSV-G tag were used to detect a coprecipitation of NUDE. As a size control, a protein standard is given (lane M). Panel B, coimmunoprecipitation experiments with purified proteins. Proteins were pulled out with protein G-agarose. Crude extracts containing no antibody were incubated with protein G-agarose and detected with antibodies against the VSV-G tag (Prot.G). Bound proteins were separated sequentially by SDS-PAGE and detected with antibodies against the VSV-G tag of NUDE. Note that α-, β-, and γ-tubulins are not separated in this electrophoresis system. Panel a, lane M, the massie-stained protein G band is shown.

2 C. Ahn, unpublished.

3 V. P. Efimov, unpublished.
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Fig. 3. Purification and interactions among dynein, dynactin, and microtubule subunits of A. nidulans. Panel A, SDS-PAGE of affinity-purified tagged proteins. Extraction buffers contained 0.5–2% Tween 20 to prevent binding of associated proteins. The purified proteins were characterized by SDS-PAGE and stained with Coomassie Blue. The identity of each purified protein is indicated above the lanes. Lane M shows marker proteins. Panel B, interaction of NUDE with NUDG (left). 1 μg of purified NUDE was incubated with the same amount of purified NUDG protein. As controls, each protein was incubated by itself. Complexes were isolated by adding affinity agarose against the FLAG tag of NUDE (FLAG-agarose) or the protein C tag of NUDG (ProteinC-agarose). Proteins were eluted under native conditions according to the manufacturer’s instruction manuals and analyzed by Western blot hybridization with antibodies against the FLAG and Protein C tags. 1 μg of NUDE and NUDK (ARP1) or NUDE and NUDG (CDLC) were mixed and incubated for 1 h on ice (+) or loaded directly (−) on an 8% acrylamide/bisacrylamide gel (pH 7.8) native separation gel. A 5% gel (pH 6.8) was used as stacking gel. After electrophoresis proteins were detected with silver staining.

Panel A

A. nidulans neurokinin subunits. Neurospora RO2, RO3, and RO10 proteins did not interact with NUDF or NUDE, the human homologues or the Aspergillus RO2, RO3, and RO10 proteins did not interact with NUDF or NUDE, the human homologues or the Aspergillus NUDF or NUDE constructs. These were placed under the control of the alcA inducible promoter, transfected into Aspergillus, expressed, and affinity purified under mild conditions and without detergents as described under “Experimental Procedures.” These proteins and their putative binding partners were used in the following pull-down and coimmunoprecipitation experiments. NUDE was pulled down from crude protein extracts by the purified tagged NUDG LC8 dynein light chain and by purified tagged NUDK ARP1 protein bound to agarose beads (Fig. 2A). Similarly, VSV-G-tagged NUDE pulled down both NUDG and NUDK as well as other not yet unidentified proteins (data not shown). Antibodies to α- and γ-tubulin coimmunoprecipitated both NUDE and NUDF from wild-type crude protein extracts (Fig. 2B). Purified NUDE (FLAG-tagged) and purified NUDF (S-tagged) also pulled out α- and γ-tubulins (Fig. 2A). These experiments confirmed most of the interactions seen in our two-hybrid experiments. Only the NUDI dynein intermediate chain, which interacted with NUDE and NUDF in the two-hybrid system, failed to exhibit an obvious interaction with these proteins.

The Purified NUDE and NUDF Proteins Interact Directly with Specific Dynein, Dynactin, and Tubulin Subunits—The yeast two-hybrid, coimmunoprecipitation, and pull-down experiments, although for the most part consistent with each other, nevertheless left open the possibility that the observed interactions could be indirect, i.e. mediated by a third protein. We therefore retested each of the interactions observed in the crude systems using purified proteins to determine whether the purified proteins would interact in the same way. Each purified protein produced a single band of appropriate molecular mass (Fig. 3A), except for NUDE, which purified as a split band with a molecular mass of about 74 kDa in which both dynein heavy chain and tubulin proteins (Fig. 4A). Purified NUDE protein interacted directly with purified NUDG (Fig. 2A). NUDE also interacted directly with purified NUDK, α-tubulin, and γ-tubulin in pull-down experiments (Fig. 3B, right; see also Fig. 5A). Similarly, purified NUDE pulled down the first P-loop of the dynein heavy chain and tubulin proteins (Fig. 4A). These results were retested in protein shift experiments performed with purified proteins under native electrophoresis conditions. Under these conditions when the purified NUDF and γ-tubulin

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proteins were coincubated and subjected to electrophoresis, a new band appeared when the purified NUDE protein was incubated by itself. The experiments described above show that several subunits of dynein, dynactin, and tubulins can bind directly to the NUDE protein. To determine whether these interactions are additive or competitive, we performed in vitro protein binding assays. Most of the proteins that produced strong two-hybrid interactions also interacted in the coimmunoprecipitation and pull-down experiments, and most importantly by assaying the ability of the purified NUDE proteins to interact with subunits of dynein, dynactin, and tubulins in a variety of systems, the yeast two-hybrid system, coimmunoprecipitation and pull-down experiments, and most importantly by assaying the ability of the purified NUDE proteins to interact directly with each other. NUDE and NUDF exhibited distinctly different interaction patterns. The NUDE protein interacted only with the part of the dynein heavy chain which spans the first ATP P-loop binding site and with α- and γ-tubulins; however, it did not interact with the middle third of the heavy chain, which contains the P-loop. The interaction of the first third of NUDE with the NUDG dynein light chain of A. nidulans NUDF and NUDE proteins to interact with subunits of dynein, dynactin, and tubulins in a variety of systems, the yeast two-hybrid system, coimmunoprecipitation and pull-down experiments, and most importantly by assaying the ability of the purified NUDE proteins to interact directly with each other. NUDE and NUDF exhibited distinctly different interaction patterns. The NUDE protein interacted only with the part of the dynein heavy chain which spans the first ATP P-loop binding site and with α- and γ-tubulins; however, it did not interact with the middle third of the heavy chain, which contains the P-loop. The interaction of the first third of NUDE with the NUDG dynein light chain and of the last third with α- and γ-tubulins (data not shown) indicated that these fragments were folded properly (confirming known data from mammalian systems, 41), but we have no such evidence to demonstrate that the middle fragment folded correctly. Incorrect folding of the middle fragment containing the P-loop could explain the different binding affinity of NUDE to this fragment versus the P-loop fragment. NUDE also interacted with α- and γ-tubulin. In contrast to NUDF it did not bind to the dynein heavy chain but rather interacted with the NUDG dynein light chain and with the NUDK actin-related protein of dynactin. Most of the proteins that produced strong two-hybrid interactions also interacted in the coimmunopre-

DISCUSSION

We have analyzed the ability of the A. nidulans NUDE and NUDF protein plus either α- or γ-tubulin caused the formation of new electrophoretically supershifted bands (Fig. 5A). Similar results were obtained when α-tubulin and NUDG were tested for their ability to interact simultaneously with NUDE. The NUDG dynein light chain and the NUDK actin-related subunit of dynactin could also bind simultaneously to NUDE, as shown
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The interactions between NUDE, NUDF, and the various components of dynein and dynactin coincide with the locations of these proteins in the cell. We have shown by GFP labeling that NUDF, NUDE, and the cytoplasmic dynein heavy chain (NUDA) appear as comet-like structures at the plus ends of cytoplasmic microtubules in *Aspergillus* (12–14). We have determined recently that much of the GFP-tagged NUDA, NUDF, and NUDE protein is associated with membranous vesicles in *Aspergillus*, suggesting that not all interactions are conserved between *Aspergillus* and higher eukaryotic dynein/dynactin-related proteins.

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One of the most intriguing features of NUDF and NUDE, seen in both *Aspergillus* and animal systems, is their interac-

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4. B. Hoffmann and N. R. Morris, manuscript in preparation.
tion with γ-tubulin. In animal cells NUDF and NUDE interact with γ-tubulin and colocalize with it at the centromere (20, 22). Moreover, overexpression of NUDE causes γ-tubulin to become dissociated from centromeres, suggesting that there is a functional interaction between these proteins (22). Although we have no direct functional information to connect NUDE and NUDF with the spindle pole body, the centromere equivalent, or with γ-tubulin in Aspergillus, a recent observation made in Schizosaccharomyces pombe suggests a possible link to NUDE, NUDF, and dynein. An unusual cold-sensitive γ-tubulin mutation (SL1) identified during a search for genes synthetically lethal with Pkl1p kinesin causes a mitotic block with an apparent impairment of chromosome attachment to kinetochore microtubules at low temperatures (45). It also causes excessive polymerization of microtubules. These phenocopy the effects of dynein deficiency seen in other organisms. Dynein deficiency has been shown to affect the interaction between spindle microtubules and kinetochores in Drosophila (46, 47) and is well known to cause microtubule hyperpolymerization in Aspergillus and other fungi (7, 12, 13, 48). Synthetic lethality between NUDF and NUDE mediated by NUDE and/or NUDF need to be tested by direct experimentation.

spindle pole bodies (the fungal equivalent of the centrosome) in cells. NUDE, NUDF, and the dynein heavy chain are abundant in the cytoplasm in both A. nidulans (50). It could be argued that the lack of colocalization of NUDF, a recent observation made in animal cells NUDF and NUDE interact with dynein/Dynactin and tubulins and in mammalian cells needs to be followed up with a functional explanation for this interaction. Whether γ-tubulin affects dynein function and whether this is mediated by NUDE and/or NUDF need to be tested by direct experimentation.

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WITHDRAWN