The LIS1-related NUDF Protein of Aspergillus nidulans Interacts with the Coiled-Coil Domain of the NUDE/RO11 Protein

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Abstract. The nudF gene of the filamentous fungus Aspergillus nidulans acts in the cytoplasmic dynein/dynactin pathway and is required for distribution of nuclei. NUDF protein, the product of the nudF gene, displays 42% sequence identity with the human protein LIS1 required for neuronal migration. Haploinsufficiency of the LIS1 gene causes a malformation of the human brain known as lissencephaly. We screened for multicopy suppressors of a mutation in the nudF gene. The product of the nudE gene isolated in the screen, NUDE, is a homologue of the nuclear distribution protein RO11 of Neurospora crassa. The highly conserved NH-terminal coiled-coil domain of the NUDE protein suffices for protein function when overexpressed. A similar coiled-coil domain is present in several putative human proteins and in the mitotic phosphoprotein 43 (M43) of X. laevis. NUDF protein interacts with the Aspergillus NUDE coiled-coil in a yeast two-hybrid system, while human LIS1 interacts with the human homologue of the NUDE/RO11 coiled-coil and also the X enus MP43 coiled-coil. In addition, NUDF coprecipitates with an epitope-tagged NUDE. The fact that NUDF and LIS1 interact with the same protein domain strengthens the notion that these two proteins are functionally related.

Key words: dynein • dynactin • lissencephaly • nudF • LIS1

Introduction

During growth of the filamentous fungi Aspergillus nidulans and Neurospora crassa, nuclei migrate into the germ tube and distribute evenly along the cell length (reviewed in Fischer, 1999; Xiang and Morris, 1999). Analysis of mutations that affect nuclear distribution in these two fungi and in S. cerevisiae has identified microtubules and cytoplasmic dynein and dynactin complexes as the main components of the nuclear distribution machinery (Oakley and Morris, 1980; Plamann et al., 1994; Xiang et al., 1994; Tinsley et al., 1996; Gieser et al., 1997). Other proteins whose functions are less well understood are also required for nuclear migration. One such protein, NUDF of A. nidulans (Xiang et al., 1995a), is particularly interesting because it is 42% identical to LIS1, a human protein whose haploinsufficiency causes a devastating brain malformation known as lissencephaly (smooth brain; Reiner et al., 1993; Attori et al., 1994). Lissencephaly is characterized by a smooth cerebral cortex, which is thought to be the result of impaired neuronal migration to the cortex during brain development (Hirotsune et al., 1998; reviewed in Walsh, 1999; Walsh and Goffinet, 2000).

Genetic analysis indicates that nudF and its S. cerevisiae homologue PAC1 function in the dynein/dynactin pathway (Xiang et al., 1995a; Gieser et al., 1997; Williams et al., 1997). Particularly intriguing is that mutations in the cytoplasmic dynein heavy chain (CDHC)1 can suppress nudF mutations (Williams et al., 1997). However, none of the proteins identified so far as components of the purified dynein or dynactin complexes from animal sources appear to be homologues of NUDF/Pac1p or LIS1, and the role of NUDF/Pac1p protein in dynein/dynactin function is unclear. Recent studies of a LIS1 homologue in Drosophila (Liu et al., 1999; Swan et al., 1999) revealed that LIS1 acts as a cortical anchor for dynein during Drosophila oogenesis (Swan et al., 1999).

Additional evidence that the NUDF and LIS1 proteins are functionally related comes from their interaction with NUDC. The nudF gene was cloned fortuitously as an extra copy suppressor of the nudC3 mutation in the nudC gene (Xiang et al., 1995a). Remarkably, a mammalian homologue of the NUDC protein, which is known to be functional in A. nidulans (Morris et al., 1997), binds LIS1 pro-

1Abbreviations used in this paper: CDHC, cytoplasmic dynein heavy chain; GFP, green fluorescent protein; nud, nuclear distribution defective; ts, temperature sensitive; VSV-G, Vesicular Stomatitis Virus Glycoprotein.
tein (Morris et al., 1998b). Unfortunately, besides the detrimental effect of the A. nidulans nudC mutation on the NUDF protein level, little is known about the function of the nudC gene. In A. spergillus, the nudC null mutant is phenotypically very different from nudF or CDHC null mutants (Chiu et al., 1997), and thus nudC does not necessarily function only in the dynein/dynactin pathway. Other proteins that have been reported to bind LIS1 include platelet-activating factor acetylhydrolase (Hattori et al., 1995), pleckstrin homology domains (Wang et al., 1995), platelet-activating factor acetylhydrolase (Hattori et al., 1995), and tubulin and microtubules (Sapir et al., 1999). It is not known if functional homologues of LIS1 are involved in similar interactions.

Here we identify nudE, the A. nidulans homologue of the N. crassa nuclear distribution gene ro-11, as a multiplicity suppressor of a mutation in the nudF gene. A scoring to genetic data, the R O11 protein functions in the cytoplasmic dynein pathway, but its precise role is not clear (Minke et al., 1999). We show that A. nidulans NUDE and NUDF proteins interact in a two-hybrid system and in A. nidulans protein extracts and demonstrate the generality of this interaction by showing that a similar interaction occurs between the human NUDE/RO11 homologue and human LIS1.

Materials and Methods

Strains and Plasmids

A. nidulans strains used were 20.3.10 (argB2; pyrG89; pabaA1; fwA1; G R 5 (pyrG89; pyroA4; awA2) (made by G. May), A O1 (nudC3; pyrG89; pabaA1; nicA2; waA2) (O’Smien et al., 1990), SF-2.9 (NudE::argB; argB2; pyrG89; pabaA1; fwA1) (this work), XX3 (nudA1; pyrG89; chA1) (Xiang et al., 1994), XX20 (nudE6; pyrG89; yaA2), XX21 (nudE7; pyrG89; yaA2) (Xiang et al., 1995a), XX21x6-17 (nude:::GATC) (pyrG89; nudF7; pyrG89; yaA2) (this work), ΔP54 (NudE::pyrG89; waA2; pyroA4) (Williams et al., 1995). Construction of the pAid vector has been described (Harlow and Lane, 1988). Antigens were detected on immunoblots using a 1:5,000 dilution of a 1:500 dilution of IgG) were coupled with dimethylpimelimidate as described (Willims et al., 1995). The constructs used for the experiments shown in Fig. 1 B all carry the same 4.8-kb region of the A. nidulans genomic DNA at the Smal site of pAid. The nudE gene is approximately in the middle of the insert and is oriented toward the AMA1 insert. Changes within the nudE gene were made as follows. The COOH-terminal part of NUDE (residues 216–586) was deleted by excising the BglII—MfeI fragment (539 bp after filling in). This disrupts the ORF after residue 215 of NUDE and adds 16 new amino acid residues following a stop codon. Hybridizations to genomic DNA revealed copy numbers similar to the intact nudE gene. The region of the nudE gene encoding residues 279–182 was replaced with the fragments of human CDNA clone (accession number U 95097). The replacing fragments encode 152-residue-long domains similar to the NUDE coiled-coil. The sequence of the used portion of the A A 424443 clone was identical to the consensus derived from overlapping ESTs A A 4422443, R 55738, AA 442918, and H 24090.

Cloning of the nudE Gene

The screen for nudF7 multicopy suppressors was based on the method previously used by us to clone A. nidulans genes (Efimov and Morris, 1998). Genomic DNA fragments (5–20 kb) from the X X20 strain were ligated to the pAid vector, which is the replicating vector. NRLGK, which is recognized by the monoclonal antibody P5D4, was done as follows. First, the plasmid pP5D4 x6-3 was constructed. This carries the epidermis encoding sequence GTA GTCT (TA C A C C G A C A T C G A G T A G T C C T G C T G C G A A G G A G T C T C) (TA C A C C G A C A T G A T C G A C A T C G C T G C G A A G G A G T C T C C) flanked by NcoI sites in the pGM XS2 vector. Second, plasmid pSH1 was made by subcloning the NruI-A ccl A. nidulans genomic fragment (2.62 kb), which contains the truncated nudE gene (resides 45–586 of the NUDE gene), at the EcoRV—Cial sites of the pXX1 vector (Xiang et al., 1995b). The epidermis encoding NcoI—NcoI fragment from pP5D4 x6-3 was cloned at the unique NcoI site of the pSH1-1 frame with the nudE gene to produce plasmid pSH x6-3. The XX21 strain was transformed with the circular pSH x6-3 plasmid and pyrG89 transformants were analyzed by PCR. Four transformants, designated XX21x6-48, 12, 15, 17, were found that had undergone a single recombination event at the nudE locus resulting in the insertion of the epidermis sequence in the intact nudE gene.

Domain Analysis of the NUDE Protein

The affinity-purified polyclonal antibody against the NUDE protein was made by Xiang et al. (1995a). P5D4 mouse monoclonal antibody and protein G-coated Sepharose 4B were from Sigma-Aldrich. The protein G-Sepharose (packed volume) and 60 mg of P5D4 antibody solution (6.5 mg/ml of IgG) were coupled with dimethylpimelimidate as described (Harlow and Lane, 1988). A nitigens were detected on immunoblots using an appropriate alkaline phosphatase conjugate and BCIP/NBT as a chromogenic substrate.

To make total protein extracts, A. nidulans cells from 18 h cultures were disrupted by grinding in liquid nitrogen and resuspended in 50 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 0.5% SDS. 1 ml of extraction buffer was added per gram of dry-pressed mycelium. The extracts were cleared by centrifugation for 10 min at 16,000 g and adjusted to the same protein concentration using the Bradford assay. Alternatively, the ground mycelium was resuspended in the SDS-PAGE loading buffer, boiled and cleared by centrifugation.

For immunoprecipitations, XX21 and XX21x6-17 strains were grown at 32°C for 18 h. 0.45 g of dry-pressed mycelium was disrupted by grinding in liquid nitrogen followed by addition of 0.45 ml of PBS containing protease inhibitor cocktail for fungal extracts (Sigma-Aldrich) and vortexing with glass beads. Aliquots of the next samples were performed on ice or at 4°C. Extracts were cleared by centrifugation for 5 min at 10,000 g and incubated.
for 2 h with 40 µl (packed volume) of protein G-Sepharose beads coupled to PSE d monoclonal antibody in a total volume of 0.6 ml on a rocker. Beads were washed five times with 1 ml of PBS, boiled in 60 to P5D4 monoclonal antibody in a total volume of 0.6 ml on a rocker.

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temperature sensitive terbuck, 1997) to screen for multicopy suppressors of the vector (Gems and Clutterbuck, 1993; Aleksenko and Clutterbuck, 1997). We employed an Other Conditional nud Mutants

Results

Extra Copies of the nudE Gene Suppress the nudF7 and Other Conditional nud Mutants

We employed an A. nidulans autonomously replicating vector (Gems and Clutterbuck, 1993; A Icksenko and Clutterbuck, 1997) to screen for multicopy suppressors of the temperature sensitive nudF7 mutation in A. nidulans. This vector maintains its extrachromosomally in A. nidulans at ~10 copies per nucleus (Gems et al., 1991). The screen was based on partial complementation of the XX21 (nudF7; pyrG89; yaA) conidiation defect by genomic DNA fragments ligated to the A. nidulans replicating vector pAid. The XX21 strain is temperature sensitive due to the presence of the nudF7 mutation. It grows like wild-type and produces yellow conidia (asexual spores) at the permissive temperature of 32°C, but forms smaller colonies and fails to produce conidia at the restrictive temperature of 43°C. Under semi-restrictive conditions, such as intermediate temperature (37°C) or at 43°C on high salt medium (0.6 M KCl), this strain grows almost to the same size as wild-type, but fails to conidiate and hence has the brownish color of unconidiated mycelium. We expected that a multicopy suppressor that improved the growth of the XX21 would also improve its conidiation. This would render the mycelium with the suppressor plasmid a yellow color that should be noticeable in the background of brownish, unconidiating mycelium.

Two genes were repeatedly identified in the screen. One was the nudF6 allele of the nudF7 gene (the nudF6 mutant was used as a source of DNA for the screen). A nother gene was similar to the N. crassa nuclear migration gene ro-11 (MInke et al., 1999). The ro-11-like gene was named nudE. Note, that nud mutations assigned to loci B, D, and E in the original screen (M Oorris, 1976) were subsequently mapped to the nudA gene (M Oorris, N.R., unpublished data). Multicopy plasmids with the nudE gene improve the growth, conidiation and nuclear distribution of the nudF7 mutant (Fig. 1A; Table I). Suppression of the nudF6 mutation, which is tighter than nudF7 (Xiang et al., 1995a), was very weak. Extra copies of the nudE gene also suppressed the conidiation defect of the nudA1 CDHC mutant grown under mildly restrictive conditions (32°C, no KCl, Fig. 1A), but suppression of the nudA1 mutant and of the nudC3 mutant was barely detectable under the most

Other Techniques

A Gal4p-based yeast two-hybrid system Matchmaker 3, which includes S. cerevisiae strain A H109 and plasmids pG BKT7 and pGA DT7, was purchased from CLONTECH Laboratories and used according to supplied instructions.

DA PI staining of nuclei and quantitation of nuclear migration was done essentially as described (Willins et al., 1995). The spores of the nudF7 mutant were transformed with different multicopy plasmids, germinated under the most restrictive conditions (43°C, no KCI), and stained with DA PI when the majority of cells had 8–16 nuclei. Nuclear migration was scored for germlings with 8–16 nuclei. If a spore had several germtubes, only the one with the most nearly normal nuclear distribution was considered. Nuclear migration was scored as failed if fewer than three nuclei had entered the germtube. If three or more nuclei were observed in the germtube, but nuclei were not evenly distributed or an abnormally large cluster was present in the spore remnant, nuclear migration was scored as intermediate.

Figure 1. (A) Suppression of nud mutants by extra copies of the nudE and nudF6 genes. The indicated mutants (all are conditional, temperature sensitive) were transformed with either the empty vector pAid or pAid clones bearing nudE and nudF6 genes (plasmids recovered in the multicopy suppressor screen) and grown at 32°C. Transformants were gridded on YAG plates with or without KCl and incubated at 43°C or 32°C for 2 d. All strains have different color of conidia: yellow for nudF7, green (wild-type) for nudF6, chartreuse for nudA1 and white for nudC3. The intensity of the colony color is proportional to the number of conidia produced. Four independent transformants of the nudF7 mutant are shown for each plasmid to demonstrate reproducibility of phenotypes. (B) Complementation of the nudE deletion and the nudF7 mutant by extra copies of the nudE, the nudE NH2-terminal domain, and nudE chimeras carrying coiled-coil regions from human and frog proteins, respectively. Strains were transformed with nudE variants in pAid vector and grown at 43°C. Numbers refer to amino acid residues of nudE protein expressed by the constructs (see Fig. 3 for detailed amino acid sequences). Due to the presence of a fawn color marker, conidiating and unconidiating AnudE colonies have very similar colors at this temperature.

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restrictive conditions (43°C, no KCl). The conidiation defect of the nudC3 mutant grown under mildly restrictive conditions was not suppressed, nor was the conidiation defect of another mutation that affects nuclear migration and conidiation, apsA5 (Fischer and Timberlake, 1995; data not shown).

The amount of NUDF protein is reduced at the restrictive temperature in nudF6 and nudF7 mutants (Xiang et al., 1995a). However, suppression by nudE was not due to the affect on NUDF concentration. Extra copies of the nudE gene did not increase the level of NUDF protein in the nudF7 mutant (Fig. 2). Predictably, extra copies of the nudF6 gene increase the amount of the mutant NUDF protein (Fig. 2). Interestingly, we consistently observed inhibition of the nudC3 mutant by extra copies of the nudF6 allele (Fig. 1 A). This is opposite to the effect of the wild-type nudF gene, which is a multicopy suppressor of the nudC3 mutation (Xiang et al., 1995a). A possible interpretation is that the overexpressed mutant NUDF protein sequesters mutant NUDC.

The nudE null mutant was viable, but displayed impaired nuclear migration, reduced colony growth, and a conidiation defect. All these defects were less severe than in the ∆nudF or ∆nudA mutants (Table I and Fig. 1 B), which have identical phenotypes (Williams et al., 1995). The double mutants ∆nudE:∆nudF and ∆nudE::nudA were similar to the ∆nudF and ∆nudA single mutants (data not shown), indicating that nudE affects the same pathway as nudF and CDHC. To make sure that differences between the ∆nudE and ∆nudF strains were not caused by background mutations, we crossed the ∆nudE strain to GR5, the wild-type parent of the ∆nudF strain. All progeny obtained from the cross were either wild-type or ∆nudE-like.

Only the NH2-terminal Coiled-Coil Domain of the NUDF Protein Is Absolutely Required for Its Function

The NUDE protein closely resembles the R011 protein of N. crassa (Minke et al., 1999). The similarity is strongest within the first 190 residues, which are predicted to form a continuous coiled-coil structure (Fig. 3 A, see also Minke et al., 1999). The COOH-terminal region that follows the coiled-coil is poorly conserved between NUDF and R011. In both proteins, however, it has an excess of positively charged residues (calculated pI = 12) and a high serine content (>18%). Other similar features of the COOH-terminal domain include a short central coiled-coil or an α-helix and the sequence DLGET(F,Y) at the very end.

A search of the public database for proteins similar to NUDF/R011 turned up multiple sequences that had extensive homology with the coiled-coil in the NH2-terminal part of the NUDF/R011 but little or no homology with the rest of the molecule (Fig. 3 B). One was the mitotic phosphoprotein 43 (M43) of Xenopus laevis (Stunkenberg et al., 1997). Multiple human and mouse ESTs, apparently coming from at least two different genes, were also identified. The translation product of the human EST AA 424443 that we chose to work with is almost identical (94% identity for the sequence shown in Fig. 3 B) to the rabbit endoligopeptidase A-related protein (Hayashi et al., 2000). A human cDNA encoding a similar coiled-coil (72% identity with AA 424443) was recently annotated as HOM_TES-87 tumor antigen mRNA (accession number A F124431).

We constructed two chimeric nudE genes by substituting the sequence encoding the most conserved region of the NUDF protein. We detected a NUDE-NUDF interaction in a Gal4p-based yeast two-hybrid system (Fig. 4). The same

The NUDE Coiled-Coil Domain Interacts with the NUDF Protein and this Interaction Is Evolutionary Conserved

Multicycop suppressors often identify physically interacting proteins. We detected a NUDE-NUDF interaction in a Gal4p-based yeast two-hybrid system (Fig. 4). The same
results were obtained with the full-length NUDE and its NH2-terminal coiled-coil fragment (data not shown). The interaction appears to be weak as it can be detected only with the most sensitive reporter gene HIS3. The use of animal homologues of the NUDE and NUDF proteins provides a rigorous control for the specificity of this interaction. No interaction can be detected between A. nidulans NUDE and human LIS1 or between chimeras of NUDE carrying the coiled-coil regions from human or frog proteins and A. nidulans NUDF. On the other hand, the frog and human NUDE chimeras interact with human LIS1. The latter interactions are readily observed with both the HIS3 and ADE2 reporter genes. As expected, all coiled-coil constructs interact with themselves. Interestingly, the Aspergillus NUDE coiled-coil does not interact with the Xenopus or human coiled-coils, while the Xenopus and human coiled-coils interact with each other. These results demonstrate that the region delimited by amino acid residues 25–183 of the A. nidulans NUDE coiled-coil specifically binds NUDF protein, while the corresponding region of the human and frog NUDE/R11 homologues binds human LIS1.

Finally, we tested whether NUDE and NUDF proteins interacted with each other physically in A. nidulans protein extracts. To facilitate detection of the NUDE protein, we tagged the nudE gene in the A. nidulans genome with six copies of the Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope sequence, which is recognized by the monoclonal antibody P5D4 (Soldati and Perriard, 1991). The tagged NUDE protein differs from the native protein by the extra sequence RS[YTDIEMNRLGK(GS)]6TMG following glycine 569. Four independently obtained tagged strains were indistinguishable from the parent strain, indicating that the tagged gene was functional. The tagged NUDE::(VSV-G)6 protein was readily detected in total protein extracts by Western blotting with the P5D4 antibody (Fig. 5 A). The protein is likely to be modified as it often migrates as a closely spaced doublet and can be further separated into at least four distinct bands (Fig. 5 B). Immunoprecipitations were performed with the P5D4 antibody using protein extracts from the tagged nudE::(VSV-G)6 strain and, as control, the parent nudE1 strain.
NUDF proteins were found to coprecipitate (Fig. 5 C), thus corroborating the results of the two-hybrid assay.

**Discussion**

We conducted a multicopy suppressor screen in the filamentous fungus *A. nidulans* to identify genes interacting with the nudF gene, a putative homologue of the human gene LIS1, which is implicated in brain development. The nudE gene, an apparent homologue of *N. crassa ro-11*, was obtained from the screen. Multicopy suppressors often correspond to physically interacting proteins. We observed a specific interaction between NUDE and NUDF in a yeast two-hybrid system (Fig. 4). In addition, an epitope-tagged NUDE coprecipitated with NUDF in *A. nidulans* protein extracts (Fig. 5 C). Thus, the simplest explanation for the suppression of conditional nud mutants by extra copies of the nudE gene is that increasing the level of the NUDE protein results in a more efficient formation of the NUDE-NUDF complex. NUDE-NUDF association is consistent with our observations that in live *A. nidulans* cells, full-length NUDE- and NUDF-GFP fusions localize to similar comet-like structures that move toward the hyphal tips (Xiang, X., V.P. Efimov, and N.R. Morris, manuscript in preparation). Similar structures are also observed with the CDHC-GFP fusion (Xiang et al., 2000). Given the size of the NUDE coiled-coil that binds NUDF (≈170 residues or 25 nm in length) and its conservation between fungal, frog and human proteins (Fig. 3 B), it is likely to bind other proteins in addition to NUDF.

The closest match to the NUDE protein is the *N. crassa* nuclear migration protein RO11. Deletion of the ro-11 gene in *N. crassa* causes the same nuclear distribution defect that is characteristic of dynein/dynactin mutants, without affecting cytoplasmic microtubules, dynein/dynactin accumulation at hyphal tips or the levels of CDHC (RO1) and p150Glued (RO3) (Minke et al., 1999). Deletion of the *A. nidulans* nudE gene similarly produced a viable strain with a nuclear distribution defect. The sequence similarity between NUDE and RO11 proteins and the involvement of both proteins in nuclear distribution indicate that NUDE and RO11 are functional homologues. Both proteins consist of two distinct domains: a highly conserved NH₂-terminal coiled-coil and a poorly conserved COOH-terminal tail. The importance of the NH₂-terminal coiled-coil domain is underscored by the fact that it is sufficient

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**Figure 4.** NUDE and NUDF proteins interact in a two-hybrid system. *S. cerevisiae* strain AH109 was transformed with pairwise combinations of plasmids expressing indicated proteins as fusions with either the Gal4p DNA binding (Gal4pDBD) or Gal4p activating (Gal4pAD) domains. NUDE, AA424443 and MP43 refer to the NH₂-terminal domains of *A. nidulans* NUDE protein (residues 1-195) and corresponding fusions with human and *X. laevis* homologues, respectively with the nudF gene, a putative homologue of the human gene LIS1, which is implicated in brain development. The nudE gene, an apparent homologue of *N. crassa ro-11*, was obtained from the screen. Multicopy suppressors often correspond to physically interacting proteins. We observed a specific interaction between NUDE and NUDF in a yeast two-hybrid system (Fig. 4). In addition, an epitope-tagged NUDE coprecipitated with NUDF in *A. nidulans* protein extracts (Fig. 5 C). Thus, the simplest explanation for the suppression of conditional nud mutants by extra copies of the nudE gene is that increasing the level of the NUDE protein results in a more efficient formation of the NUDE-NUDF complex. NUDE-NUDF association is consistent with our observations that in live *A. nidulans* cells, full-length NUDE- and NUDF-GFP fusions localize to similar comet-like structures that move toward the hyphal tips (Xiang, X., V.P. Efimov, and N.R. Morris, manuscript in preparation). Similar structures are also observed with the CDHC-GFP fusion (Xiang et al., 2000). Given the size of the NUDE coiled-coil that binds NUDF (≈170 residues or 25 nm in length) and its conservation between fungal, frog and human proteins (Fig. 3 B), it is likely to bind other proteins in addition to NUDF.

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**Figure 5.** NUDE protein tagged with VSV-G epitopes coprecipitates with NUDF in *A. nidulans* protein extracts. (A) Total protein extracts from four independently isolated NUDE::(VSV-G)⁶ strains (8, 12, 15, 17) and the parent strain with the wild-type nudE gene were analyzed by SD-S-PAGE (4-20%) and immunoblotting with P5D4 antibody (left). Ponceau S staining of the membrane after protein transfer is shown on the right. (B) Same samples as in A (tagged strain #17 and untagged control) after better separation on a 10% SD-S-PAGE. (C) The left panel shows immunoblotting of proteins precipitated by P5D4 antibodies coupled to Sepharose beads from nudE::(VSV-G)⁶ and nudE* extracts with P5D4 and anti-NUDF antibodies. The right panel shows silver staining of the same samples.
for the biological activity of NUDE protein when expressed from a multicyclic plasmid. However, it is possible that the extra copies of the gene make up for the lack of the COOH-terminal domain.

We selected the coiled-coil regions encoded by the X. laevis protein MP43 (Stukenberg et al., 1997) and by a human EST (accession number A A 424443) as potential homologues of the NUDE/R011 NH2-terminal domain (Fig. 3 B). However, they were not functional in A. nidulans (Fig. 1 B). Also, we were not able to complement the A. nidulans nudF mutations with the human LIS1 gene (Xiang, X., V.P. Efimov, and N.R. Morris, unpublished data), despite the 42% sequence identity between the two proteins. As an alternative, we used a yeast two-hybrid system to analyze interactions between A. nidulans nuDF and itself, while NUDE chimeras carrying the coiled-coil regions from human or frog homologues interact with human LIS1 and themselves. The above interactions are extremely specific as A. nidulans NUDE does not interact with human LIS1 or human and frog NUDE homologues, nor does NUDE interact with the human or frog NUDE homologues.

While this work was in progress, we learned that a mouse NUDE/R011-like protein was identified by Dr. S. Hirotunde’s lab in a two-hybrid screen with the mouse LIS1 protein (Sasaki, S., A. Shionoya, M. Ishida, Y. Sugimoto, and S. Hirotunde, manuscript submitted for publication). At the amino acid level, the human EST fragment used by us (sequence in Fig. 3 B) has 99.5% identity with the mouse sequence. A slightly different sequence has been identified in the two-hybrid screen with the human LIS1 protein by Dr. C. Walsh’s lab (Feng, Y., and C. Walsh, personal communication).

The region of the MP43 protein that follows the coiled-coil is much shorter than the equivalent region in the NUDE protein (160 residues vs. 394) and shows little sequence homology to NUDE. The salient features of this region in NUDE and R011, an excess of positively charged residues and serines and the presence of a short COOH-terminal domain. It has a pI of 9.8, a serine content of 15%, and a predicted central α-helical region of ~50 residues. Considering all the facts, it is very likely that MP43 is a homologue of the NUDE/R011 protein that functions in the cytoplasmic dynein pathway and interacts with the X. laevis homologue of LIS1 protein. As the MP43 protein is phosphorylated specifically during mitosis (Stukenberg et al., 1997), an exciting possibility is that its modification may be related to dynein/dynactin regulation. Cytoplasmic dynein drives the movement of membranous networks in X enopos interphase extracts, and this movement ceases during mitosis, presumably because dynein dissociates from membranes (Niclas et al., 1996).

The sequence similarity between NUDE and LIS1, and the fact that both NUDE and LIS1 interact with the same domain of NUDE and its human homologue, respectively, support the idea that NUDE and LIS1 are functionally related. The documented involvement of fungal NUDE/Pac1p and NUDE/R011 proteins in the cytoplasmic dynein pathway suggests that LIS1 exerts its effect through cytoplasmic dynein. Several hypothetical roles for cytoplasmic dynein in lissencephaly have been discussed (Sapir et al., 1997; Morri et al., 1998a,b; Vallee et al., 2000). They include a role in the regulation of microtubule dynamics, in mitosis, in nuclear translocation, and in retrograde transport of signaling molecules. Of particular interest is the recent finding that in axons cytoplasmic dynein generates a force counterbalancing contractile forces generated by myosin (A hamd et al., 2000).

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