NUDF, a Fungal Homolog of the Human LIS1 Protein, Functions as a Dimer in Vivo*

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The NUDF protein is required for nuclear migration through the mycelium of the filamentous fungus Aspergillus nidulans. It is of particular interest, because it closely resembles a human protein, LIS1, that is required for development of the cerebral cortex. Both are ~50-kDa proteins with a short N-terminal predicted coiled coil and seven WD-40 domains in the C-terminal half of the molecule. They also interact with homologous proteins, suggesting that they may have similar biochemical functions. Here we describe the purification to homogeneity of NUDF protein in a single step from a cell-free extract of A. nidulans. We demonstrate that NUDF is a homodimer, that its dimerization occurs via the N-terminal coiled coil region of the molecule, and that it must be a dimer to support the growth of A. nidulans.

The nud genes of Aspergillus nidulans and the ropy genes of Neurospora crassa encode proteins required for nuclear migration through the fungal mycelium. Many of these proteins are subunits of the microtubule-dependent motor cytoplasmic dynein and of its activator, dynactin (1–6). In A. nidulans these include the dynein heavy chain (nudA), an intermediate chain (nudD), and a light chain (nudG) (6–8). NudK encodes the ARP1 actin-related protein component of dynactin (9). Components of dynactin and dynactin are also found among the N. crassa ropy genes. Thus the dynactin/dynactin system is believed to be the main motor for nuclear migration in these fungi. Some of the nud genes of A. nidulans encode proteins that are not immediately recognizable as components of dynein or dynactin. Of these the NUDF protein is particularly interesting, because it closely resembles a human protein, LIS1, required for development of the cerebral cortex (10, 11). NUDF encodes a 49-kDa protein that is 42% identical to LIS1 in its amino acid sequence. It also has a short predicted coiled coil region near its N terminus and seven WD-40 repeats in the C-terminal half of the molecule, as does LIS1. NUDF and LIS1 interact either genetically or physically with the A. nidulans and mammalian homologs of two other proteins, NUDC and NUDE (11–14). The close sequence similarity between NUDF and LIS1 and the fact that NUDF and LIS1 interact with homologous proteins suggest that they may have a similar biochemical function.

Genetic evidence from A. nidulans has linked NUDF to cytoplasmic dynein. Loss of function mutations in components of either dynein or its activator complex, dynactin, in A. nidulans, N. crassa, and other filamentous fungi inhibit nuclear migration through the fungal mycelium. As a consequence growth of the mycelium is severely inhibited. This dynein-related mutant phenotype is phenocopied by loss of function mutations in the nudF gene, suggesting that NUDF protein is required for dynein to mediate nuclear migration. Strains doubly mutant for NUDF and the cytoplasmic dynein heavy chain are no more severely affected than their singly mutant parental strains, and mutations causing loss of NUDF function are suppressed by mutations in the cytoplasmic dynein heavy chain (15). These data mean that NUDF and dynein are on the same biochemical pathway, and they imply that NUDF and the dynein heavy chain interact in some way. Similarly, perturbing LIS1 concentration, either by deletion or overexpression, phenocopies effects caused by perturbation of dynein and dynactin function in both mammalian cells and Drosophila melanogaster (16–19).

LIS1 colocalizes in developing brain with components of cytoplasmic dynein and also coinmunoprecipitates from mammalian extracts with components of dynein and dynactin (20, 21). Like LIS1 cytoplasmic dynein intermediate chains have an N-terminal predicted coiled coil domain and seven C-terminal WD-40 domains. Although they are about 25 kDa larger than LIS1, this similarity has raised the possibility that LIS1 could be a variant cytoplasmic dynein intermediate chain (22). There appear to be two cytoplasmic dynein intermediate chains in each cytoplasmic dynein complex (23). Sucrose gradient centrifugation and gel filtration data have shown that LIS1 synthesized in vitro has a molecular mass of almost twice the molecular mass predicted from its amino acid sequence. These results suggested that LIS1 might be a dimer (24). Our preliminary physical data suggested that NUDF might also be a dimer, as it sedimented faster from crude extracts of A. nidulans than expected from its calculated molecular mass of 49 kDa (11). In this paper we describe the purification of NUDF and its characterization as a homodimer. We then provide evidence that NUDF functions as a dimer in the living cell by showing that dimer formation is required for NUDF to mediate normal growth and nuclear migration.

EXPERIMENTAL PROCEDURES

Construction of Strains Carrying an S-tagged nudF Gene—A DNA sequence encoding a 15-amino acid S-tag peptide (Novagen) from the small subtilisin fragment of RNase A was added to the 3′-end of the coding region of the nudF gene. The nudF cDNA and its 3′-untranslated region were used as templates in PCR reactions using the following primer sets: 5′-CCGCTCTCAGATACATCGGTCGCATCGG-3′ (P1) and 5′-CTGCGTCTCCATGTCGCGGCCTCAGAATTTAGCAACACGGCTTT-3′ (P2). The abbreviations used are: PCR, polymerase chain reaction; MEK, methyl ethyl ketone; PAGE, polyacrylamide gel electrophoresis; kb, kilobase pair(s); PIPES, 1,4-piperazinediethanesulfonic acid.

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NUDF Functions as a Dimer

Gene Perturbation Chromatography—The purified NUDF protein was concentrated 10-fold and applied to a Superdex 200 HR 10/30 FPLC column, which had been equilibrated with 50 mM sodium phosphate (pH 7.0) buffer containing 150 mM NaCl and calibrated with apoferritin (443 kDa), albumin (66 kDa), and lysozyme (14.3 kDa). The column was eluted using the same buffer at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected. Aliquots (20 μl) of each fraction were directly subjected to SDS-PAGE followed by Western blotting with alkaline phosphatase-conjugated anti-protein. The density of the NUDF protein bands was measured using an ImageMaster™ (Amersham Pharmacia Biotech) densitometer.

Analytical Ultracentrifugation—Equilibrium ultracentrifugation was performed using a Beckman Optima XL-I analytical ultracentrifuge with an interference optical system and an An-60Ti rotor. The molecular mass of NUDF protein was estimated at 4°C using short column techniques at four different loading concentrations (0.16, 0.32, 0.4, and 0.48 mg/ml) and at three different rotor speeds: 20,000, 26,000, and 30,000 rpm using the program NONLIN.

RESULTS

Purification of NUDF Protein—Purification was performed by homogeneity by S-protein affinity chromatography using the S-tag/S-protein system (25). We first made a strain (CA1) in which the wild-type chromosomal nudF gene was replaced by a C-terminally S-tagged nudF′ gene (Fig. 1A) and demonstrated that this strain grew as well as the wild-type strain, indicating that the S-tagged NUDF protein was functional in vivo (Fig. 1B). Because only very small amounts of protein could be purified from this strain, we engineered a second strain (CA1[pAAFS]) to overexpress the S-tagged NUDF from a high copy number plasmid. CA1[pAAFS] was grown under inducing conditions, fragmented frozen by grinding in liquid nitrogen, and a cell-free extract was prepared. The S-tagged NUDF protein was then purified to homogeneity in a single step directly from the extract by adsorption to S-protein linked to agarose beads (Novagen), followed by elution with an excess of the S-peptide (Fig. 1C). Nothing was eluted when the same purification procedure was performed on S-protein that did not contain a S-tagged NUDF protein.

NUDF Protein Is a Homodimer—We have previously shown by sucrose gradient ultracentrifugation that the molecular mass at which wild-type NUDF protein sedimented was greater than its calculated molecular mass (11). The protein from crude extracts had an S value consistent with a molecular mass of 120 kDa rather than with the molecular mass of 49

CTTTGAAACCCTGCCGAGAGT-3′ (P2), 5′-CAAGAACGCCCTGGTCTGTA-3′ (P4). P1 and P2 (carrying the S-tag sequence) were used to add the S-tag to the 5′-end of the 900-nucleotide downstream sequence. The two PCR products were then annealed to be used as template in a second PCR reaction with P1 and P4. The second PCR product was cloned into the Xhol-KpnI sites of a vector pRGI to generate a plasmid pCA1 containing the truncated nudF-S-tag-S-protein system (25). We first made a strain (CA1) in which amino acid residue 72 in the coiled coil motif was replaced by MP3/P5 and MP4/P6 primer sets were used to make the mutant NUDF gene. The 0.7-kb DNA fragment containing the S-tag, a stop codon, and the coding and 3′-untranslated region was made by PCR using pCA2 as a template. 0.8 kb of DNA containing the S-tag sequence was cloned into the SphI site of pCA1 to make the mutant nudF′ gene. The resulting plasmid, named pAAMFS1, was then transformed into competent E. coli cells and grown in an M9 minimal medium containing 1% glycerol as carbon source. E. coli strain CA1[pAAMFS1] was grown in a 1.5-l conical flask in a shaking incubator at 37°C. The cell density of the culture was monitored using an Eppendorf Biophotometer.

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kDa calculated from the sequence (11). The purified S-tagged NUDF protein also gave an apparent molecular mass of 140 kDa by gel permeation chromatography on Superdex G-200 (Fig. 2, A and B). These data suggested that it might be complexed with another protein(s). However, when the purified S-tagged material was analyzed by SDS-PAGE electrophoresis it gave only a single 50-kDa band (Fig. 1 C), indicating that it was a homopolymer. The molecular mass of LIS1, the mammalian homolog of NUDF, calculated from sedimentation and gel filtration data, is close to that expected for a dimer (24). This led us to suspect that NUDF might also be a dimer. To determine whether NUDF was a dimer or was a trimer, as suggested by its migration on gel permeation chromatography, we cross-linked the purified S-tagged NUDF protein with disuccinimidyl suberate and analyzed the denatured, cross-linked protein by SDS-PAGE (Fig. 3). The uncross-linked 50-kDa protein band seen at the start of the experiment was converted with time to a broad band migrating at an apparent molecular mass of 120 kDa. The amounts of NUDF starting material and of the putative dimer band that was produced were approximately equal, and no aggregated protein was seen in the wells. If NUDF-S were a trimer we would have expected to see an intermediate band representing the dimer appear between the monomer and the 120-kDa band. No such intermediate band was detected, indicating that NUDF is a homodimer. The fact that the dimer gave a broad band is presumably due to the fact that different cross-linking between molecules causes a disperse set of cross-linked dimers that migrate differently on SDS gels after denaturation (Fig. 3). The result of the cross-linking experiment was confirmed by equilibrium ultracentrifugation, which showed that the purified NUDF-S protein is a dimer with a monomer molecular mass of 48,154 kDa ($\pm 4.78\%$ error) and a dissociation constant of $3.63 \times 10^{-7}\text{M}$ (Fig. 4). As NUDF protein from crude cell extracts gave the same apparent native molecular mass as the S-tagged protein, we conclude that dimerization is a property of NUDF rather than an effect of the attached S-tag peptide. The fact that both NUDF and
LIS1 appear to be dimeric adds an additional structural feature to the previously observed similarities between these proteins. The slower than expected migration of the NUDF dimer by sedimentation and gel permeation chromatography suggests that it is an extended molecule.

The predicted coiled coil region of NUDF is near the N terminus, between amino acids 60 and 86 (Fig. 5A). It seemed likely that this coiled coil region of the protein would be implicated in formation of the NUDF homodimer; however, WD-40 motifs are also believed to be involved in protein-protein interactions. To learn which parts of the molecule are involved in dimer formation, we asked whether overexpression of the coiled coil region or the WD-40 region interferes with NUDF function, whether either of these regions is sufficient to form the dimer, and whether the ability to form a dimer is required for NUDF function.

**Overexpression of the Coiled Coil Region Affects Colony Growth**—We used a set of alcAlp:nuDF chimeras to determine whether overexpression of the coiled coil region or the WD-40 region of NUDF affects NUDF function. NUDF is required for normal colony growth of A. nidulans. If formation of a NUDF

![Fig. 3. Time course of cross-linking of NUDF with disuccinimidyl suberate. The purified S-tagged NUDF protein was cross-linked for various times as indicated and analyzed by SDS-PAGE and silver staining.](http://www.jbc.org/Downloaded from)

![Fig. 4. Sedimentation equilibrium analysis of NUDF-S protein. The bottom panel shows the fringe gradient in the centrifuge cell after attaining sedimentation equilibrium. The solid line is the result of fitting to a monomer-dimer system, and the circles, triangles, and squares are the experimental values. The data correspond to a global fit for three independent experiments performed at 20,000, 26,000, and 34,000 rpm. A starting protein concentration of 0.32 mg/ml was used. The top panel shows the difference in the fitted and experimental value as a function of radial position (residuals).](http://www.jbc.org/Downloaded from)

![Fig. 5. Inhibition of the growth of A. nidulans by overexpression of N-terminal fragment. A, schematic representation of NUDF protein. B, the cells were grown for 48 h. The repression medium was YAG. The induction medium was minimal medium containing glycerol (4 mM) and MEK (50 mM). C, Western blot showing overexpression of NUDF and the fragments. The cells were grown on liquid minimal medium containing MEK. The cells were disrupted, and protein extracts were subjected to SDS-PAGE followed by Western blotting. Lane M shows marker proteins. Lane 1 is wild-type protein. Lane 2 shows the overexpressed full-length S-tagged NUDF. Lane 3 shows the overexpressed coiled coil fragment. Lane 4 shows the overexpressed WD-40 fragment.](http://www.jbc.org/Downloaded from)
NUDF Functions as a Dimer

Mutations That Interfere with the Coiled Coil Interaction

The Coiled Coil Region Binds Full-length NUDF Protein—

The preceding experiment indicated that overexpression of the coiled coil region of NUDF had a significant effect on NUDF function as measured by colony growth. This suggested that the overexpressed coiled coil region was either competing with the endogenous coiled coil region on the full-length NUDF molecule and thereby inhibiting dimer formation or that it was interfering with an interaction between NUDF and some other protein whose interaction with NUDF was necessary for normal growth. To determine whether the isolated coiled coil domain of the molecule was able to participate in the dimerization of NUDF, we asked whether the coiled coil region would bind the full-length NUDF protein. We transformed the plasmid bearing the N-terminal fragment (pAATFS1) into a wild-type strain (GR5), overexpressed it by induction on methyl ethyl ketone, and purified it from a cell-free extract of an A. nidulans protein on S-protein beads (Novagen) as described above for the full-length protein. Note that this strain (GR5) contains the full-length, endogenous, wild-type, non-S-tagged NUDF protein, which is necessary to sustain growth. SDS-PAGE analysis of the material eluted by the S-peptide showed that the N-terminal fragment was purified to homogeneity and that the N-terminal fragment interacted strongly and specifically with NUDF protein, suggesting that the WD-40 portion of the molecule plays little or no role in NUDF dimer formation (Fig. 6). As there was no NUDF protein seen in the gels of either the full-length NUDF protein or if the WD-40 also made a significant contribution to the interaction between monomers.

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Mutations That Interfere with the Coiled Coil Interaction

The Coiled Coil Interaction

The coiled coil interaction takes place between hydrophobic portions of the interacting coils, specifically between leucines or isoleucines in positions “a” and “d” of the heptamer repeat (Fig. 7). We generated two types of mutations that we thought would interfere with NUDF coiled coil formation. In one, L72E, we replaced the d position leucine 72 in the coiled coil S-tagged fragment by glutamic acid.
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The strong negative charge repulsion between the opposed glutamic acid residues should destabilize the helix-helix interaction. In the second, Ala<sup>a+d</sup>, we replaced all the leucines and isoleucines in the a and d positions between amino acids 62 and 83 in the S-tagged coiled coil fragment with alanines. This was expected to have an even larger effect on formation of the coiled coil structure. We then tested the ability of the mutated S-tagged coiled coil fragments to interact with full-length NUDF protein directly. Plasmids containing the mutant coiled coil fragment genes were transformed into <i>A. nidulans</i> (GR5), and their S-tagged protein products were purified on S-protein beads and eluted with S-peptide as above. The amount of full-length NUDF protein that bound to the S-tagged proteins and protein fragments was determined by SDS-PAGE (Fig. 8).

Even though the mutated fragments were overproduced in relation to the unmutated coiled coil fragment, they pulled down much less of the full-length NUDF protein than the unmutated fragment, and as expected, the Ala<sup>a+d</sup> fragment pulled down an even smaller amount of NUDF protein than the L72E fragment. This experiment together with the previous experiments suggest strongly that the coiled coil interaction is largely responsible for formation of the NUDF dimer.

Presumably NUDF exists <i>in vivo</i> in an equilibrium between the monomer and the dimer forms. Since the coiled coil region is involved in dimer formation, if dimer formation were required for NUDF function, mutations that interfere with the coiled coil interaction might be expected to inhibit growth. Accordingly, we have mutated the coiled coil region in ways expected to interfere with dimer formation and have assayed the effect of these mutations on the ability of the mutated proteins to support growth in the absence of endogenous NUDF. Specifically we have asked whether full-length NUDF proteins bearing the Ala<sup>a+d</sup> and L72E mutations are able to complement the growth defect of the temperature-sensitive nudF6 mutation at restrictive temperature. When transformed into <i>A. nidulans</i> the wild-type nudF gene fully complemented the nudF6 mutation (Fig. 9). In contrast neither the Ala<sup>a+d</sup> nor the L72E mutant nudF genes were able to reverse the slow growth phenotype of nudF6. These data suggest strongly that NUDF must be able to form a dimer to function.

**DISCUSSION**

In this paper we describe the affinity purification of NUDF protein in one step from a crude cell-free extract of <i>A. nidulans</i>. We also show that the purified protein is a dimer. This finding agrees with prior sucrose gradient sedimentation experiments on crude extracts of <i>A. nidulans</i>, which showed that NUDF sediments more rapidly than expected from its predicted molecular mass of 49 kDa (11), indicating that dimer formation was not an artifact of purification. It is also consistent with a previous study of LIS1, the mammalian homolog of NUDF, which suggested, but did not demonstrate conclusively, that LIS1 is a dimer (24). Our data show not only that the predicted N-terminal coiled coil domain of NUDF is the primary determinant of dimer formation, but that dimerization is required for NUDF function. The evidence is 3-fold. We have shown that overexpression of the coiled coil region acts as a dominant negative, as would be expected if it interfered with the interaction between NUDF coiled coil domains during dimer formation. This experiment by itself is ambiguous as we cannot rule out the possibility that growth inhibition might result from an interaction between the coiled coil domain and some other protein required for growth. It nevertheless is consistent with the idea that NUDF functions as a dimer <i>in vivo</i>. We have also shown directly that the coiled coil domain is involved in the interaction between the NUDF monomers by demonstrating that it binds tightly to the full-length NUDF protein, whereas the WD-40 domain does not. Finally, we have shown that mutations in the coiled coil region, which prevent the coiled coil interaction, fail to complement a temperature-sensitive loss of function nudF mutation. It is possible that preventing the N-terminal domain from forming a coiled coil blocks its interaction with some protein other than NUDF. It seems more likely, however, that the inability of the Ala<sup>a+d</sup> and L72E mutant proteins to support growth is the result of their inability to form a NUDF dimer.

The identification of NUDF as a dimer adds additional support to the idea that NUDF and LIS1 are very similar proteins and strongly suggests that LIS1 may also need to dimerize to function, particularly in light of the evidence suggesting that LIS1 is also a dimer (24). Because LIS1 communoprecipitates with components of dynein and its structure resembles that of the mammalian cytoplasmic dynein intermediate chains, it and NUDF could be variant intermediate chains (22). The fact that NUDF is a dimer is consistent with such a role, as there are two copies of the heavy and intermediate chains per cytoplasmic dynein complex. The intermediate chains of cytoplasmic dynein bind to the N-terminal region of the molecule, and they interact with dynactin (22). However, there is as yet no convincing evidence that either LIS1 or NUDF interacts directly with the dynein heavy chain.

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**REFERENCES**

1. Minke, P. F., Lee, I. H., Tinsley, J. H., Bruno, K. S., and Plamann, M. (1999) <i>Mol. Microbiol.</i> 32, 1065–1076
2. Plamann, M., Minke, P. F., Tinsley, J. H., and Bruno, K. S. (1994) J. Cell Biol. 127, 139–149
3. Tinsley, J. H., Minke, P. F., Bruno, K. S., and Plamann, M. (1996) Mol. Biol. Cell 7, 731–742
4. Morris, N. R. (2000) J. Cell Biol. 146, 1097–1101
5. Osmani, A. H., Osmani, S. A., and Morris, N. R. (1999) J. Cell Biol. 111, 543–551
6. Xiang, X., Beckwith, S. M., and Morris, N. R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2100–2104
7. Beckwith, S. M., Roghi, C. H., Liu, B., and Morris, N. R. (1998) J. Cell Biol. 143, 1239–1247
8. Xiang, X., and Morris, N. R. (1999) Curr. Opin. Microbiol. 2, 636–640
9. Xiang, X., Liu, B., Winklemann, D., Zhuo, W., and Morris, N. R. (2000) Curr. Biol. 10, 603–606
10. Hirotsune, S., Fleck, M. W., Gambello, M. J., Bix, G. J., Chen, A., Clark, G. D., Ledbetter, D. H., McEain, C. J., and Wynshaw-Boris, A. (1998) Nat. Genet. 19, 333–339
11. Xiang, X., Osmani, A. H., Osmani, S. A., Xin, M., and Morris, N. R. (1995) Mol. Biol. Cell 6, 297–310
12. Efimov, V. P., and Morris, N. R. (2000) J. Cell Biol. 150, 681–688
13. Kitagawa, M., Umezu, M., Aoki, J., Koizumi, H., Arai, H., and Inoue, K. (2000) FEBS Lett. 479, 57–62
14. Morris, S. M., Albrecht, U., Reiner, O., Eichele, G., and Yu-Lee, L. Y. (1998) Curr. Biol. 8, 603–606
15. Williams, D. A., Liu, B., Xiang, X., and Morris, N. R. (1997) Mol. Gen. Genet. 255, 194–200
16. Lei, Y., and Warrior, R. (2000) Dev. Biol. 226, 57–72
17. Liu, Z., Xie, T., and Steward, R. (1999) Development (Camb.) 126, 4477–4488
18. Liu, Z, Steward, R., and Luo, L. (2000) Nat. Cell Biol. 2, 776–783
19. Swan, A., Nguyen, T., and Suter, B. (1999) Nat. Cell Biol. 1, 444–449
20. Faulkner, N. E., Dujardin, D. L., Tai, C.-Y., Vaughan, K. T., O’Connell, C. B., Wang, Y., and Vallee, R. B. (2000) Nat. Cell Biol. 2, 784–791
21. Smith, D. S., Nethammer, M., Ayala, R., Zhou, Y., Gambello, M. J., and Wynshaw-Boris, A., and Tsai, L.-H. (2000) Nat. Cell Biol. 2, 767–775
22. Vallee, R. B., Faulkner, N. E., and Tai, C. (2000) Biochim. Biophys. Acta 1496, 89–98
23. Vallee, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8769–8772
24. Garcia-Higuera, I., Fenoglio, J., Li, Y., Lewis, C., Panchenko, M. P., Reiner, O., Smith, T. F., and Neer, E. J. (1996) Biochemistry 35, 13985–13994
25. Kim, J. S., and Raines, R. T. (1993) Protein Sci. 2, 348–356
26. Waring, R. B., May, G. S., and Morris, N. R. (1989) Gene (Amst.) 79, 119–130
