Molecular Biology of the Cell
Vol. 6, 297–310, March 1995

NudF, a Nuclear Migration Gene in *Aspergillus nidulans*, Is Similar to the Human *LIS-1* Gene Required for Neuronal Migration

Xin Xiang,* Aysha H. Osmani,† Stephen A. Osmani,† Mei Xin,* and N. Ronald Morris*‡

*Department of Pharmacology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854–5635; and †Weis Center for Research, Geisinger Clinic, Danville, Pennsylvania 17822

Submitted December 12, 1994; Accepted February 13, 1995
Monitoring Editor: David Botstein

During a study of the genetics of nuclear migration in the filamentous fungus *Aspergillus nidulans*, we cloned a gene, *nudF*, which is required for nuclear migration during vegetative growth as well as development. The NUDF protein level is controlled by another protein NUDC, and extra copies of the *nudF* gene can suppress the *nudC3* mutation. *nudF* encodes a protein with 42% sequence identity to the human LIS-1 (Miller-Dieker lissencephaly-1) gene, which is required for proper neuronal migration during brain development. This strong similarity suggests that the LIS-1 gene product may have a function similar to that of NUDF and supports previous findings to suggest that nuclear migration may play a role in neuronal migration.

INTRODUCTION

Nuclear migration is essential for proper growth, development, and cellular function in both higher and lower eukaryotes. For example, during mouse embryo development, the peripherally located nuclei migrate toward the base of each cell before the 8-cell stage; this displacement may be critical for the generation of blastomeres of different sizes in the 16-cell embryo (Reeve and Kelly, 1983). Intermitotic nuclear migration occurs during brain epithelial cell proliferation, and is believed to mediate epithelial folding (Smith and Schoenwolf, 1988). Another very well known nuclear migration phenomenon occurs during early embryonic development in *Drosophila melanogaster*, when nuclei migrate from deep within the egg to the cortex before cellularization (Zalokar and Erk, 1976). In muscle cells, nuclei migrate to form clusters beneath acetylcholine receptors in neuromuscular junctions, a process thought to play a role in maintaining the position of the receptors (Englander and Rubin, 1987). In virus-induced cell syncytia, nuclei migrate and assemble into tightly packed rows (Wang *et al.*, 1979). Nuclear migration is also required for the fusion of pronuclei in eggs during fertilization (Schatten, 1982) and for karyogamy in fungi (Dutcher and Hartwell, 1983; Rose and Fink, 1987).

Although nuclear migration is involved in many biological processes, the mechanisms and controls of nuclear migration are only beginning to be understood. Microtubules have been shown to be required for nuclear migration in a wide variety of plants and animals (Doonan *et al.*, 1986; Lloyd *et al.*, 1987) as well as in *A. nidulans* (Oakley and Morris, 1980) and *Saccharomyces cerevisiae* (Palmer *et al.*, 1992). The astral microtubules, which extend from the spindle pole into the cytoplasm in *S. cerevisiae*, have been specifically identified as essential for this process (Palmer *et al.*, 1992). Cytoplasmic dynein, a microtubule-dependent, minus end–directed motor (reviewed by Vallee, 1993), apparently provides the motive force for vegetative nuclear migration. The heavy chain of cytoplasmic dynein has been shown to be required for nuclear migration during vegetative growth in *A. nidulans*, *Neurospora crassa*, and *S. cerevisiae* (Eshel *et al.*, 1993; Li *et al.*, 1993; Plamann *et al.*, 1994; Xiang et al. 1994). Actin-related protein in the dynactin complex (reviewed by Vallee, 1993; Schroer, 1994) is also required

‡ Corresponding author.

for nuclear migration in *N. crassa* and *S. cerevisiae* (Li *et al.*, 1994; Plamann *et al.*, 1994). The yeast KAR3 protein, which is also a minus end–directed motor (Endow *et al.*, 1994), catalyzes the migration involved in nuclear fusion during mating (Meluh and Rose, 1990). We have initiated a study of nuclear migration using the multinuclear, filamentous fungus *A. nidulans* as a model system. In *A. nidulans*, nuclei migrate actively into the growing germ tube in a microtubule-dependent fashion (Oakley and Morris, 1980, 1981). We have isolated a set of mutants in *A. nidulans* that are defective in nuclear migration and distribution (Morris, 1976 and our unpublished data). When these mutant cells are incubated at the restrictive temperature of 42°C, the nuclei divide but fail to migrate into the growing germ tube, forming nuclear clusters in the spore end of the germ tube. If the mutants are germinated at permissive temperature followed by a shift to restrictive temperature, they develop nuclear clusters at various locations along the germ tube, leaving long stretches of mycelium anucleate (Morris, 1976). Two *nud* genes, *nudA* and *nudC*, have been previously cloned by complementation of their temperature-sensitive mutant phenotypes. The *nudA* gene sequence showed that it encodes a cytoplasmic dynein heavy chain (Xiang *et al.*, 1994). This result provides direct evidence that cytoplasmic dynein is involved in nuclear migration. The *nudC* gene encodes a protein of 22 kDa with no obvious homology to any protein whose function is known (Osmani *et al.*, 1990). However, it does exhibit strong sequence similarity to an expressed sequence tag of *Caenorhabditis elegans* in the GenBank database. Sequences resembling *nudC* have also been identified in *Drosophila* (J. Cunniff and R. Warrior, personal communication) and rat (Y-L. Li, personal communication), suggesting that its function is evolutionarily conserved. In this report, we describe the phenotype of the *nudF* mutants, the cloning and sequencing of the *nudF* gene, and show that it is an extracopy suppressor of the *nudC3* mutation. We also demonstrate that the NUDF protein level declines in the *nudC3* mutant grown at restrictive temperature, and that this also occurs when the *nudF* gene expression is induced from an exogenous promoter, indicating that the change in NUDF protein level in the *nudC3* mutant is not dependent on transcription from the *nudF* promoter. The sequence of the NUDF protein is similar to the Miller-Dieker lissencephaly (LIS-1) gene product (Reiner *et al.*, 1993) with an identity of 42% throughout the whole protein. This result suggests that the Miller-Dieker lissencephaly (LIS-1) gene product may have a function similar to that of the NUDF protein.

MATERIALS AND METHODS

Strains Used

Strains used were: AO1 (*nudC3; pabaA1; wA2; nicA2; pyrG89*); SH2E9 (*nudF6; riboA1; yA2; nicA2*); BB22A (*nudF7; pabaA1; yA2*); Uvts768 (*nudC3; adE20; biA1; wA2; cnxE; sC12; methG1; nicA2; lacA1; choA1; chaA1*); Uvts320 (*nudA2*; the other markers are the same as *Uvts768*); XX3 (*nudA1; chaA1; pyrG89*); XX20 (*nudF6; pyrG89*); XX21 (*nudF7; pyrG89; yA2*); SJ002 (*pyrG89*); FGSC122 (*riboA1; nicB8; yA2*); XX19 (*nudA2; pyrG89; chaA1; nicA2* and/or *nicB8*); XX8 (*nudA4; pyrG89; wA2; chaA1*); XX10 (*nudA5; pyrG89; wA2; chaA1*); XX24 (*pabaA1; yA1*); XX25 (*alcA(p)::nudF; nudC3; pabaA1; wA2; nicA2; pyrG89*); XX41 (*alcA(p)::nudF; pabaA1; chaA1*); GR5 (*pyrG89; wA2; pyroA4*).

Growth Media

YAG (Morris, 1976) + UU (0.12% uridine and 0.12% uracil) plates were used for colony growth. For some experiments, *Aspergillus* strains were grown in appropriately supplemented minimal medium (Waring *et al.*, 1989) containing either 20 g/l glucose, 10 ml/l glycerol,or 20 ml/l 95% ethanol. For the ts$^-$ *nud* mutants, permissive temperature for growth is 32°C and restrictive temperature is 42°C.

Nuclear Staining

For nuclear staining of the germlings, 10^6 asexual spores (conidia) were inoculated onto coverslips in a petri dish containing 30 ml YG (YAG without agar) + UU medium. After 8 h of incubation at 42°C, the cells were fixed and the DNA was stained with DAPI (4′, 6-diamidine 2-phenylindole dihydrochloride), and photographed using a Zeiss epifluorescence microscope. For nuclear staining of conidiophores, about 10^7 spores were mixed with 15 ml top agar, poured, and incubated at the semipermissive temperature of 38°C for 14.5 to 19.5 h. Two milliliters of DAPI fix (4% glutaraldehyde, 0.2% NP-40, 50 mM phosphate buffer, pH 6.5) was layered on top of the sporulating culture. Conidiophores were harvested by scraping the top carefully with a razor blade; they were then pipetted into a 1.5-ml tube and kept at 4°C. Two microliters was mixed with 80 μl of Citifluor, 20 μl H_2O, and 1 μl DAPI (2 μg/ml) before microscopic examination.

Southern Hybridization

Southern blots were prepared according to the alkaline blotting method described in the Bio-Rad instruction manual for ζ-Probe membranes (Bio-Rad, Chemical Division, Richmond, CA). The blots were hybridized with ^{32}P-labeled probe in 0.5 M $NaHPO_4$ (pH 7.2) and 7% sodium dodecyl sulfate (SDS) at 65°C overnight and washed with 40 mM NaHPO4 (pH 7.2) and 1% SDS at 65°C for 1 h.

Cloning and Sequencing of nudF

Aspergillus transformation, the plasmid library (Osmani *et al.*, 1987), the Charon 4a λ phage library (provided by Dr. W. E. Timberlake, Myco Pharmaceuticals, Cambridge, MA), and the cDNA library were as previously described (Osmani *et al.*, 1988). Subclones were made to identify the region with *nudC3* suppressor activity and with *nudF*-complementing activity. There is a subtle difference between the ability of a subclone to complement the *nudF* mutations and to suppress the *nudC3* mutation. To complement *nudF*, because transformation in *A. nidulans* is integrative, only a fragment large enough to repair the mutation by a double crossover or gene conversion event is required. In contrast, integration of a complete gene is required for extracopy suppression of *nudC3*. The 3.9-kb *Eco*RI fragment in the *nudF* region (see Figure 2) (which did not suppress *nudC3* because it only contains part of the *nudF* gene) was able to complement *nudF6* and *nudF7*, indicating that the *nudF6* and *nudF7* mutations lie within the 1.3-kb *Pst*I-*Eco*RI region (Figure 2). The 3.9-kb fragment was used to screen an *Aspergillus* cDNA library

(Osmani *et al.*, 1988), and four clones were obtained. One of them hybridized to the 2.6-kb *Eco*RI-*Pst*I region, and the other three hybridized to the 1.3-kb *Pst*I-*Eco*RI region (see Figure 2). The three cDNA clones in the *nudF* region that hybridized to the 1.3-kb *Pst*I-*Eco*RI fragment were analyzed further. Preliminary restriction digest and sequencing data showed that they represented overlapping sequences. Two of them contained the same 5′ end; the third one had a truncated 5′ end. Subcloning was performed using standard methods (Sambrook *et al.*, 1989). Double stranded sequencing was done using the Sequenase version 2.0 kit (United States Biochemical Corp. Cleveland, OH). Computer analysis of sequence data was performed using the University of Wisconsin GCG package (Devereux *et al.*, 1984).

Construction of the alcA(p):: nudF Strain

For the construction of the *alcA(p)::nudF* construct (named pX1), the 0.9-kb *Eco*RI-*Bam*HI fragment in 5′ of *nudF* cDNA was subcloned into pBluescript KS(+). Then the *Kpn*I-*Bam*HI fragment (which has restriction sites 5′ of the *Eco*RI site) was cloned into the *Kpn*I-*Bam*HI sites of the *pyr4*$^+$ plasmid pAL5 (Doonan *et al.*, 1991) downstream of the *alcA* promoter. To limit the work of screening transformants with this homologous integration event, the pX1 construct was transformed into the temperature sensitive *nudC3* strain AO1. Because extra copies of *nudF* can suppress *nudC3*, we anticipated that overexpression of a single copy of *nudF* should have the same effect. *Pyr*$^+$ transformants were gridded to ethanol and glucose plates and incubated at restrictive temperature to determine whether the *nudC3* phenotype was suppressed. One transformant (XX25), which appeared to be ts$^+$ on ethanol and ts$^-$ on glucose, was isolated, and its genomic DNA was analyzed by Southern hybridization. The *alcA* promoter can be induced by growth on ethanol to give high expression of the downstream gene and can be repressed by glucose. If glycerol is used as a carbon source, the promoter is neither induced nor repressed, giving an intermediate level of expression. Homologous integration of the *nudF* sequence on the pX1 plasmid into the genomic *nudF* sequence generates two copies of *nudF*, a truncated, presumably inactive *nudF* gene with its own promoter and a full length *nudF* gene under the control of the *alcA* promoter (Figure 6A). The DNA hybridization pattern confirmed that the truncated *nudF* gene had integrated into the *nudF* locus (Figure 6B). This strain is named "*alcA(p)::nudF/nudC3*." The *alcA(p)::nudF/nudC3* strain was ts$^-$ at 42°C on a glycerol plate. Therefore, on glycerol media, the *alcA* promoter gives a level of *nudF* expression sufficient for *nudF* gene function but insufficient to suppress the *nudC3* mutation. To obtain an *alcA(p)::nudF* strain without the *nudC3* mutation, we crossed *alcA(p)::nudF/nudC3* with a *pyrG89* uridine auxotrophic strain SJ002. The *pyr*$^+$ progeny (which contained the integrated plasmid) from the cross were gridded on the glycerol plates and incubated at 42°C. The progeny containing *alcA(p)::nudF* with the ts$^-$ *nudC3* mutation were expected to be temperature sensitive at 42°C whereas the *alcA(p)::nudF* strain without the *nudC3* mutation should grow like the wild type. The genotype was further confirmed by Western blot using affinity-purified NUDF antibody (see below).

NudF Antibody Production and Western Blotting

The 0.9-kb *Ssp*I-*Eco*RI fragment from the 5′ region of the *nudF* cDNA was cloned into the *Eco*RV-*Eco*RI sites of the plasmid pBluescript KS(+). The *Sal*I-*Eco*RI fragment was then isolated from this plasmid. This fragment, combined with the 0.6-kb *Eco*RI-*Hin*dIII fragment at 3′ of the *nudF* cDNA, was ligated to the *Sal*I-*Hin*dIII digested vector pUR290 (for the production of *lacZ* fusion protein) (Sambrook *et al.*, 1989) or pATH2 (for the production of *typE* fusion protein) (Koerner *et al.*, 1991). *LacZ-nudF* and *TrpE-nudF* fusion proteins were induced and purified as previously described (Sambrook *et al.*, 1989; Koerner *et al.*, 1991). Both fusion proteins contain the full length *nudF* sequence minus the first three 5′ amino acids. The *LacZ-nudF* fusion protein was used to inject rabbits (Hazelton, Denver, PA) and the *trpE-nudF* fusion protein was used to purify the antiserum using the nitrocellulose absorption method (Sambrook *et al.*, 1989). Mycelial protein preparation (Mirabito and Morris, 1993) and Western blotting (Sambrook *et al.*, 1989) were performed as previously described. To show that the NUDF antibody recognizes the NUDF protein in *A. nidulans*, we used the *AlcA(p)::nudF* strain as a control. The NUDF protein level increases in the inducing medium and decreases in the repressing medium, as shown by Western blotting using affinity-purified NUDF antibody.

Sucrose Density Gradient Centrifugation

Wild-type *Aspergillus* cells were grown at 37°C for 20 h, and total protein was isolated (Mirabito and Morris, 1993) using Tris/KCl buffer (Collins and Vallee, 1989) with 1% NP-40 and 2 μg/ml each of soybean trypsin inhibitor, pepstatin, leupeptin, and aprotinin (Sigma Chemical, St. Louis, MO). Four milligrams of total protein was loaded on an 11-ml linear 5–20% sucrose gradient in Tris/KCl buffer (Collins and Vallee, 1989). Marker proteins of known S values were run in parallel. These were cytochrome *c* (2.1 S, 12.5 kDa), bovine serum albumin (4.4 S, 68 kDa), aldolase (8.5 S, 158 kDa), and catalase (11.2 S, 240 kDa) (Sigma Chemical). The gradients were centrifuged at 35,000 rpm for 16 h in an SW-41 rotor (Beckman Instruments, Fullerton, CA) at 4°C and seventeen 0.65-ml fractions were taken from the top of the centrifuge tube. The absorbance values at 280nm were determined for the fractions. The fractions containing cytochrome *c* and catalase can also be observed directly because of their colors.

RESULTS

Identification of a New nud Locus: nudF

Two new ts$^-$ (temperature sensitive) nuclear distribution (*nud*) mutants, were isolated after UV mutagenesis. Both showed severely reduced growth at the restrictive temperature of 42°C. After outcrossing to a wild-type strain, both appeared to be phenotypically similar to other previously isolated *nudA* and *nudC* mutants at 42°C. One of them, *nud6*, showed partially reduced growth even at 32°C whereas the other, *nud7*, grew as well as wild type at 32°C. The colonies of *nud6* and *nud7* produced at 42°C were minute and without conidia (asexual spores). Examination under the microscope of spores germinated at 42°C showed that the nuclei can divide, but they fail to migrate, leading to a cluster of nuclei in the spore end of the germ tube (Figure 1). The length of the germ tubes is similar in the mutants and the wild-type cells after 8 h of incubation at 42°C. Some of the mutant cells have one germ tube and some, like the mutant cell in Figure 1A, have two germ tubes. The *nud* mutants appear to branch more frequently than wild-type cells during further hyphal growth, presumably as compensation for the inability of the nonmigrating nuclei to support the growth of distant hyphal tips. Although not quantitated, the migration of mitochondrial DNA (visualized by DAPI staining) into the germ tube does not seem to be impaired.

Nud6 and *nud7* are very tightly linked and are probably allelic. Crosses between *nud6* and *nud7* were of low fertility, yielding cleistothecia with very few or no ascospores. However, one fertile cleisto-

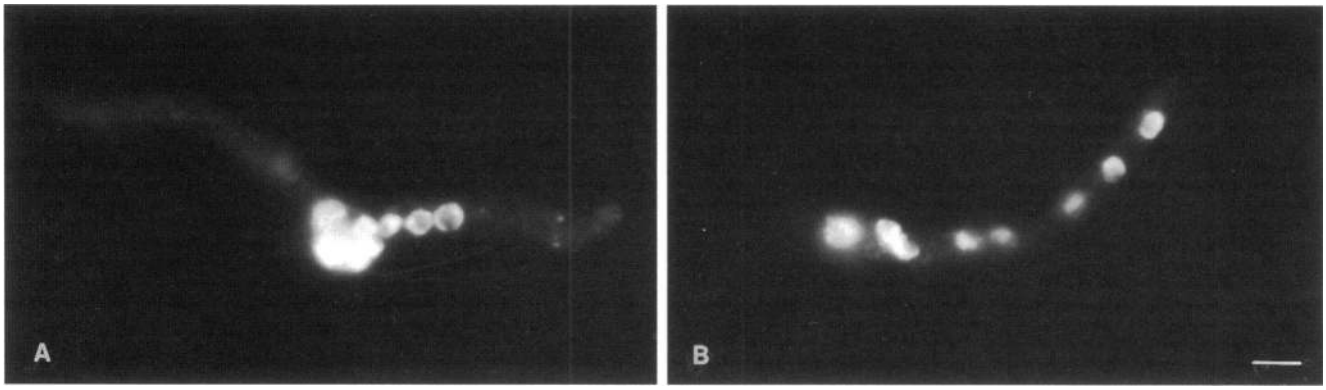

Figure 1. The phenotype of *nud7* mutant germlings at 42°C. Nuclear staining of a *nud7* mutant cell (A) and a wild-type cell (B) germinated at 42°C for 8 h. Bar, approximately 5 μm. This mutant cell has produced two germ tubes. The original spore is represented by the swollen region filled with nuclei between the two germ tubes.

thecium from the cross of *nud6* to *nud7* gave a normal number of ascospores. From this cleistothecium, no ts^+ progeny were produced of 1284 progeny examined, although other parental markers segregated normally. The *nud6* and *nud7* mutants were crossed to strains carrying temperature sensitive *nudA* or *nudC* mutations (Table 1). Approximately 25% ts^+ progeny were produced from either *nud6* or *nud7* crossed with *nudA1* or *nudC3*, indicating that *nud6* and *nud7* mutations are not allelic to *nudA* or *nudC*. We concluded from these results that we had identified a new *nud* locus, which we named *nudF*. The two new *nud* mutants were accordingly designated *nudF6* and *nudF7*.

Molecular Cloning of the nudF Gene as an Extra Copy Suppressor of nudC3

During the cloning of the *nudC* gene, a transformant (TR7) was isolated that grew better than the *nudC3* mutant strain but not as well as the wild type at restrictive temperature. It also failed to conidiate at restrictive temperature. A plasmid (TR7.SC) was recovered from the transformant. The *Aspergillus* genomic insert from this plasmid did not hybridize even at low stringency with the DNA fragment containing the wild-type *nudC* gene, indicating that the sequence was unrelated to *nudC*. TR7.SC partially reverts the temperature sensitivity of *nudC3*, so we concluded that it contains an extracopy suppressor of *nudC3*. To determine whether the *nudC3* extracopy suppressor could also suppress or complement the *nudA* and/or *nudF* mutations, we transformed the plasmid TR7.SC into *pyrG89* ts^- *nudA1* (XX3), *nudA2* (XX19), *nudA4* (XX8), *nudA5* (XX10), *nudF6* (XX20), and *nudF7* (XX21) strains. Ten of ten pyr^+ transformants from each *nudA* strain were ts^-, indicating that the *nudC3* extracopy suppressor cannot suppress the *nudA* mutations. However, transforming TR7.SC into *nudF6* and *nudF7* gave rise to 9 of 10 or 10 of 10 ts^+ transformants. These ts^+ transformants grew and conidiated as well as wild-type cells at the restrictive temperature, suggesting that the TR7.SC plasmid contained the sequence of the *nudF* gene. We showed that the TR7.SC plasmid contains the *nudF* sequence by demonstrating that it integrates site specifically at the *nudF* locus. The recombinatory event leading to site-specific plasmid integration generates a tandem duplication having one wild-type and one mutant sequence flanking the plasmid sequence. Recombination between the tandemly duplicated sequences causes the plasmid sequence and either the wild-type or the mutant sequence to be lost. It is therefore expected to generate both wild-type and mutant segregants. Two strongly growing ts^+ transformants of *nudF6* were treated with 5-fluoroorotic acid (FOA, 1 g/l) to select for loss of the integrated *pyr4* plasmid marker. Both ts^+ and ts^- segregants were found in each case. To show that the integrated locus was identical with the *nudF* locus, we crossed two of the ts^+ segregants with a wild-type strain. If integration had been at a

Table 1. Crosses between different *nud* mutants

Cross	Ts^+ progeny (%)
nud6 × *nudC3*	27.2
nud6 × *nudA1*	24.3
nud7 × *nudC3*	20.0
nud7 × *nudA1*	28.1
nud6 × *nud7*	0

Crosses between the two new *nud* mutants *nud6* and *nud7* and with *nudA* and *nudC* mutants. 1284 progeny were analyzed for the cross between *nud6* and *nud7* to be *more* sure that they are allelic. About 50 to 100 progeny were analyzed for the other crosses.

locus other than *nudF*, e.g. at an extragenic suppressor locus, these crosses would have generated both ts^- and ts^+ progeny. In fact all progeny (432 from one cross and 708 from another) were ts^+, indicating that the TR7.SC sequence had integrated site specifically into the *nudF* gene. Similar results were obtained from the *nudF7* transformants, confirming that the two new *nud* mutants, *nudF6* and *nudF7*, are allelic. Overall, this evidence shows that the extracopy suppressor of *nudC3* is *nudF*.

TR7.SC was used as a probe to screen a wild-type *Aspergillus* genomic library, and three clones were obtained, named λ3, λ5, and λ11 (see Figure 2). All three of these λ clones were able to complement the *nudF6* and *nudF7* mutations, and partially suppress the *nudC3* mutation. Subclones of λ3 were made and transformed into the mutant strains. The results are summarized in Figure 2. The smallest fragment (2.3 kb *Pst*I-*Eco*RI*) that suppressed the *nudC3* mutation also complemented both the *nudF6* and *nudF7* mutations. One of the longer cDNA clones in this region (see MATERIALS AND METHODS) was able to complement the *nudF6* and *nudF7* mutations, confirming that it represented the *nudF* gene.

The nudF6 and nudF7 Mutations Affect Both Asexual and Sexual Development of A. nidulans

At restrictive temperature the *nudF* mutants form minute colonies that lack conidia (asexual spores). At permissive temperature (32°C), strains carrying the *nudF7* allele conidiate normally, but those carrying the *nudF6* allele show little or no conidiation. The conidiation (asexual spore development) defects of both alleles were fully remedied in the ts^+ *nudF* transformants, demonstrating that the conidiation defect is caused by the *nudF* mutations. To quantitate the conidiation defect of the *nudF* mutants, pairs of ts^- and ts^+ FOA segregants from the ts^+ *nudF7* transformants (see above) were point inoculated on nutrient agar and allowed to form a mycelial mat at the permissive temperature. Except

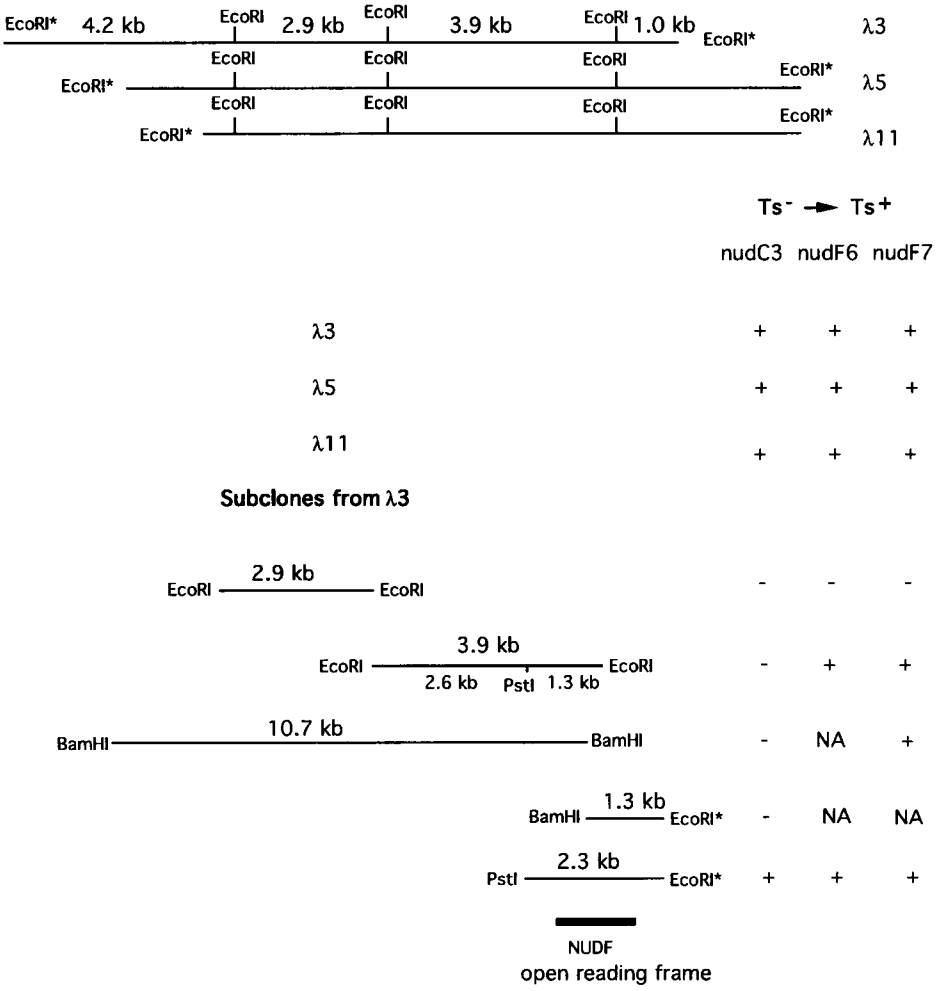

Figure 2. The genomic map of the *nudF* locus and the results of complementation of the *nudF* mutants with different genomic subclones. λ3, λ5, and λ11 are three λ clones. "EcoRI*" is the site in the phage vector λ Charon 4A. The rest of the subclones are in plasmid pBluescript. The ability to complement the mutation is presented as "+". Inability to complement the mutation is presented as "−". "NA" means "not attempted". The position of the *nudF* open reading frame is indicated as a black bar.

Table 2. Number of ascospore and conidia from *nudF* mutants

	conidia × 10^{-4}/0.2 cm^2		ascospores × 10^{-3}/cleistothecium			
	nudF7		nudF6		nudF7	
2	ts$^-$	ts$^+$	ts$^-$	ts$^+$	ts$^-$	ts$^+$
1	7	450	0	84	1.4	40
2	11	500	0	77	0.2	70
3	35	800	0.2	29	9.6	28

The effect of *nudF* on conidiation and cleistotheciation. Isogenic ts$^+$ and ts$^-$ FOA sectors from the *nudF* transformants (see RESULTS) were allowed to form mycelial mats at permissive temperature. After 2 days, the plates were shifted to a semi-restrictive temperature of 37°C and sealed for 10 days to allow cleistothecia formation. Cleistothecia from ts$^+$ and ts$^-$ colonies were analyzed for their content of ascospores. All of the conidia from a 0.2 cm^2 area were harvested into 1 ml 0.1% Tween 80 and counted in a Haemocytometer chamber. The data are from three different colonies. The ascospore number was determined by crushing a cleistothecium into 20 μl of water, and counted the same way. The ascospore numbers are from three different cleistothecia.

for the presence or absence of the *nudF* mutation, these paired segregants are isogenic and grow equally well at the permissive temperature of 32°C. After 30 h, the cultures were shifted to the restrictive temperature of 42°C. The effect on conidiation was monitored by harvesting and counting conidia from equal areas of both ts$^+$ and ts$^-$ colonies. Conidiation was decreased (Table 2) more than 20-fold in the *nudF7* mutant strain.

These data suggest, but do not demonstrate, that nuclear migration is important for the development of asexual spores (conidia) in *A. nidulans*. The process of conidiation has been thoroughly described in *A. nidulans* (Timberlake and Marshall, 1988) and is illustrated by Figure 3. The first step in conidiation in the wild type is the differentiation of specialized cells, called foot cells, in the mycelial mat. Each foot cell generates a thickened aerial hypha or stalk, that swells to form a vesicle at the tip (Figure 3, a and g). Nuclei undergo several divisions and increase their number many fold as they migrate into the vesicle. The mature vesicle then synchronously produces a layer of buds (Figure 3, b and h). A single nucleus migrates from the vesicle into each bud, forming the uninucleate primary sterigmatum or metula (Figure 3, c and i). Each metula then produces another bud at its tip (Figure 3, d and j). The metular nucleus divides once and one daughter nucleus moves into the secondary sterigmatum, or phialide (Figure 3, e and k). The phialide then produces a string of as many as 100 conidia by repeated budding and nuclear division. As each conidial bud is formed at the tip of the phialide, the nucleus of the phialide divides and sends one daughter nucleus into the bud, which then seals off to form a conidium (Figure 3, f and l).

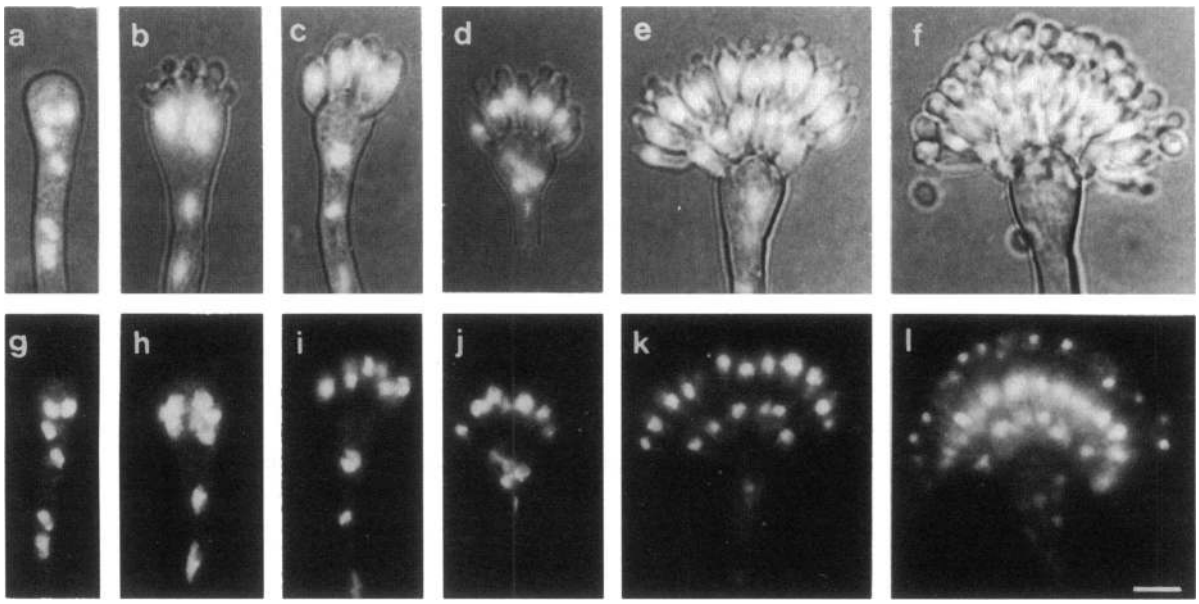

Figure 3. The developmental process of wild-type conidiophores. Different stages of conidiophore were stained with DAPI and observed by phase plus fluorescence (a–f) or fluorescence (g–l) microscopy. (a and g) Vesicle swelling at the tip of the conidiophore. (b and h) Initiation of metular buds. (c and i) Fully developed, nucleated metular buds. (d and j) Initiation of phialide buds on nucleated metulae. (e and k) Fully developed phialides and the initiation of conidial buds on nucleated phialides. (f and l) Formation of conidia. Bar, approximately 5 μm.

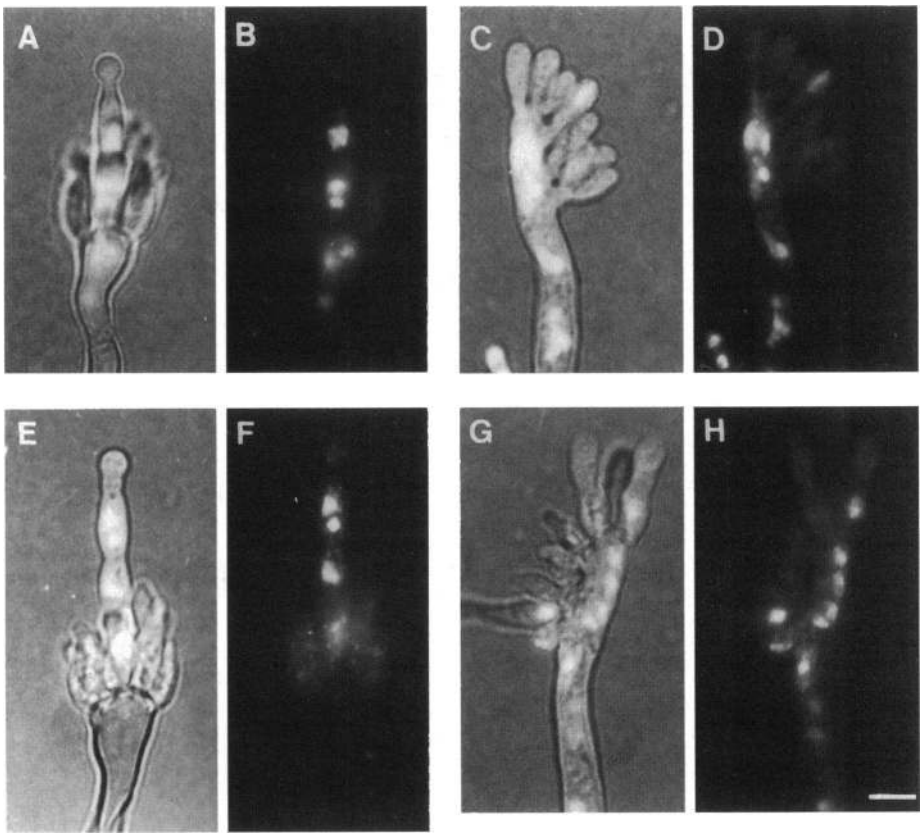

Figure 4. The abnormal conidiophores of the *nudF7* mutant at the semipermissive temperature of 38°C. Conidiophores were stained with DAPI and observed with phase plus fluorescence (A, C, E, and G) or fluorescence (B, D, F and H) microscopy. (A, B, E, and F) Conidiophores with mostly anucleate metulae; the one metula that received the nucleus continued the developmental pathway. (C, D, G and H) Abnormal conidiophores with asymmetrically developed metulae. Bar, approximately 5 μm.

Conidiophore development of the *nudF7* mutant is greatly retarded at the full restrictive temperature of 42°C, and is highly abnormal at the semipermissive temperature of 38°C (Figure 4). Although the timing of differentiation at 38°C is the same in the wild type fective in the mutant. Most of the metulae in the mutant strain are anucleate. The anucleate metulae fail to form fully developed phialides. If a nucleus leaks into the metula, the developmental sequence continues; however, the full sequence, ending with the production of a chain of conidia, is only rarely completed (Figure 4, A, B, E, and F). Occasionally, metular buds develop asymmetrically from the conidiophore vesicle (Figure 4, C, D, G, and H). This could be because the nuclei distribute asymmetrically in the vesicle. This suggests that the position of the nuclei in the vesicle determines the sites at which metulae bud. Alternatively, *nudF* may play a direct role in the determination of the sites of metular bud initiation.

Even at the permissive temperature of 32°C, the *nudF* mutants often gave rise to cleistothecia (spore sacs) with low ascospore (sexual spore) yields (Table 2). The low ascospore yield cosegregated with the *nudF* mutations.

NUDF Protein Level Declines in the nudC3, nudF6, and nudF7 Mutants

Extra copies of *nudF* can suppress the *nudC3* mutation, so it is likely that the functions of the NUDC and NUDF proteins are in some way connected. To examine whether the levels of NUDC and NUDF protein were affected by mutations in the *nudA*, *nudC*, or *nudF* genes, total cellular proteins were extracted from wild-type and mutant strains grown at restrictive temperature. Western blot analysis was performed using affinity-purified NUDC (Osmani *et al.*, 1990) and NUDF antibodies (see MATERIALS AND METHODS). The NUDF antibody recognized a 49-kDa protein, as predicted by the *nudF* sequence. The level of the NUDC protein was unaffected by the mutations in the *nudA*, *nudC*, and *nudF* genes; however, the level of the NUDF protein declined dramatically in the *nudC3*, as well as the *nudF6* and *nudF7* strains (Figure 5). Since a linear *nudF* wild-type cDNA fragment can complement both the *nudF6* and *nudF7* alleles, these mutations are not in the promoter region. Thus, the change in the NUDF protein level in the *nudF* mutants must be due to a posttranscriptional mechanism.

To examine whether the effect of the *nudC3* mutation on the level of NUDF is *nudF* promoter-de-

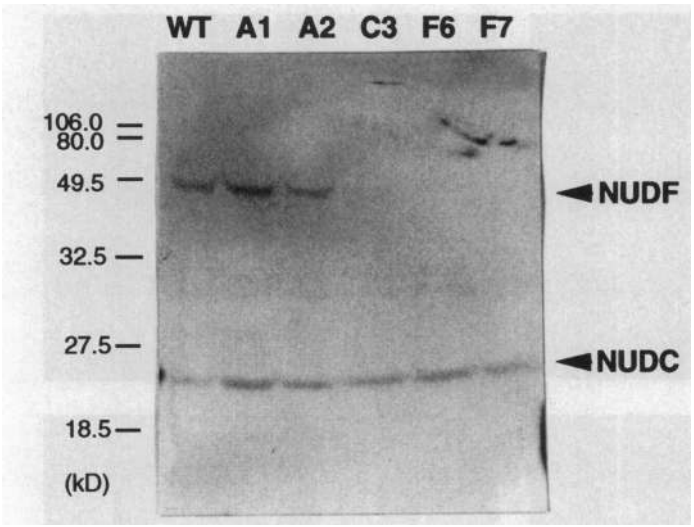

Figure 5. Western blot analysis of the NUDF protein level in different *nud* mutants. The wild type strain and the mutant strains were grown at restrictive temperature for 42 h. The inoculation was 10^6/50 ml for the wild type and 10^7/50 ml for the mutants. This blot was probed with affinity-purified NUDF antibody (1:100) and NUDC antibody (1:500) overnight. Approximately 30 μg protein was loaded in each lane. Equal loading of the different samples was insured by Coomassie blue staining of the gel and Ponceau S staining of the blot. WT: wild type; A1: *nudA1*; A2: *nudA2*; C3: *nudC3*; F6: *nudF6*; F7: *nudF7*.

pendent or not, we linked the *nudF* gene to an exogenous promoter and examined the NUDF protein level in the presence or absence of the *nudC3* mutation. This was done by using the strain *alc:A(p)::nudF* (see MATERIALS AND METHODS and Figure 6, A and B) in which the only copy of *nudF* is controlled by the *alcA* (alcohol dehydrogenase I) promoter (Waring *et al.*, 1989), which is induced by ethanol to give high expression of the downstream gene and is repressed by glucose. The amount of NUDF protein was dramatically reduced in the *alcA(p)::nudF* strain with glucose as carbon source (Figure 6C). The NUDC protein level was not affected by this reduction in the NUDF protein (Figure 6C).

We examined the NUDF protein level in *alcA(p)::nudF* strains with or without the *nudC3* mutation. The cells were grown at restrictive temperature for 40 h with glycerol as a noninducing and nonrepressing carbon source, and shifted to ethanol, the inducing medium, for 3 h. The result of the Western blot analysis is shown in Figure 6D. The *nudC3* mutation was still able to reduce the NUDF protein amount even when the *nudF* gene was up-regulated by the *alcA* promoter. Therefore, it is likely that the *nudC3* mutation affects the NUDF protein level in a posttranscriptional fashion. The *alcA(p)::nudF/nudC3* strain had a NUDF protein level similar to that of the wild type and higher than that of the *nudC3* strain (Figure 6D), consistent with our previous observation that extra-copies of *nudF* can suppress the *nudC3* mutation.

Microscopic examination of the glucose-grown *alcA(p)::nudF* cells showed a defect in nuclear migra-

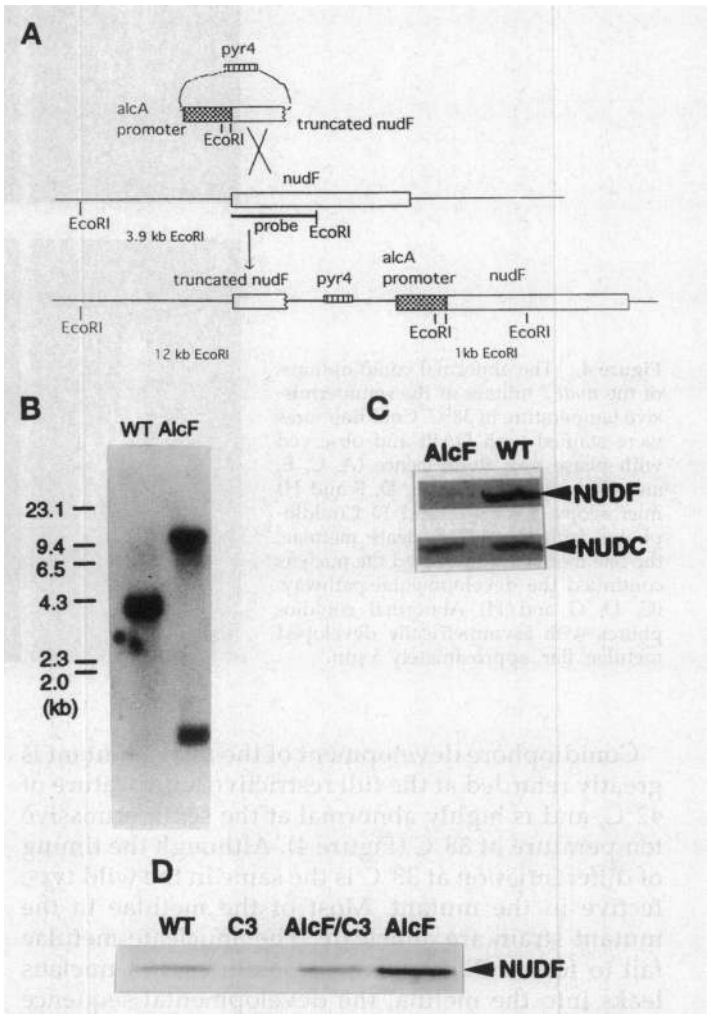

Figure 6. The *alcA(p)::nudF* strain. (A) Illustration of the plasmid integration event that leads to a single copy of the full length *nudF* gene under the control of the *alcA* promoter and to a tandem truncated copy of the endogenous *nudF* gene. (B) A Southern blot that demonstrates the plasmid integrated into the *nudF* locus. Genomic DNA from the wild type (WT) and the transformant strain (AlcF) were prepared and digested with *Eco*RI. Southern blot analysis of a site-specific integration was performed using the *Eco*RI fragment 5′ of the *nudF* cDNA as probe (black bar in A). (C) The Western blot shows that the *nudF* expression is repressed by glucose in the *alcA(p)::nudF* strain. This blot was probed with affinity-purified NUDF antibody and NUDC antibody. WT: wild type; AlcF: *alcA(p)::nudF*. (D) The Western blot shows the NUDF protein level in different strains. Strains were grown with glycerol as a carbon source for 40 h, then shifted to ethanol medium for 3 h before protein isolation. Approximately 30 μg protein was loaded in each lane. Equal loading of the different samples was insured by Coomassie blue staining of the gel and Ponceau S staining of the blot. The blot was probed with affinity-purified NUDF antibody. WT: wild type; C3: *nudC3*; AlcF/C3: *alcA(p)::nudF/nudC3*; AlcF: *alcA(p)::nudF*.

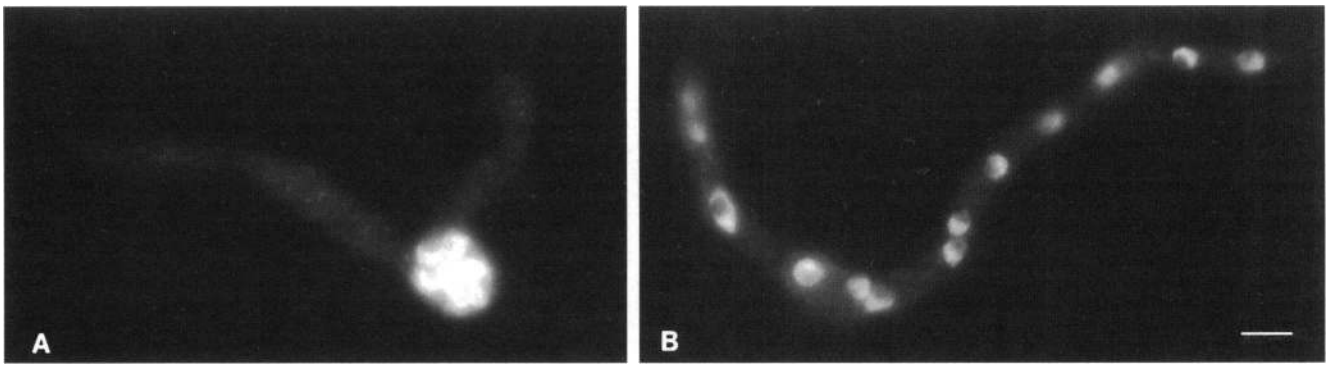

Figure 7. The phenotype of the *alcA(p)::nudF* strain. Nuclear staining of a *alcA(p)::nudF* cell (A) and a wild type cell (B) germinated at 37°C for 8 h in glucose medium. Bar, approximately 5 μm.

tion similar to that observed in the *nudF6* and *nudF7* mutants (Figure 7). This result, and the fact that the *nudF6* and *nudF7* mutations are recessive, suggests that they, like the *alcA(p)::nudF* grown on glucose, are loss of function mutations. We have not observed any obvious phenotype linked to *nudF* overexpression, as the *alcA(p)::nudF* strain grows as well as the wild-type strain on ethanol-containing plates, and microscopic examination of the *alcA(p)::nudF* strain with ethanol as a carbon source did not reveal any obvious defect in nuclear distribution.

NUDF and NUDC Proteins Do Not Cosediment in a Sucrose Gradient

One possible explanation of the fact that the NUDF protein level declines in the *nudC3* mutant is that NUDF and NUDC normally form a complex that is disrupted by the *nudC3* mutation, with consequent degradation of unpaired NUDF. To test this, we determined whether the wild-type NUDF and NUDC proteins cosediment in a sucrose gradient.

Experiments using Western blots to detect the location of the NUDF and NUDC proteins in sucrose gradients (Figure 8) showed that the NUDF protein sediments at about 7.8 S. The apparent mass of NUDF, as determined from its sequence and confirmed by SDS gel analysis, is 49 kDa. However, it sediments faster than bovine serum albumin, which has a mass of 68 kDa and a sedimentation coefficient of 4.4 S. This suggests that NUDF is sedimenting as a high molecular weight complex. However, the NUDC protein did not cosediment with the NUDF protein (Figure 8).

The nudF Gene Is Homologous to the Miller-Dieker Lissencephaly Gene

The sequence of *nudF* is shown in Figure 9. The *nudF* gene encodes a 444 aa protein with six β-transducin-like repeats (also called WD-40 repeats (Voorn and Ploegh, 1992); however, its sequence identity to the human and other mammalian β-transducins is only about 24% (Table 3). The sequence of the NUDF protein was found to be much more similar to that of the human Miller-Dieker lissencephaly syndrome gene (LIS-1) product (Figure 9B). The overall amino acid sequence identity and similarity between

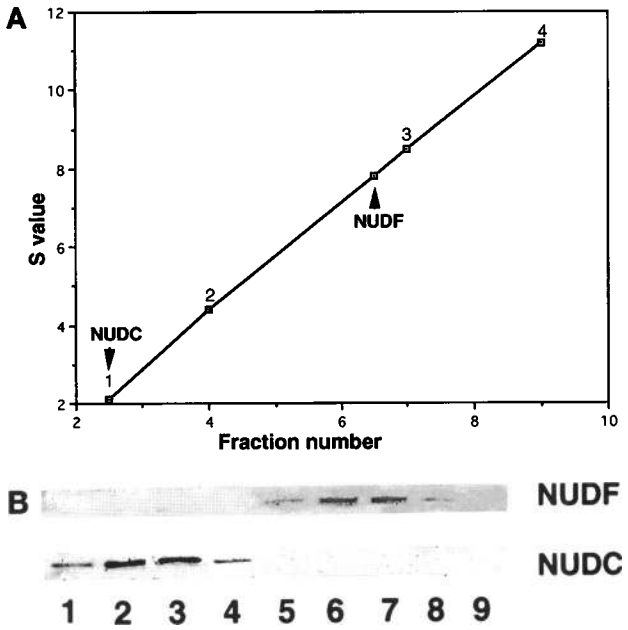

Figure 8. Analysis of the fractions of the sucrose gradients. (A) Standard curve with the position of NUDF and NUDC indicated. S value markers are cytochrome *c* (1), bovine serum albumin (2), aldolase (3), and catalase (4). (B) Western blot showing the fractions containing NUDF and NUDC proteins. The part of the blot that showed positive signals was presented. NUDF peaked at fractions 6 and 7 (we chose 6.5 as the fraction number to estimate its S value) and its estimated S value is about 7.8 S. NUDC peaked at fractions 2 and 3 (we chose 2.5 as the fraction number to estimate its S value) and its estimated S value is about 2.1 S.

A

```
  1  MSQILTAPQA EALHKAMLAY LSVINAPQTA ETLREELHFD ESYNEATCKK
 51  FEGVLEKKWT GIARLQRRIN DLEAEVRSLQ AELEASPSAA RAKNQDPTNW
101  LPKPSSTHTL TSHRDAVTCV AFHPVFTSLA SGSEDCTIKI WDWELGEIER
151  TLKGHIRGVS GLDYGGQKGN TLLASCSSDL TIKLWDPSKD YANIRTLSGH
201  DHSVSSVRFL TSNDNHLISA SRDGTLRIWD VSTGFCVKVI KSATESWIRD
251  VSPSFDGKWL VSGGRDQAIT VWEVSSAEPK AALLGHENFI ECCVFAPPAS
301  YEHLATLAGL KKPPPATSSC EFVATGARDK TIKLWEARGR LIKTLHGHDN
351  WVRGLVFHPG GKYLFSVSDD KTIRCWDLSQ EGRLVKTISG AHEHFVSCIR
401  WAPSPNTDNP DPAGEKAGKK DAVKPSYRCV IATGCADNSV RVFS
```

B

```
NUDF   M S Q I L T A P Q A E A L H K A M L A Y L S V I N A P Q T A E T L R E E L H F D E S Y N E A T C K K    50
LIS1   - - M V L S Q R Q R D E L N R A I A D Y L R S N G Y E E A Y S V F K K E A E L D - - V N E E L D K K    46

NUDF   F E G V L E K K W T G I A R L Q R R I N D L E A E V R S L Q A E L E A S P S A A R A K N Q D P T N W   100
LIS1   Y A G L L E K K W T S V I R L Q K K V M E L E S K L N E A K E E F - - T S G G P L G Q K R D P K E W    94

NUDF   L P K P S S T H T L T S H R D A V T C V A F H P V F T S L A S G S E D C T I K I W D W E L G E I E R   150
LIS1   I P R P P E K Y A L S G H R S P V T R V I F H P V F S V M V S A S E D A T I K V W D Y E T G D F E R   144

NUDF   T L K G H I R G V S G L D Y G G Q K G N T L L A S C S S D L T I K L W D P S K D Y A N I R T L S G H   200
LIS1   T L K G H T D S V Q D I S F - - D H S G K L L A S C S A D M T I K L W D - F Q G F E C I R T M H G H   191

NUDF   D H S V S S V R F L T S N D N H L I S A S R D G T L R I W D V S T G F C V K V I K S A T E S W I R D   250
LIS1   D H N V S S V A I M - P N G D H I V S A S R D K T I K M W E V Q T G Y C V K T F T G H R E - W V R M   239

NUDF   V S P S F D G K W L V S G G R D Q A I T V W E V S S A E P K A A L L G H E N F I E C C V F A P P A S   300
LIS1   V R P N Q D G T L I A S C S N D Q T V R V W V V A T K E C K A E L R E H E H V V E C I S W A P E S S   289

NUDF   Y E H L A T L A G L K K P P P A T S S C E F V A T G A R D K T I K L W E A R G R L - I K T L H G H D   349
LIS1   Y S S I S E A T G S E T K K S G K P G - P F L L S G S R D K T I K M W D V S T G M C L M T L V G H D   338

NUDF   N W V R G L V F H P G G K Y L F S V S D D K T I R C W D L S Q E G R L V K T I S G A H E H F V S C I   399
LIS1   N W V R G V L F H S G G K F I L S C A D D K T L R V W D Y K N K - R C M K T L - N A H E H F V T S L   386

NUDF   R W A P S P N T D N P D P A G E K A G K K D A V K P S Y R C V I A T G C A D N S V R V F S - - -       444
LIS1   D F H K T A - - - - - - - - - - - - - - - - - - - - P Y V V T G S V D Q T V K V W E C R -           410
```

Figure 9. The sequence of the *nudF* gene and the sequence comparison of NUDF to the Miller-Dieker lissencephaly gene product. (A) The deduced protein sequence of *nudF*. The coiled-coil domain is underlined. The position of the WD (or WE) at the end of each repeat is marked with bold characters. (B) Pretty plot (GCG program) demonstrating the homology between NUDF and the LIS-1 gene product.

NUDF protein and the LIS-1 gene product are 42% and 64%, respectively, which is much higher than that between either of these proteins and other WD-40 proteins (Table 3). Because authentic G-protein β-subunits have been noted to have a coiled-coil motif N-terminal to the WD-40 repeats (Lupas *et al.*, 1992), we analyzed the predicted *nudF* gene products for this motif using the program devised by Lupas *et al.* (1991). Amino acids 60–86 were identified as four repeats (heptads) of the coiled-coil motif.

DISCUSSION

The *nudF* gene was isolated serendipitously during the cloning of *nudC* as a sequence that partially suppressed the temperature sensitivity of the *nudC3* mutation. The ts$^-$ mutations in *nudF*, like ts$^-$ mutations in the other *nud* genes, inhibit nuclear migration in vegetative mycelia and during asexual spore formation. The *nudF* mutations also have a profound affect on sexual sporulation, presumably because coordinated nuclear migration is required for

Table 3. The percentage of sequence identity between "WD" proteins

	NUDF	LIS1	TUP1	CDC4	STE4	β-transducin
NUDF	—	42.1	22.1	25.5	21.8	24.0
LIS1	—	—	24.8	26.2	21.7	25.7
TUP1	—	—	—	22.5	25.1	24.3
CDC4	—	—	—	—	21.6	25.4
STE4	—	—	—	—	—	41.3
β-transducin						—

Percentage of identity between NUDF and the other WD proteins.

maintenance of dikaryotic ascogenous hyphae and/or for karyogamy.

An adequate level of *nudF* protein appears to be crucial for nuclear migration. In contrast to vegetative growth, spore production (both sexual and asexual) is sensitive to the *nudF* mutations even at permissive temperatures. This implies that the *nudF6* and *nudF7* mutations either reduce NUDF proteins at permissive temperature in spore-forming tissues to levels similar to those that block vegetative migration at restrictive temperature or that the processes of conidiation and ascosporogenesis are more sensitive to intermediate levels of reduction of NUDF protein. Our results also show that the effect of the *nudC3* mutation is possibly exerted via its effect on the level of NUDF protein. This suggested that the NUDF and NUDC proteins might form a complex; however, sucrose gradient sedimentation data do not support this hypothesis.

The sequence of the *nudF* protein contains WD-40 repeats and a putative coiled coil region N-terminal to the repeat, features commonly found in the β-subunits of heteromeric G-proteins (reviewed in Birnbaumer, 1990; Clapham and Neer, 1993; Conklin and Bourne, 1993). A data base search of translated protein sequences, looking for similarities to the NUDF protein, generated a long list of WD-40 proteins, of which the human LIS-1 sequence (Reiner *et al.*, 1993) showed a much higher degree of similarity to NUDF (42% identity) than any other gene. The WD-40 proteins (reviewed in Neer *et al.*, 1994), which include the yeast proteins *TUP1p, CDC4p, STE4p, CDC20p, PRP4p, Dictyostelium* protein *coronin* (reviewed in Voorn and Ploegh, 1992), plant protein *Cop1* (Deng *et al.*, 1992), and the mammalian protein TFIID subunit $dTAF_{11}80$ (Dynlacht *et al.*, 1993), are involved in a wide variety of biological processes. The *STE4* gene encodes a protein with 41% sequence identity to the human β-subunit of heterotrimeric G protein. This high sequence identity is not surprising because STE4 was shown by genetics to interact with SCG1 and STE18, which are homologous to the α and γ subunits of the heterotrimeric G protein. On the basis of the sequence analysis (41% identity between STE4 and β-transducin and 42% between NUDF and LIS-1), and the fact that the STE4 and the human β-transducin interact with similar proteins, it is reasonable to propose that NUDF and the LIS-1 gene product also interact with similar protein partners.

LIS-1 has been identified as the gene that is deleted in Miller-Dieker (Type 1) lissencephaly in human patients (Reiner *et al.*, 1993). Type I lissencephaly in humans is a neuronal migration disease characterized by a profound underdevelopment of the cerebral cortex as well as by olivary and cerebellar heterotopias. Patients may also exhibit an abnormal facial appearance, may have visceral abnormalities, are severely mentally retarded, and usually die young. The disease is thought to be caused by an arrest of neuronal migration at about 10 to 14 wk of gestation (Dobyns *et al.*, 1992).

Cytological observations have suggested that nuclear translocation is an essential feature of neuronal migration both in the cerebral cortex (Morest, 1970; Book and Morest, 1990) and in the cerebellum (Rakic, 1971). Neuronal migration in the cortex apparently occurs in two steps. First the neuron sends out a very long process toward its target position in the brain and then the nucleus with accompanying perikaryon translocate through this process. In the cerebellum, granule cell neurons extend a vertical process through the molecular layer, and the nucleus and perikaryon move downward within this process. Similar observations of nuclear translocation have also been made using brain explants in tissue culture (Book *et al.*, 1991) and by infrared video microscopy of living cerebellar slices (Hager *et al.*, 1994). This type of nuclear-involved cell locomotion also occurs during the translocation of human lung adenocarcinoma cells in tissue culture (Klominek *et al.*, 1991). If nuclear migration is essential to neuronal migration, it is not unreasonable to propose that a defect in nuclear migration is the basic cause of the defects in neuronal distribution that are observed in Miller-Dieker lissencephaly.

The sequence identity (42%) between the NUDF protein and the Miller-Dieker lissencephaly gene (LIS-1) is strikingly higher than that between either gene and the other known WD-40 proteins. The similarity between these sequences coupled with the similarity between the *nudF* mutant phenotype and the appearance of the lissencephalic brain suggests that *nudF* may be a functional homologue in *A. nidulans* of the LIS-1 gene in humans. We have shown that the effect of the ts *nudF* mutations is mediated via a deficiency of NUDF protein. Because Miller-Dieker lissencephaly is caused by a heterozygous deletion of the LIS-1 gene, the gene dosage effect presumably also leads to a deficiency of the LIS-1 protein in the brain. Interestingly, the neuronal migration defect is not absolute. Neurons do migrate in lissencephaly, albeit not all the way to their correct positions. On the basis of the differences in sensitivity to the *nudF* mutations

between different cell types (vegetative vs. sporulative) in *A. nidulans*, it is possible that different types of neurons in the brain could be differentially affected.

The biochemical mechanism by which the *LIS-1* gene product acts to mediate neuronal migration is not known. The recent finding, however, that the human *LIS-1* gene product is 99% identical in amino acid sequence to the 45-kDa subunit of bovine brain platelet-activating factor acetylhydrolase (PAFAH) has provided a potentially important clue (Hattori *et al.*, 1994). PAFAH inactivates platelet-activating factor (PAF; 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), which is known to act in other systems as a lipid second messenger (reviewed by Liscovitch and Cantley, 1994). PAFAH has been purified to homogeneity. It has three subunits of 29, 30, and 45 kDa (Hattori *et al.*, 1993). The 45-kDa subunit, when purified away from the other two subunits, has no detectable PAFAH activity. The catalytic activity is retained by the remaining 29/30 subunit complex. Thus, it has been proposed that the 45-kDa protein may be regulatory (Hattori *et al.*, 1994). The similarity of the NUDF amino acid sequence to the *LIS-1* and the PAFAH 45-kDa proteins suggests that the NUDF protein may function as part of an *A. nidulans* PAFAH enzyme.

Our genetic results show that *Aspergillus* cytoplasmic dynein is involved in nuclear migration (Xiang *et al.*, 1994). One possible function for NUDF protein is that it in some way affects cytoplasmic dynein. Cytoplasmic dynein is a 20 S multisubunit complex (Collins and Vallee, 1989). Experiments using Western blots to detect the location of NUDF protein in sucrose gradients indicate that the NUDF protein sediments at about 7.8 S. This does not rule out the possibility that NUDF regulates cytoplasmic dynein, but does make it unlikely that it is a component of the cytoplasmic dynein complex.

It is known that the cytoplasmic dynein motor moves along microtubules. Nuclear migration also requires a device to couple the nucleus to the motor. Evidence from yeast indicates that spindle pole microtubules are involved in nuclear migration (Palmer *et al.*, 1992) and may serve as part of the coupling between the nucleus and the motor protein. Therefore it is also possible that wild-type NUDF protein is required for proper microtubule assembly or function. Immunofluorescent staining of *nudC3* germlings with antitubulin antibodies has shown that the microtubules of the mutant are indistinguishable from those of the wild type (Osmani *et al.* 1990). Thus, if depletion of NUDF protein is affecting microtubules, the effect is subtle and not revealed as a structural change.

The sequence homology between the *A. nidulans* NUDF and the human LIS-1 gene products suggests that these two proteins may be involved in similar regulatory pathways. The notion that nuclear migration may play a role in the neuronal migration process provides an exciting potential connection between these two biological systems, which are evolutionarily so divergent. We know of no direct evidence, however, that a defect in nuclear migration causes the neuronal migration defect characteristic of lissencephaly. A defect in some other cytoskeletal transport mechanism that involves the LIS-1 gene product could equally well be the primary cause of the neuronal migration defect. Because of the rarity of lissencephaly and the difficulty of studying it in humans, we will probably have to wait for the development of a transgenic lissencephaly mouse model before the question of whether nuclear migration is affected by deletion of LIS-1 can be answered.

ACKNOWLEDGMENTS

We thank B. Burnside and S. Hughes for isolation of the *nudF* mutants; the Morris lab members, especially Drs. P. Mirabito, M. O'Connell, M. Mischke, M. Mahajan, and S. Beckwith for much technical advice during this project; and Dr. D. Willins for critical reading of the manuscript. This research was supported by the Institute of General Medical Sciences of the National Institutes of Health. The GenBank accession number of the *nudF* sequence is U22009.

REFERENCES

Birnbaumer, L. (1990). G proteins in signal transduction. Annu. Rev. Pharmacol. Toxicol. *30*, 675–705.

Book, K.J., Howard, R., and Morest, D.K. (1991). Direct observation in vitro of how neuroblasts migrate: medulla and cochleovestibular ganglion of the chick embryo. Exp. Neurol. *111*, 228–243.

Book, K.J., and Morest, D.K. (1990). Migration of neuroblasts by perikaryal translocation: role of cellular elongation and axonal outgrowth in the acoustic nuclei of the chick embryo medulla. J. Comp. Neurol. *297*, 55–76.

Clapham, D.E., and Neer, E.J. (1993). New roles for G-protein $\beta\gamma$-dimers in transmembrane signalling. Nature *365*, 403–406.

Clutterbuck, A.J. (1977). The genetics of conidiation in *Aspergillus nidulans*. In: Genetics and Physiology of *Aspergillus*, ed. J.E. Smith and J.A. Pateman, London, UK: Academic Press, 305–317.

Collins, C.A., and Vallee, R.B. (1989). Preparation of microtubules from rat liver and testis: cytoplasmic dynein is a major microtubule associated protein. Cell Motil. Cytoskeleton *14*, 491–500.

Conklin, B.R., and Bourne, H.R. (1993). Structural elements of Gα subunits that interact with G$\beta\gamma$, receptors, and effectors. Cell *73*, 631–641.

Deng, X.W., Matsui, M., Wei, N., Wagner, D., Chu, A.M., Feldmann, K.A., and Quail, P.H. (1992). Cop1, an *Arabidopsis* regulatory gene, encodes a protein with both a Zinc-binding motif and a Gβ homologous domain. Cell *71*, 791–801.

Devereux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. *12*, 387–395.

Dobyns, W.D., Elias, E.R., Newlin, A.C., Pagon, R.A., and Ledbetter, D.H. (1992). Causal heterogeneity in isolated lissencephaly. Neurology *42*, 1375–1388.

Doonan, J.H., Jenkins, G.I., Cove, D.J., and Lloyd, C.W. (1986). Microtubules connect the migrating nucleus to the prospective di-

vision site during side branch formation in the moss *Physcomitrella patens*. Eur. J. Cell Biol. *41*, 157–164.

Doonan, J.H., Mackintosh, C., Osmani, S., Cohen, P., Bai, G., Lee, E.Y.C. and Morris, N.R. (1991). A cDNA encoding rabbit muscle protein phosphatase 1α complements the *Aspergillus* cell cycle mutation, *bimG11*. J. Biol. Chem. *266*, 18889–18894.

Dutcher, S.K., and Hartwell, L.H. (1983). Genes that act before conjugation to prepare the *Saccharomyces cerevisiae* nucleus for karyogamy. Cell *33*, 203–210.

Dynlacht, B.D., Weinzierl, R.O.J., Admon, A., and Tjian, R. (1993). The $dTAF_{II}$ 80 subunit of *Drosophila* TFIID contains β-transducin repeats. Nature *363*, 176–179.

Endow, S.A., Kang, S.J., Satterwhite, L.L., Rose, M.D., Skeen, V.P., and Salmon, E.D. (1994). Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends. EMBO J. *13*, 2708–2713.

Englander, L.L., and Rubin, L.L. (1987). Acetylcholine receptor clustering and nuclear movement in muscle fibers in culture. J. Cell Biol. *104*, 87–95.

Eshel, D., Urrestarazu, L.A., Vissers, S., Jauniaux, J.-C., van Vliet-Reedijk, J.C., Planta, R.J., and Gibbons, I.R. (1993). Cytoplasmic dynein is required for normal nuclear segregation in yeast. Proc. Natl. Acad. Sci. USA *90*, 11172–11176.

Hager, G., Dodt, H.U., Zieglgansberger, W., and Liesi, P. (1994). Novel forms of neuronal migration in the rat cerebellum. J. Neurosci. Res. (*in press*).

Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994). Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor. Nature *370*, 216–218.

Hattori, M., Arai, H., and Inoue, K. (1993). Purification and characterization of bovine brain platelet-activating factor acetylhydrolase. J. Biol. Chem. *268*, 18748–18753.

Klominek, J., Sundqvist, K.G., and Robert, K.H. (1991). Nucleokinesis: distinct pattern of cell translocation in response to an autocrine motility factor-like substance or fibronectin. Proc. Natl. Acad. Sci. USA *88*, 3902–3906.

Koerner, T.J., Hill, J.E., Myers, A.M., and Tzagoloff, A. (1991). High expression vectors with multiple cloning sites for construction of *trpE* fusion genes: pATH vectors. Methods Enzymol. *194*, 477–490.

Li, M., Karpovar, T.S., and Copper, J.A. (1994). A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration. Cell *78*, 669–679.

Li, Y.-Y., Yeh, E., Hays, T., and Bloom, K. (1993). Disruption of mitotic spindle orientation in a yeast dynein mutant. Proc. Natl. Acad. Sci. USA *90*, 10096–10100.

Liscovitch, M., and Cantley, L.C. (1994). Lipid second messengers. Cell *77*, 329–334.

Lloyd, C.W., Pearce, K.J., Rawlins, D.J., Ridge, R.W., and Shaw, P.J. (1987). Endoplasmic microtubules connect the advancing nucleus to the tip of legume root hairs, but F-actin is involved in basipetal migration. Cell Motil. Cytoskeleton *8*, 27–36.

Lupas, A., Dyke, M.V., and Stock, J.B. (1991). Predicting coiled coils from protein sequences. Science *252*, 1162–1164.

Lupas, A.N., Lupas, J.M., and Stock, J.B. (1992). Do G protein subunits associate via a three-stranded coiled coil? FEBS Lett. *314*, 105–108.

Meluh, P.B., and Rose, M.D. (1990). KAR3, a kinesin-related gene required for yeast nuclear fusion. Cell *60*, 1029–1041.

Mirabito, P.M., and Morris, N.R. (1993). BIMA, a TPR-containing protein required for mitosis, localizes to the spindle pole body in *Aspergillus nidulans*. J. Cell Biol. *120*, 959–968.

Morest, D.K. (1970). A study of neurogenesis in the forebrain of opossum pouch young. Z. Anat. Entwicklungsgesch. *130*, 265–305.

Morris, N.R. (1976). Mitotic mutants in *Aspergillus nidulans*. Genet. Res. *26*, 237–254.

Neer, E.J., Schmidt, C.J., Nambudripad, R., and Smith, T.F. (1994). The ancient regulatory-protein family of WD-repeat proteins. Nature *371*, 297–300.

Oakley, B.R., and Morris, N.R. (1980). Nuclear movement is β-tubulin-dependent in *Aspergillus nidulans*. Cell *19*, 255–262.

Oakley, B.R., and Morris, N.R. (1981). A β-tubulin mutation in *Aspergillus nidulans* that blocks microtubule function without blocking assembly. Cell *24*, 837–845.

Osmani, S.A., May, G.S., and Morris, N.R. (1987). Regulation of the mRNA levels of *nimA*, a gene required for the G2-M transition in *Aspergillus nidulans*. J. Cell Biol. *104*, 1495–1504.

Osmani, A.H., Osmani, S.A., and Morris, N.R. (1990). The molecular cloning and identification of a gene product specifically required for nuclear movement in *Aspergillus nidulans*. J. Cell Biol. *111*, 543–551.

Osmani, S.A., Pu, R.T., and Morris, N.R. (1988). Mitotic induction and maintenance by overexpression of a G2-specific gene that encodes a potential protein kinase. Cell *53*, 237–244.

Palmer, R.E., Sullivan, D.S., Huffaker, T.C., and Koshland, D. (1992). Role of astral microtubules and actin in spindle orientation and migration in the budding yeast, *Saccharomyces cerevisiae*. J. Cell Biol. *119*, 583–594.

Plamann, M., Minke, P.F., Tinsly, J.H., and Bruno, K. (1994). Cytoplasmic dynein and centractin are required for normal nuclear distribution in filamentous fungi. J. Cell Biol. *127*, 139–149.

Rakic, P. (1971). Neuron-glia relationship during granule cell migration in developing cerebellar cortex: a Golgi and electron microscopic study in macacus rhesus. J. Comp. Neurol. *141*, 283–312.

Reeve, W.J., and Kelly, F.P. (1983). Nuclear position in the cells of the mouse early embryo. J. Embryol. Exp. Morphol. *75*, 117–139.

Reiner, O., Carrozzo, R., Shen, Y., Wehnert, M., Faustinella, F., Dobyns, W.B., Caskey, C.T., and Ledbetter, D.H. (1993). Isolation of a Miller-Dieker lissencephaly gene containing G protein β-subunit-like repeats. Nature *364*, 717–721.

Rose, M.D., and Fink, G.R. (1987). *KAR1*, a gene required for the function of both intranuclear and extranuclear microtubules in yeast. Cell *48*, 1047–1060.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Schatten, G. (1982). Motility during fertilization. Int. Rev. Cytol. *79*, 59–163.

Schroer, T.A. (1994). New insights into the interaction of cytoplasmic dynein with the actin-related protein, Arp1. J. Cell Biol. *127*, 1–4.

Smith, J.L., and Schoenwolf, G.C. (1988). Role of cell cycle in regulating neuroepithelial cell shape during bending of the chick neural plate. Cell Tissue Res. *252*, 491–500.

Sullivan, D.S., and Huffaker, T.C. (1992). Astral microtubules are not required for anaphase B in *Saccharomyces cerevisiae*. J. Cell Biol. *119*, 379–388.

Timberlake, W.E., and Marshall, M.A. (1988). Genetic regulation of development in *Aspergillus nidulans*. Trends Genet. *4*, 162–169.

Vallee, R. (1993). Molecular analysis of the microtubule motor dynein. Proc. Natl. Acad. Sci. USA *90*, 8769–8772.

Voorn, L.V.D., and Ploegh, H.L. (1992). The WD-40 repeat. FEBS Lett. *307*, 131–134.

Wang, E., Cross, R.K., and Choppin, P.W. (1979). Involvement of microtubules and 10-nm filaments in the movement and positioning of nuclei in syncytia. J. Cell Biol. *83*, 320–337.

Waring, R.B., May, G.S., and Morris, N.R. (1989). Characterization of an inducible expression system in *Aspergillus nidulans* using *AlcA* and tubulin-coding genes. Gene *79*, 119–130.

Xiang, X., Beckwith, S., and Morris, N.R. (1994). Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. Proc. Natl. Acad. Sci. USA *91*, 2100–2104.

Zalokar, M., and Erk, I. (1976). Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. J. Microbiol. Cell *25*, 97–106.