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Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*

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ABSTRACT **Nuclear migration plays an important role in the growth and development of many organisms including the multinuclear fungus *Aspergillus nidulans*. We have identified four genes, *nudA*, *nudC*, *nudF*, and *nudG*, in which temperature-sensitive mutations affect nuclear distribution. In this report, we describe the cloning of the *nudA* gene by complementation of the mutant phenotype by using a chromosome VIII-specific cosmid library. A genomic fragment of *nudA* hybridized to an mRNA of ≈14 kb. Sequencing analysis of *nudA* revealed four ATP-binding sites that are characteristic of the cytoplasmic dynein heavy chain. The amino acid sequence of the *nudA* gene product shows 52% overall identity with the rat brain cytoplasmic dynein heavy chain. Our study provides *in vivo* evidence that dynein, a microtubule motor molecule, plays a role in the nuclear migration process.**

In addition to the obvious role of nuclear migration in fusion of pronuclei during fertilization (1), nuclear movement is critical for proper growth and development in both higher and lower eukaryotes. For example, intracellular nuclear migration occurs during brain epithelial development and may mediate epithelial folding (2). Nuclei migrate in muscle cells and form clusters beneath acetylcholine receptors in neuromuscular junctions (3). Nuclei also assemble into tightly packed rows in virus-induced cell syncytia (4). During early embryonic development in *Drosophila melanogaster*, nuclei migrate from deep within the egg to the cortex prior to cellularization (5). Nuclear movement to an asymmetric position can generate unequal daughter cells and determine cellular polarity (6–8).

Although nuclear migration is of general importance in biology, little is known about the force used to generate the movement and the signals that regulate this process. We have initiated a study of nuclear movement using the multinuclear filamentous fungus *Aspergillus nidulans* as a model system because of its powerful genetics and the ease with which nuclear migration can be observed. In *A. nidulans*, nuclei migrate actively into the mycelium in a microtubule-dependent fashion (9, 10). We have isolated a set of mutants in *A. nidulans* that are defective in nuclear migration (11). Genetic analysis of these *nu*clear *d*istribution (*nud*) mutants identified four genes called *nudA*, *nudC*, *nudF*, and *nudG* (ref. 11 and unpublished results). The *nudC* gene has been cloned by complementation of the mutant phenotype and encodes a protein of 23 kDa with no obvious homology to any protein whose function is known (12). Here we report the cloning of the *nudA* gene and show that *nudA* encodes a cytoplasmic dynein heavy chain.† This result provides direct evidence that cytoplasmic dynein is involved in nuclear migration.

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MATERIALS AND METHODS

Strains Used. XX3 (*nudA1, pyrG89, chaA1*), SJ002 (*pyrG89*), MO73 (*nimT23, pabaA1*), XX33 (*nimT23, nudA1, pabaA1, chaA1*), XX19 (*nudA2, pyrG89, chaA1, nicA2,* and/or *nicB8*), XX8 (*nudA4, pyrG89, wA2, chaA1*), XX10 (*nudA5, pyrG89, wA2, chaA1*), and XX24 (*pabaA1, yA1*) were used.

Growth Media and Nuclear Staining. YAG (13) + UU (0.12% uridine and 0.12% uracil) plates were used for colony growth. For nuclear staining, 10^6 asexual spores (conidia) were inoculated on coverslips in a Petri dish containing 30 ml of YG (YAG without agar) + UU medium. After 7 h of incubation at 44°C, the cells were fixed, and the DNA was stained with 4′,6-diamidino-2-phenylindole dihydrochloride (12) and photographed using a Zeiss epifluorescence microscope.

Cloning and Sequencing of *nudA*. The chromosome VIII-specific cosmid library was obtained from the Fungal Genetics Stock Center (Kansas City, KS). DNA isolation and *A. nidulans* transformations were performed as described (13) and by standard methods (14). The *nudA1* strain was transformed with seven cosmid pools; each pool contained 48 cosmid clones (except for the seventh pool, which contained 40 cosmid clones). One pool was able to complement the temperature sensitivity of this mutation. This pool was subsequently divided and eventually a single cosmid clone was identified that complemented the mutation. This cosmid was subcloned, and genomic fragments were tested in *nudA1* and *nudA2* strains for complementation activity. A 2.8-kb *Hin*dIII fragment that complemented the *nudA2* mutation was then used to screen an *A. nidulans* cDNA library (13). A 4.3-kb cDNA clone was isolated, and double-stranded sequencing was performed on the cDNA and upstream genomic DNA. For some clones, nested deletions were made with exonuclease III using the Exo/Mung deletion kit from Stratagene. Sequencing was done using standard procedures (14) and analyzed using the GCG program package (15).

Northern and Southern Blot Hybridization. Total RNA (30 μg) was electrophoresed on a denaturing 1% formaldehyde/agarose gel (14) and transferred to Zeta-Probe blotting membranes (Bio-Rad) according to the instruction manual from Bio-Rad. The blot was hybridized with ^{32}P-labeled probe in 0.5 M sodium phosphate, pH 7.2/7% (wt/vol) SDS at 65°C overnight and washed with 40 mM sodium phosphate, pH 7.2/1% SDS at 65°C for 1 h. Southern blots were prepared by the alkaline blotting method described in the Bio-Rad instruction manual for Zeta-Probe membranes. All the Southern blot hybridizations and washings were performed under the conditions described above except for one low-stringency genomic Southern blot, on which the hybridization and washing were performed at 55°C.

Abbreviation: ts, temperature sensitive.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. U03904).

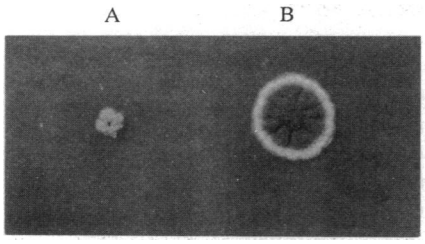

FIG. 1. Colony morphology of the *nudA1* mutant at a restrictive temperature (44°C). The *nudA1* mutant (*A*) and wild-type strain (*B*) were grown at 44°C for 3 days on YAG + UU plates.

RESULTS

Four recessive temperature-sensitive (ts) alleles of *nudA* (*nudA1*, *nudA2*, *nudA4*, and *nudA5*) have been identified. The *nudA* mutations are not lethal at the restrictive temperature of 44°C, but mutants form very small colonies (Fig. 1*A*) easily distinguishable from wild-type colonies (Fig. 1*B*). After germination of *nudA* conidia (asexual spores) at the restrictive temperature of 44°C, nuclei divide but fail to migrate into the germ tube (Fig. 2*A*). In contrast, wild-type nuclei distribute along the entire germ tube (Fig. 2*B*). When *nudA* conidia are germinated at the permissive temperature of 32°C and later shifted to the restrictive temperature, nuclear migration stops. This results in clustering of nuclei within the extending germ tube (11). Thus, the migration defect is independent of the position of the nuclei within the developing germ tube.

Rather than having a nuclear migration defect, it is possible that the *nudA* nuclei are unable to disconnect from each other after division. This could result in a large multinucleate mass that is unable to move due to space constraints in the germ tube. To address this possibility, we constructed a double mutant containing both the *nudA1* and *nimT23* ts mutations. *nimT* is the *A. nidulans* homolog of the *Schizosaccharomyces pombe cdc25* phosphatase gene, and the *nimT23* mutation causes cells to be blocked with a single G_2 nucleus (11, 16). At restrictive temperatures, the single nucleus of the *nimT23* mutant migrates into the germ tube, indicating that nuclear migration is independent of mitosis (Fig. 2*D*). In the *nudA1* *nimT23* double mutant, nuclear migration is blocked at restrictive temperatures (Fig. 2*C*). This result demonstrates that the *nudA1* defect affects nuclear migration *per se*.

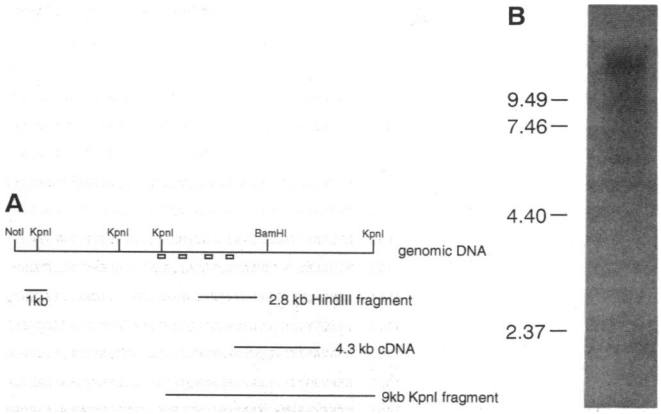

FIG. 3. Map of *nudA* clones and a Northern blot showing the *nudA* mRNA. (*A*) DNA clones of *nudA* used for complementation assays and Northern blot analysis. The genomic map shown on top was obtained by combining two overlapping clones. One clone contains the sequence between the *Not* I site (which is at the insert–vector border of the cosmid W14C05) and the *Bam*HI site. The other clone is the 9-kb *Kpn* I clone shown at the bottom of *A*. The 2.8-kb *Hin*dIII genomic fragment complemented the *nudA2* mutation. The 4.3-kb cDNA clone was isolated using the 2.8-kb *Hin*dIII genomic fragment as a probe. This cDNA clone also complemented the *nudA2* mutation. The 9-kb *Kpn* I genomic fragment is able to complement the *nudA1*, *nudA2*, *nudA4*, and *nudA5* mutations. The locations of the four ATP-binding consensus motifs are represented by open boxes. (*B*) Northern blot analysis of the nudA mRNA. The 2.8-kb *Hin*dIII fragment that complemented the *nudA2* mutation was used as a probe. The autoradiogram revealed a hybridization signal that represents an mRNA of ≈14 kb.

We mapped the *nudA* mutation to chromosome VIII of *A. nidulans* by parasexual genetic analysis (17, 18). Linkage analysis shows that *nudA* is linked to the color marker *chaA* (data not shown). Previous studies have shown that *nudA* is not allelic to *benA* (β-tubulin) or *tubA* (α-tubulin), which are also located on chromosome VIII (9). To clone the *nudA*

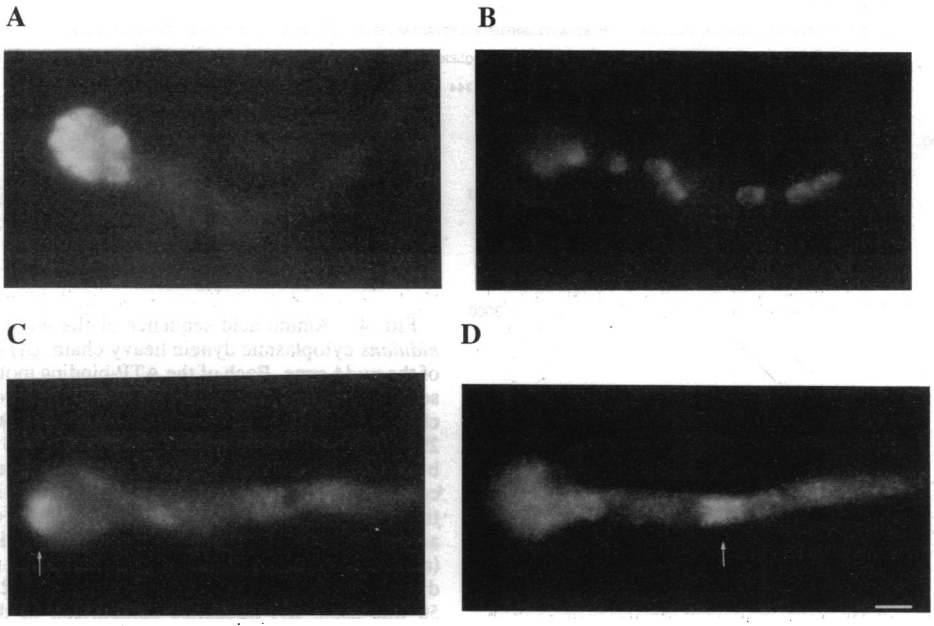

FIG. 2. Phenotype of *nudA1* mutant germlings at 44°C. Nuclear staining of *nudA1* mutant cells (*A*) and wild-type cells (*B*) germinated at 44°C. Nuclear staining of *nimT23 nudA1* double mutant cells (*C*) and *nimT23* cells (*D*) germinated at 44°C. The arrows point to the single nuclei in these cells. (Bar = 5 μm.)

A

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   1 MEVASSGVPDGATAISQGPLVDADAVVEYLADVLRVTLGALRSELENAGSLLSKTKYSETAQRCLRFASESQVALYVQKDLVASDHTNGTADSEEPPAEY  100
 101 VYTLSAEISSSSTTVASVAFIKRPAAIDPSLPISSQIQVMNLPGPAALNNTQAPKGTSLSPYEILHLLVHHGLSPYFEANTRNQDGAAKAKTDSEVKTGV  200
 201 PGTKKKFAELELGLLHLQQNVEIPALNLPLHEVVQAALVEAEKRGVKPSVDLIDSTLLESSAFINSIQNNVNAWIKSIQTITKMSRDADSGSAAQEINFW  300
 301 LSMETALEGIENQLRGDGVQLTMDILRHAKRYQATLSFVADTGLREATDLVQKYNQLMRDFPLDELLSATSLQKVRESLVLIFNHLNKKLKICPYPIKRA  400
 401 LALVEAISGDLDSQIHSLLNGRTIMHLDYREFRTLMKTDGSIWRTWDDNLKEFTNVARESTRRRNEKFIPIKINARHDKTQERLKYINTFRVNHEQLQRT  500
 501 IANVLGPKSYSAEDAAAGAAADGAVIVEEIGDVDAVEEVAQAYAALKSVDVLDVSPTGTQNWITAEIAYNERTSRVENSIIARLRDRLATAKNANEMFRV  600
 601 FSKFNALLVRPKIRGAIGEYQTQLIDNVKQDISALHERFKQQYGHSEAHAMAQLRDLPPVSGAIVWARQIERQLDGYMRKVEDVLGEDWHLHTEGQKLQA  700
 701 ESNLFRKKLDTRPVFETWLHDVQKRRITISGRLFNIIRNRAARNTLELAVNFDAQIIALFKEIRNLIWLNFQVPHAVSNISKEAKRVYPYAISLMESVRT  800
 801 LLQTNRSILSMTDVAILLNGYMNDAQSMIVRGIPLRWESFVHSYELHVKQAALVNGALDSVIPSRGESKHVQFVREFAASAAVLQHKTAVLASINDTIQK  900
 901 AIHELKTCPYEFSAFKQRLDAIQAAVDKLNLENYVNLGFWGHNLNQKIEGILSERLHKAIREWMNSFQESQSGQPMQKVNGNGDTDTTAYNIEFPGLTHE 1000
1001 ISMRNQVLHLDPPLQYARATWFSHFDNWLGIICNLEKIKSSRYQISIEVQKVQLSESCFADLPQHCTNELILVYSAIETRLKEVSEYVDKWLQFQSLWDL 1100
1101 QSEQVYDILGDDLSQWLQLLQEIRKSRATFDTSEVSRSFGNIKIDYEQVQTRVNAKYDQWQHEILLKFGSKLGNRMREVHSEIATARHDLEGKSLDSAST 1200
1201 AHAVAFITSVQQCKRKAKVWEPEVDLFRQGQATLVRQRYQFPSDWLHVENVDGEWAALNELLGRKGKIVEEQTEALRAKIAAEDKVINDKITEAIAQWNE 1300
1301 EKPVSGTIPPEEASRSLSMFQTRLESLQSEFEMVSKAKEALDLPPSAESSLPAILEEVQDFMSVWAALSTIWRSLNDLRDTLWTSIQPRKLRQSIDGLIK 1400
1401 MTKEMPSRMRQYAAFEHIQNVLKQLLKVNPLLSDMKSEAVRERHWLKIYKALKPGMRFSLVSLTLGDVWDLQLAASETVIRNIIAQAQGEMALEEFLKSV 1500
1501 RETWQNYSLDLVNYQNKCRLIRGFDDLFAKCSENLNSLQAMRHSPYYKEFEEDASSWEDKLNRVHVLFDVWIDVQRQWVYLEGVFTGNADIKHLLPLESS 1600
1601 RFQNINSEFFAVMKKVYKSPFVLDVLAINGVQKSLERLAELLNKIQKALGEYLERERVSFPRFYFVGDEDLLEIIGNSNDIFRVAKHFKKMFAGLSGVLM 1700
1701 DDDNNIVGFTSKEGEEVRLKKEVNLFKTPRINDWLTALESNMKLTLAELLAEAIBQFEPIYNSAEVDRTAFDDFIANYPAQIVVLASQVVWTNEVQKSLE 1800
1801 NSGTTLPTLYDAQVRILELLAVTVLGDLDPISRKKCEHLITEFVHQRDTISKLIASNATSATHYLWLLQMRYVYQADGDFLQRLYVHMANAKLNYGFEYL 1900
1901 GVPERLVRTPLTDRCFLTLTQALCQRLGGSPYGPAGTGKTESVKALGLQLGRFTLVFCCDDTFDFQAMGRIFLGICQVGAWGCFDEFNRLEERILSAVSQ 2000
2001 QIQNIQIGLRNKETDEKSQIDLVGRRLTVNMNTGIFITMNPGYAGRSNLPDNLKKLFRSVAMSKPDKELIAEVMLFSQGFKQAKRLSQQTVPFFDHCSTR 2100
2101 LSKQAHYDFGLRALKSVLVSSGGLKRARIANSDGDLGPDEIVEPQIIVQSLRETIAPKLVREDVATMLQIQEQDFAGVEYVPANYEALTAAIREIAREQH 2200
2201 FVDSEMWITKILQLYQIQSIHHGVMMVGKSGSGKSSAWKILLQALQRIEGVEGVSHIIDSKVMSKEALYGSLDSTTREWTDGLFTGILRKIVDNLRGEDT 2300
2301 KRHWIVFDGDVDPEWVENLNSVLDDNKLLTLPNGERLNLPPNVRIMFEVESLKYATLATVSRCGMVWFNEDTVTPSMIITNYVESLKTKTPEDLDDDSVP 2400
2401 SGQSAVKTQDCQDMLSTILSQLLQTDELVHKSLGEAKKYNHIMEFTEIRALNTLFSLLNKACRNILEYNIQHVDFPLEYEQIESYISKKLLLALVWSFTG 2500
2501 DCPLGDRKSFGEFVSGLTTIDLPIETNSSIIDFDVTLPKGTWSSWQSQVPTIDVNTHSITQTDVVIPTVDTVRHEDVLYSWLAEHKPLLLCQPPGSGKTM 2600
2601 TLFAALRKLPNMEVVGLNFSSATTPDLLIKTFEQYCEYKKTLSGVVMSPNQIGRWLVIFCDEINLPAPDKYGTQRAISFLRRVEQNGFWRTSDKTWVSLD 2700
2701 RIQFVGACNPPTDAGRPLAERFLRHSPLVMVDYPGEISLNQIYGTFNSAILKILPLLRGYSESLTKAMVQFYLESQQRFTPKIQPHYVYSPRELTRWVRG 2800
2801 VYEAIKPLESLSVEGLVRIWAHEALRLFQDRLVTEEERAWTADAVRRIALEHFPTIDQEAALKGPILFSNWLSRNYVPVEQEQLRDFVKARLKTFCEEEV 2900
2901 DVPLVLFNDVLEHALRIDRVFRQPQGHLILIGVSGSGKTTLSRFVAWMNGLKVFQIKVHGKYSSEDFDDDLRSVLRRAGCKGEKICFIMDESNVLDSGFL 3000
3001 ERMNTLLANAEVPGLFEGDEFSSLMTACKEGPQRQGLIPDSQEELYKWFTQQIVKNLHVVFTMNPPEEGLSSKAATSPALFNRCVLNWMGDWSDQALFQV 3100
3101 GSELTQSVDLDKPGFVAPDSIPVAYRELSLPASHRDTVINAMVYIHHSLQRFNQRLQKQQGKTTYLTPRHYLDFVAQYVKLFNEKREDLEEQQRHLNVGL 3200
3201 EKLRDTVEKVSDLRGSLAQKKMQLEKKDAERNEKLQRMVADQREAEQRKAVSLEVQAALEKQEKEVALRKDVVLHDLARAEPAVLEAQKSVSNIKRQHLT 3300
3301 EVRSMGNPPAGVRLALEAVCTLLGHKVDSWKTIQGIVRRDDFIASIVNYDNEKQMTKNHRLKMQNEFFSKEDFTYERVNRASKACGPLVQWVEPQVNYSA 3400
3401 ILDRVGPLRDEVGQLEEQALQTKAEAQAIENTINDLESSIATYKSEYTALISETQAIKAEMERVQFKVDRSVRLLDSLSSERTRWEEGSKSFETQISTLI 3500
3501 GDVLIAAAFLAYAGFYDQQFRKAMTEDWVQHLVQSGISLKPHNPITEYLSNADERLAWQAHSLPVDDLSTENAIFLKRYNRYPLIIDPSGRVTEFLQKES 3600
3601 SDRKLTVTSFLDDSFVKQLESALLSETRFIIQDAEHLDPILNHVLNKEYQKTGGRVLIQLGKQEIDFSPSFKLFLSTRDPSATFAPDVCSRTTFVWYNFT 3700
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3801 IIARSCSAVFAVLEQLHHINHFYQFSLQYFTDIFESVLHGNPHLENSGLRKMEDYQHRIQIILRDLFVTTYQRTSLGVIQKDRITLAMLLAQAAPYPMDK 3900
3901 SIIDTILDESVEGTDLSANPEAKVQVMSAFGNMSLFKAHLPSVTAEQWDQFLGEELAENFVPKVWDENTSELDKLLRSLLLVKLCRMDRFVPAAERFIVA 4000
4001 VFGRELYEGSTDLKDIVGQVTATAPISLSSSPGIPHASYKVDALVERTTHMPNIAMGSNEGLESADKAISNAASAGTWVLVKNVHLAPSWLQSLEKRLAS 4100
4101 LKPHKDFRLFLSMESSPKIPVNLIRASRVLMYEQPAGVRGNMKDSLSSLSTRASKAPVEKARVYLLLCFLHAVVQERLRYAPSLGWKGFWEFNDSDYECS 4200
4201 ANIIDHWVHVVAQGRSNVAPQKLPWDMIRTLITEMYGGKVDDSDDFQQLERLVHSFLTPATFEADYKLVEGVENDECLILPGETGLPAFVEWVNKLPERE 4300
4301 PPTYLGLPANAEKLLLVGHGKKMISDLARITSLLDEGEQLMIDA 4344
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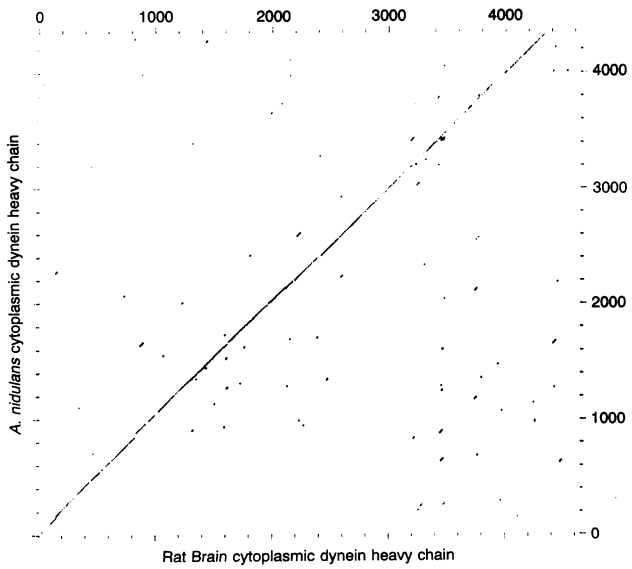
B



C

A. nidulans	GPAGTGKT	GKSGSGKS	GPPGSGKT	GVSGSGKT
Rat	GPAGTGKT	GPSGSGKS	GPPGSGKT	GVSGAGKT
Dictyostelium	GPAGTGKT	GPSGGGKT	GPPGSGKT	GVSGGGKS
Flagellar	GPAGTGKT	GNAGTGKS	GNAGLGKS	GVGGSGKQ

FIG. 4. Amino acid sequence of the *nudA* gene product, an *A. nidulans* cytoplasmic dynein heavy chain. (*A*) Amino acid sequence of the *nudA* gene. Each of the ATP-binding motifs is underlined. The sequence after amino acid 2949 is obtained from the 4.3-kb cDNA clone that contains the poly(A) tail. The sequence before amino acid 2949 is obtained from the genomic clones (see Fig. 3*A*). One intron between amino acids 94 and 95 was spliced out by sequence comparison with cytoplasmic dynein in other species and the identification of 5′ and 3′ splicing sites. (*B*) Dot plot matrix comparing the amino acid sequence of *A. nidulans* cytoplasmic dynein heavy chain (amino acids 1–4344) with the sequence of the rat brain cytoplasmic dynein (amino acids 1–4645). A stringency of 25 in a window size of 50 was used. (*C*) Sequence comparison of the four ATP-binding motifs among the *A. nidulans*, rat brain, and *Dictyostelium* cytoplasmic dynein heavy chains and the sea urchin flagellar dynein β heavy chain.

gene, a chromosome VIII-specific genomic cosmid library (19) was divided into several pools. DNA from each pool was transformed into the *nudA1* strain to complement the ts mutation. A single cosmid clone (W14C05) was isolated that was able to complement the *nudA1* mutation. The cosmid clone was digested into smaller fragments that were tested for the ability to complement either the *nudA1* or *nudA2* mutation. A 2.8-kb *Hin*dIII fragment was isolated that complemented the *nudA2* mutation (Fig. 3*A*). The 2.8-kb *Hin*dIII fragment hybridized to an mRNA of ≈14 kb on a Northern blot (Fig. 3*B*).

The 2.8-kb *Hin*dIII genomic fragment was used as a probe to screen an *A. nidulans* cDNA library (13). A positive cDNA clone with a 4.3-kb insert was isolated (Fig. 3*A*) and found to complement the *nudA2* mutation at restrictive temperatures for growth, conidiation, and nuclear migration. This indicates that the cDNA encodes the *nudA* gene rather than an extracopy suppressor of *nudA* (which would require the entire promoter and coding region for complementation). This 4.3-kb cDNA clone was shown to correspond to the 2.8-kb *Hin*dIII genomic clone (see Fig. 3*A*) by sequence analysis. The sequence obtained from this cDNA clone showed strong homology to the cytoplasmic dynein heavy chain genes from *Dictyostelium discoideum* (20) and rat brain (21, 22). Genomic sequences upstream and downstream of this cDNA clone were identified within the same cosmid clone by restriction enzyme digestion and Southern blot analysis. Sequence analysis of the genomic clones was performed. The entire protein sequence of the *A. nidulans* cytoplasmic dynein heavy chain is shown in Fig. 4*A*. The overall sequence identity between *A. nidulans* and rat brain (21, 22) and *Dictyostelium* (20) cytoplasmic dynein heavy chains, and sea urchin flagellar dynein β heavy chain (23, 24) is 52%, 49%, and 27%, respectively. Among the cytoplasmic dyneins, sequence conservation is not limited to the region containing the four ATP-binding sites; rather, it extends to both the C-terminal and N-terminal regions of the proteins (Fig. 4*B*). However, there is very little sequence conservation between the N-terminal 1000 amino acids of *A. nidulans* cytoplasmic dynein and sea urchin flagellar dynein β heavy chain (data not shown). The first ATP-binding motif is conserved in all of the above dynein sequences. It is interesting to note that the third ATP-binding motif is absolutely conserved only in the cytoplasmic dyneins (Fig. 4*C*).

A 9-kb *Kpn* I genomic fragment that extends from the first ATP-binding site to the 3′ end of dynein (Fig. 3*A*) was isolated from the cosmid clone and ligated into a plasmid containing the *pyr4* marker. This plasmid was found to complement all four *nudA* ts mutant alleles. We showed that this plasmid contains the *nudA* sequence by demonstrating that it integrates site specifically at the *nudA* locus. The recombinatory event leading to site-specific plasmid integration generates a tandem duplication of wild-type and mutant sequences. The reverse event that causes plasmid elimination causes the plasmid and one or the other of these sequences to be lost and is, therefore, expected to generate both wild-type and mutant segregants. A ts^+ transformant of *nudA5* was treated with 5-fluoroorotic acid to select for loss of the integrated *pyr4* plasmid marker. Both ts^+ and ts^- segregants appeared after 5-fluoroorotic acid selection. To show that the integration was at the *nudA* locus, we crossed one of the ts^+ segregants with a wild-type strain. If integration had been at a locus other than *nudA* (i.e., at an extragenic suppressor locus), this cross would have generated both ts^- and ts^+ progeny, with the fraction of ts^- progeny depending on the genetic linkage between the two sites. In fact, all progeny (600 analyzed) were ts^+. This result indicates that the integration site and the wild-type *nudA* sequence are tightly linked. This linkage analysis combined with the cDNA complementation of the *nudA2* mutation provides strong evidence that the *A. nidulans* *nudA* gene encodes a cytoplasmic dynein heavy chain.

DISCUSSION

We have cloned the *nudA* gene in *A. nidulans* by complementing the *nudA* mutant phenotype. The *nudA* gene encodes a cytoplasmic dynein heavy chain that shows strong sequence homology to the cytoplasmic dynein heavy chains from *Dictyostelium discoideum* (20) and rat brain (21, 22). Southern blot analysis at low stringency was performed using two genomic fragments that cover the four ATP-binding sites as probes. Only one hybridization signal was detected (data not shown), suggesting that there is only one cytoplasmic dynein heavy chain gene in *A. nidulans*. This result is consistent with the notion that organisms generally have a single cytoplasmic dynein heavy chain gene (25).

Cytoplasmic dynein is a complex molecule consisting of heavy, intermediate, and light chains (for review, see ref. 26). It is believed to behave as a microtubule-dependent motor that mediates retrograde organelle transport in cells such as neurons (27–29). Nuclear migration in *A. nidulans* has been shown to be microtubule-dependent (9, 10). In this paper we demonstrate an *in vivo* function for cytoplasmic dynein in nuclear migration. Interestingly, another microtubule-dependent motor protein, the kinesin-related *KAR3* gene product, mediates a specialized type of nuclear movement, karyogamy, in the yeast *Saccharomyces cerevisiae* (30). Thus, nuclear movement may require the coordinated effort of several molecular motors or may utilize different motors for different purposes, microtubule polarities, or directions.

In addition to a role in neuronal transport, cytoplasmic dynein is also thought to be involved in mitosis (31). Microinjection of dynein-heavy-chain-inactivating antibody into mammalian cells prevents separation of the spindle poles at an early stage in mitosis (32). In contrast, none of the four *nudA* alleles exhibited any detectable mitotic defect at a restrictive temperature. This does not necessarily mean that cytoplasmic dynein has no role in mitosis in *A. nidulans*, as the criteria for identification of *nud* mutants require that nuclear division continues. Other, as yet unidentified, mutations in the *A. nidulans* cytoplasmic dynein heavy chain gene might affect both mitosis and nuclear migration or even mitosis alone.

Alternatively, cytoplasmic dynein may have no role in mitosis in *A. nidulans*. Cytoplasmic dynein appears not to be involved in mitosis in *Saccharomyces cerevisiae*. Rather, it appears to play a role in spindle orientation (33, 34). There is evidence to suggest that the mammalian mitotic function of cytoplasmic dynein may be taken over by the kinesin-related proteins in fungi. Mutation of the *bimC* kinesin-related protein of *A. nidulans* causes a failure of spindle pole separation similar to that caused by inactivation of cytoplasmic dynein in mammalian cells (35). Deletion of *klpA*, another kinesin-related gene similar to *Saccharomyces cerevisiae KAR3*, partially suppresses the *bimC3* mutation, suggesting that it is also involved in spindle pole separation (36). The kinesin-related *CIN8*, *KIP1* (37), and *KAR3* (30) genes have similarly been shown to be involved in mitosis in *Saccharomyces cerevisiae* (37). It is also possible that functional redundancy among motor proteins could explain the lack of an obvious mitotic defect in the fungal dynein mutants.

There appear to be a number of genes involved in nuclear migration in *A. nidulans*. These include the *nudC* gene, which encodes a 23-kDa protein of unknown function (12), that exhibits strong sequence homology to an expressed sequence tag of *Caenorhabditis elegans* in the GenBank data base. Homologs of *nudC* have also been identified in *Drosophila* (J. Cunniff and R. Warrior, personal communication) and rat

(Y.-L. Li, personal communication), suggesting that it is a conserved protein. The *nudF* gene has been cloned as an extracopy suppressor of the *nudC3* mutation. The *nudF* gene encodes a protein with an N-terminal coiled-coiled structure and six repeats characteristic of the β subunit of heterotrimeric guanine nucleotide binding proteins (unpublished data). This suggests that *nudF* may play a role in a signal transduction pathway that regulates the dynein-mediated nuclear migration process. Because the *nud* gene products are conserved in other species, we believe that additional components of the nuclear migration process may also be conserved among all eukaryotes.

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