

The Molecular Cloning and Identification of a Gene Product Specifically Required for Nuclear Movement in *Aspergillus nidulans*

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Abstract. A temperature-sensitive mutation in the *nudC* gene (*nudC3*) of *Aspergillus nidulans* specifically prevents the microtubule-based movement of nuclei in this organism at the restrictive temperature. The mutation does not affect short term growth, nuclear division, or the movement of other subcellular organelles. Immunofluorescence analysis of cells blocked at the restrictive temperature, using antitubulin antibodies, shows that the inability of nuclei to move under these conditions is not related to an inability of a particular class of microtubule to form. The inability to move nuclei in this mutant is also shown to be independent of both mitosis and the number of nuclei in the cell as a double mutant carrying

both *nudC3* and a cell cycle-specific mutation blocks with a single immotile nucleus at the restrictive temperature. The molecular cloning of the *nudC* gene and sequence analysis reveal that it encodes a previously unidentified protein of 22 kd. Affinity-purified antisera reactive to the *nudC* protein cross reacts to a single protein of 22 kd in *Aspergillus* protein extracts. This purified sera failed to reveal a subcellular location for the *nudC* protein at the level of indirect immunofluorescence. The data presented suggest that the 22-kD *nudC* gene product functions by interacting between microtubules and nuclei and/or is involved in the generation of force used to move nuclei during interphase.

NUCLEAR migration plays a fundamental role in many developmental processes in both higher and lower eukaryotes. It is essential for fusion of pronuclei in eggs during fertilization (Schatten, 1982) and for karyogamy in yeast (Dutcher and Hartwell, 1983; Rose and Fink, 1987). There is a mass migration of nuclei to the egg cortex during early development of *Drosophila* (Zolaker and Erk, 1976), and nuclei migrate into the preformed bud during budding in *Saccharomyces cerevisiae* (Byers, 1981). In plants and animals, the nucleus establishes the site of the cleavage furrow during cell division, and migration of the nucleus to an eccentric cellular position is known to be responsible for the unequal nuclear divisions that are part of normal development (Gunning, 1982; Reeve and Kelly, 1983; Schroeder, 1987). In filamentous fungi, nuclear migration maintains a more or less regular distribution of nuclei along the mycelium and is required for colony development, since temperature-sensitive mutants defective for nuclear migration fail to form colonies (Morris, 1976). In *Aspergillus*, nuclear migration is also required for the entry of nuclei into asexual spore generation structures (sterigmata) and asexual spores (conidia) (Timberlake and Marshall, 1988).

The biochemistry of nuclear migration is very incompletely understood. Few of the molecules involved in nuclear motility have been identified, and almost nothing is known

about the signals that control the onset of nuclear movement or the ultimate position of the nucleus. Studies in a variety of organisms have indicated that nuclear migration is microtubule mediated (Lloyd et al., 1987; Doonan et al., 1986; Lutz and Inouye, 1982; Schatten et al., 1982; Wolf, 1978). This is also true of *Aspergillus*, since nuclear migration in *Aspergillus* is inhibited by the antimicrotubule drug benomyl (Oakley and Morris, 1981). Actin (Lloyd et al., 1987; Schatten et al., 1986) and a kinesin-related protein, the KAR3 gene product of *S. cerevisiae*, have also been shown to be involved in nuclear movement (Meluh and Rose, 1990).

To apply the power of genetics to the study of nuclear motility we have previously isolated a unique class of recessive temperature-sensitive mutants in the filamentous fungus *Aspergillus nidulans* that specifically affect nuclear movement (Morris, 1976). At the restrictive temperature, strains carrying a *nud* (for nuclear distribution) mutation are unable to transport nuclei into growing mycelia and are therefore severely restricted for growth and differentiation under these conditions. The genes defined by these mutations have been shown to be involved specifically in nuclear movement as only nuclei are rendered immotile at the restrictive temperature whereas other subcellular organelle transport, such as mitochondrial transport, carries on (Oakley and Rinehart, 1985). The molecular nature of the *nud* gene products is at present unknown but they could presumably be involved in the mechanism of force generation used to move nuclei, be associated with microtubule function, and/or interactions (as

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nuclear movement has been shown to require beta-tubulin function in *Aspergillus*; Oakley and Morris, 1980, 1981), or be part of an intracellular signaling system that specifies nuclear movement should occur. The molecular cloning of the *nud* genes, as well as genes that interact with them, and a functional analysis of their products will help lead to an understanding of the molecular mechanisms of nuclear movement.

The most obvious manner by which the *nud* mutations could render microtubule-based nuclear motility inoperative would be to cause the selective loss of a particular type of microtubule population that is specifically required for nuclear movement, for example, cytoplasmic microtubules or astral microtubules, at the restrictive temperature. We have therefore carried out an immunofluorescence study to determine if the *nud* phenotype of the *nudC3* mutation is caused by the loss of a particular subpopulation of microtubules. We have also investigated the dependence of the *nud* phenotype on the completion of mitosis. In addition we describe the cloning of the *nudC* gene by complementation of the mutant phenotype using a genomic plasmid library. The sequence of several cDNA clones of the gene and the genomic clone reveal that the *nudC* gene encodes a previously unknown 22-kD protein. Finally, affinity-purified antibodies reactive to the *nudC* gene product have been produced and used to identify this previously unknown protein.

Materials and Methods

Aspergillus Strains and Growth

The strains used in this study were AO1 (*nudC3*, *wA2*, *nicA2*, *pabaA1*, *pyrG89*), AO7 (*nudC3*, *nimA5*, *yA2*, and possibly other markers from SO14), SO7 (*nimA5*, *wA2*), and SO14 (*nimA5*, *riboA2*, *nicB8*, *yA2*). The permissive temperature for growth was 25°C and the restrictive temperature 42°C using YAG media (0.5% yeast extract, 2% glucose, trace elements, 2% agar). Standard growth and genetic techniques were used to generate and test double mutant strains (Pontecorvo et al., 1953; Clutterbuck, 1974; Cove, 1977).

Staining Techniques and Microscopy for *Aspergillus nidulans*

To visualize DNA-containing organelles, cells were fixed in a solution containing 16% Glycerol, 4% Glutaraldehyde, 0.16% Triton-X-100, 40 mM PO₄, pH 6.5, 0.02 µg/ml 4'-diamido-2-phenylindole (DAPI) and viewed under epifluorescence as described previously (Bergen et al., 1984). Indirect immunofluorescence of *Aspergillus nidulans* was carried out as described previously (Osmani et al., 1988a).

Molecular Cloning of the *nudC* Gene

The temperature sensitivity and uridine requirement of strain AO1 were complemented by DNA-mediated transformation using the plasmid library and techniques as described in Osmani et al. (1987). DNA from such doubly complemented transformants was analyzed by Southern blotting using nick-translated vector DNA (May et al., 1985) as a probe to identify those transformants containing a single site of plasmid integration in their genome. Plasmids from such a transformant were obtained from genomic DNA isolated from the strain by cutting the DNA with Bgl II followed by ligation and transformation of *E. coli* to ampicillin resistance.

Plasmids were isolated that could complement the temperature sensitivity of strain AO1. Several similar plasmids were isolated and used to probe a genomic lambda Charon4A library. Subcloning of the genomic clones isolated was carried out using standard molecular biological techniques (Maniatis et al., 1982) until a fragment was isolated that retained the ability to complement *nudC3* and hybridized to a single RNA species in Northern blot experiments (Osmani et al., 1987). This fragment was used to screen

a cDNA library constructed from *Aspergillus nidulans* total polyA⁺ mRNA as described previously (Osmani et al., 1988a). The smallest genomic fragment able to complement *nudC3* was sequenced on both strands as were the *nudC* cDNAs using the dideoxynucleotide chain termination procedure (Sanger et al., 1977).

Production and Affinity Purification of Anti-*nudC* Antisera

To raise antibodies to the product of the *nudC* gene we subcloned a 633-bp Alu I fragment from *nudC* cDNA into the Sma I site of the pATH11 vector to produce a fusion protein between *nudC* and the amino terminus of *E. coli* *trpE* (Crivellone et al., 1988). The fusion protein was induced and isolated in an insoluble fraction before final purification by preparative SDS polyacrylamide electrophoresis (Crivellone et al., 1988). 250 µg of the purified fusion protein in Freund's complete adjuvant was used to immunize guinea pigs which were then boosted at 10-d intervals with 100 µg of antigen in incomplete adjuvant. Blood was collected at 2-wk intervals by heart puncture and the isolated sera tested for reactivity to total *Aspergillus nidulans* protein by Western blotting (Towbin et al., 1979). Affinity purification was carried out on positive sera by reacting the sera to the fusion protein that had been cross linked to Affi-Gel according to the manufacturer's instructions (Bio-Rad Laboratories, Richmond, California). After binding, the matrix was washed extensively with TBS before elution of the antibodies using 100 mM glycine, pH 2.8. Fractions were collected into sufficient 1 M Tris, pH 8.0, to neutralize the glycine, and those fractions with significant adsorption at 280 nm were dialyzed against TBS.

Results

The *nudC* Gene Function Is Specifically Required for Nuclear Migration

If conidia (uninucleate asexual spores) of *Aspergillus* are inoculated into growth medium they germinate and send out a germ tube. The single nucleus present in the conidia normally undergoes a division before the germ tube has grown and the nuclei migrate into the growing germ tube as it begins to emerge. Subsequent nuclear divisions occur as the cell grows at the tip of the germ tube, and the resulting nuclei are maintained at a fairly constant distance from one another in the growing mycelial tube. Strains carrying the *nudC3* mutation are specifically temperature sensitive for the process that normally moves nuclei into the germ tube and maintains nuclei at a distinctive distance from one another during growth. The phenotype is best visualized by comparing nuclear movement during germination of wild type conidia (Fig. 1, left panel) to *nudC3* carrying conidia (Fig. 1, right panel) when both are germinated at the restrictive temperature (Fig. 1). The nuclei in the wild type strain shown in Fig. 1 (left panels) have undergone four divisions. The resulting nuclei are maintained at regular intervals in the growing germ tube. In the strain carrying *nudC3* it can be seen that this mutation does not prevent the short term processes of germination, cellular growth, or mitosis, and the mutant cells in Fig. 1 (right panel) have also undergone four nuclear divisions. However, in these mutant cells, the resulting nuclei are unable to move into the germ tube. Upon prolonged incubation, cellular growth in *nudC3* mutants does become inhibited at the restrictive temperature and very restricted colonies are eventually formed that are unable to undergo the normal program of differentiation to form conidia (data not shown).

Does the *nudC3* Mutation Affect a Particular Class of Microtubule?

The movement of nuclei in *Aspergillus* has been shown to be

1. Abbreviation used in this paper: DAPI, 4'-diamido-2-phenylindole.

dependent on a functional microtubule system (Oakley and Morris, 1980, 1981). We have therefore studied the appearance of microtubule cytoarchitecture in a *nudC3* strain grown at the restrictive temperature to determine if the inability to move nuclei under these conditions is related to any gross defects in, or absence of, a particular microtubule structure.

A strain carrying the *nudC3* mutation was germinated at the restrictive temperature and stained with DAPI and antitubulin antibodies by indirect immunofluorescence to visualize both microtubules and the position of nuclear DNA (Fig. 2). The DAPI staining pattern of these cells showed that nuclear division could take place but that the resulting nuclei remained in the germ tube head (Fig. 2, right panel) demonstrating that the *nud* phenotype was being expressed.

We then studied the samples to ascertain if these mutant cells were able to form cytoplasmic microtubules, mitotic spindles, and astral microtubules. A cytoplasmic network of microtubules was apparent throughout cells during interphase (three germlings to the far left of Fig. 2). The pattern of staining during interphase was indistinguishable from that of wild type cells (data not shown, see Osmani et al., 1988a; Engle et al., 1988; Doonan and Morris, 1989) indicating that cytoplasmic microtubules could form in the *nudC3* mutant at restrictive temperature.

At mitosis spindles were observed to form in the bulb of germlings (Fig. 2, arrowhead) and in the mitotic cells cytoplasmic microtubules were largely disassembled (Fig. 2, arrowed cell) in a manner similar to wild type cells at mitosis. Therefore, the microtubule cycle (from cytoplasmic microtubules to spindle and back to cytoplasmic microtubules) does not appear to be affected by the *nudC3* mutation nor is there an inability to form mitotic spindles and undergo nuclear division.

We also studied mitotic cells for the presence of the astral microtubules normally associated with the spindle pole bodies of *Aspergillus* during part of mitosis. The cell depicted in Fig. 3 can be seen to have sent out three germ tubes with the result that the spindles present in the cell are spread out and the astral microtubules can be clearly seen (Fig. 3, arrows) demonstrating that this third class of microtubule structure can form in this mutant at the restrictive temperature.

The above data demonstrate that the *nudC3* mutant phenotype is not caused by a major defect in tubulin assembly into any identifiable class of microtubule structure (cytoplasmic, spindle, or astral), the absence of which could affect nuclear migration.

Is the nudC3 Mutant Phenotype Dependent on Mitosis?

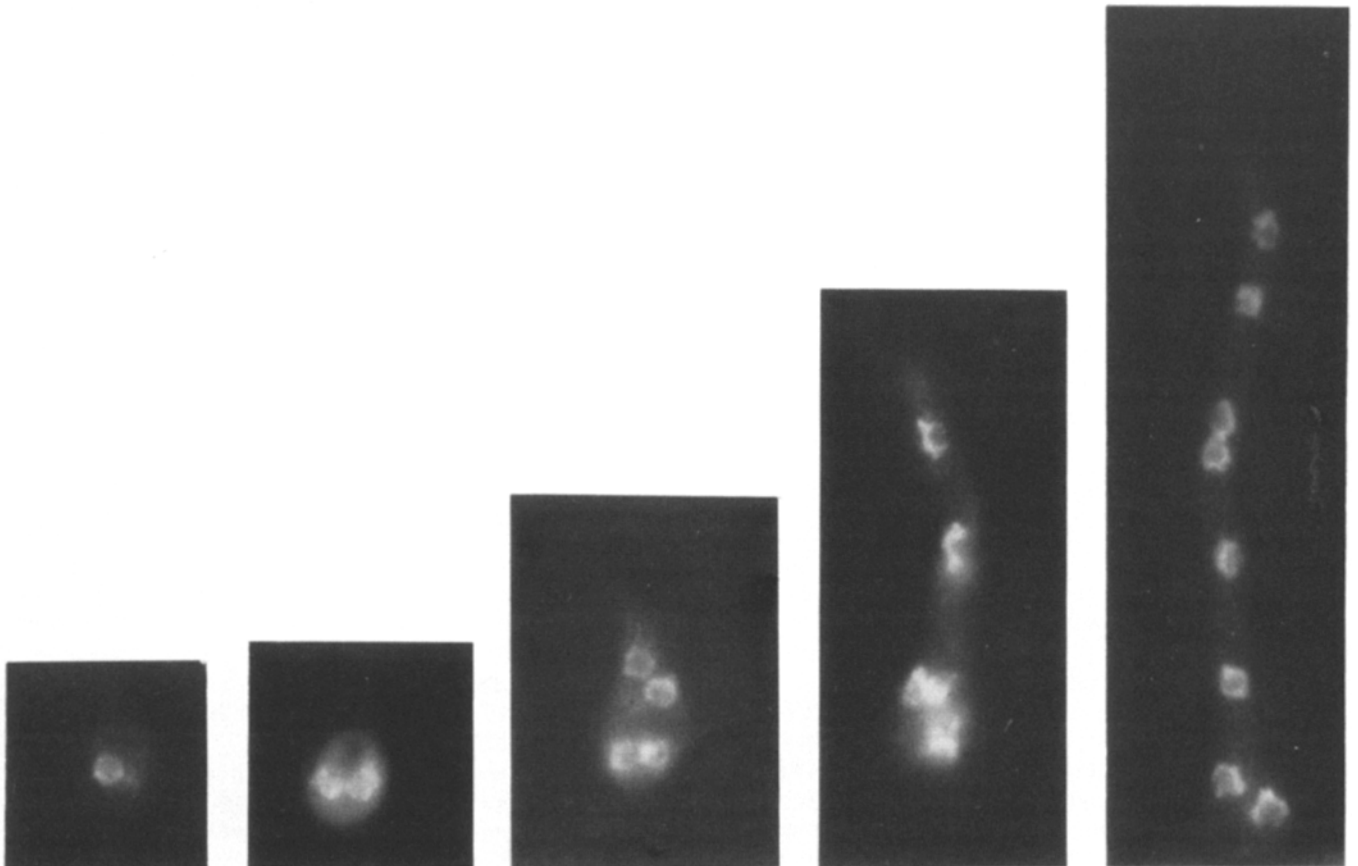
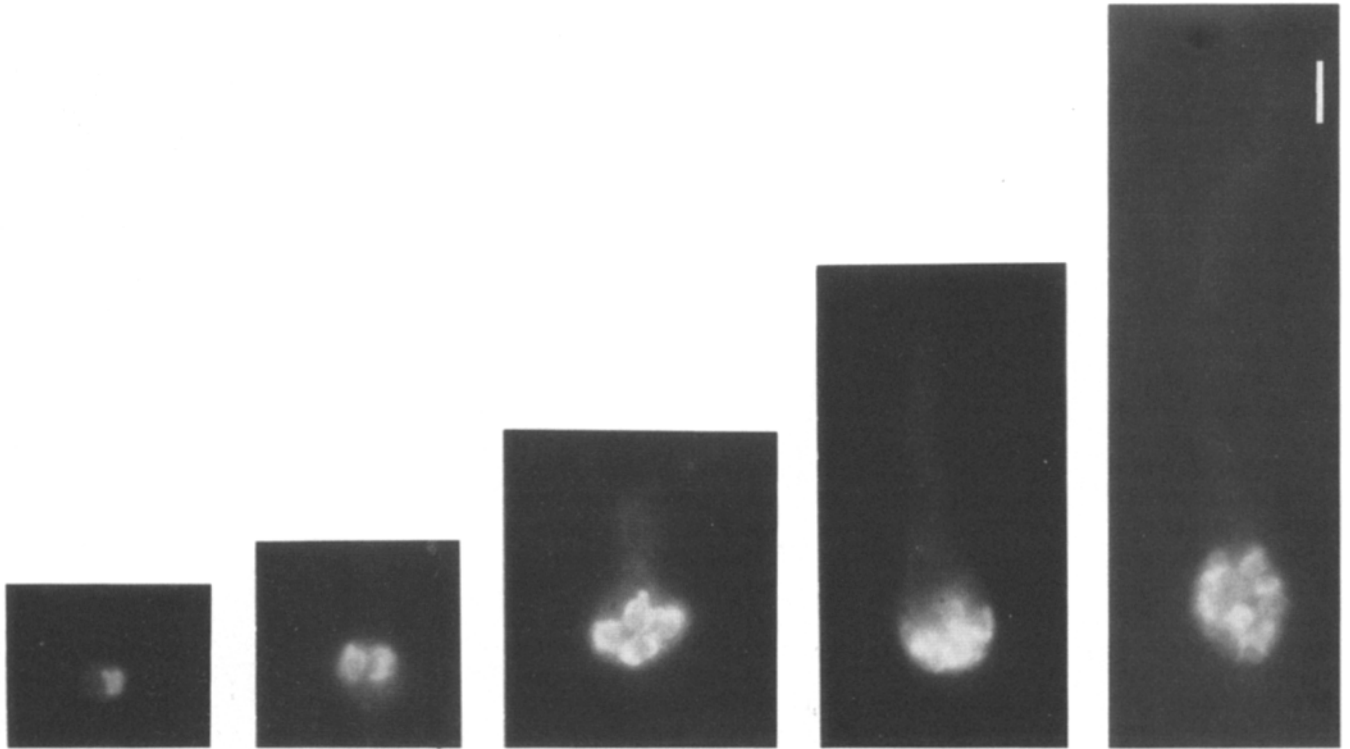
During the process of spore germination one mitosis normally occurs before germ tube extension. It is possible that the presence of two nuclei in the swollen spore before germ tube extension could play a role in hindering normal nuclear migration in the *nud* mutants and hence contribute to the *nud* phenotype. For example, steric hindrance could be a factor if nuclei could not separate correctly at the end of mitosis in the *nudC3* mutant cells. In addition, as it is known that significant nuclear movement is normally associated with mitosis in *Aspergillus*, particularly during telophase (Gambino et al., 1984), we were interested to ascertain if absence

of nuclear division would modify the *nudC3* mutant phenotype. To this end we constructed a double mutant strain carrying both the *nudC3* mutation and the temperature-sensitive *nimA5* mutation. The *nimA5* mutation causes a G2 block at the restrictive temperature (Oakley and Morris, 1983; Bergen et al., 1984). By germinating conidia of the *nudC3*, *nimA5* double mutant at the restrictive temperature we have asked whether a single nucleus in a germinated cell is able to move into the germ tube when the *nudC3* mutation is imposed. If conidia of a *nimA5* mutant strain are grown at the restrictive temperature for a period to allow germ tube extension, the single nucleus present in the cell is able to move into the growing germ tube (Fig. 4). The double *nimA5*, *nudC3* mutant strain also has a single nucleus but in this case the nucleus is not able to migrate into the germ tube (Fig. 4). These data demonstrate that the *nudC3* mutant phenotype (inability to move nuclei) is independent both of mitosis and the number of nuclei present in the germling before germ tube extension.

Molecular Cloning of the nudC Gene

We have cloned the *nudC* gene by DNA-mediated complementation of the *nudC3* mutant phenotype using techniques outlined previously (Osmani et al., 1987). The mutant *nudC3* *ts*⁻ phenotype was complemented by integrative transformation using a genomic plasmid library (Osmani et al., 1987) in a vector that contains the *pyr4* gene of *Neurospora* which is able to complement the *pyrG89* mutation of *Aspergillus* (Ballance et al., 1983). Several transformants were identified that were no longer temperature sensitive for growth. Because the transformants could reflect either integration of wild type *nudC* sequence or a suppressor of *nudC3*, the transformants were analyzed by the two step gene replacement method (Miller et al., 1985). Several temperature-resistant *nudC3* transformants were shown by Southern blot analysis to contain a single integrated copy of the transforming plasmid. One of these transformants was put through a self cross to replace the endogenous genomic sequence with the integrated wild type sequence. Because site-specific integration creates a tandem duplication of the target sequence with one wild type and one mutant copy, when a site-specific transformant is put through a genetic cross with itself, the tandem sequences recombine in 5–10% of the progeny to eliminate either the wild type or the mutant *nudC3* sequence and the intervening *pyr4* containing plasmid sequence. Temperature-resistant *pyr4*⁻ progeny from the self cross that contained the wild type sequence were identified, and these were crossed to a wild type strain to determine whether integration was at the *nudC* locus or some other (suppressor) locus. If the gene replacement occurred at the *nudC* locus, a cross back to a wild type strain should not reveal any *ts*⁻ progeny. If, on the other hand, a suppressor had been gene replaced, a cross back to wild type should uncover the original *ts*⁻ mutation. In five gene-replaced strains crossed back to a wild type strain we found no *ts*⁻ progeny from a total of 278 progeny tested. We therefore conclude that we had complemented the *ts*⁻ phenotype with the wild type copy of the *nudC* gene and not a suppressor.

Plasmids were recovered from the *nudC*⁺ transformant described above by partial restriction digestion of genomic DNA followed by ligation and transformation of *E. coli* to ampicillin resistance. A common fragment from the isolated



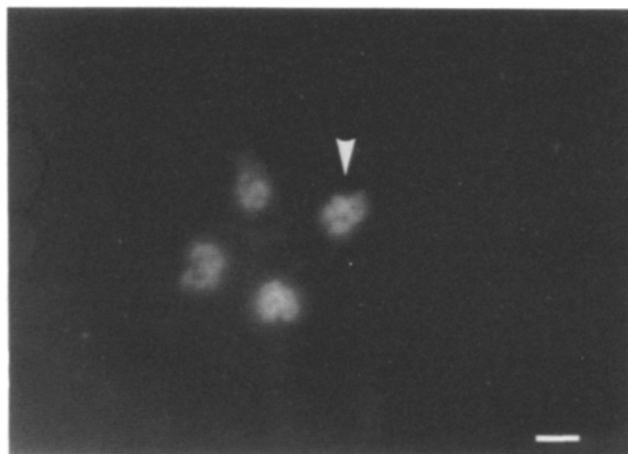
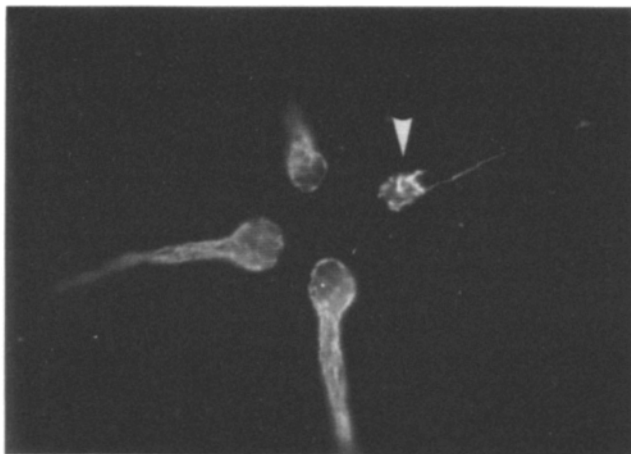


Figure 2. Effect of the *nudC* mutation on microtubule structures. A strain carrying the *nudC3* mutation was grown from conidia for a period of 8 h at the restrictive temperature of 42°C. Cells were double stained to visualize both microtubule structures using antitubulin antibodies (*left panel*) and nuclei using DAPI (*right panel*). Cells are shown both in mitosis (*arrowhead*) and during interphase. Bar, 5 μ m.

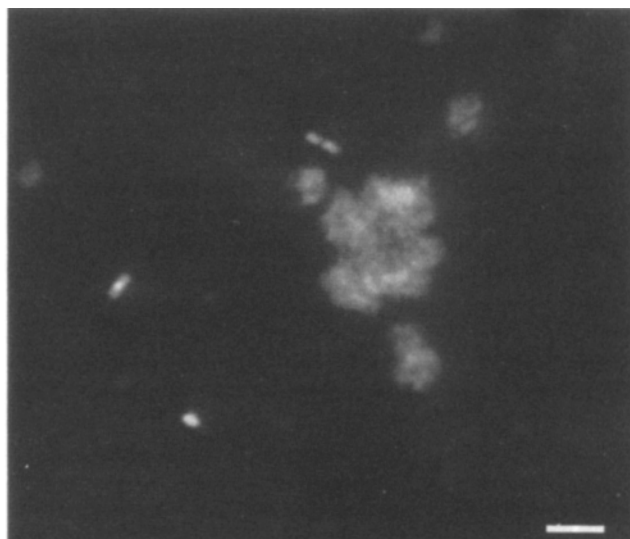
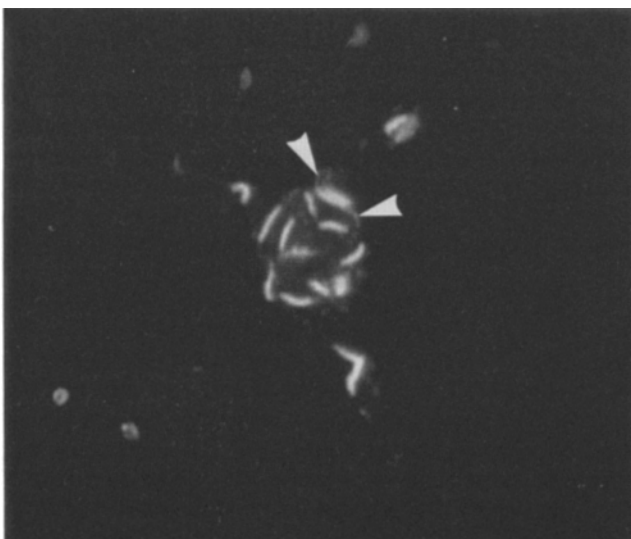


Figure 3. Astral microtubules are present in cells blocked by the *nudC3* mutation. The cell depicted has been grown at the restrictive temperature of 42°C for a period of 10 h. Several of the spindles present in the conidial bulb have astral microtubules protruding from the poles of the mitotic spindle (*arrowheads*). This particular cell had grown three germ tubes. (*left panel*) Tubulin; (*right panel*) DAPI. Bar, 5 μ m.

plasmids was used to probe a lambda Charon 4A genomic library. Six Eco RI fragments from the resulting genomic clones were tested for their ability to complement the *nudC3* mutation, and a 6.4-kb fragment was identified that contained this activity (Fig. 5). Subcloning experiments were performed on this genomic Eco RI fragment and an analysis of these data is given in Fig. 5. The results demonstrate that the Eco RI–Acc I fragment C is capable of complementing the *nudC3* mutation and that it hybridizes to a RNA species of \sim 850 bases.

Sequence Analysis of the *nudC* Gene

Fragment C was used as a specific probe for the *nudC* gene to probe an *Aspergillus* cDNA library (Osmani et al., 1988b) to isolate cDNA copies of the gene for sequence analysis. Four different *nudC* cDNAs were isolated, ranging in size from 333 to 826 bp. The larger cDNAs were subcloned into a pUC plasmid vector and tested in transformation experiments for their ability to complement the *nudC3* mutant phenotype relying on gene conversion to correct the *nudC3*

Figure 1. The effect of the *nudC3* mutation on nuclear migration in germlings of *Aspergillus nidulans*. The series on the left show a wild type strain and the series to the right a strain carrying the *nudC3* mutation. Growth of conidia (asexual uninucleate spores) was at the restrictive temperature of 42°C and nuclei have been visualized by staining with the DNA-specific dye DAPI. The temperature of growth has no effect on nuclear motility in the wild type strain but has severely restricted nuclear migration in the *nudC3* strain. Bar, 5 μ m.

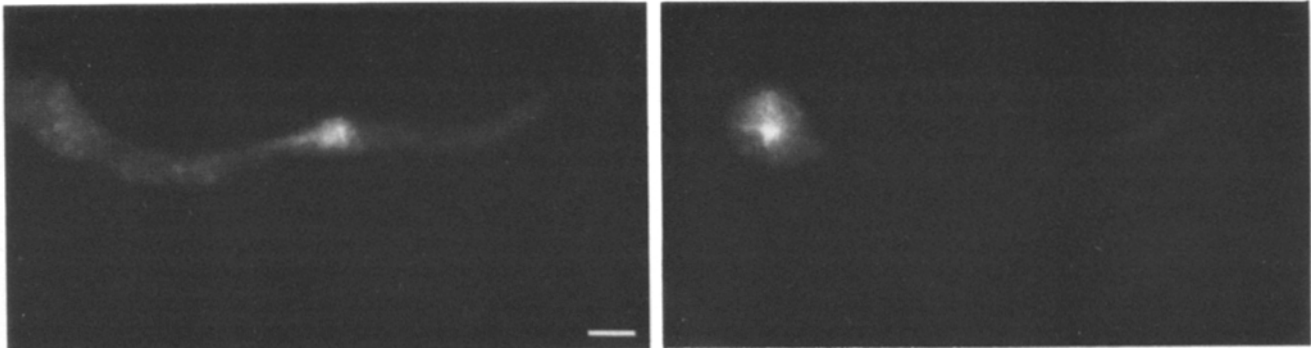


Figure 4. Absence of nuclear division does not affect the *nud* phenotype of *nudC3*. Conidia of a strain containing the *nimA5* mutation (*left panel*) and a double *nimA5 nudC3* mutant (*right panel*) were germinated at the restrictive temperature of 42°C and stained with DAPI to visualize the number and position of nuclei. Bar, 5 μ m.

mutation. These were found to be able to complement the *nudC3* mutation, confirming they contain the *nudC* gene. Initial sequence data showed that all the cDNAs were of a common origin, the *nudC* locus, and were derived from within a single Hind III fragment. Sequence analysis was performed on this Hind III fragment to obtain the genomic sequence of the gene and all four cDNAs were sequenced to derive the *nudC* translational unit (Fig. 6).

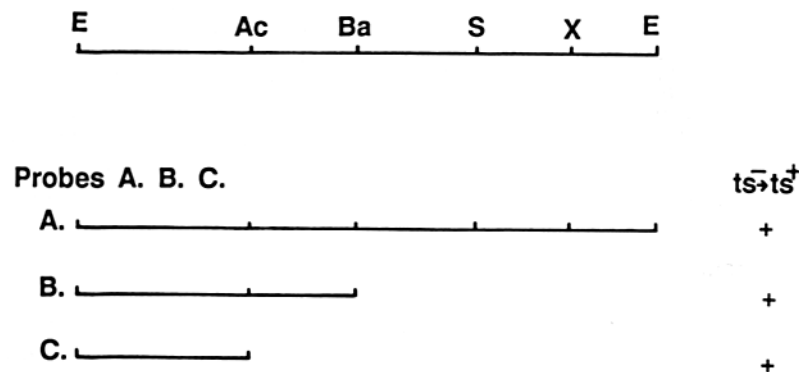
A comparison of the *nudC* cDNA sequences to that of the genomic reveals that there are two introns present (Fig. 6). Interestingly, one of the cDNAs isolated did not have these introns spliced out, leading to the formal possibility that the *nudC* gene encodes two different proteins based on differential splicing (but see below).

Theoretical translation of the cDNA that extended furthest to the 5' end identified an open reading frame initiating at the first ATG present in the sequence after 76 nucleotides.

This open reading frame is preceded by a stop codon and is open for 198 codons and could encode a protein of 22.4 kD (Fig. 6). Translation of the cDNA derived from unspliced mRNA could potentially initiate at this same codon. This reading frame is maintained through the first intron, which would introduce an extra 21 amino acids. The reading frame is continued through the second exon and remains open into the second intron. After translation of 24 amino acids encoded in the second intron the reading frame terminates. This second potential protein would contain 233 amino acids and have a computed molecular weight of 26 kD.

Identification of the *nudC* Gene Product in *Aspergillus*

A fusion protein between the *trpE* gene of *E. coli* and a portion of the *nudC* gene was produced in *E. coli* and purified to use as antigen to raise a polyclonal antisera against the *nudC* gene product (see Materials and Methods). This sera,



Hybridization of probes A, B, and C to polyA⁺ mRNA.

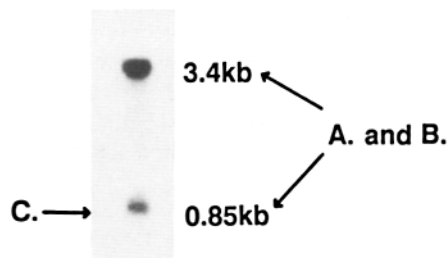


Figure 5. Subcloning of a genomic clone containing the *nudC* gene. The top line diagram shows a partial restriction map of a 6.4-kb genomic fragment able to complement the *nudC3* mutation (*E*, Eco RI; *Ac*, Acc I; *Ba*, Bam HI; *S*, Sal I; *X*, Xho I). Fragments *A*, *B*, and *C* were tested for their ability to complement *nudC3* and were hybridized to polyA⁺ mRNA on Northern blots. Shown is mRNA probed with *A*. Only probe *C* hybridized exclusively with the 0.85-kb species.

GCTAAATCCAACTTTCTCCATTTCCCTTCTTCTGCTCTCTCGAACTTACTTACTCAAGGTCAATCTATACCGCAAG

5 10 15 20
 ATG TCG GAA CAA GAA CCG TCT TCT GCC GAC CTC GCC GCC CGC GAG GCC GAA GAA AAG CAA
 M S E Q E P S S A D L A A R E A E E K Q

25 30 35 40
 CGC AAA GCC GCC GAA GAA GCT GAG CAG GCG ACC CTC CCC TAC AAA TGG ACA CAG ACG ATC
 R K A A E E A E Q A T L P Y K W T Q T I

45 50 55 60
 CGC GAC GTG GAC GTC ACG ATA CCC GTC TCT GCG AAC CTG AAG GGA CGC GAT CTG GAC GTC
 R D V D V T I P V S A N L K G R D L D V

65 70 75 80
 GTG CTC AAA AAG GAC AGC ATT AAG GTT AAG GTC AAG GGC GAG AAC GGG GAG GTC TTT ATT
 V L K K D S I K V K V K G E N G E V F I

GAC GTACTACAACCATCTCCCCAAATCTCCACGCAGCGAGGGCTAACCAAGGGTTTGATAATAAAG GGC CAA TTT
 D G Q F

85 90 95 100
 CCG CAC CCC ATC AAA CCG TCT GAG TCT TCC TGG ACG CTT GAA ACA ACG TCT AAA CCT CCC
 P H P I K P S E S S W T L E T T S K P P

105 110 115 120
 GGC AAG GAA GTC AGC ATC CAC CTT GAC AAA GTC AAC CAG ATG GAG TGG TGG GCG CAC GTT
 G K E V S I H L D K V N Q M E W W A H V

125 130 135 140
 GTC ACC ACC GCG CCG AAG ATC GAT GTC AGC AAG ATC ACG CCG GAG AAC TCG AGT CTG AGC
 V T T A P K I D V S K I T P E N S S L S

145 150 155 160
 GAC CTG GAC GGT GAG ACC AGG GCG ATG GTT GAG AAG ATG ATG TAT GAT CAG CCG CAG AAG
 D L D G E T R A M V E K M M Y D Q R Q K

165 170 175 180
 GAG ATG GGA GCG CCG ACC AGT GAT GAG CAG AGG AAG ATG GAT ATT TTG AAG AAG TTC CAG
 E M G A P T S D E Q R K M D I L K K F Q

185
 AAG GAA CAT CCT G GTATGTTGATCTATGCATCTGCGCGTCTTATTGTTCTGAGTGAAGGTGGAATGATGC
 K E H P

190 195
 GAGCATTGTGCTAATGTTACTGAGCTTTTGCAG AG ATG GAC TTT TCG AAT GCG AAG ATT GGT TAG TGA
 E M D F S N A K I G - -

* * * *
 CAGGCTAATGCTAATGAAGTTTATGATTTCCCTACCTCGACTTAATGTGCTCTATATAAGCATCTACTTTGTATGGA
 CAGGCTAATTAATCCAGTGTAAATCAGGCTGAATGAGGCTGAAGAATGCACGGGACGCCAA

Figure 6. Genomic sequence of *nudC*. The sequence begins at the 5' end defined by the largest cDNA isolated and extends 3' to all cDNAs isolated. The intron positions were derived by comparing the genomic sequence to that of cDNA sequence. The four asterisks show the position of polyadenylation sites for the four cDNAs isolated. These sequence data are available from EMBL/GenBank/DBJ under accession number X52565.

when reacted to *Aspergillus* protein, identified a protein of some 22 kD and a high molecular weight smear on Western blots. The preimmune sera did not react with the 22-kD protein nor with the smear. We affinity purified the immune sera against the primary antigen. This purified sera only reacted with the 22-kD protein, and the wash through from the affinity column reacted with the high molecular weight smear (Fig. 7). These data indicate that the unspliced mRNA, detected by the isolation of genomic sequence containing cDNA, does not get translated in vivo because, if it did, one would expect to detect a second protein significantly larger than 22 kD, at 26 kD. Therefore, because the high molecular weight material detected by the immune sera is not reactive in the affinity-purified sera, and because the molecular weight

of the reactive protein to the affinity-purified antibody is that predicted from the sequence analysis, we think it most likely that the 22-kD protein represents the only protein product of the *nudC* gene. However, there is a formal possibility that a second protein is produced from *nudC* at a level below our detection limits.

Preliminary immunofluorescence studies to localize the *nudC* gene product in actively growing germlings using the affinity-purified *nudC* specific antisera did not identify a particular subcellular location for the *nudC* gene product.

Discussion

In the present work we have tested the hypothesis that the

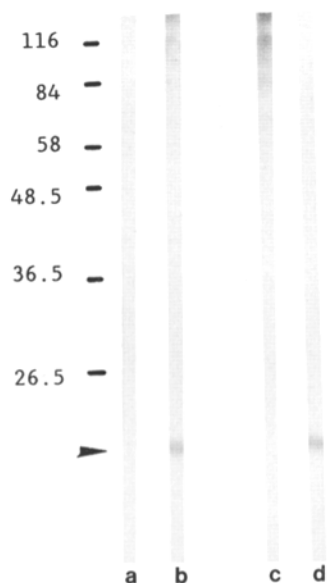


Figure 7. Western blot analysis of anti-*nudC* antisera. Four immunoblots are shown that contain *Aspergillus* protein reacted with (in order, left to right) preimmune sera, immune sera, wash through from affinity column, and affinity-purified sera. Relative size (kD) of marker proteins is shown to the left.

temperature-sensitive *nudC3* mutation of *Aspergillus* disrupts the microtubule-based motility system responsible for nuclear movement in this organism by selectively destroying a particular class of microtubule. The results indicate that this notion is not correct as all three classes of microtubules visible by indirect immunofluorescence (spindle, cytoplasmic, astral) could be readily detected in cells carrying the *nudC3* mutation when they were grown at the restrictive temperature and unable to move nuclei. We conclude that the molecular defect caused by the *nudC3* mutation that leads to an inability to transport nuclei is not associated with the loss of a particular class of microtubule. During the course of this work a similar conclusion has been reached, using EM, for another *Aspergillus nidulans nud* mutation, *nudB2* (Mayer et al., 1988).

We have also determined the effect of preventing nuclear division on the *nud* phenotype caused by the *nudC3* mutation by constructing a double mutant between *nudC3* and another temperature-sensitive mutation that causes a cell cycle-specific block at the restrictive temperature. The phenotype of the double mutant at the restrictive temperature is a *nud* phenotype in a cell containing a single division-blocked nucleus. This result clearly demonstrates that the *nudC3* mutant phenotype is independent of both mitosis and the number of nuclei present in the cell.

If the *nudC* gene product does not affect the structural integrity of microtubules or disrupt mitosis, how else might it function? Since actin, tubulin, and a kinesin-related protein have all been implicated to play a role in nuclear movement (Lloyd et al., 1987; Schatten et al., 1986), it could be a protein that interacts with, or regulates the activity of, one of these proteins. Although we know from its predicted amino acid sequence and from Western blots with antibody generated against a *nudC* fusion protein that the gene encodes a protein of 22 kD, comparison of the *nudC* protein sequence to known sequences in the standard databases has not provided any clues about the identity or activity of *nudC*. We do, however, now know that the *nudC* gene product is a newly discovered protein that plays a role in nuclear migration in addition to the already known proteins actin, tubulin, and

kinesin. How, or if, the *nudC* gene product interacts with these other components will be the focus of future studies.

Elucidation of the function of the gene products defined by the unique *nud* mutations of *Aspergillus* should shed considerable light on the molecular mechanisms by which eukaryotes normally move nuclei and possibly cytoplasmic motility in general. The cloning and sequence of *nudC* is the first step towards this analysis.

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