Cytoplasmic Dynein and Actin-related Protein Arp1 Are Required for Normal Nuclear Distribution in Filamentous Fungi

Michael Plamann, Peter F. Minke, John H. Tinsley, and Kenneth S. Bruno

Department of Biology, Texas A&M University, College Station, TX 77843-3258

Abstract. Cytoplasmic dynein is a multisubunit, microtubule-dependent mechanochemical enzyme that has been proposed to function in a variety of intracellular movements, including minus-end–directed transport of organelles. Dynein-mediated vesicle transport is stimulated in vitro by addition of the Glued/dynactin complex raising the possibility that these two complexes interact in vivo. We report here that a class of phenotypically identical mutants of the filamentous fungus *Neurospora crassa* are defective in genes encoding subunits of either cytoplasmic dynein or the Glued/dynactin complex. These mutants, defined as *ro*py, have curled hyphae with abnormal nuclear distribution. *ro-1* encodes the heavy chain of cytoplasmic dynein, while *ro-4* encodes an actin-related protein that is a probable homologue of the actin-related protein Arp1 (formerly referred to as actin-RPV or centractin), the major component of the glued/dynactin complex. The phenotypes of *ro-1* and *ro-4* mutants suggest that cytoplasmic dynein, as well as the Glued/dynactin complex, are required to maintain uniform nuclear distribution in fungal hyphae. We propose that cytoplasmic dynein maintains nuclear distribution through sliding of antiparallel microtubules emanating from neighboring spindle pole bodies.

Axonemal and cytoplasmic dyneins are large, multisubunit complexes that function as microtubule-dependent mechanochemical enzymes (Porter and Johnson, 1989; Vallee, 1993; Walker and Sheetz, 1993). Axonemal dynein is the motor that powers movement of cilia and flagella by sliding adjacent doublet microtubules. Cytoplasmic dynein has been implicated in a number of intracellular transport processes including retrograde transport of organelles in axons, the endocytic pathway, and organization of the Golgi (Walker and Sheetz, 1993). In addition, cytoplasmic dynein has been localized to kinetochores, spindle fibers and centrosomes, indicating a likely role in microtubule-dependent mitotic processes (Pfarr et al., 1990; Steuer et al., 1990).

Cytoplasmic dynein has a mass of 1.2×10^6 kD and consists of two heavy chains (~500 kD), three or four intermediate (~70 kD) chains, and four light (59–53 kD) chains (Paschal et al., 1987; Schroer et al., 1989). This complex is sufficient to support the movement of microtubules in vitro (Lye et al., 1987; Paschal et al., 1987); however, it does not support efficient in vitro microtubule-dependent movement of vesicles (Schroer et al., 1989). A second protein complex referred to as the Glued or dynactin complex (~6 subunits) is required for cytoplasmic dynein to mediate efficient microtubule-dependent transport of organelles in vitro (Schroer and Sheetz, 1991). Three subunits of the Glued/dynactin complex (150-, 50-, and 45-kD subunits) partially copurify with cytoplasmic dynein (Gill et al., 1991; Schroer and Sheetz, 1991; Paschal et al., 1993). The 150-kD subunit is homologous to the product of the *Drosophila* Glued gene and the 45-kD subunit has been identified as the actin-related protein Arp1 that was originally named actin-RPV or centractin (Gill et al., 1991; Holzbaur et al., 1991; Schroer and Sheetz, 1991; Lees-Miller et al., 1992*a*; Fyrberg et al., 1994; Schafer et al., 1994).

Genetic analysis of dynein has, until recently, been restricted to axonemal dynein. The unicellular alga *Chlamydomonas reinhardtii* has been used extensively for the analysis of flagellar structure, and many mutants with altered motility have been identified as defective in either inner or outer arm dyneins which generate the force required for flagellar movement (Dutcher and Lux, 1989). The absence of a system for the genetic analysis of cytoplasmic dynein has been due in part to the unknown phenotype of cytoplasmic dynein mutants. Recently, the gene encoding cytoplasmic dynein heavy chain has been isolated from a number of organisms including *Dictyostelium discoideum*, rat, *Saccharomyces cerevisiae*, and *Aspergillus nidulans* (Koonce et al., 1992; Eshel et al., 1993; Li et al., 1993; Mikami et al., 1993; Zhang et al., 1993; Xiang et al., 1994). With the availability of the cloned gene, cytoplasmic dynein mutants have been constructed in *S. cerevisiae* (Eshel et al., 1993; Li et al., 1993). Yeast dynein mutants show a reduced growth rate and a disruption in mitotic spindle orientation such that ~25% of budded cells have a binucleate mother cell and an enucleated

Address all correspondence to Michael Plamann, Dept. of Biology, Texas A&M University, College Station, TX 77843. Tel.: (409) 845-2982. Fax: (409) 845-2891.

bud. A screen of temperature-sensitive mutants of *A. nidulans*, a filamentous fungus, led to the identification of four mutants that are defective in nuclear distribution (*nud* mutants) (Morris, 1976). Recently, the *nudA* gene of *A. nidulans* was shown to encode cytoplasmic dynein heavy chain (Xiang et al., 1994). This result implicates cytoplasmic dynein as playing a role in the distribution and movement of nuclei in filamentous fungi.

In this paper, we report the characterization of a class of *Neurospora crassa* mutants that identify genes encoding subunits of cytoplasmic dynein and the Glued/dynactin complex. We determined previously that the *cot-1* gene of the filamentous fungus *N. crassa* encodes a serine/threonine protein kinase required for hyphal elongation (Yarden et al., 1992). In an effort to identify either the substrate(s) or specific process(es) regulated by Cot1 kinase, we have isolated mutants that show suppression of the hyphal growth defect of a *cot-1* mutant. We now describe a group of eight identical, but unlinked, mutants known as *ro*py (*ro*) that partially suppress a *cot-1* defect. All *ro* mutants have curled hyphal (*ro*py) growth and altered nuclear distribution. Our analysis of two of eight known *ro* genes shows that *ro-1* encodes the heavy chain of cytoplasmic dynein and *ro-4* encodes the actin-related protein Arp1. We propose that all mutants defective in genes specific for either cytoplasmic dynein or the Glued/dynactin complex will exhibit a *ro* phenotype. The ease with which *ro* mutants can be isolated and *ro* genes cloned makes *N. crassa* an ideal organism for the genetic dissection of cytoplasmic dynein.

Materials and Methods

Strains and Media

N. crassa strains used in this study were obtained from the Fungal Genetic Stock Center (FGSC), Department of Microbiology, University of Kansas Medical Center, Kansas City, KS. The wild-type *N. crassa* strain was 74-OR23-1A (FGSC 987). *cot-1*(C102t) (FGSC 4065) was used to isolate mutants containing *cot-1* suppressors (see below). The *ro* mutants were *ro-1* (FGSC 146), *ro-1* (FGSC 4351), *ro-2* (FGSC 44), *ro-3* (FGSC 43), *ro-4* (FGSC 2981), *ro-4* (FGSC 1511), *ro-6* (FGSC 3627), *ro-7* (FGSC 3322), *ro-7* (FGSC 4025), *ro-10* (FGSC 3619), *ro-11* (FGSC 3911), and *ro(P904)* (FGSC 1669). The construction of *ro*; *cot-1* double mutants and the formation of heterokaryotic strains was done using standard genetic procedures (Davis and de Serres, 1970). Media and growth conditions are as described (Davis and de Serres, 1970).

Identification of ro Mutations as Partial Suppressors of cot-1

cot-1 suppressors were isolated by plating 10^5 conidia of the temperature-sensitive *cot-1* mutant on a sucrose supplemented minimal medium and incubating at 37°C for 3–7 d. *cot-1* conidia germinate under these conditions and produce colonies that are ~0.1 mM in diameter (Fig. 1 *B*). Fully suppressed *cot-1* mutants grew very rapidly and covered a plate within 24 h, while partially suppressed *cot-1* mutants produced colonies that were 1–5 mM in diameter. Approximately 90% of partially suppressed *cot-1* mutants had curled hyphal growth when incubated at 25°C. Because curled hyphal growth is characteristic of a group of *N. crassa* hyphal growth mutants known as "ropy" mutants (Garnjobst and Tatum, 1967), we used heterokaryon analysis to determine if any of these mutants were alleles of known *ro* mutants. Analysis of two of these mutants, *ro*(PFM23) and *ro*(PFM30), showed them to be allelic with *ro-1* and *ro-10*, respectively. *ro*; *cot-1* double mutants were constructed for all eight nonallelic *ro* mutations that were available from the FGSC and all were determined to partially suppress *cot-1*.

Cloning and Sequence Analysis

Cosmids complementing the *ro-1* and *ro-4* mutants were identified by sib-selection (Vollmer and Yanofsky, 1986). Pools of 96 cosmid clones from the Orbach/Sachs cosmid library (FGSC) were used to transform *ro* mutants to hygromycin resistance. Hyg^R transformants were examined microscopically at 18–24 h after plating to identify transformants that showed straight hyphal growth instead of the characteristic curled hyphal growth of *ro* mutants. Pools of cosmids containing complementing clones were further subdivided until single cosmids were identified that complemented the *ro-1* or *ro-4* mutant. Complementing cosmid clones were digested separately with various restriction endonucleases, and *ro* mutants were cotransformed with the digested DNA and pMP6, a plasmid coding for Hyg^R (Plamann and Yanofsky, unpublished observations). (*N. crassa* is efficiently transformed with linear DNA.) The smallest fragment that efficiently complemented a *ro* mutant was subcloned. The map location of cloned DNA was determined by restriction fragment length polymorphism analysis (Metzenberg et al., 1985).

DNA sequencing was performed by the dideoxy-chain termination method (Sanger et al., 1977), using Sequenase version 2.0 (United States Biochemical Corp., Cleveland, OH) and custom oligonucleotide primers synthesized by the Gene Technologies Laboratory at Texas A&M University. DNA sequences were analyzed using programs of The University of Wisconsin Genetics Computer Group (Devereux et al., 1984). GenBank searches were performed at the National Center for Biotechnology Information (Bethesda, MD), using the BLAST network service (Altschul et al., 1990).

Standard procedures for Southern analyses, restriction endonuclease digestions, agarose gel electrophoresis, purification of DNA from agarose gels, DNA ligations, and other cloning-related techniques were performed as described (Sambrook et al., 1989). Genomic DNA was isolated from *N. crassa* as described (Yarden et al., 1992).

Isolation and Analysis of cDNA Clones

cDNA clones of *ro-1* and *ro-4* were isolated by screening ~360,000 plaques from a λ ZAP® II *N. crassa* cDNA library constructed by R. H. Garrett (University of Virginia, Charlottesville, VA) and available through the FGSC (Sambrook et al., 1989). Restriction fragments covering the distal one-third of *ro-1* and the proximal one-half of *ro-4* were used as probes. Four positive *ro-1* cDNAs clones and 10 *ro-4* cDNA clones were identified and rescued as phagemid (Stratagene Inc., La Jolla, CA). The DNA sequence of one of the *ro-1* cDNA clones was determined to verify the position of the distal intron (Fig. 3). The DNA sequence of an apparently full-length *ro-4* cDNA clone was determined to identify the 5′ end of the gene and verify the positions of three introns that were predicted, from analysis of the *ro-4* genomic DNA sequence, to interrupt the *ro-4* structural gene (Fig. 5).

Analysis of Hyphal Morphology and Nuclear Distribution in Wild-type, cot-1, and ro Mutants

Hyphal morphologies of wild-type, *cot-1*, various *ro* mutants, and *ro*; *cot-1* double mutants were determined by inoculating sucrose minimal agar media with conidia and incubating plates at the designated temperature for 2 d. Conidia from *cot-1* were plated with 100 μl of 1 M sorbitol solution to help prevent lysing of *cot-1* spheres. Pictures were taken with TMAX 100 or TMAX 400 film on an Olympus binocular dissection microscope at 70× magnification with transmitted light.

Nuclear distribution was examined by staining cells with 4′6-diamidino-2-phenylindole (DAPI).[1] Coverslips were placed on an agar surface and ~100 conidia, suspended in sucrose minimal liquid media, were placed on each coverslip and the plates were incubated at 34°C for 16 h. Hyphal material on coverslips was treated for 1 min with fixing solution (3.7% formaldehyde, 67% ethanol), rinsed for 5 min with wash solution (50 mM KPO_4, pH 7.0), and then soaked in wash solution containing 0.5 μg/ml DAPI for 10 min. Vaseline was placed around the edges of the coverslip which was then pressed to a slide. Pictures were taken with TMAX 400 film on an Olympus microscope using a SPlan Apo 60X oil objective and differential interference contrast optics. The sample was illuminated with UV light to view the DAPI stain.

1. *Abbreviations used in this paper*: DAPI, 4′,6-diamidino-2-phenylindole; MTOC, microtubule-organizing center; ORFs, open reading frames; uORF1, upstream ORF1; SPB, spindle pole bodies.

Results

Ropy (ro) Mutants Are Partial Suppressors of cot-1

cot-1 is predicted to encode a serine/threonine protein kinase, and inactivation of *cot-1* results in cessation of hyphal elongation and massive induction of branching (Yarden et al., 1992). To learn more about the role of Cot1 kinase, *cot-1* suppressors were identified by plating conidia (asexually derived spores) of a *cot-1*^{ts} mutant strain at 37°C and screening for colonies with increased radial growth rates. The majority of mutants (>95%) were partially suppressed for *cot-1* function and showed only slightly improved radial growth rates as compared to wild type (Fig. 1, *C* vs. *B*), while a small minority (<5%) were fully suppressed. Most of the full suppressor mutations had no visible effect when mutants were grown at 25°C, but many of the partial suppressor mutants had an unusual hyphal growth phenotype consisting of curled and distorted hyphae (Fig. 1 *D*). This unusual hyphal growth phenotype was independent of the

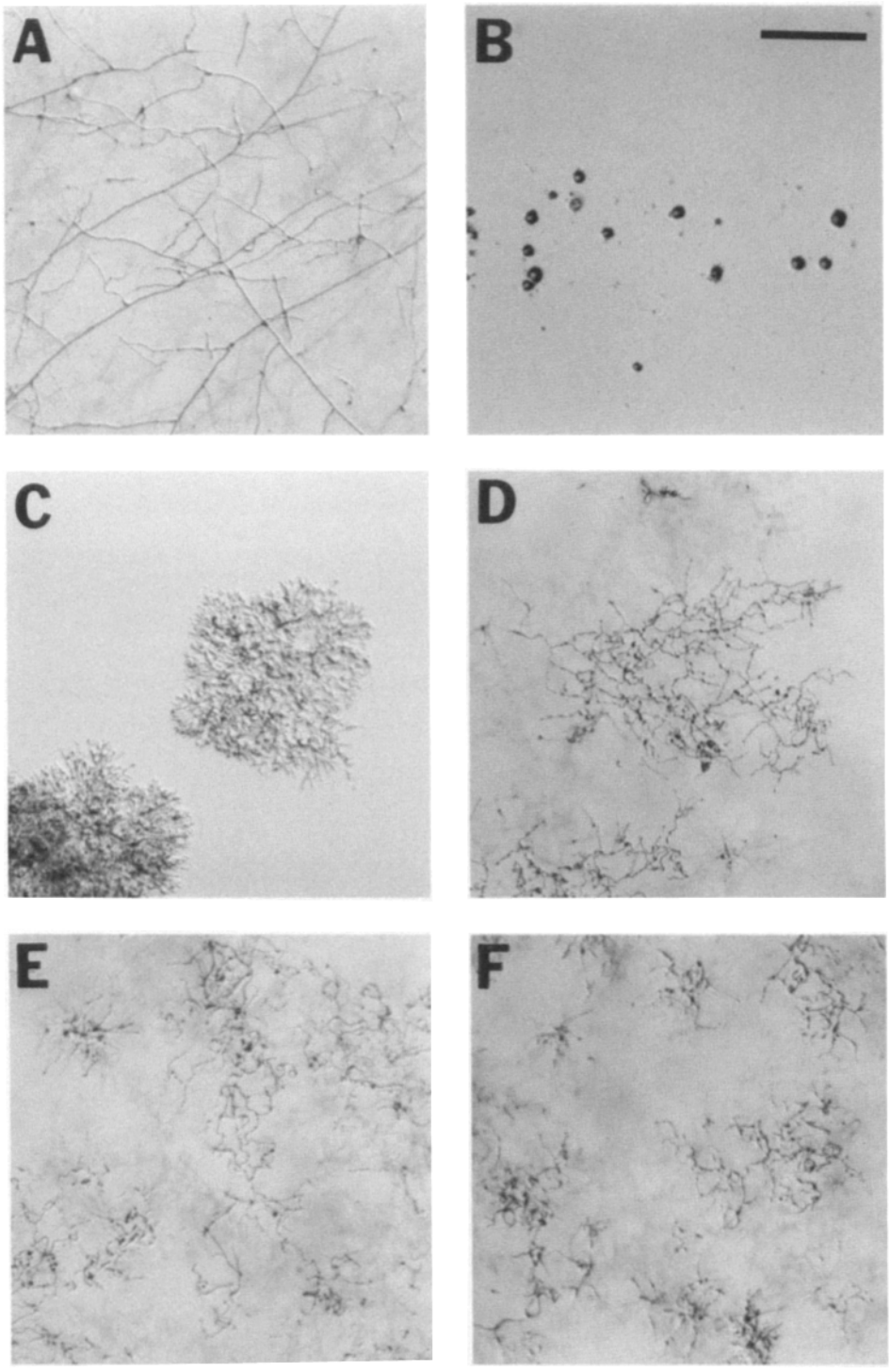

Figure 1. Partial suppression of a *cot-1* mutant by *ro-1* and *ro-4* mutations. Conidia from *N. crassa* *cot-1*, *ro* and *ro*; *cot-1* mutants were inoculated on sucrose minimal agar media and incubated at either 25 or 37°C for 2 d. (*A* and *B*) *cot-1* growing at 25 and 37°C, respectively. (*C* and *D*) *ro-1*; *cot-1* growing at 37 and 25°C, respectively. Note the increased colony size of a *ro-1*; *cot-1* double mutant (*C*) vs. a *ro*$^{+}$; *cot-1* strain (*B*). (*E* and *F*) *ro-1* and *ro-4* mutants growing at 37°C, respectively. Bar, 0.5 mM.

original *cot-1* mutation (Fig. 1, *E* and *F*). Because these mutants appeared similar to a class of hyphal growth mutants known as "ropy" mutants, we tested the genetic relationship between partial suppressors and the eight previously described *ro* mutants (Garnjobst and Tatum, 1967). The first two *cot-1* suppressor mutants we isolated were determined to be alleles of *ro-1* and *ro-10*, respectively, and all eight *ro* mutants (*ro-1*, *-2*, *-3*, *-4*, *-6*, *-7*, *-10*, *-11*) partially suppressed *cot-1* in the same manner (not shown).

Ro Mutants Are Defective in Nuclear Distribution, Hyphal Growth and Sexual Development

Nuclei are relatively evenly distributed in *N. crassa* and other coencytial fungi (Fig. 2 *A*). In *ro* mutants nuclear distribution is highly asymmetric (Fig. 2, *B* and *C*; *ro-1* and *ro-4* mutants, respectively). Large numbers of nuclei are observed in some regions of hyphae while other regions have none. The clumpy nuclear distribution found in *ro-1* and *ro-4* is typical for all *ro* mutants (not shown). Along with the unusual nuclear distribution, we find that hyphal tips grow in a curled fashion and are able to extend a considerable distance (>200 μm) without nuclei (Fig. 2, *B* and *C*). In addition, these long enucleate hyphae are able to branch which indicates that the formation of a new hyphal tip can occur in the absence of close proximity to a nucleus. We have also examined the ability of *ro* mutants to participate in crosses and we find that all are able to function as males, but only strains containing weak (nearly normal hyphal growth) *ro* alleles are able to function as females. All *ro* mutants have nearly normal conidiation (asexual reproduction).

ro-1 Encodes the Heavy Chain of Cytoplasmic Dynein

ro mutants define at least eight genes that when mutated give rise to abnormal nuclear distribution and hyphal growth. The *ro-1* and *ro-4* genes were isolated by complementation of the respective *ro* mutants with a *N. crassa* genomic cosmid library (Materials and Methods). Complementation was defined as restoration of straight hyphal growth and rapid radial extension of individual colonies (Fig. 1, *A* vs. *E* and *F*). Complementing DNA was subcloned by identifying restriction endonuclease-generated fragments that were able to complement the respective mutant (*N. crassa* is efficiently transformed with linear DNA). The smallest complementing DNA fragment was 18 kb for *ro-1* and 4.0 kb for *ro-4*. RFLP mapping was used to determine the map location of the clones (the RFLP data will be presented in the *Fungal Genetics Newsletter*). The map position of *ro-1*– and *ro-4*–complementing DNA coincided with that of the respective mutations indicating that complementing DNAs were likely to contain functional copies of the genes and not extracopy suppressors.

ro-1 has a 4367–codon open reading frame that is interrupted by two short introns and is predicted to encode a polypeptide with a calculated mol wt of 495,574 (Fig. 3). Rol is 53 and 49% identical to the cytoplasmic dynein heavy chain of rat and *Dictyostelium*, respectively, and 29% identical to sea urchin flagellar dynein β heavy chain (Fig. 4, *A* and *B*). Based on extensive similarity with the rat and *Dictyostelium* proteins, and the absence of flagella and cilia in *N. crassa*, we conclude that *ro-1* encodes the heavy chain subunit of cytoplasmic dynein. It is likely *N. crassa* has only a single cytoplasmic dynein heavy chain, because probing Southern blots of genomic DNA and cDNA libraries with *ro-1* sequences at low stringency did not identify additional cytoplasmic dynein heavy chain-like sequences (data not shown). *N. crassa* Rol is also 72% identical to the *A. nidulans* cytoplasmic dynein heavy chain. Interestingly, the cytoplasmic dynein heavy chain of *S. cerevisiae* is only ~32% identical

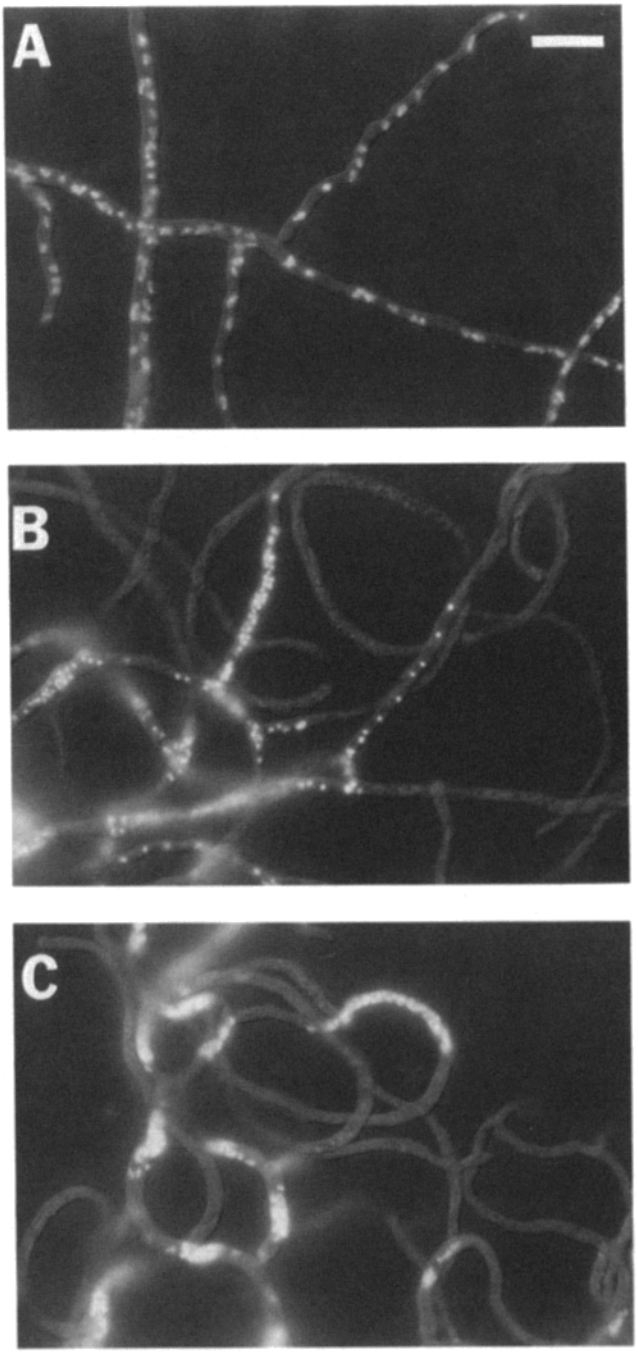

Figure 2. The effect of *ro* mutations on nuclear distribution. (*A*) Wild-type (74-OR23-1A), (*B*) *ro-1*, and (*C*– *ro-4 N. crassa* mutants were grown overnight in sucrose minimal media at 34°C, and nuclei were visualized by staining with the DNA-specific dye DAPI. Bar, 20 μm.

Figure 3. Deduced amino acid sequence of the *N. crassa ro-1* gene product. Four ATP-binding consensus sequences are in bold and underlined. *ro-1* is proposed to contain two introns: a 66-bp intron that interrupts codon 104; and a 72-bp intron that is located between codons 4205 and 4206 (the respective amino acids are in bold). The introns were predicted by identification of 5′ and 3′ splice junction consensus sequences and comparison of the Rol sequence with cytoplasmic dyneins of other species. The presence of the second intron was verified by DNA sequence analysis of a *ro-1* cDNA clone. These sequence data are available from EMBL/GenBank/DDBJ under accession number L31504.

with either *N. crassa* or *A. nidulans*. This indicates that cytoplasmic dynein heavy chain of yeast has significantly diverged from that of other organisms, including the filamentous fungi.

The region of highest sequence identity among dynein heavy chains surrounds four P-loop motifs (GXXXXGKT/S), characteristic of many ATP-binding sequences (Walker et al., 1982; Fry et al., 1986), that are clustered in the middle third of the protein (Fig. 3). All four of these sites are conserved in both cytoplasmic and flagellar dynein heavy chains. The function(s) of these sites have not yet been determined; however, the first site has been proposed to be the primary site of ATP binding and hydrolysis (Gibbons et al., 1991). Rol, as well as the respective polypeptides of *A. nidulans* and *Saccharomyces cerevisiae*, differ from other dynein heavy chains in that they lack a COOH-terminal ~280-residue segment that is present in both cytoplasmic and flagellar forms (Fig. 4, *A* and *B*). DNA sequence analysis of a *ro-1* cDNA indicated that the absence of this region is not due to a DNA rearrangement of *ro-1* sequences during the cloning procedure (data not shown). In addition, Southern analyses indicated that the restriction map of the *ro-1*–containing cosmid clone was identical with the corresponding region of the *N. crassa* genome (data not shown).

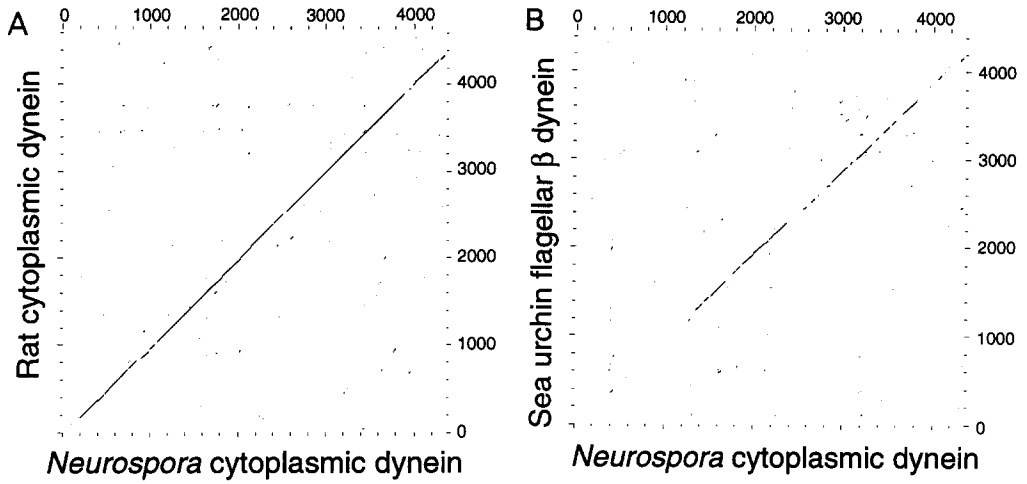

Figure 4. Dot plot matrix comparing the amino acid sequence of *N. crassa* cytoplasmic dynein heavy chain with the amino acid sequences of rat cytoplasmic dynein heavy chain (*A*) and sea urchin flagellar dynein β heavy chain (*B*). The plots were made using the UWGCG program COMPARE with a window size of 50 and a stringency of 25. Note that the last ~280 residues of cytoplasmic and flagellar dynein heavy chains are not found in Rol (see upper right hand corner of each plot).

It is possible that the absence of this conserved region (40% identity between rat and *Dictyostelium* proteins) indicates a functional difference between cytoplasmic dynein of fungi and dynein heavy chains of other organisms.

ro-4 Encodes a Member of the Arp1 Family of Actin-related Proteins

The DNA sequence and gene organization of *ro-4* is shown in Fig. 5. The *ro-4* structural gene is interrupted by three introns and encodes a 380–amino acid polypeptide that has a predicted mol wt of 42,382. Analysis of a *ro-4* cDNA clone indicates that the 5′ end of *ro-4* mRNA is ~374 bases upstream of the proposed translation initiation codon (Fig. 5). Within the unusually long 5′ leader region are three open reading frames (ORFs) of 3, 3, and 13 codons, respectively. Curiously, upstream ORF1 (uORF1) overlaps uORF2, and uORF3 overlaps the proposed translation initiation codon of *ro-4* (Fig. 5). The preferred sequence context of *N. crassa* translation initiation codons is 5′-CNNNCAMVATGGC-3′, where M = A or C and V = A or C or G (Bruchez et al., 1993*b*). None of the four ATG initiator codons have flanking sequences that are ideal matches to the consensus sequence, although the context of the proposed ATG initiator codon for *ro-4* has the best match. The presence of ORFs within the leader region of *ro-4* may indicate that *ro-4* gene expression is translationally regulated (Yoon and Donahue, 1992).

The predicted amino acid sequence of Ro4 has similarity to actin-related proteins and actin (Fig. 6). Two classes of actin-related proteins have been identified in vertebrates, centractin and Act2 (now referred to as the Arp1 and Arp3 families; Fyrberg et al., 1994). A comparison of Ro4 with centractin (Arp1), human cytoplasmic γ-actin and the bovine, *Drosophila* and *Schizosaccharomyces pombe* Act2 proteins (members of the Arp3 family) shows that Ro4 is an apparent homologue of centractin. Ro4 and centractin are identical at 42 positions where these proteins differ from both actin and Arp3 proteins (Fig. 6). Ro4 is 65% identical with centractin, 50–55% identical with conventional actins, and ~38% identical with members of the Arp3 family. In addition, the Arp2 and Arp3 families differ from conventional actins in having insertions of amino acids at specific positions while members of the Arp1 family are nearly collinear with actin (Fyrberg et al., 1994). Based on this criteria and the sequence similarity between Ro4 and centractin, we conclude that Ro4 is a member of the Arp1 family (Fig. 6). As noted previously for centractin (Lees-Miller et al., 1992*a*), most residues that are required for actin polymerization and binding of ATP and Ca^{2+} are conserved in Ro4 (Fig. 6), while residues thought to interact with the myosin head are not conserved. Centractin has been identified as the major component of the Glued/dynactin complex, which is an activator of cytoplasmic dynein-driven vesicle movement (Lees-Miller et al., 1992*a*).

Discussion

We showed previously that *N. crassa cot-1* encodes a serine/threonine protein kinase that is required for hyphal tips to elongate, but is not required for hyphal tips to form. In this study, we have shown that recessive mutations at eight different loci are able to partially suppress the effects of a *cot-1* mutation and result in a *r*opy phenotype. The basis of this suppression is unknown, but it is unlikely that Cot1 kinase directly regulates cytoplasmic dynein activity because recessive mutations in multiple genes encoding subunits of cytoplasmic dynein or the Glued/dynactin complex can partially suppress a *cot-1* defect. It is more likely that mutations resulting in decreased dynein activity partially bypass the need for Cot1 kinase. Microscopic analysis of a *cot-1* mutant has led to the suggestion that *cot-1* may be required for transport of precursors and enzymes, needed for cell wall synthesis, to hyphal tips (Steele and Trinci, 1977). If Cot1 kinase is a regulator of long-range transport of cell wall vesicles or a step in the secretory pathway, then it may be that a mutation affecting the endocytic pathway, as would be predicted for a cytoplasmic dynein mutant (Aniento et al., 1993), partially compensates for the loss of Cot1 kinase activity. Alternatively, a disruption in distribution of nuclei, as is seen in all of the *ro* mutants, may in some unknown way partially suppress a *cot-1* mutant.

ro Mutants Define Genes Required for Cytoplasmic Dynein and Glued/Dynactin Complex Activity

We have determined that *ro-1* is a single-copy gene that en-

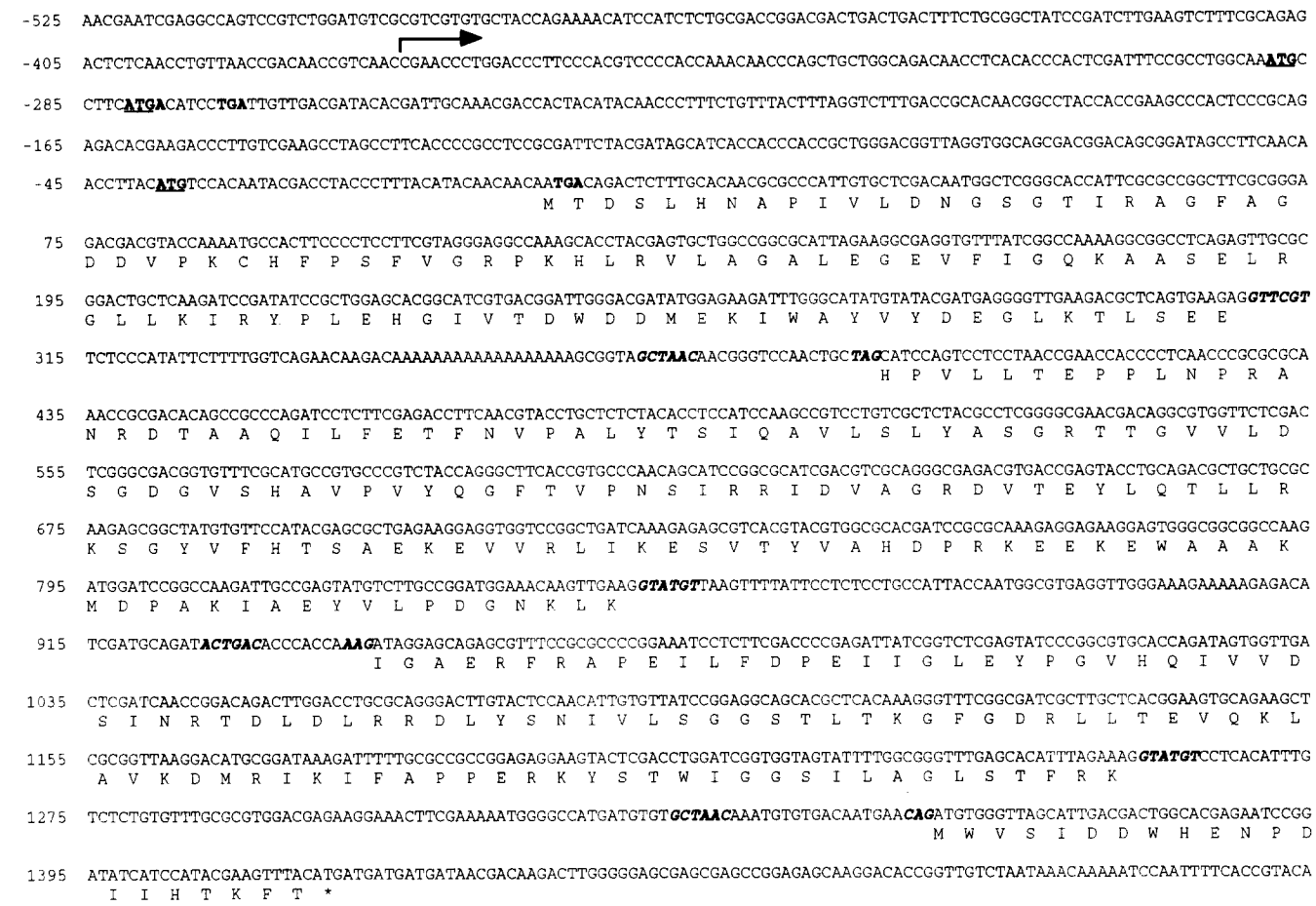

Figure 5. Genomic DNA sequence of *N. crassa ro-4*. The *ro-4* structural gene is 380 codons long and is interrupted by three introns. +1 defines the A residue of the predicted translation initiation codon for *ro-4*. The 5′ and 3′ splice junctions and the internal conserved sequences for each intron are in bold and italics (Bruchez et al., 1993*a*). The 5′ end of an apparently full-length cDNA is indicated by the arrow above the DNA sequence. Three short open reading frames within the 374-bp 5′ leader region are indicated. The ATG sequence initiating each uORF is underlined and in bold, and the respective translation termination codon for each uORF is also in bold. Note that uORF1 overlaps uORF2 and uORF3 overlaps the predicted translation initiation codon for *ro-4*. These sequence data are available from EMBL/GenBank/DDBJ under accession number L31505.

codes the heavy chain of cytoplasmic dynein and *ro-4* encodes an actin-related protein that is an apparent homologue of the vertebrate protein centractin, a member of the Arp1 family. Recently, a centractin-like protein has been identified in *Pneumocystis carinii* (GenBank accession number L21184). This protein is 75% identical to Ro4, 69% identical to centractin, and has conserved 36 of the 42 residues that are specific to Ro4 and centractin. The high level of conservation between these three proteins relative to actin and other actin-related proteins suggests that they are homologues. Centractin has been determined to be the most abundant subunit of the Glued/dynactin complex, which is required for cytoplasmic dynein to efficiently transport vesicles along microtubules in vitro (Gill et al., 1991; Schroer and Sheetz, 1991). The finding that recessive mutations affecting cytoplasmic dynein or the Glued/dynactin complex exhibit the same phenotype provides the first genetic evidence that these two complexes are required for the same cellular process(es).

All of the *ro* mutants are phenotypically identical. We propose that all genes encoding polypeptides that are nonredundant subunits of cytoplasmic dynein or the Glued/dynactin complex will give a *ro* phenotype when mutated. In addition, we also predict that regulatory genes that are required specifically for expression of genes encoding dynein subunits, assembly of the cytoplasmic dynein and Glued/dynactin complexes, or regulation of dynein activity will also exhibit a *ro* phenotype when mutated. In support of this hypothesis, we have recently determined that the *ro-3* gene encodes an apparent homologue of p150^{Glued} which is the largest subunit of the Glued/dynactin complex (Plamann, M., P. F. Minke, J. H. Tinsley, and K. S. Bruno, unpublished observations). Therefore, the ability to isolate an unlimited number of *ro* mutants by the identification of partial suppressors of *cot-1* should allow the genetic identification of all nonredundant genes that are specifically required for cytoplasmic dynein or Glued/dynactin complex activity. However, even if all genes that are specifically required for dynein activity give a *ro* phenotype when mutated, they may represent only a subset of all *ro* mutants. One of the most striking phenotypic effects of a *ro* mutation is the disruption of nuclear distribution. As stated above, if alteration of nu-

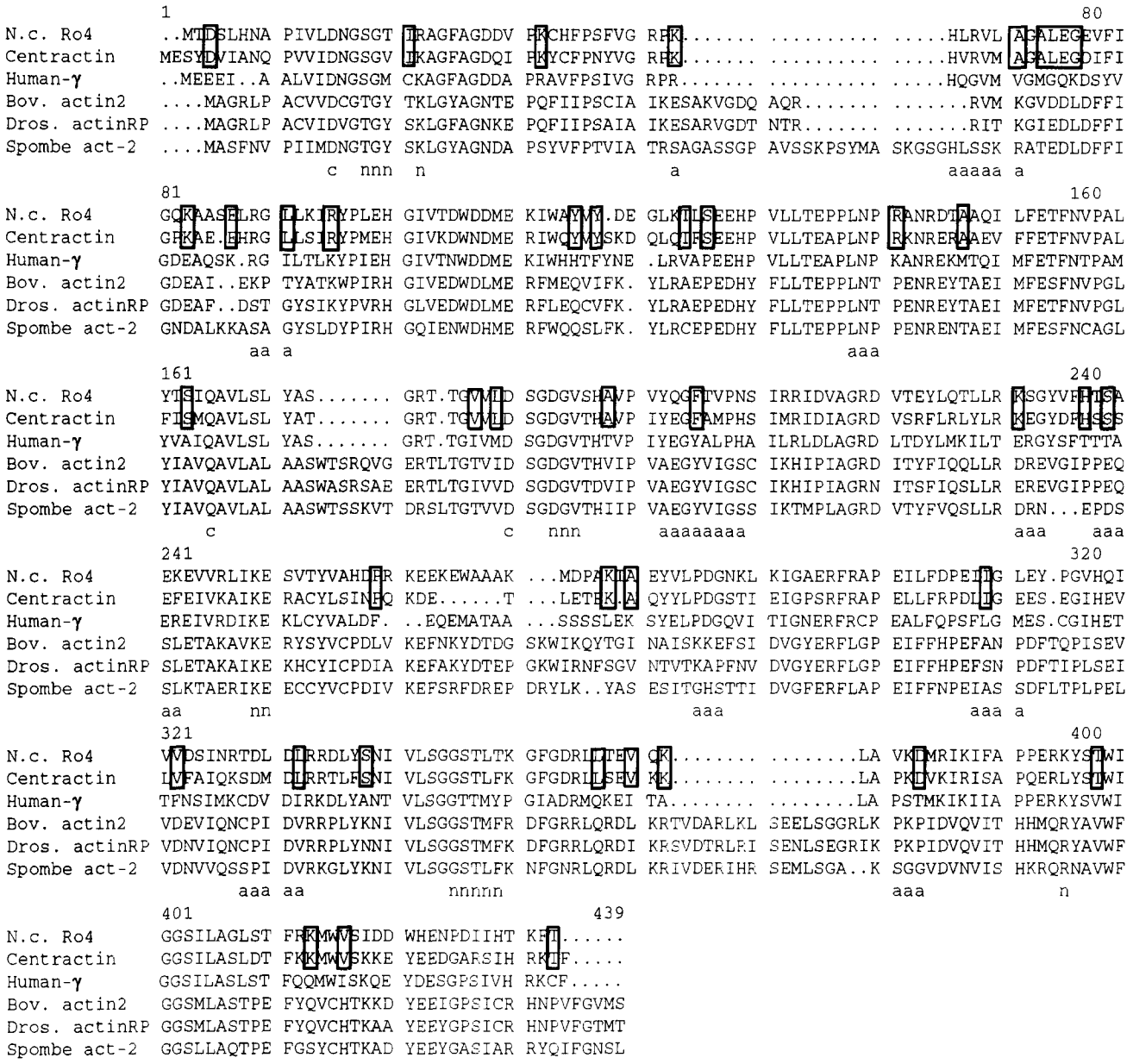

Figure 6. Comparison of the predicted amino acid sequence of Ro4 with human centractin, human cytoplasmic γ-actin, and the products of the *act2* genes of bovine, *Drosophila* and *S. pombe* (Erba et al., 1986; Lees-Miller et al., 1992*a*,*b*; Tanaka et al., 1992; Fyrberg and Fyrberg, 1993). Alignments were made using the GCG program PILEUP. Dots that interrupt sequences indicate gaps that were introduced to maximize the alignment. Ro4 and centractin sequences that are boxed indicate specifically conserved residues. The residues in actin that interact with ATP and Ca^{+2} are indicated by the *n*'s and *c*'s below the sequence. The residues involved in actin-actin polymerization are indicated by the *a*'s below the sequence (Holmes et al., 1990; Kabsch et al., 1990).

clear distribution leads to partial suppression of *cot-1* and the *ro* phenotype, then any mutation that alters nuclear distribution will be identified as a *ro* mutant. One would expect that there exist genes that are required for nuclear distribution but are not required for cytoplasmic dynein activity.

Nuclear Distribution and Hyphal Growth in N. crassa Cytoplasmic Dynein Mutants

Cytoplasmic dynein has been proposed to be involved in retrograde transport of organelles in axons, the endocytic pathway, organization of the Golgi and microtubule-dependent mitotic processes (Walker and Sheetz, 1993). Cytoplasmic dynein mutants have now been constructed in *S. cerevisiae* and isolated in *A. nidulans* and *N. crassa* (Eshel et al., 1993; Li et al., 1993; Xiang et al., 1994). In *S. cerevisiae*, cytoplasmic dynein mutants are defective in orientation of the mitotic spindle, and in *A. nidulans* and *N. crassa* (filamentous fungi) cytoplasmic dynein mutants are defective in nuclear distribution. It is noteworthy that hyphal tips of *N. crassa* cytoplasmic dynein mutants are able to grow considerable distances (>200 μm) without nuclei. Transport of apical vesicles, containing precursors and enzymes required for cell wall synthesis, to hyphal tips has

been proposed to be dependent on cytoplasmic microtubules (Gooday, 1983). We have determined that enucleate hyphae of *ro* mutants contain abundant cytoplasmic microtubules that extend from hyphal tips to the nearest clump of nuclei (Minke and Plamann, unpublished observations). Therefore, the presence of cytoplasmic microtubules in enucleate hyphae and the absence or reduction of cytoplasmic dynein activity in *ro* mutants suggests that microtubule-dependent transport of apical vesicles does not require minus-end–directed cytoplasmic dynein activity.

A Model for Establishment and Maintenance of Nuclear Distribution in Filamentous Fungi

In coenocytic fungi, nuclei are relatively evenly distributed (Fig. 2 *A*). The division of nuclei within each hypha is synchronized such that internuclear distances are at a minimum immediately after nuclear division and at a maximum just before nuclear division (Rosenberger and Kessel, 1967; Robinow and Caten, 1969; Morris, 1976). As a hyphal tip grows, nuclei migrate towards the tip while maintaining their position relative to each other. Nuclear migration has been shown to be dependent upon microtubules (Oakley and Morris, 1980), and in this study we show that nuclear movement is also dependent upon cytoplasmic dynein (Fig. 2, *B* and *C*). We propose that nuclear distribution and movement could be maintained through the action of cytoplasmic dynein on cytoplasmic microtubules (Fig. 7). In our model, cytoplasmic microtubules radiate out from spindle pole bodies (SPB; a fungal microtubule-organizing center (MTOC) that is associated with each nucleus). For simplicity, we show a single microtubule extending in each direction from each SPB. Only cytoplasmic microtubules originating from adjacent SPBs (i.e., microtubules of opposite polarity) are cross-linked through the action of cytoplasmic dynein. Cytoplasmic dynein anchors to one microtubule and the dynein motor heads exert force towards the minus-end of an antiparallel microtubule. Therefore, adjacent SPBs, and thus nuclei, will be pulled towards each other due to the motor activity of dynein. When nuclei enter mitosis (Fig. 7 *B*), cytoplasmic microtubules disassemble (Gambino et al., 1984; Osmani et al., 1988, 1990; Salo et al., 1989), and after nuclear division, cytoplasmic microtubules reform and nuclear positioning is re-established (Fig. 7, *C* and *D*). Assuming a uniform number of dynein motors per given length of overlapping, antiparallel cytoplasmic microtubules, nuclei that are far apart will be more strongly pulled towards each other than nuclei that are close together, because they will have a larger region of overlapping cytoplasmic microtubules and hence more force-producing dynein motors drawing the two nuclei together. Equilibrium is achieved when internuclear distances are uniform.

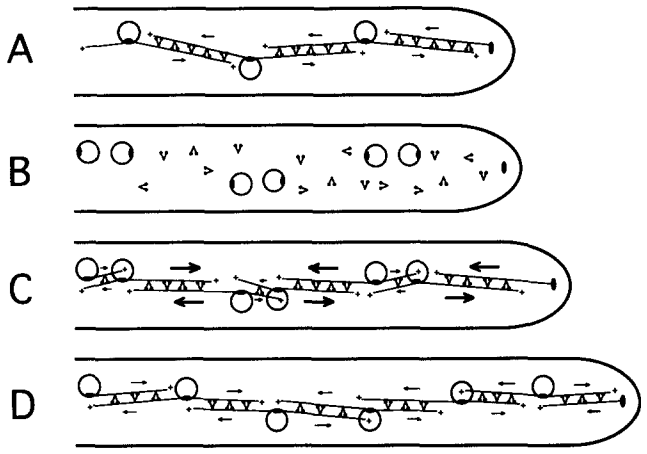

Figure 7. Model for the establishment and maintenance of nuclear distribution in coenocytic fungi. Cytoplasmic dyneins, represented by V-shaped structures, are proposed to cross-link antiparallel cytoplasmic microtubules that emanate from each SPB (MTOC). Minus-end directed motor activity of cytoplasmic dynein slides the microtubules across each other resulting in the generation of an attractive force between neighboring SPBs (see Discussion). Attractive force between SPBs is indicated by arrows. SPBs are represented by dark ovals associated with each nucleus (*large open circles*). For simplicity single cytoplasmic microtubules, represented by lines with fast growing ends identified as (+), are shown extending out in each direction from each SPB. The base of each cytoplasmic dynein is anchored to one cytoplasmic microtubule while the motor heads contact an antiparallel cytoplasmic microtubule. A MTOC (*dark oval*) is proposed to be located at the tip of each hypha and serves to link the end SPB (nucleus) with the growing tip (see Discussion). (*A*) As a hyphal tip extends, balanced force-generation between adjacent SPBs maintains equal internuclear distances. (*B*) During mitosis, cytoplasmic microtubules break down. (*C* and *D*) After mitosis, cytoplasmic microtubules reform and cytoplasmic dynein cross-links antiparallel microtubules. Note that nuclei that are close together have a smaller region of overlapping microtubules than nuclei that are far apart. Assuming an even distribution of cytoplasmic dynein per unit length of overlapping cytoplasmic microtubule, there will be greater attractive force (*larger arrows*; *C*) between nuclei that are farther apart (see Discussion). Equilibrium is reached when nuclei are repositioned at approximately equal distances.

The generation of attractive force between adjacent nuclei requires that a string of nuclei be anchored at the hyphal tip and at the distal-end of the hypha. At the distal-end of a hypha, we propose a static tethering of the end nucleus to the cell membrane, a septum or a fixed component of the cytoskeleton. At the hyphal tip, we propose the existence of an anchored MTOC from which cytoplasmic microtubules originate. Cross-linking of these cytoplasmic microtubules by dynein with cytoplasmic microtubules originating from the SPB of the terminal nucleus would provide a means to link nuclear movement with tip extension and the distance between the tip of a hypha and a terminal nucleus would be maintained in the same manner described for adjacent nuclei.

Our model for maintenance of nuclear distribution in filamentous fungi is based on a number of observations. The cross-linking function we are proposing for cytoplasmic dynein is analogous to the role of outer and inner arm dynein in the movement of flagella and cilia (Porter and Johnson, 1989). In the axoneme, dyneins slide microtubules relative to each other by attaching to the A subfiber of one microtubule doublet and the respective motor heads "walk" along the B subfiber of an adjacent parallel microtubule in an ATP-dependent manner. We are proposing that in filamentous fungi, the attachment of cytoplasmic dynein to one microtubule restricts the motor heads to interact only with a microtubule of the opposite polarity, therefore, differing from axonemal dyneins.

In the fungus *Basidiobolus magnus*, a single large nucleus

is present in each cell and the position of the nucleus relative to the hyphal tip is constant. Disruption of cytoplasmic microtubules with an ultraviolet light microbeam at any position anterior to the nucleus causes the nucleus to move backwards, while disruption of cytoplasmic microtubules anywhere posterior to the nucleus causes the nucleus to move forward (McKerracher and Heath, 1986). Analysis of cytoplasmic microtubules surrounding the nucleus indicates that microtubules move with the nucleus as opposed to the nucleus moving with respect to microtubules or independently of microtubules. The conclusion of this study, that nuclear positioning is maintained by the action of opposing forces that are in equilibrium, is consistent with our model (McKerracher and Heath, 1986).

As a part of our model, we propose that MTOCs are located at hyphal tips. In the fungus *Uromyces phaseoli*, repolymerization of cytoplasmic microtubules, following depolymerization with anti-tubulin agents, occurs first at the hyphal apex and not near the nuclei or SPBs, suggesting that a MTOC is located in the apical region of hyphae (Hoch and Staples, 1985). In the fission yeast *S. pombe*, examination of microtubule distribution during all phases of the cell cycle has led to the suggestion that non-SPB MTOCs are associated with the cell equator (Hagan and Hyams, 1988). The observation that γ-tubulin (Oakley et al., 1990; Oakley, 1992), a component of MTOCs, is also found to localize to the cell equator (Horio et al., 1991), in addition to the SPB, supports the hypothesis that non-SPB MTOCs are associated with the ends of fungal cells.

While our model provides a possible mechanism for the maintenance of nuclear distribution during vegetative growth, fungal nuclei also exhibit directed movements during other phases of growth suggesting the requirement for other motor proteins. In *S. cerevisiae*, *KAR3* encodes a kinesin-related protein required for nuclear fusion during mating (Meluh and Rose, 1990). A model has been proposed whereby Kar3 cross-links antiparallel microtubules emanating from the SPBs of the two nuclei that will undergo karyogamy. Predicted minus-end–directed motor activity of Kar3 is suggested to draw nuclei together through the sliding of antiparallel microtubules in the same manner we have proposed for cytoplasmic dynein.

Finally, the role of the Glued/dynactin complex in nuclear migration is unknown. *ro-4* and *ro-3* encode apparent homologues of centractin and Glued/dynactin (Plamann, M., P. F. Minke, J. H. Tinsley, and K. S. Bruno, unpublished observation), two subunits of the Glued/dynactin complex which has been proposed to stimulate the ability of cytoplasmic dynein to conduct microtubule-dependent organelle movement in vitro (Gill et al., 1991; Holzbaur et al., 1991; Lees-Miller et al., 1992*a*; Schroer and Sheetz, 1991). The observation that mutations affecting this complex have the same phenotype as mutations affecting cytoplasmic dynein indicate that both complexes are required to maintain nuclear distribution. We propose that cytoplasmic dynein is anchored to one microtubule and the dynein motor heads interact with an antiparallel microtubule. It is possible that the Glued/dynactin complex is required for either the establishment or maintenance of the anchoring of dynein to one microtubule. In addition, if nuclear distribution is maintained as described, uniform spacing of dynein motors along microtubules may contribute to the efficiency of the process and it is possible that the Glued/dynactin complex could anchor cytoplasmic dynein to microtubules at discrete intervals. Analysis of additional *ro* genes and the interaction of their gene products will allow a better understanding of the cellular roles of cytoplasmic dynein and the Glued/dynactin complex.

We thank Dr. Tom Adams and Dr. Lynda Plamann for critically evaluating the manuscript.

This work was supported by Texas Advanced Research Program grant 010366-79 to M. Plamann.

Received for publication 23 May 1994 and in revised form 15 July 1994.

References

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.

Aniento, F., N. Emans, G. Griffiths, and J. Gruenberg. 1993. Cytoplasmic dynein-dependent vesicular transport from early to late endosomes. *J. Cell Biol.* 123:1373–1387.

Bruchez, J. J. P., J. Eberle, and V. E. A. Russo. 1993*a*. Regulatory sequences in the transcription of *Neurospora crassa* genes: CAAT box, TATA box, introns, poly(A) tail formation sequences. *Fungal Genetics Newsl.* 40:89–96.

Bruchez, J. J. P., J. Eberle, and V. E. A. Russo. 1993*b*. Regulatory sequences involved in the translation of *Neurospora crassa* mRNA: Kozak sequences and stop codons. *Fungal Genetics Newsl.* 40:85–88.

Davis, R. H., and F. J. de Serres. 1970. Genetic and microbiological research techniques for *Neurospora crassa*. *Methods Enzymol.* 27A:79–143.

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

Dutcher, S. K., and F. G. Lux. 1989. Genetic interactions of mutations affecting flagella and basal bodies in Chlamydomonas. *Cell Motil. Cytoskeleton.* 14:104–117.

Erba, H. P., P. Gunning, and L. Kedes. 1986. Nucleotide sequence of the human gamma cytoskeleton actin mRNA: anomalous evolution of vertebrate non-muscle actin genes. *Nucleic Acids Res.* 14:5275–5294.

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

Fry, D. C., S. A. Kuby, and A. S. Mildvan. 1986. ATP-binding sites of adenylate kinase: mechanistic implications of its homology with *ras*-encoded p21, F1-ATPase, and other nucleotide-binding proteins. *Proc. Natl. Acad. Sci. USA.* 83:907–911.

Fyrberg, C., and E. Fyrberg. 1993. A *Drosophila* homologue of the *Schizosaccharomyces pombe act2* gene. *Biochem. Genetics.* 31:329–341.

Fyrberg, C., L. Ryan, M. Kenton, and E. Fyrberg. 1994. Genes encoding actin-related proteins of *Drosophila*. *J. Mol. Biol.* In press.

Gambino, J., L. G. Bergen, and N. R. Morris. 1984. Effects of mitotic and tubulin mutations on microtubule architecture in actively growing protoplasts of *Aspergillus nidulans*. *J. Cell Biol.* 99:830–838.

Garnjobst, L., and E. L. Tatum. 1967. A survey of new morphological mutants in *Neurospora crassa*. *Genetics.* 57:579–604.

Gibbons, I. R., B. H. Gibbons, G. Mocz, and D. J. Asai. 1991. Multiple nucleotide-binding sites in the sequence of dynein β heavy chain. *Nature (Lond.).* 352:640–643.

Gill, S. R., T. A. Schroer, I. Szilak, E. R. Steuer, M. P. Sheetz, and D. W. Cleveland. 1991. Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J. Cell Biol.* 115:1639–1650.

Gooday, G. W. 1983. The hyphal tip. *In* Fungal Differentiation. J. E. Smith, editor. Marcel Dekker, New York. 315–356.

Hagan, I. M., and J. S. Hyams. 1988. The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* 89:343–357.

Hoch, H. C., and R. C. Staples. 1985. The microtubule cytoskeleton in hyphae of *Uromyces phaseoli* germlings: its relationship to the region of nucleation and to the F-actin cytoskeleton. *Protoplasma.* 124:112–122.

Holmes, K. C., D. Popp, W. Gebhard, and W. Kabsch. 1990. Atomic model of the actin filament. *Nature (Lond.).* 347:44–49.

Holzbaur, E. L. F., J. A. Hammarback, B. M. Paschal, N. G. Kravit, K. K. Pfister, and R. B. Vallee. 1991. Homology of the 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued*. *Nature (Lond.).* 351:579–583.

Horio, T., S. Uzawa, M. K. Jung, B. R. Oakley, K. Tanaka, and M. Yanagida. 1991. The fission yeast γ-tubulin is essential for mitosis and is localized at microtubule organizing centers. *J. Cell Sci.* 99:693–700.

Kabsch, W., H. G. Mannherz, D. Suck, E. F. Pai, and K. C. Holmes. 1990. Atomic structure of the actin: DNAse I complex. *Nature (Lond.).* 347: 37–44.

Koonce, M. P., P. M. Grissom, and J. R. McIntosh. 1992. Dynein from *Dictyostelium*: primary structure comparisons between a cytoplasmic motor enzyme and flagellar dynein. *J. Cell Biol.* 119:1597–1604.

Lees-Miller, J. P., D. M. Helfman, and T. A. Schroer. 1992*a*. A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-base vesicle motility. *Nature (Lond.).* 359:244–247.

Lees-Miller, J. P., G. Henry, and D. M. Helfman. 1992*b*. Identification of *act2*, an essential gene in the fission yeast *Schizosaccharomyces pombe*. *Proc. Natl. Acad. Sci. USA.* 89:80–83.

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

Lye, R. J., M. E. Porter, J. M. Scholey, and J. R. McIntosh. 1987. Identification of a microtubule-based cytoplasmic motor in the nematode *C. elegans*. *Cell.* 51:309–318.

McKerracher, L. J., and I. B. Heath. 1986. Fungal nuclear behavior analyzed by ultraviolet microbeam irradiation. *Cell Motil. Cytoskeleton.* 6:35–47.

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

Metzenberg, R. L., J. N. Stevens, E. U. Selker, and E. Morzycka-Wroblewska. 1985. Identification and chromosomal distribution of 5S rRNA genes in *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA.* 82:2067–2071.

Mikami, A., B. M. Paschal, M. Mazumdar, and R. B. Vallee. 1993. Molecular cloning of the retrograde transport motor cytoplasmic dynein (MAP1C). *Neuron.* 10:787–796.

Morris, N. R. 1976. Mitotic mutants of *Aspergillus nidulans*. *Genet. Res.* 26:237–254.

Oakley, B. R. 1992. γ-tubulin: the microtubule organizer? *Trends Cell Biol.* 2:1–5.

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

Oakley, B. R., C. E. Oakley, Y. Yoon, and M. K. Jung. 1990. γ-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. *Cell.* 61:1289–1301.

Osmani, A. H., S. A. Osmani, and N. R. Morris. 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., D. B. Engle, J. H. Doonan, and N. R. Morris. 1988. Spindle formation and chromatin condensation in cells blocked at interphase by mutation of a negative cell cycle control gene. *Cell.* 52:241–251.

Paschal, B. M., H. S. Shpetner, and R. B. Vallee. 1987. MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties. *J. Cell Biol.* 105:1273–1282.

Paschal, B. M., E. L. F. Holzbaur, K. K. Pfister, S. Clark, D. I. Meyer, and R. B. Vallee. 1993. Characterization of a 50-kDa polypeptide in cytoplasmic dynein preparations reveals a complex with p150^{GLUED} and a novel actin. *J. Biol. Chem.* 268:15318–15323.

Pfarr, C. M., M. Coue, P. M. Grissom, T. S. Hays, M. E. Porter, and J. R. McIntosh. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature (Lond.).* 345:263–265.

Porter, M. E., and K. A. Johnson. 1989. Dynein structure and function. *Annu. Rev. Cell Biol.* 5:119–151.

Robinow, C. F., and C. E. Caten. 1969. Mitosis in *Aspergillus nidulans*. *J. Cell Sci.* 5:403–437.

Rosenberger, R. F., and M. Kessel. 1967. Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans*. *J. Bacteriol.* 94:1464–1469.

Salo, V., S. S. Niini, I. Virtanen, and M. Raudaskoski. 1989. Comparative immunocytochemistry of the cytoskeleton in filamentous fungi with dikaryotic and multinucleate hyphae. *J. Cell Sci.* 94:11–24.

Sambrook, J., E. H. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:54638-5467.

Schafer, D. A., S. R. Gill, J. A. Cooper, J. E. Heuser, and T. A. Schroer. 1994. Ultrastructural analysis of the dynactin complex: an actin-related protein is a component of a filament that resembles F-actin. *J. Cell Biol.* 126:403–412.

Schroer, T. A., and M. P. Sheetz. 1991. Two activators of microtubule-based vesicle transport. *J. Cell Biol.* 115:1309–1318.

Schroer, T. A., E. R. Steuer, and M. P. Sheetz. 1989. Cytoplasmic dynein is a minus end-directed motor for membranous organelles. *Cell.* 56:937–946.

Steele, G. C., and A. P. J. Trinci. 1977. Effect of temperature and temperature shifts on growth and branching of a wild type and a temperature sensitive colonial mutant (*cot-1*) of *Neurospora crassa*. *Arch. Microbiol.* 113:43–48.

Steuer, E. R., L. Wordeman, T. A. Schroer, and M. P. Sheetz. 1990. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature (Lond.).* 345:2668-268.

Tanaka, T., F. Shibasaki, M. Ishikawa, N. Hirano, R. Sakai, J. Nishida, T. Takenawa, and H. Hirai. 1992. Molecular cloning of bovine actin-like protein, actin2. *Biochem. Biophys. Res. Commun.* 187:1022–1028.

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

Vollmer, S. J., and C. Yanofsky. 1986. Efficient cloning of genes of *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA.* 83:4869–4873.

Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:945–951.

Walker, R. A., and M. P. Sheetz. 1993. Cytoplasmic microtubule-associated motors. *Annu. Rev. Biochem.* 62:429–451.

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

Yarden, O., M. Plamann, D. J. Ebbole, and C. Yanofsky. 1992. *cot-1*, a gene required for hyphal elongation in *Neurospora crassa*, encodes a protein kinase. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2159–2166.

Yoon, H., and T. F. Donahue. 1992. Control of translation initiation in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 6:1413–1419.

Zhang, Z., Y. Tanaka, S. Nonaka, H. Aizawa, H. Kawasaki, T. Nakata, and N. Hirokawa. 1993. The primary structure of rat brain (cytoplasmic) dynein heavy chain, a cytoplasmic motor enzyme. *Proc. Natl. Acad. Sci. USA.* 90:7928–7932.