p150^{Glued}, the Largest Subunit of the Dynactin Complex, Is Nonessential in *Neurospora* but Required for Nuclear Distribution

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

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

Submitted December 13, 1995; Accepted February 5, 1996
Monitoring Editor: J. Richard McIntosh

Dynactin is a multisubunit complex that is required for cytoplasmic dynein, a minus-end-directed, microtubule-associated motor, to efficiently transport vesicles along microtubules in vitro. p150^{Glued}, the largest subunit of dynactin, has been identified in vertebrates and *Drosophila* and recently has been shown to interact with cytoplasmic dynein intermediate chains in vitro. The mechanism by which dynactin facilitates cytoplasmic dynein-dependent vesicle transport is unknown. We have devised a genetic screen for cytoplasmic dynein/dynactin mutants in the filamentous fungus *Neurospora crassa*. In this paper, we report that one of these mutants, *ro-3*, defines a gene encoding an apparent homologue of p150^{Glued}, and we provide genetic evidence that cytoplasmic dynein and dynactin interact in vivo. The major structural features of vertebrate and *Drosophila* p150^{Glued}, a microtubule-binding site at the N-terminus and two large α -helical coiled-coil regions contained within the distal two-thirds of the polypeptide, are conserved in Ro3. *Drosophila* p150^{Glued} is essential for viability; however, *ro-3* null mutants are viable, indicating that dynactin is not an essential complex in *N. crassa*. We show that *N. crassa* cytoplasmic dynein and dynactin mutants have abnormal nuclear distribution but retain the ability to organize cytoplasmic microtubules and actin in anucleate hyphae.

INTRODUCTION

Cytoplasmic dynein is a microtubule-associated, mechanochemical enzyme that has been implicated in minus-end-directed, intracellular transport of various membranous organelles (Walker and Sheetz, 1993; Holzbaur and Vallee, 1994). In vertebrates, cytoplasmic dynein has been proposed to be required for retrograde axonal transport (Paschal and Vallee, 1987), organization of Golgi (Corthésy-Theulaz *et al.*, 1992), formation of endoplasmic reticulum networks (Allan, 1995), the endocytic pathway (Aniento *et al.*, 1993), apical transport of vesicles in polarized intestinal epithelial cells (Fath *et al.*, 1994), and formation of the mitotic spindle (Vaisberg *et al.*, 1993). In fungi, cytoplasmic dynein has been implicated in nuclear migration, nuclear distribution, and anaphase chromosome segregation (Eshel *et al.*, 1993; Li *et al.*, 1993; Plamann

et al., 1994; Xiang *et al.*, 1994; Yeh *et al.*, 1995). The regulatory mechanisms controlling the various transport activities of cytoplasmic dynein are not known; however, dynein function has been proposed to be regulated by modulating dynein-organelle interaction and dynein motor activity (for review see Holzbaur and Vallee, 1994). A multisubunit complex known as dynactin (*dynein activator*) has been shown to be required for cytoplasmic dynein to efficiently transport vesicles along microtubules in vitro (Gill *et al.*, 1991; Schroer and Sheetz, 1991). The mechanism by which dynactin stimulates dynein-mediated transport of vesicles is unknown; however, dynactin has been proposed to regulate interaction of cytoplasmic dynein with organellar cargo (Gill *et al.*, 1991; Schroer and Sheetz, 1991). Dynactin consists of approximately nine polypeptides, the largest of which is a 150-kDa polypeptide known as p150^{Glued}, the product of the essential *Drosophila melanogaster* *Glued* gene (Swaroop *et al.*, 1987; Gill *et al.*, 1991; Holzbaur *et al.*, 1991;

* Corresponding author.

Schroer and Sheetz, 1991; Schafer *et al.*, 1994). p150^{Glued} of vertebrates and *Drosophila* contains a conserved microtubule-binding domain first identified in the endocytic vesicle linker protein CLIP-170 (Holzbaur *et al.*, 1991; Pierre *et al.*, 1992; Holzbaur and Vallee, 1994). This domain mediates binding of p150^{Glued} to microtubules in vivo (Waterman-Storer *et al.*, 1995). The most abundant subunit of the dynactin complex is the actin-related protein Arp1, which forms a 37-nm long filament (Schafer *et al.*, 1994). p150^{Glued}, as well as additional dynactin subunits, associate with the Arp1 filament (Lees-Miller *et al.*, 1992; Schafer *et al.*, 1994; Waterman-Storer *et al.*, 1995). p150^{Glued} has been shown to bind to cytoplasmic dynein intermediate chains in vitro, suggesting that p150^{Glued} may mediate interaction of the dynactin complex with cytoplasmic dynein in vivo (Vaughan *et al.*, 1995).

Genetic evidence for a common cellular role for cytoplasmic dynein and the dynactin complex has been obtained from studies of cytoplasmic dynein heavy chain mutants and Arp1 mutants in the budding yeast *Saccharomyces cerevisiae* (Eshel *et al.*, 1993; Li *et al.*, 1993; Clark and Meyer, 1994; Muhua *et al.*, 1994) and the filamentous fungus *Neurospora crassa* (Plamann *et al.*, 1994; Robb *et al.*, 1995). In both fungi, recessive mutations in either gene result in a defect in nuclear movement. Recently, genetic and cytological evidence for functional association of cytoplasmic dynein and dynactin has been obtained in *Drosophila* (McGrail *et al.*, 1995). Cytoplasmic dynein heavy chain mutations were identified in *Drosophila* that act as dominant suppressors or enhancers of a dominant p150^{Glued} mutation, and the localization of p150^{Glued} (dynactin) during oogenesis in *Drosophila* was found to be dependent on cytoplasmic dynein function.

Mutations at eight previously identified loci in *N. crassa* result in a curled hyphal growth phenotype described as ropy (*ro*) (Garnjobst and Tatum, 1967; Perkins *et al.*, 1982). *N. crassa* is a coenocytic organism that maintains even distribution of nuclei within hyphae growing by apical extension. We showed that *ro* mutants exhibit highly asymmetric nuclear distribution (Plamann *et al.*, 1994). *ro-1* and *ro-4* were cloned and shown to encode cytoplasmic dynein heavy chain and Arp1, respectively (Plamann *et al.*, 1994; Robb *et al.*, 1995). From this observation, we proposed that mutations in other genes encoding subunits of dynein or dynactin would also result in a *ro* phenotype. We devised a genetic screen for the isolation of additional *ro* mutants and used this screen to isolate >1000 *ro* mutants (Bruno *et al.*, 1996). Approximately 10% of the *ro* mutants are unusual because even though they contain a single, recessive *ro* mutation, they are unable to complement two or more recessive *ro* mutants defining unlinked loci. This phenomenon, referred to as unlinked noncomplementation, often indicates that

the respective gene products interact at some level (Stearns and Botstein, 1988; Vinh *et al.*, 1993). The unlinked loci most frequently involved were *ro-1* and *ro-3*, suggesting that *ro-3* encodes a protein that interacts with cytoplasmic dynein. In this paper, we report the isolation and initial characterization of the *ro-3* gene, which encodes the first putative homologue of p150^{Glued} identified in fungi.

MATERIALS AND METHODS

Strains, Growth Conditions, and Genetic Techniques

N. crassa strains used in this study are listed in Table 1. Some of the strains were obtained from the Fungal Genetic Stock Center (FGSC), Department of Microbiology, University of Kansas Medical Center, Kansas City, KS. Media, growth conditions, crosses, and the use of heterokaryon tests for complementation analysis are as described (Davis and de Serres, 1970). DNA-mediated transformations were carried out as described (Vollmer and Yanofsky, 1986), and where appropriate, the medium was supplemented with hygromycin B (Calbiochem, San Diego, CA) at 200 µg/ml.

Isolation of *ro-3* Genomic and cDNA Clones, and DNA Sequence Analysis

Sib-selection was used to identify cosmid clone X11C6 of the Orbach/Sachs cosmid library (FGSC), which complements a *ro-3* mutant (Vollmer and Yanofsky, 1986). Cosmid X11C6 was digested separately with various restriction endonucleases and the digested DNA was used to transform a *ro-3* mutant. (*N. crassa* is efficiently transformed with linear DNA). Restriction endonucleases that cut within insert sequences of cosmid X11C6, but did not inactivate complementing activity, were used to subclone the *ro-3* gene. A 6.5-kbp *EcoRV*-generated fragment, containing the entire *ro-3* gene, was cloned into pBS SK⁺ (Stratagene, La Jolla, CA) to generate pJT15 (Figure 1A). Restriction endonuclease sites within the 6.5-kbp

Table 1. *N. crassa* strains used in this study

Strains	Genotype*	Source
FGSC2489	74-OR23-1VA wild type; A	FGSC
FGSC4096	<i>cot-1</i> (C102t); A	FGSC
FGSC146	<i>ro-1</i> (B15); a	GFSC
FGSC43	<i>ro-3</i> (R2354); a	FGSC
MP150	<i>ro-1</i> (B15); <i>trp-4</i> (Y2198); a	Bruno <i>et al.</i> , 1995
MP152	<i>ro-3</i> (R2354); <i>trp-4</i> (Y2198); a	Bruno <i>et al.</i> , 1995
MPRO39	<i>ro-1</i> (A59); a	Bruno <i>et al.</i> , 1995
MPRO50	<i>ro-1</i> (B10); a	Bruno <i>et al.</i> , 1995
MPRO184	<i>ro-3</i> (C99); a	Bruno <i>et al.</i> , 1995
MPRO246	<i>ro-1</i> (D34); a	Bruno <i>et al.</i> , 1995
MPRO258	<i>ro-1</i> (D47); a	Bruno <i>et al.</i> , 1995
MPRO262	<i>ro-3</i> (D53); a	Bruno <i>et al.</i> , 1995
MPRO337	<i>ro-1</i> (E88); a	Bruno <i>et al.</i> , 1995
MPRO359	<i>ro-3</i> (E112); a	Bruno <i>et al.</i> , 1995
MPRO389	<i>ro-3</i> (F22); a	Bruno <i>et al.</i> , 1995
MPRO413	<i>ro-3</i> (F54); a	Bruno <i>et al.</i> , 1995
MPRO423	<i>ro-1</i> (F67); a	Bruno <i>et al.</i> , 1995
JT21	$\Delta ro-3::trpC-hph$; <i>pan-1</i> ; a	This work
JT27	$\Delta ro-4::trpC-hph$; <i>pan-1</i> ; a	This work

*A and a signify the mating type of the respective strains. All MPRO strains contain *cot-1*(C102t) and *his-3*(Y269M614) mutations.

EcoRV fragment, which when cleaved inactivate complementing activity, were mapped to define the location of the *ro-3* gene within pJT15. The map location of the cloned DNA was determined by restriction fragment length polymorphism analysis (RFLP) (Metzenberg *et al.*, 1985).

cDNA clones of *ro-3* were isolated by screening a λ ZAP II *N. crassa* cDNA library (available from the FGSC) constructed by R.H. Garret (University of Virginia, Charlottesville, VA). Restriction fragments containing the distal one-half of *ro-3* were used as probes (Sambrook *et al.*, 1989).

DNA sequencing was performed by the dideoxy-chain termination method (Sanger *et al.*, 1977), using Sequenase version 2.0 (United States Biochemical, 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 (UWGCG) (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). Prediction of α -helical coiled-coil domains were made using the Lupas algorithm (Lupas *et al.*, 1991; Knight and Kendrick-Jones, 1993) programmed to run on a Macintosh personal computer (Stripe version 1.3; Alex Knight, Whitehead Institute, Cambridge, MA).

Genomic DNA and total RNA were isolated from *N. crassa* as described (Yarden *et al.*, 1992). Northern and Southern analyses were performed as described (Sambrook *et al.*, 1989).

Disruption of the *N. crassa ro-3* and *ro-4* Genes

Plasmids pPM5 and pJT17 contain the *ro-3* and *ro-4* genes, respectively, disrupted by a *Hyg^r* marker (Figures 3A and 4A). The *ro-3* disruption plasmid, pPM5 (Figure 3A), was constructed by replacing a 3.9-kbp *AflIII-SmaI* fragment of the *ro-3* structural gene with a 2.3-kbp *SmaI-NcoI* fragment containing the *trpC-hph* gene (Cullen *et al.*, 1987). The *AflIII* site cuts within the predicted ATG translation initiation codon of the *ro-3* structural gene. The *ro-4* disruption plasmid, pJT17 (Figure 4A), was constructed by replacing a 1.3-kbp *PvuII-BamHI* fragment of the *ro-4* structural gene with a 2.3-kbp *SmaI-BamHI* fragment containing the *trpC-hph* gene (Cullen *et al.*, 1987). The *PvuII* site cuts 325-bp upstream of the predicted ATG translation initiation codon of the *ro-4* structural gene. This site is located within DNA sequences corresponding to the 5'-untranslated leader region of the *ro-4* mRNA, and therefore, unlikely to cut within an adjacent gene (Plamann *et al.*, 1994).

N. crassa strains containing disrupted *ro-3* or *ro-4* genes were constructed by transformation of a *cot-1^{ts}* mutant with linear DNA containing *ro-3* or *ro-4* disrupted by a selectable marker (*trpC-hph*) conferring resistance to hygromycin. *cot-1* is predicted to encode a serine/threonine protein kinase that is required for hyphal tip extension (Yarden *et al.*, 1992), and loss of Cot1 kinase activity results in very small colonies (~0.1 mm in diameter). We showed previously that recessive mutations in *ro* genes partially suppress the *cot-1* growth defect (Plamann *et al.*, 1994). Most DNA-mediated transformation in filamentous fungi occurs by ectopic integration of transforming sequences (Fincham, 1989). To identify the relatively rare *Hyg^r* transformants disrupted for *ro-3* or *ro-4*, we transformed spheroplasts of a *cot-1^{ts}* strain (FGSC4096) with linearized DNA of the respective disruption construct (Figures 3A and 4A), and screened the single-colony purified transformants at 37°C to identify those *Hyg^r* transformants that showed an improved radial growth rate relative to the *cot-1^{ts}* strain (Plamann *et al.*, 1994). Putative *ro-3* and *ro-4* null mutants (~5% of all *Hyg^r* transformants) were identified as those *Hyg^r* transformants showing curled hyphal growth and partial suppression of the *cot-1* growth defect at 37°C. Disruption of the *ro-3* and *ro-4* genes was verified by Southern analysis using DNA from two independent *ro-3* and *ro-4* null mutants (Figures 3B and 4B). The *ro-3* and *ro-4* null mutations segregated 1:1 in crosses.

Microscopy

Microscopic examination of *N. crassa* hyphae was conducted by first plating fresh conidia on a small piece of cellulose filter (GN-6 membrane filter; Gelman Sciences, Ann Arbor, MI) placed on the surface of a sucrose agar plate and incubating overnight at 25°C. Filters were plunge-frozen in liquid propane cooled to -180°C, using a Reichert-Jung KF-80 Cryoimmersion system, and transferred to fixative (3.7% formaldehyde in 95% ethanol) precooled to -80°C. Samples were maintained at -80°C for 2-3 days, then transferred to -20°C for 2 h to allow fixation to proceed. Samples were transferred to 4°C for 1 h, then allowed to warm for 1 h at room temperature. The filters were rehydrated by transferring through a series of ethanol solutions of descending concentration starting at 90% and ending in a transfer from 10% ethanol to a solution of phosphate-buffered saline (PBS; 150 mM NaCl, 10 mM Na₂HPO₄, pH 7.2). After several rinses in PBS, the samples were soaked for 30-60 min in a 0.1 mg/ml solution of Novozyme 234 LP (Novo Nordisk Biotech, Danbury, CT) containing protease inhibitors (leupeptin at 10 μ g/ml, trypsin inhibitor at 0.1 mg/ml, pepstatin A at 5 μ g/ml) to partially digest the fungal cell wall. The filters were soaked in a 1% bovine serum albumin solution in PBS containing 0.25% Triton-X 100 for 10 min to block nonspecific binding of the filter. Samples were immersed in the respective primary antibody for 8 h, washed twice in a solution of PBS containing 0.25% Triton-X 100, and immersed in the appropriate secondary antibody for 8 h. To examine nuclear distribution, samples were immersed for 30 min in PBS containing 1 μ g/ml 4',6-diamidino-2-phenylindole (DAPI), and allowed to incubate for 1 h in PBS to remove excess DAPI. Samples were observed on an Olympus BH-2 microscope with the appropriate epi-illumination filter cube using a 1.4 N.A. 60 $\times$ oil immersion lens. Pictures were taken using TMAX 3200 black and white film. Primary antibodies used in this study were as follows: DM1-A monoclonal anti- α -tubulin mouse IgG antibody (used at a 1:1100 dilution; ICN Biochemicals, Costa Mesa, CA); and C4 monoclonal anti-actin mouse IgG antibody (used at a 1:400 dilution; ICN Biochemicals). Secondary antibodies were as follows: sheep anti-mouse IgG conjugated to fluorescein isothiocyanate (used at a 1:200 dilution; Sigma, St. Louis, MO), or sheep anti-mouse IgG conjugated to Cy3 (used at a 1:200 dilution; Sigma). Cy3 was observed using a rhodamine filter set. SlowFade-Light (Molecular Probes, Eugene, OR) was used to reduce photo-bleaching of fluorescein isothiocyanate-stained samples.

RESULTS

Molecular Cloning of *ro-3*

To explore the relationship between *ro-1*, encoding cytoplasmic dynein heavy chain, and *ro-3*, we used a sib-selection protocol to isolate a cosmid clone from the Orbach/Sachs cosmid library of *N. crassa* that complements a *ro-3* mutant (Vollmer and Yanofsky, 1986; Orbach, 1994). *ro* mutants grow as slowly spreading colonies with curled hyphal growth. Complementation of a *ro* mutant results in restoration of straight hyphal growth and rapid radial extension of individual colonies. The map position of the cosmid clone was determined by RFLP analysis (Metzenberg *et al.*, 1985) and found to be coincident with the map position of the *ro-3* mutation, suggesting that the complementing DNA contains a functional copy of the *ro-3* gene and not a gene capable of extracopy suppression. (The RFLP data is available from the *Fungal Genetics Newsletter*). Complementing DNA was localized to a 6.5-kbp *EcoRV* fragment (pJT15; Figure 1A). *N. crassa*

Figure 1. Localization and organization of the *N. crassa ro-3* gene. (A) Diagram of a 6.5-kb region of genomic DNA that it is able to complement a *ro-3* mutant (pJT15). Relevant restriction endonuclease sites within this fragment are indicated. Cleavage at restriction endonuclease sites that do (-) or do not (+) eliminate complementation are indicated. Restriction sites: B, *Bam*HI; EV, *Eco*RV; M, *Mlu*I; S, *Sal*I; Sm, *Sma*I; and X, *Xba*I. The *ro-3* structural gene (open box), the position of an intron (shaded region) located within the structural gene, and the direction of *ro-3* transcription (arrow) are marked. (B) Northern analysis of *ro-3* in a wild-type strain and in *ro-1*, *ro-3*, and $\Delta ro-3$ mutants of *N. crassa*. Total RNA was isolated from the four cultures grown at 25°C for 8 h. Twenty-five micrograms of RNA was loaded in each lane and probed with labeled pJT15. Note the absence of the 4.5-kb *ro-3* mRNA in the $\Delta ro-3$ strain. (C) A 14-codon upstream open reading frame overlaps the predicted translation initiation codon for the *ro-3* structural gene. The predicted amino acid sequences of the uORF and the N-terminus of Ro3 are indicated below the DNA sequence. The ATG sequence initiating each ORF is underlined and in bold, and the translation termination codon for the overlapping uORF is also in bold. These sequence data are available from EMBL/GenBank/DBJ under accession number L48661.

is efficiently transformed with linear DNA; therefore, to localize the *ro-3* gene within the 6.5-kbp *Eco*RV fragment, pJT15 was digested with several restriction endonucleases before transformation of a *ro-3* mutant. Cleavage at restriction endonuclease sites spanning a 3.4-kbp region was found to eliminate high-frequency complementation (Figure 1A). pJT15 was used as probe for Northern analysis and a 4.5-kb transcript was observed (Figure 1B). This transcript was not observed in a *ro-3* null mutant (Figure 1B; see below).

DNA sequence analysis of the first 5.4 kbp of the 6.5-kbp *Eco*RV fragment revealed a 1300-codon open reading frame, interrupted by a 293-bp intron (Figure 1A; DNA sequence data are available from EMBL/GenBank/DBJ under accession number L48661). DNA sequence analysis of *ro-3* cDNA clones verified the direction of transcription and the 3' end of the *ro-3* gene. An unusual feature of *ro-3* is that a short 14-codon upstream open reading frame (uORF) spans the proposed translation initiation codon of *ro-3* (Figure 1C). The preferred sequence context of *N. crassa* translation initiation codons is 5'-CNNNCAMVATGGC-3', where M = A or C and V = A, C, or G (Bruchez *et al.*, 1993). The sequences flanking the ATG initiator codon of the 14-codon uORF are a poor match to the consensus sequence; however, the context of the proposed ATG initiator codon for *ro-3* matches the consensus sequence at six of seven positions (Figure 1C). A 13-codon uORF also spans the proposed translation initiation codon of the *ro-4* gene (Plamann *et al.*, 1994).

Ro3 Is Homologous to p150^{Glued}

ro-3 is predicted to encode a 1300-amino acid polypeptide with a calculated mass of 145 kDa. Ro3 is overall 29% and 26% identical to p150^{Glued} of rat and *Drosophila*, respectively (using the UWGCG program BESTFIT; Figure 2A). Most of the identities between Ro3 and rat and *Drosophila* p150^{Glued} are in the first 600 residues (Figure 2A). The most highly conserved features of rat and *Drosophila* p150^{Glued} are a microtubule-binding domain at the N-terminus (Waterman-Storer *et al.*, 1995) and two large regions predicted to comprise α -helical coiled-coil domains; both regions are well conserved in Ro3 (Lupas *et al.*, 1991; Figure 2, B and C). Based on these observations and the finding that *ro-3* mutants are phenotypically identical to *N. crassa ro-4* (Arp1/dynactin) mutants (see below), we conclude that *ro-3* encodes the *N. crassa* equivalent of p150^{Glued}. It is noteworthy that while the extent of amino acid sequence identity between Ro3 and either rat or *Drosophila* p150^{Glued} is not extremely high, it is similar to the amount of identity between the rat and *Drosophila* p150^{Glued} sequences (32%; Holzbauer *et al.*, 1991).

Dynactin Is Not Essential for Viability in *Neurospora*

Cytoplasmic dynein, along with associated dynactin complex, has been implicated in many vital microtubule-dependent cellular processes. The *Drosophila* gene encoding p150^{Glued} is essential, with most homozygous *Glued* mutants dying at the first-instar stage (Harte and Kankel, 1982; Garen *et al.*, 1984). Recently, the *Drosophila* gene encoding cytoplasmic dynein has

Figure 2. Domain structure of *N. crassa* Ro3 and comparison with rat p150^{Glued} and *Drosophila* p150^{Glued}. (A) Schematic representation of the predicted amino acid sequence of the *N. crassa* ro-3 gene product and the relative locations of the predicted microtubule-binding domain (MB) and α -helical coiled-coil domains. Below the illustration are indicated the percent identities of the N-terminal half and the C-terminal half of Ro3 with the respective segments of rat p150^{Glued} and *Drosophila* p150^{Glued} as determined by the UWGCG program BEST-FIT. (B) Alignment of the N-terminal segments of Ro3, rat p150^{Glued}, and *Drosophila* p150^{Glued} that contain microtubule-binding domains and their similarity to the consensus CAP-Gly domain (Riehemann and Sorg, 1993). Residues that are identical in two of the three sequences are in bold. The numbers on the right represent the position of the last residue shown within the respective full-length polypeptides. (C) Alignment of plots of probability of the formation of α -helical coiled-coil versus residue position for Ro3, rat p150^{Glued}, and *Drosophila* p150^{Glued}. Predictions were made using the Lupas algorithm (Lupas *et al.*, 1991).

been reported to be essential as well (McGrail *et al.*, 1995). To determine whether dynactin is an essential complex in *N. crassa*, we constructed strains disrupted for either the ro-3 or ro-4 gene, encoding p150^{Glued} and Arp1, respectively (Figures 3 and 4). For each gene, a plasmid was constructed in which a segment of DNA spanning the ATG initiation codon and a large part of the respective structural gene was replaced with DNA containing a Hyg^r selectable marker (Cullen *et al.*, 1987; Figures 3A and 4A). The plasmids were linearized and used to transform *N. crassa* to Hyg^r (see MATERIALS AND METHODS). In *N. crassa*, as in other filamentous fungi, most transformants result from ectopic integration of DNA containing a selectable marker; however, homologous integration of

transforming DNA is observed (Fincham, 1989). We observed that approximately 5% of the single-colony purified Hyg^r transformants displayed the curled hyphal growth characteristic of ro mutants. Two putative ro-3 and two putative ro-4 null mutants were analyzed by Southern hybridization and found to have replaced the respective ro gene with the constructed null allele (Figures 3B and 4B). Because we are able to obtain viable homokaryotic ro-3 and ro-4 disruptants, dynactin is not essential for viability of *N. crassa*. In addition, each disruption strain produced viable spores when crossed with a wild-type strain, again indicating that dynactin null mutants are viable and that dynactin is not required for germination of sexually derived spores.

Figure 3. Disruption of the *ro-3* gene. (A) Restriction map of a 6.5-kbp *EcoRV* fragment containing the *ro-3* gene. Relevant restriction sites are indicated as follows: A, *AflIII*; E, *EcoRI*; EV, *EcoRV*; N, *NcoI*; and Sm, *SmaI*. Plasmid pPM5 was constructed for disruption of *ro-3* by replacing a 3.9-kbp *AflIII*–*SmaI* fragment of the *ro-3* structural gene with a 2.3-kbp *SmaI*–*NcoI* fragment containing the *trpC-hph* gene. (B) Southern analysis of $\Delta ro-3::trpC-hph$ transformants. Five micrograms of total DNA from a wild-type strain and two putative *ro-3* disruptants were digested with *EcoRI* or *EcoRV* and subjected to Southern analysis using as probe the 6.5-kbp *EcoRV* fragment containing the *ro-3* gene.

Genetic Evidence for Interaction between Cytoplasmic Dynein and Dynactin

A large-scale screen for *ro* mutants led to the isolation of 1187 *ro* mutants defining 23 complementation groups (Bruno et al., 1996). Approximately 10% of the *ro* mutants could not be assigned unambiguously to a single complementation group because they were noncomplementing with two or more mutants defining unlinked *ro* loci. Many *ro* mutants showing unlinked noncomplementation do not complement the FGSC tester strains defining the *ro-1* and *ro-3* complementation groups, *ro-1*(B15) and *ro-3*(R2354) (Bruno et al., 1996). For this study, we mapped three *ro* mutants that show unlinked noncomplementation with the *ro-1*(B15) and *ro-3*(R2354) tester strains and found that one of the mutants (B10) represents a *ro-1* mutant, and the other two (F22 and F54) represent *ro-3* mutants. We examined the genetic interaction between *ro-1* and

Figure 4. Disruption of the *ro-4* gene. (A) Restriction map of a 6.3-kbp *EcoRI* fragment containing the *ro-4* gene. Relevant restriction sites are indicated as follows: B, *BamHI*; C, *ClaI*; E, *EcoRI*; EV, *EcoRV*; P, *PvuII*; S, *SacI*; and Sm, *SmaI*. Plasmid pJT17 was constructed for disruption of *ro-4* by the replacement of a 1.3-kbp *PvuII*–*BamHI* fragment of the *ro-4* structural gene with a 2.3-kbp *SmaI*–*BamHI* fragment containing the *trpC-hph* gene. (B) Southern analysis of $\Delta ro-4::trpC-hph$ transformants. Five micrograms of total DNA from a wild-type strain and two putative *ro-4* disruptants were digested with *ClaI* or *EcoRV* and subjected to Southern analysis using as probe the indicated *SacI*–*EcoRV* fragment of pJT15 (A).

ro-3 mutants in greater detail by conducting a series of complementation tests between the atypical *ro-1*(B10), *ro-3*(F22), and *ro-3*(F54) mutants and a group of *ro-1* and *ro-3* mutants (Table 2). *ro-1*(B10) is noncomplementing with four of six *ro-3* mutants, while *ro-3*(F22) and *ro-3*(F54) are noncomplementing with two of seven *ro-1* mutants (Table 2). Unexpectedly, heterokaryons formed between either *ro-3*(F22) or *ro-3*(F54) and *ro-1*(B15), and between *ro-3*(R2354) and *ro-1*(B10) are much slower growing than any of the homokaryotic mutants by itself (Table 2).

We also conducted complementation tests between *ro-3* and *ro-4* null mutants and the *ro-1* mutants shown in Table 2. (The *ro-4* null mutant complements all *ro-3* mutants tested to date, including those shown in Table 2). We predicted that the *ro-3* and *ro-4* null mutants

Table 2. Complementation analysis between *ro-1* (cytoplasmic dynein heavy chain) and *ro-3* (p150^{Glued}) mutants

	<i>ro-1</i> mutants						
	<i>ro-1</i> (A59)	<i>ro-1</i> (B10)	<i>ro-1</i> (B15)	<i>ro-1</i> (D34)	<i>ro-1</i> (D47)	<i>ro-1</i> (E88)	<i>ro-1</i> (F67)
<i>ro-3</i> (C99)	+	–	+	+	+	+	+
<i>ro-3</i> (D53)	+	+	+	+	+	+	+
<i>ro-3</i> (E112)	+	–	+	+	+	+	+
<i>ro-3</i> (F22)	+	+	–*	+	+	–	+
<i>ro-3</i> (F54)	+	–	–*	+	+	+	+
<i>ro-3</i> (R2354)	+	–*	+	+	+	+	+
$\Delta ro-3$	+	+	–*	+	+	+	+
$\Delta ro-4$	+	+	–*	+	+	+	+

Complementation analysis was conducted by pair-wise formation of heterokaryons. (+) signifies that a heterokaryon formed with two *ro* mutants grows as straight, rapidly extending hyphae, while (–) indicates that the heterokaryon grows as a *ro* mutant (see Figure 5, A vs. C, E, and G). (*) indicates that a heterokaryon grows more slowly than either of the two respective homokaryons.

would complement all *ro-1* mutants; however, no complementation was seen between either null mutant and the *ro-1*(B15) strain. As with three of the heterokaryons described above, the heterokaryons formed between either the *ro-3* or *ro-4* null mutant and the *ro-1*(B15) strain grow much more slowly than any one of the three mutants by itself (Table 2).

Hyphal Growth, Nuclear Distribution, Cytoplasmic Microtubules, and Actin Organization in *ro* Mutants

N. crassa is a rapidly growing filamentous fungus with wild-type hyphae extending out at rates approaching 6 mm/h under optimal conditions. In *ro* mutants, hyphae grow in a curled fashion, resulting in colonies with slow radial growth rates (Figure 5, A versus C, E, and G). *ro-1*(B15) and the *ro-3* and *ro-4* null mutants shown in Figure 5 have radial growth rates that are approximately one-fourth that of wild-type. Nuclei are uniformly distributed within wild-type hyphae (Figure 5I). However, in *ro* mutants, nuclear distribution is severely altered; nuclei tend to be clumped together and regions of hyphae exist that are completely devoid of nuclei (Figure 5, K, M, and O).

In wild-type *N. crassa*, cytoplasmic microtubules are oriented primarily parallel to the direction of hyphal growth and are located in all areas of the cytoplasm (Figure 5J). There are typically 5–15 cytoplasmic microtubule tracks located in any one region of a hypha. It is not known if cytoplasmic microtubule tracks consist of a single microtubule or represent a bundle of microtubules. Some cytoplasmic microtubule tracks are >50 μ m in length and extend from distal regions to the hyphal tip (Figure 5J). SPBs represent the microtubule organizing centers of fungi (for review see McKerracher and Heath, 1987), and can be observed in DAPI-stained hyphae of *N. crassa* as a brightly staining region on the nuclear envelope (Thompson-Coffe and

Zickler, 1994). Careful examination of the relative positions of SPBs and cytoplasmic microtubules revealed that most microtubule tracks do not appear to contact SPBs of the nuclei located within a given region of hyphae (Figure 5J; our unpublished results).

Cytoplasmic microtubules of filamentous fungi have been proposed to be required for transport of apical vesicles, which contain enzymes and precursors required for cell wall synthesis, to hyphal tips (Howard, 1981). All organelle movements within *N. crassa* hyphae, observed by computer contrast-enhanced video-DIC microscopy, have been shown to be dependent on cytoplasmic microtubules (Steinberg and Schliwa, 1993). In *ro* mutants, hyphal tips are able to grow >200 μ m beyond the nearest nucleus (unpublished observations; Plamann *et al.*, 1994); therefore, one would predict that cytoplasmic microtubule tracks would be present in *ro* mutants to support long-range transport required to form anucleate hyphae. In agreement with this hypothesis, we find that anucleate hyphae of *ro-1* (cytoplasmic dynein) and *ro-3* and *ro-4* (dynactin) mutants (shown in Figure 5, K, M, and O) have long cytoplasmic microtubule tracks similar to those found in wild-type hyphae (Figure 5, L, N, and P). The polarity of these cytoplasmic microtubules is unknown. Regions of *ro* hyphae containing dense clusters of nuclei have extensive cytoplasmic microtubule networks and lack the distinct long cytoplasmic microtubule tracks observed in hyphae of wild-type *N. crassa* and anucleate hyphae of *ro* mutants (Figure 5, Q and R).

We also examined actin organization in wild-type *N. crassa* and *ro* mutants and in both observed a concentration of actin patches and filaments at actively growing hyphal tips (Figure 5, B, D, F, and H). At the level of resolution afforded by indirect immunofluorescence, it appears that actin organization is indepen-

Figure 5. Microscopic analysis of an *N. crassa* wild-type strain and cytoplasmic dynein and dynactin mutants. Colony morphology: conidia from each strain were centrally inoculated onto sucrose minimal agar media and the plates were incubated overnight at 25°C. Pictures depict colony edges for (A) wild type, (C) *ro-1*(B15), (E) $\Delta ro-3$, and (G) $\Delta ro-4$. Bar, 0.4 mm. Immunolocalization of actin and microtubules: conidia were inoculated onto filters placed on sucrose minimal agar media and the plates were incubated at 25°C for 10–14 h. The samples were processed for indirect immunofluorescence as described (see MATERIALS AND METHODS). Pictures depict actin localization at tips of hyphae for (B) wild type, (D) *ro-1*(B15), (F) $\Delta ro-3$, and (H) $\Delta ro-4$. Bar, 5 μ m. Pictures depict nuclear distribution (visualized by DAPI staining) for (I) wild type, (K) *ro-1*(B15), (M) $\Delta ro-3$, and (O) $\Delta ro-4$, and the corresponding view of microtubules in (J), (L), (N), and (P). Bar, 10 μ m. Note that microtubules in the *ro* mutants extend to the tip of anucleate hyphae and lose their parallel arrangement in regions of clumped nuclei. A view of *ro-1*(B15) hyphae showing microtubule organization in a region of densely packed nuclei, (Q) and (R). Bar, 5 μ m.

Figure 5 (cont).

dent of cytoplasmic dynein or dynactin activity, and the close proximity of a nucleus.

DISCUSSION

We showed previously that *N. crassa ro-1* and *ro-4* genes encode cytoplasmic dynein heavy chain and Arp1, respectively (Plamann *et al.*, 1994), and predicted that other *ro* genes likely encode additional subunits of cytoplasmic dynein or dynactin. In support of this hypothesis, we report here that *ro-3* encodes a putative fungal homologue of p150^{Glued}, the largest subunit of the dynactin complex. Overall sequence identity between Ro3 and vertebrate and *Drosophila* p150^{Glued} is not exceptionally high (29% and 26%, respectively); however, the major structural features of p150^{Glued} are present in Ro3. p150^{Glued} has been shown to physically interact with microtubules, Arp1, and cytoplasmic dynein intermediate chains (McGrail *et al.*, 1995; Vaughan *et al.*, 1995; Waterman-Storer *et al.*, 1995). While the domain within p150^{Glued} that mediates interaction with dynein intermediate chains has not been identified, the domains required for interaction with microtubules and Arp1 are located at the N- and C-termini, respectively (Waterman-Storer *et al.*, 1995). As shown in Figure 2B, the microtubule-binding domain is highly conserved between vertebrate and *Drosophila* p150^{Glued} and Ro3. A conserved motif at the C-terminus of p150^{Glued} mediates binding to Arp1 in vitro (Waterman-Storer *et al.*, 1995). In rat and chicken p150^{Glued} this motif consists of a cluster of charged residues (KKEK), and in *Drosophila* p150^{Glued} and Ro3 similar sequences are observed (LKEK and KKEA, respectively). The largest structural features of p150^{Glued} are two regions (~350 and 150 residues) predicted to form α -helical coiled-coils (Lupas *et al.*, 1991) both α -helical coiled-coil domains are present in Ro3 (Figure 2C).

The regulatory mechanism(s) controlling cytoplasmic dynein/dynactin subunit gene expression are not known. It does not appear that dynactin gene transcription directly responds to the need for increased dynein/dynactin activity because *ro-3* mRNA levels do not increase in *ro-1* or *ro-3* mutants (Figure 1B), and *ro-4* mRNA levels do not increase in a *ro-4* mutant (Robb *et al.*, 1995). An unusual feature of both the *ro-3* and *ro-4* genes is a short ORF (14 and 13 codons, respectively) that overlaps the respective proposed translation initiation codon (Figure 1C; Plamann *et al.*, 1994). There is no similarity between the putative peptides encoded by the uORFs or between the DNA sequences that make up the two uORFs. The presence of open reading frames within the leader regions of the *ro-3* and *ro-4* genes may indicate that expression of some of the *ro* genes is translationally regulated (Yoon and Donahue, 1992). A common translational control

mechanism could be one method of ensuring coordinate production of dynein/dynactin subunits.

In *S. cerevisiae* and *N. crassa*, cytoplasmic dynein heavy chain and Arp1 mutants are phenotypically identical, suggesting that dynein and dynactin are required for common cellular process(es) (Eshel *et al.*, 1993; Li *et al.*, 1993; Clark and Meyer, 1994; Muhua *et al.*, 1994; Plamann *et al.*, 1994). Our observation of unlinked noncomplementation between various *ro-1* and *ro-3* mutants provides genetic evidence that cytoplasmic dynein and dynactin interact in vivo (Table 2). Similar results have been obtained in *Drosophila* where cytoplasmic dynein heavy chain mutants were identified that can act as dominant suppressors or enhancers of a dominant p150^{Glued} mutant (*Gl¹*) (McGrail *et al.*, 1995). Unexpectedly, neither a *ro-3* nor *ro-4* null mutant is able to complement the *ro-1*(B15) mutant, while either null mutant complements the other six *ro-1* mutants (Table 2). To determine the nature of the genetic lesion in *ro-1*(B15), we localized the mutation to the distal one-third of *ro-1* (our unpublished results). The function of the C-terminal domain of cytoplasmic dynein heavy chain is unknown (Holzbaur and Vallee, 1994). One possible interpretation of noncomplementation between *ro-1*(B15) and dynactin null mutants is that the *ro-1*(B15) mutant produces a defective cytoplasmic dynein heavy chain that acts as a *trans*-dominant negative in the absence of sufficient dynactin complex. Cytoplasmic dynein motors containing one or two mutant *ro-1*(B15) heavy chains, and lacking association with dynactin, could in some way either block the activity of functional dynein/dynactin or interfere with a dynein-independent cellular process. Alternatively, noncomplementation between dynactin null mutants and *ro-1*(B15) may result from a decrease in the effective dosage of dynein/dynactin activity. If the *ro-1*(B15) mutation results in a greater loss of dynein activity than the other *ro-1* alleles examined here, then a drop in dosage of dynactin activity may be sufficient to drop dynein/dynactin activity below a critical level required for normal growth.

In *Drosophila*, p150^{Glued} and cytoplasmic dynein heavy chain are essential (Harte and Kankel, 1982; McGrail *et al.*, 1995). We determined that *ro-3* and *ro-4* null mutants are viable, indicating that dynactin is not an essential complex in *N. crassa* (Figures 3 and 4). At this time, we have been unable to construct a strain disrupted for *ro-1*; however, in *Aspergillus nidulans*, a *nudA* (cytoplasmic dynein heavy chain) null mutant has been constructed and found to be viable (Xiang *et al.*, 1995). It has been suggested that the inability of *Drosophila* to tolerate total loss of cytoplasmic dynein/dynactin activity is because this activity is required for an essential cellular process (Harte and Kankel, 1982). If this is true, either this essential process is not present in fungi or there is sufficient redundancy of motor activities in fungal cells such that dynein/dynactin

activity is not critical for viability. Alternatively, *Drosophila* development is dependent upon cell differentiation and interaction between numerous cell types and tissues, and loss of cytoplasmic dynein/dynactin activity may prevent or interfere with formation of multicellular structures (Harte and Kankel, 1982).

The complete range of functions of cytoplasmic dynein/dynactin in fungi has not been determined. Cytoplasmic dynein/dynactin is required for nuclear movement in filamentous fungi and in budding yeast (Eshel *et al.*, 1993; Li *et al.*, 1993; Clark and Meyer, 1994; Muhua *et al.*, 1994; Plamann *et al.*, 1994; Xiang *et al.*, 1994), and in yeast it is one of three redundant motors required for anaphase chromosome segregation (Saunders *et al.*, 1995). Recently, cytoplasmic dynein heavy chain was determined to be concentrated to tips of growing hyphae of *A. nidulans* (Xiang *et al.*, 1995). Growing hyphal tips also contain an abundance of vesicles that are presumed to contain enzymes and precursors required for cell wall growth (Grove and Bracker, 1970). Whether this correlation indicates that dynein is also required for vesicle trafficking in filamentous fungi is unknown. In *N. crassa* cytoplasmic dynein (*ro-1*) and dynactin (*ro-3* and *ro-4*) mutants, hyphal tips can extend >200 μ m beyond the nearest nucleus, indicating that loss or reduction in dynein/dynactin activity does not completely block long-range transport of materials/organelles required for tip growth (Figure 5; Plamann *et al.*, 1994). Organelle movements in *N. crassa* have been shown to be mediated by cytoplasmic microtubules (Steinberg and Schliwa, 1993); therefore, one would predict that anucleate hyphae of *ro* mutants contain cytoplasmic microtubules. In agreement with this hypothesis, we show that there are numerous cytoplasmic microtubule tracks in anucleate hyphae (Figure 5). It appears that cytoplasmic microtubule and actin organization in anucleate hyphae is independent of cytoplasmic dynein/dynactin activity or the close proximity of a nucleus. Determining the polarity of these microtubules will be an important prerequisite to understanding tip-directed, microtubule-based transport and the movement of nuclei in filamentous fungi. Future studies are directed at determining the intracellular locations of cytoplasmic dynein and dynactin relative to each other, cytoplasmic microtubules, and membranous organelles. The availability of hundreds of cytoplasmic dynein/dynactin mutants will be an invaluable resource in determining the cellular roles of cytoplasmic dynein/dynactin in fungi and the specific role of dynactin in cytoplasmic dynein-mediated cellular processes.

ACKNOWLEDGMENTS

This work was supported by grant GM-51217 from the National Institutes of Health.

REFERENCES

- Allan, V. (1995). Protein phosphatase 1 regulates the cytoplasmic dynein-driven formation of endoplasmic reticulum networks in vitro. *J. Cell Biol.* 128, 879–891.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.
- Aniento, F., Emans, N., Griffiths, G., and Gruenberg, J. (1993). Cytoplasmic dynein-dependent vesicular transport from early to late endosomes. *J. Cell Biol.* 123, 1373–1387.
- Bruchez, J.J.P., Eberle, J., and Russo, V.E.A. (1993). Regulatory sequences involved in the translation of *Neurospora crassa* mRNA: kozak sequences and stop codons. *Fungal Genet. News.* 40, 85–88.
- Bruno, K.S., Tinsley, J.H., Minke, P.F., and Plamann, M. (1996). Genetic interactions among cytoplasmic dynein, dynactin, and nuclear distribution mutants of *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* (in press).
- Clark, S.W., and Meyer, D.I. (1994). Act3: a putative centractin homologue in *S. cerevisiae* is required for proper spindle orientation of the mitotic spindle. *J. Cell Biol.* 127, 129–138.
- Corthésy-Theulaz, I., Pauloin, A., and Pfeffer, S.R. (1992). Cytoplasmic dynein participates in the centrosomal location of the golgi complex. *J. Cell Biol.* 118, 1333–1345.
- Cullen, D., Leong, S.A., Wilson, L.J., and Henner, D.J. (1987). Transformation of *Aspergillus nidulans* with the hygromycin-resistance gene, *hph*. *Gene* 57, 21–26.
- Davis, R.H., and de Serres, F.J. (1970). Genetic and microbiological research techniques for *Neurospora crassa*. *Methods Enzymol.* 27A, 79–143.
- Devereux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12, 387–395.
- Eshel, D., Urrestarazu, L.A., Vissers, S., Jauniaux, J., Vliet-Reedijk, J.C. v., Planta, R.J., and Gibbons, I.R. (1993). Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Natl. Acad. Sci. USA* 90, 11172–11176.
- Fath, K.R., Trimbur, G.M., and Burgess, D.R. (1994). Molecular motors are differentially distributed on Golgi membranes from polarized epithelial cells. *J. Cell Biol.* 126, 661–675.
- Fincham, J.R.S. (1989). Transformation in fungi. *Microbiol. Rev.* 53, 148–170.
- Garen, A., Miller, B.R., and Paco-Larson, M.L. (1984). Mutations affecting functions of the *Drosophila* gene *Glued*. *Genetics* 107, 645–655.
- Garnjobst, L., and Tatum, E.L. (1967). A survey of new morphological mutants in *Neurospora crassa*. *Genetics* 57, 579–604.
- Gill, S.R., Schroer, T.A., Szilak, I., Steuer, E.R., Sheetz, M.P., and Cleveland, D.W. (1991). Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J. Cell Biol.* 115, 1639–1650.
- Grove, S.N., and Bracker, C.E. (1970). Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. *J. Bacteriol.* 104, 989–1009.
- Harte, P.J., and Kankel, D.R. (1982). Genetic analysis of mutations at the *Glued* locus and interacting loci in *Drosophila melanogaster*. *Genetics* 101, 477–501.
- Holzbaun, E.L.F., Hammarback, J.A., Paschal, B.M., Kravitz, N.G., Pfister, K.K., and Vallee, R.B. (1991). Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued*. *Nature* 351, 579–583.

- Holzbaur, E.L.F., and Vallee, R.B. (1994). Dyneins: molecular structure and cellular function. *Annu. Rev. Cell Biol.* 10, 339–372.
- Howard, R.J. (1981). Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. *J. Cell Sci.* 48, 89–103.
- Knight, A.E., and Kendrick-Jones, J. (1993). A myosin-like protein from a higher plant. *J. Mol. Biol.* 231, 148–154.
- Lees-Miller, J.P., Helfman, D.M., and Schroer, T.A. (1992). A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-based vesicle motility. *Nature* 359, 244–247.
- Li, Y.-Y., Yeh, E., Hays, T., and Bloom, K. (1993). Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Natl. Acad. Sci. USA* 90, 10096–10100.
- Lupas, A., Dyke, M.V., and Stock, J. (1991). Predicting coiled coils from protein sequence. *Science* 252, 1162–1164.
- McGrail, M., Gepner, J., Silvanovich, A., Ludmann, S., Serr, M., and Hays, T.S. (1995). Regulation of cytoplasmic dynein function in vivo by the *Drosophila* Glued complex. *J. Cell Biol.* 131, 411–425.
- McKerracher, L.J., and Heath, I.B. (1987). Cytoplasmic migration and intracellular organelle movements during tip growth of fungal hyphae. *Exp. Mycol.* 11, 79–100.
- Metzenberg, R.L., Stevens, J.N., Selker, E.U., and Morzycka-Wroblewska, E. (1985). Identification and chromosomal distribution of 5S rRNA genes in *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* 82, 2067–2071.
- Muhua, L., Karpova, T.S., and Cooper, J.A. (1994). A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration. *Cell* 78, 669–679.
- Orbach, M.J. (1994). A cosmid with a Hy^R marker for fungal library construction and screening. *Gene* 150, 159–162.
- Paschal, B.M., and Vallee, R.B. (1987). Retrograde transport by the microtubule-associated protein MAP1C. *Nature* 330, 181–183.
- Perkins, D.D., Radford, A., Newmyer, D., and Björkman, M. (1982). Chromosomal loci of *Neurospora crassa*. *Microbiol. Rev.* 46, 426–570.
- Pierre, P., Scheel, J., Rickard, J.E., and Kreis, T.E. (1992). CLIP-170 links endocytic vesicles to microtubules. *Cell* 70, 887–900.
- Plamann, M., Minke, P.F., Tinsley, J.H., and Bruno, K.S. (1994). Cytoplasmic dynein and actin-related protein Arp1 are required for normal nuclear distribution in filamentous fungi. *J. Cell Biol.* 127, 139–149.
- Riehemann, K., and Sorg, C. (1993). Sequence homologies between four cytoskeleton-associated proteins. *Trends Biochem. Sci.* 18, 82–83.
- Robb, M.J., Wilson, M.A., and Vierula, P.J. (1995). A fungal actin-related protein involved in nuclear migration. *Mol. Gen. Genet.* 247, 583–590.
- Sambrook, J., Fritsch, E.H., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
- Saunders, W.S., Koshland, D., Eschel, D., Gibbons, I.R., and Hoyt, M.A. (1995). *Saccharomyces cerevisiae* kinesin- and dynein-related proteins required for anaphase chromosome segregation. *J. Cell Biol.* 128, 617–624.
- Schafer, D.A., Gill, S.R., Cooper, J.A., Heuser, J.E., and Schroer, T.A. (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 Sheetz, M.P. (1991). Two activators of microtubule-based vesicle transport. *J. Cell Biol.* 115, 1309–1318.
- Stearns, T., and Botstein, D. (1988). Unlinked noncomplementation: isolation of new conditional-lethal mutations in each of the tubulin genes of *Saccharomyces cerevisiae*. *Genetics* 119, 249–260.
- Steinberg, G., and Schliwa, M. (1993). Organelle movements in the wild type and wall-less *fz*; *sg*; *os-1* mutants of *Neurospora crassa* are mediated by cytoplasmic microtubules. *J. Cell Sci.* 106, 555–564.
- Swaroop, A., Swaroop, M., and Garen, A. (1987). Sequence analysis of the complete cDNA and encoded polypeptide for the *Glued* gene of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 84, 6501–6505.
- Thompson-Coffe, C., and Zickler, D. (1994). How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. *Dev. Biol.* 165, 257–271.
- Vaisberg, E.A., Koonce, M.P., and McIntosh, J.R. (1993). Cytoplasmic dynein plays a role in mammalian spindle formation. *J. Cell Biol.* 123, 849–858.
- Vaughan, K.T., Holzbaur, E.L.F., and Vallee, R.B. (1995). Subcellular targeting of the retrograde motor cytoplasmic dynein. *Biochem. Soc. Trans.* 23, 50–54.
- Vinh, D.B.N., Welch, M.D., Corci, A.K., Wertman, K.F., and Drubin, D.G. (1993). Genetic evidence for functional interaction between actin noncomplementing (Anc) gene products and actin cytoskeletal proteins in *Saccharomyces cerevisiae*. *Genetics* 135, 275–286.
- Vollmer, S.J., and Yanofsky, C. (1986). Efficient cloning of genes of *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* 83, 4869–4873.
- Walker, R.A., and Sheetz, M.P. (1993). Cytoplasmic microtubule-associated motors. *Annu. Rev. Biochem.* 62, 429–451.
- Waterman-Storer, C.M., Karki, S., and Holzbaur, E.L.F. (1995). The p150^{Glued} component of the dynactin complex binds to both microtubules and the actin-related protein cetractin (Arp1). *Proc. Natl. Acad. Sci. USA* 92, 1634–1638.
- Xiang, X., Beckwith, S.M., and Morris, N.R. (1994). Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* 91, 2100–2104.
- Xiang, X., Roghi, C., and Morris, N.R. (1995). Characterization and localization of the cytoplasmic dynein heavy chain in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* 92, 9890–9894.
- Yarden, O., Plamann, M., Ebbola, D.J., and Yanofsky, C. (1992). *cot-1*, a gene required for hyphal elongation in *Neurospora crassa*, encodes a protein kinase. *EMBO J.* 11, 2159–2166.
- Yeh, E., Skibbens, R.V., Cheng, J.W., Salmon, E.D., and Bloom, K. (1995). Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* 130, 687–700.
- Yoon, H., and Donahue, T.F. (1992). Control of translation initiation in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 6, 1413–1419.