Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 4775–4780, May 1996
Genetics

Genetic interactions among cytoplasmic dynein, dynactin, and nuclear distribution mutants of *Neurospora crassa*

KENNETH S. BRUNO, JOHN H. TINSLEY, PETER F. MINKE, AND MICHAEL PLAMANN*

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

Communicated by David D. Perkins, Stanford University, Stanford, CA, January 16, 1996 (received for review October 10, 1995)

ABSTRACT **Cytoplasmic dynein is a multisubunit, microtubule-associated, mechanochemical enzyme that has been identified as a retrograde transporter of various membranous organelles. Dynactin, an additional multisubunit complex, is required for efficient dynein-mediated transport of vesicles *in vitro*. Recently, we showed that three genes defined by a group of phenotypically identical mutants of the filamentous fungus *Neurospora crassa* encode proteins that are apparent subunits of either cytoplasmic dynein or dynactin. These mutants, designated ropy (*ro*), display abnormal hyphal growth and are defective in nuclear distribution. We propose that mutations in other genes encoding dynein/dynactin subunits are likely to result in a ropy phenotype and have devised a genetic screen for the isolation of additional *ro* mutants. Cytoplasmic dynein/dynactin is the largest and most complex of the cytoplasmic motor proteins, and the genetic system described here is unique in its potentiality for identifying mutations in undefined genes encoding dynein/dynactin subunits or regulators. We used this screen to isolate >1000 *ro* mutants, which were found to define 23 complementation groups. Unexpectedly, interallelic complementation was observed with some allele pairs of *ro-1* and *ro-3*, which are predicted to encode the largest subunits of cytoplasmic dynein and dynactin, respectively. The results suggest that the Ro1 and Ro3 polypeptides may consist of multiple, functionally independent domains. In addition, ≈10% of all newly isolated *ro* mutants display unlinked noncomplementation with two or more of the mutants that define the 23 complementation groups. The frequent appearance of *ro* mutants showing noncomplementation with multiple *ro* mutants having unlinked mutations suggests that nuclear distribution in filamentous fungi is a process that is easily disrupted by affecting either dosage or activity of cytoplasmic dynein, dynactin, and perhaps other cytoskeletal proteins or regulators.**

Cytoplasmic dynein is a microtubule-associated motor enzyme that has been implicated in a number of intracellular, microtubule-dependent transport processes, including retrograde axonal transport (1). In addition, cytoplasmic dynein plays a role in mitotic spindle formation in mammalian cells, and in *Saccharomyces cerevisiae* it participates in anaphase chromosome separation (2, 3). Dynein also is required for nuclear migration in *S. cerevisiae* and nuclear distribution in the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa* (4–7).

Cytoplasmic dynein, the largest and most complex of the cytoplasmic motor proteins, is composed of two identical heavy chains (≈500 kDa), three intermediate chains (≈70 kDa), and four light chains (53–59 kDa) (for review, see ref. 8). In addition, a multisubunit complex known as dynactin is required for efficient dynein-mediated transport of vesicles *in vitro* (9, 10). The dynactin complex consists of at least nine different subunits, which include p150^{Glued} (the largest subunit) and the actin-related protein Arp1 (the most abundant subunit) (9–12).

Mutants defective in cytoplasmic dynein/dynactin have been isolated in a number of organisms. The gene encoding p150^{Glued} was first identified in *Drosophila* (13–15). Flies homozygous for recessive Glued mutations exhibit an embryonic lethal phenotype, and flies heterozygous for dominant Glued alleles are defective in eye development and neural connections between the eye and optic lobe (13, 14). The isolation of *S. cerevisiae* genes encoding cytoplasmic dynein heavy chain and Arp1 allowed construction of dynein and dynactin mutants, both of which are viable but defective in nuclear migration (6, 7, 16, 17). Nuclear distribution (*nud*) mutants have been isolated in *A. nidulans*, and one of the *nud* genes, *nudA*, encodes cytoplasmic dynein heavy chain (4).

In *N. crassa*, mutations at eight different loci define a group of phenotypically identical mutants known as ropy (*ro*), which are defective in hyphal growth and nuclear distribution (5, 18). DNA sequence analysis of three of these genes, *ro-1*, *ro-3*, and *ro-4*, revealed that they encode cytoplasmic dynein heavy chain, p150^{Glued}, and Arp1, respectively (5). These observations led us to predict that mutations in other genes encoding subunits of cytoplasmic dynein or dynactin will also result in a ropy phenotype. Therefore, a large-scale screen for *ro* mutants of *N. crassa* may allow for the first time the identification of all nonredundant genes encoding subunits or specific regulators of cytoplasmic dynein and dynactin, and possibly of other genes that are not required for dynein/dynactin activity but are required for nuclear movement. Previously, we showed that *ro* mutants can be readily isolated as partial suppressors of *cot-1*, a gene encoding a serine/threonine protein kinase required for hyphal elongation (5, 19). In this paper, we report the isolation of >1000 *ro* mutants and the identification of 23 *ro* complementation groups. Unexpectedly, ≈10% of all the newly isolated *ro* mutants failed to complement two or more *ro* mutants defining unlinked loci. The high frequency of *ro* mutants displaying such unlinked noncomplementation may reflect the complex subunit composition of cytoplasmic dynein and dynactin and the need for coordinated activities of dynein motors and other proteins for proper distribution of nuclei within hyphae.

MATERIALS AND METHODS

Strains, Genetic Techniques, and Media. *N. crassa* strains were obtained from the Fungal Genetics Stock Center (FGSC; Department of Microbiology, University of Kansas Medical Center, Kansas City). The *ro* mutants used in this study were *ro-1*(B15) (FGSC no. 146), *ro-2*(B20) (FGSC no. 44), *ro-3* (R2354) (FGSC no. 43), *ro-4*(P904) (FGSC no. 1669), *ro-6* (R2431) (FGSC no. 3627), *ro-7*(P997) (FGSC no. 3322), *ro-10*(AR7) (FGSC no. 3619), and *ro-11*(P3053) (FGSC no.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FGSC, Fungal Genetics Stock Center; DAPI, 4′,6-diamidino-2-phenylindole.

*To whom reprint requests should be addressed. e-mail: mplamann@bio.tamu.edu.

3911). The wild-type *N. crassa* strain was 74-OR23-1A (FGSC no. 987), and a *cot-1*(C102t); *his-3* strain was constructed and used to isolate *ro* mutants as partial suppressors of *cot-1* (see below). Crosses for mapping and strain construction and the formation of heterokaryons for complementation analysis were done using standard procedures (20). Media and growth conditions were as described (20).

Isolation of *ro* Mutants and Complementation Analysis. The isolation of *ro* mutants by screening for partial suppressors of *cot-1* has been described (5). Briefly, spontaneous *ro* mutations were identified as *cot-1* suppressors by plating $\approx 2 \times 10^5$ conidia of a temperature-sensitive *cot-1* mutant on sucrose-supplemented minimal medium and incubating at 37°C for 3–7 days. *cot-1* conidia germinate under these conditions and produce colonies that are ≈0.1 mm in diameter, whereas *cot-1* mutants containing *ro* mutations produce colonies that are 1–5 mm in diameter. Newly isolated *ro*; *cot-1* strains were transferred to a fresh medium and incubated at 25°C. Each of the *ro*; *cot-1* mutants was single colony purified three times. *ro*; *cot-1* mutants that reverted rapidly to wild-type growth were not used for complementation analysis.

Heterokaryons were formed between various *ro* mutants to determine the number of complementation groups (20). A *trp-4* marker was crossed into each of the eight *ro* strains obtained from the FGSC. These strains were used to make forced heterokaryons with each of the newly isolated *ro*; *cot-1*; *his-3* strains by coinoculating on a minimal medium lacking tryptophan and histidine. Because *cot-1* (C102t) is a temperature-sensitive mutation that has no detectable effect on growth morphology at the permissive temperature (25°C), all complementation tests were conducted at 25°C. The *ro* mutants have curled hyphae; the formation of straight, rapidly extending hyphae was taken to indicate complementation while retention of curled hyphal growth was taken to indicate the lack of complementation. All of the *ro* mutations from the FGSC were found to be recessive except *ro-6*, which is semidominant in heterokaryons and could not be used in the complementation analysis. Most of the newly isolated *ro* mutants (70%) could be assigned to a complementation group defined by one of the seven *ro* mutants obtained from the FGSC (Table 1). The remaining *ro* mutants were sorted into complementation groups by coinoculating pairs of mutants on histidine-supplemented media and incubating at 25°C. Although these *ro* mutants lack nutritional markers for "forcing" formation of heterokaryons, strains containing *ro* mutations have highly restricted growth rates so that positive complementation tests could be scored unambiguously on the basis of presence of straight, rapidly extending hyphae.

Microscopic Examination of *ro* Mutants. Hyphal morphologies of wild-type and *ro* mutants were examined by inoculating sucrose minimal agar medium with conidia and incubating plates at the designated temperature for 2 days. Pictures were taken with Kodak TMAX 100 or TMAX 400 film (Eastman Kodak Company, Rochester, NY) on an Olympus (New Hyde Park, NY) binocular dissection microscope at 7× magnification with transmitted light.

Nuclear distribution was examined by staining hyphae with the DNA-specific dye 4′,6-diamidino-2-phenylindole (DAPI). Sterile dialysis membranes [1 cm × 1 cm; CELLU·SEP T1 membrane, 4000–6000 M_r cut off, Membrane Filtration Products (San Antonio, TX)] were placed on an agar surface and ≈100 conidia were spread on each membrane; the plates were then incubated at 25°C for 8 hr. Membranes with attached hyphae were quick frozen in liquid N_2, treated for 10 min with fixing solution (3.7% formaldehyde in PBS), rinsed for 30 min with wash solution (PBS) containing 0.5 μg/ml DAPI, and then soaked in wash solution without DAPI for 30 min. A membrane containing hyphal material was placed on a slide and a coverslip anchored with Vaseline was then pressed to the slide. Pictures were taken using TMAX 400 film with an Olympus microscope using a SPlan Apo 60× oil objective and differential interference contrast optics. The sample was illuminated with UV light to view the DAPI stain.

Table 1. Complementation groups of *ro* mutants

C.G.	Defining allele*	No. of mutants†	No. of screen‡
1	*ro-1*(B15)	401	24
2	*ro-2*(B20)	49	13
3	*ro-3*(R2354)	103	19
4	*ro-4*(P904)	58	17
5	*ro-7*(P997)	61	17
6	*ro-10*(AR7)	49	14
7	*ro-11*(P3053)	31	10
8	*ro*(B2)	149	22
9	*ro*(JT2)	2	1
10	*ro*-1(A59)	27	11
11	*ro*(L12)	41	12
12	*ro*(C52)	30	14
13	*ro*(K51)	13	9
14	*ro*(C18)	5	4
15	*ro*(C102)	5	5
16	*ro-3*(C115)	2	2
17	*ro*(C76)	9	3
18	*ro*(U3)	9	4
19	*ro*(I107)	2	2
20	*ro*(J1)	1	1
21	*ro*(G42)	3	3
22	*ro*(P56)	3	3
23	*ro*(M76)	13	1

C.G., complementation group.
*Defining allele signifies the *ro* allele that was used as a tester strain to define a given complementation group.
†Total number of *ro* mutants that do not complement exclusively a specific *ro* mutant tester strain.
‡The number of mutant screens, out of 24 independent screens, in which a given complementation group was identified.

RESULTS

Isolation of *ro* Mutants and Complementation Analysis. Our finding that three *ro* genes encode putative subunits of cytoplasmic dynein or dynactin led us to conduct a large-scale screen for *ro* mutants that may define additional genes required for cytoplasmic dynein/dynactin function and nuclear distribution. We showed previously that in the absence of Cot1 kinase activity, *N. crassa* forms small colonies (≈0.1 mm in diameter) consisting of masses of spheres with many short hyphal tips while *ro*; *cot-1* double mutants form slightly larger colonies (1–5 mm in diameter) that are easily distinguished from *cot-1* colonies (5). The mechanism by which *ro* mutations cause hyphal tips of a *cot-1* mutant to extend slowly is not known.

Approximately 1500 *ro* mutants were isolated from 24 independent *cot-1* cultures by isolation of partial suppressors of the *cot-1*^{ts} growth phenotype, and 1187 of these mutants were assigned to complementation groups by heterokaryon analysis (see *Materials and Methods*; Table 1). As a first step in establishing the number of *ro* complementation groups, we conducted complementation tests between the newly isolated *ro* mutants and *ro-1*, *-2*, *-3*, *-4*, *-7*, *-10*, and *-11* mutants that were obtained from the FGSC. Approximately 30% of the *ro* mutants complemented all seven of the initial tester strains, and subsequent complementation tests established that these mutants defined an additional 16 complementation groups (Table 1). Typically, the number of loci defined by complementation groups would be determined by conducting a series of pair-wise crosses between mutants defining each complementation group. However, most *ro* mutants are infertile when

used as the female (21); therefore, we were unable to define unambiguously the number of *ro* loci.

New *ro* Mutants and Nuclear Distribution. All *ro* mutants from the FGSC are phenotypically identical, consisting of curled hyphae with highly asymmetric nuclear distribution relative to a wild-type strain (Fig. 1 *A–D*). To determine if the curled hyphal growth characteristic of *ro* mutants always coincides with defective nuclear distribution, we examined nuclear distribution in the 16 tester strains defining each new *ro* complementation group. Only 12 of the 16 new *ro* complementation groups had obvious nuclear distribution defects (Fig. 1 *E* and *F*). Complementation groups 9, 15, 17, and 19 defined by *ro*(JT2), *ro*(C102), *ro*(C76), and *ro*(I107), respectively, have a typical *ro* growth defect, without the characteristic strong defect in nuclear distribution (Fig. 1*F*). Numerous mutants belonging to these four complementation groups were identified and all were found to have nearly normal nuclear distribution, suggesting that the mutants defining the four groups do not contain "leaky" alleles of the typical *ro* genes. These atypical *ro* mutants appear to have retained the ability to move nuclei into growing hyphae; however, these nuclei are not as evenly distributed as they are in a wild-type strain.

Complementation Between Alleles at *ro-1* and at *ro-3*. Although the number of loci identified by the 16 new *ro*

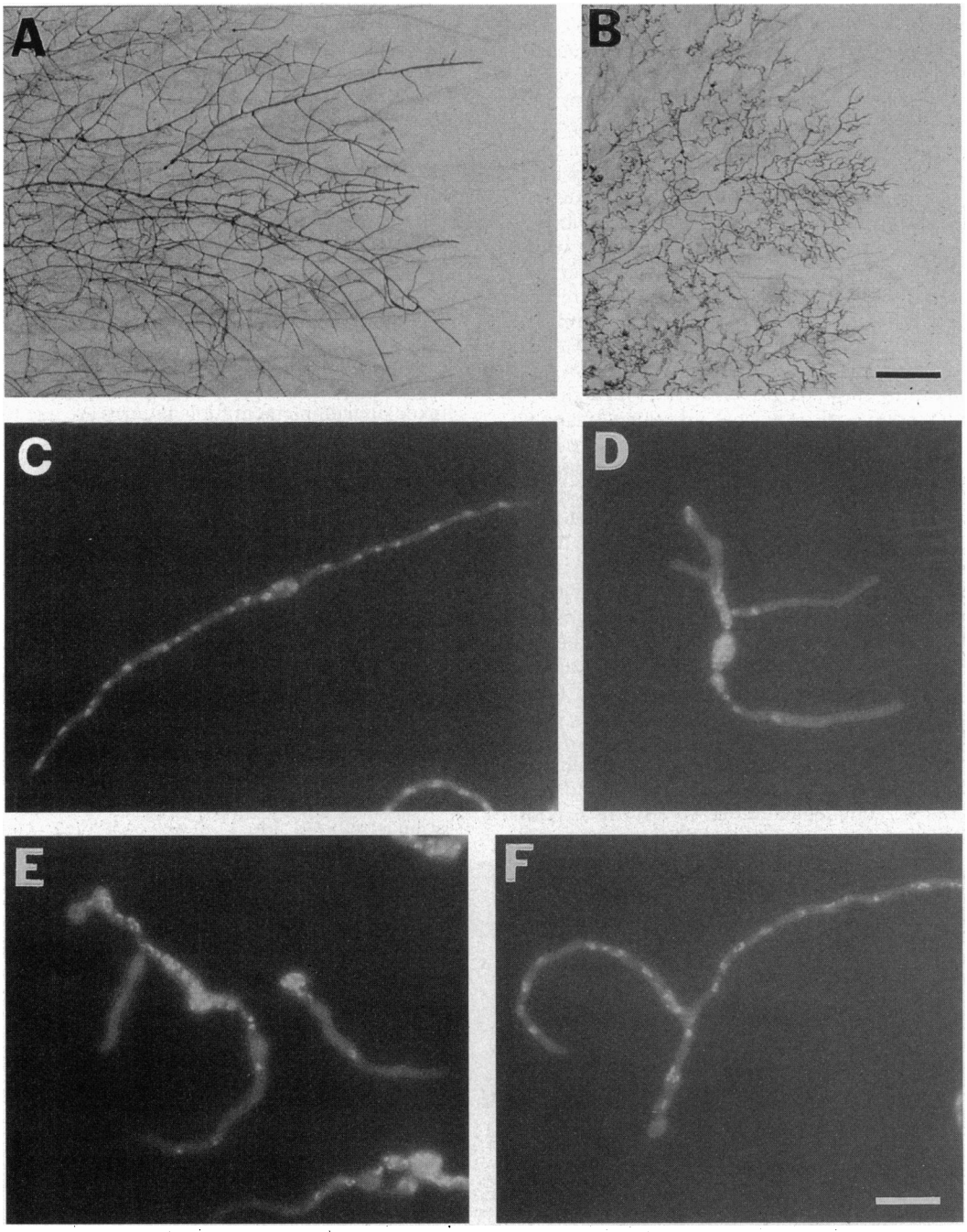

FIG. 1. Curled hyphal growth and asymmetric nuclear distribution of *ro* mutants. Conidia from wild-type (*A*) and *ro-3*(R2354) (*B*) mutants were inoculated onto sucrose minimal agar medium and incubated overnight at 25°C. Note the difference in hyphal morphologies of the wild-type strain (*A*) and the *ro-3*(R2354) (*B*) mutant. Conidia from a wild-type strain (*C*) and *ro-1*(B15) (*D*) mutant were allowed to grow for 8 hr on filters placed on sucrose minimal media at 25°C, and germinated conidia were stained with DAPI to visualize nuclei. Conidia from newly isolated *ro* mutants *ro*(P56) (*E*) and *ro*(C76) (*F*) were incubated on sucrose minimal media for 8 hr and stained with DAPI to visualize nuclei. Note that nuclear distribution in *ro*(C76) is not severely affected as it is in typical *ro* mutants. (*A* and *B*, bar = 500 μm; *C–F*, bar = 20 μm.)

complementation groups cannot be determined unambiguously by pair-wise crosses, we have isolated cosmid clones containing the *ro-1*, *-2*, *-3*, *-4*, *-7*, *-10*, and *-11* genes (5) (unpublished data) and we have used these plasmids to transform the defining mutant of each of the 16 new complementation groups. If a recessive *ro* mutant is not rescued by any of the cloned *ro* genes, then it likely defines a new *ro* locus. Most of the mutants defining the 16 new complementation groups are not complemented by any of the seven *ro* clones. However, the *ro* phenotype of complementation group 10 defined by *ro*(A59) was rescued by transformation with a plasmid containing the *ro-1* gene and complementation group 16 defined by *ro*(C115) was rescued by transformation with a plasmid containing the *ro-3* gene. This is an unexpected result considering that *ro*(A59) and *ro*(C115) clearly complement both *ro-1*(B15) and *ro-3*(R2354). Restriction endonuclease digestion of plasmid DNA containing *ro-1* or *ro-3* genes established that digestion by restriction enzymes cutting only within the respective genes eliminated rescue, while digestion with restriction enzymes cutting outside of the respective genes still allowed rescue. These results indicate that rescue is dependent on *ro-1* or *ro-3* sequences and likely results either from extracopy suppression or by introduction of a wild-type copy of the affected gene [i.e., *ro*(A59) and *ro*(C115) would represent alleles of *ro-1* and *ro-3*, respectively]. To determine if *ro*(A59) and *ro*(C115) are alleles of *ro-1* and *ro-3*, respectively, we mapped the *ro*(A59) and *ro*(C115) mutations relative to the *ro-1*-linked markers *pan-1* (<1%) and *cot-1* (1–2%) and the *ro-3*-linked marker *pyr-4* (1–2%). In both cases, the *ro*(A59) and *ro*(C115) mutations showed tight linkage (≤1%) with *ro-1*- and *ro-3*-linked markers, respectively. Additional mapping studies of five other members of complementation group 10 and the remaining member of complementation group 16, *ro*(M144), also showed tight linkage with *ro-1*- and *ro-3*-linked markers, respectively. Finally, neither *ro*(C115) nor *ro*(M144) complement a *ro-3* deletion strain, consistent with these two *ro* mutations representing mutant alleles of *ro-3*. From these results, we conclude that members of complementation group 10 represent alleles of *ro-1* that are capable of interallelic complementation with the *ro-1*(B15) tester strain, and complementation group 16 represent alleles of *ro-3* that are capable of interallelic complementation with the *ro-3*(R2354) tester strain.

Because complementation group 10 includes 27 *ro-1* mutants isolated in 11 of the 24 screens (>5% of the *ro-1* mutants isolated), we expected that other *ro-1* mutants might also be capable of interallelic complementation. To test this hypothesis, pair-wise complementation tests were conducted between 24 *ro-1* mutants from complementation group 1 where each of the *ro-1* mutants was isolated in an independent screen. As shown in Table 2, six of the independent *ro-1* mutants are capable of complementing one or more of the *ro-1* mutants.

Unlinked Noncomplementation. Most of the 1187 *ro* mutants examined by complementation analysis were assigned unambiguously to a single complementation group. However, 121 of the *ro* mutants were found to not complement more than one of the 23 tester strains. In many cases, these mutants fail to complement two or more defining members of the first seven complementation groups, which are known to map to different loci, indicating that these represent examples of unlinked noncomplementation. Five representative *ro* mutants displaying unlinked noncomplementation are shown in Table 3. Many *ro* mutants show "allele-specific" noncomplementation. For example, mutants *ro*(A40), *ro*(B10), *ro*(C81), and *ro*(E58) do not complement group 3 tester *ro-3*(R2354), but do complement group 16 tester *ro-3*(C115) (Table 3). In addition, all four of these mutants fail to complement group 1 tester *ro-1*(B15), but *ro*(A40) and *ro*(C81) are able to complement group 10 tester *ro-1*(A59) (Table 3).

Heterokaryons formed between the five representative *ro* mutants displaying unlinked noncomplementation and the complementation group testers 8–23 were not "forced" using auxotrophic markers; it is possible that complex patterns of unlinked noncomplementation observed with specific *ro* mutants are due in some cases to the inability of a *ro* mutant to form stable heterokaryons rather than an inability to restore wild-type hyphal growth once a heterokaryon has formed. Therefore, an auxotrophic marker (*pan-1*) was crossed into each of the seven complementation group testers that did complement one or more of the five representative *ro* mutants displaying unlinked noncomplementation (shown in Table 3) and the complementation tests repeated. In all cases, heterokaryons were successfully formed between the respective *ro* mutants, and each of the resulting heterokaryons exhibited a *ro* growth phenotype. The results indicate that complex unlinked noncomplementation patterns are due to an inability to restore wild-type hyphal growth rather than a general failure to form heterokaryons.

Table 2. Allelic complementation among select *ro-1* mutants from complementation group 1

	ro-1(A5)	*ro-1*(B10)	*ro-1*(C24)	*ro-1*(E11)	*ro-1*(O39)
ro-1(B10)	−				
ro-1(C24)	+	+			
ro-1(E11)	−	−	−		
ro-1(O39)	+	−	−	+	
ro-1(V22)	+	−	−	+	−

Complementation analysis was conducted by pair-wise formation of heterokaryons. + and − signify complementation or noncomplementation, respectively, based on whether a heterokaryon grows as straight, rapidly extending hyphae or grows as curled hyphae, respectively.

DISCUSSION

Cytoplasmic dynein, along with the associated dynactin complex, is the largest and most complex of the known mechanochemical enzymes operating in the cytoplasm (1, 8). Previously, we showed (5) that the *N. crassa ro-1* gene encodes the heavy chain of cytoplasmic dynein, and that the *ro-3* and *ro-4* genes encode the dynactin subunits p150^{Glued} and Arp1, respectively. An exciting implication of these results is that mutations in other genes encoding subunits or regulators of cytoplasmic dynein and dynactin may also result in a *ro* phenotype. In this study, we report the isolation and initial characterization of 1187 *ro* mutants defining 23 complementation groups (Table 1). Twelve of these complementation groups were identified in nine or more of the 24 independent screens, while the remaining *ro* mutants defined an additional 11 complementation groups identified in 1–5 screens. The number of loci defined by the 23 *ro* complementation groups has not yet been determined because most *ro* mutants do not function as the female in a cross (21). However, we are in the process of determining the number of loci by restriction fragment length mapping using cloned representatives of each group (*ro* genes are easily cloned; ref. 5). The *ro-1* structural gene consists of 4367 codons and, as expected, the *ro-1*(B15) tester defines the largest complementation group, identified in all 24 screens and representing >33% of all *ro* mutants. *ro-10* is the smallest *ro* gene we have examined to date and 49 *ro-10* mutants were identified from 14 of 24 screens (*ro-10* is nonessential for viability and encodes a 217-aa polypeptide; unpublished data). The small size of *ro-10* and the relatively large number of screens from which *ro-10* mutants were identified suggests that our genetic screen is saturating for those genes that do not present abnormally small genetic targets. The relatively rare complementation groups identified in only one to five screens may represent cases of interallelic complementation (see below) or may define genes involved in

Table 3. Representative *ro* mutants displaying unlinked noncomplementation

C.G.	Defining allele*	*ro* mutants displaying unlinked noncomplementation†				
		ro(A40)	*ro*(B10)	*ro*(C81)	*ro*(D3)	*ro*(E58)
1	*ro-1*(B15)	−	−	−	+	−
2	*ro-2*(B20)	+	+	−	+	+
3	*ro-3*(R2354)	−	−	−	+	−
4	*ro-4*(P904)	+	+	+	−	+
5	*ro-7*(P997)	+	+	−	+	−
6	*ro-10*(AR7)	−	+	+	+	−
7	*ro-11*(P3053)	+	+	−	+	+
8	*ro*(B2)	−	+	−	−	−
9	*ro*(JT2)	+	+	+	+	+
10	*ro-1*(A59)	+	−	+	+	−
11	*ro*(L12)	+	+	+	+	+
12	*ro*(C52)	+	+	+	+	+
13	*ro*(K51)	+	+	−	+	+
14	*ro*(C18)	+	+	+	+	+
15	*ro*(C102)	+	+	+	+	−
16	*ro-3*(C115)	+	+	+	+	+
17	*ro*(C76)	+	+	−	+	+
18	*ro*(U3)	+	+	+	+	+
19	*ro*(I107)	+	+	−	−	+
20	*ro*(J1)	+	+	+	+	+
21	*ro*(G42)	+	+	+	+	+
22	*ro*(P56)	−	+	+	+	−
23	*ro*(M76)	+	+	+	+	+

Complementation analysis was conducted by pair-wise formation of heterokaryons. + and − signify complementation or noncomplementation, respectively, based on whether a heterokaryon grows as straight, rapidly extending hyphae or grows as curled hyphae, respectively. C.G., complementation group.

*Defining allele signifies the *ro* allele that was used as a tester strain to define a given complementation group.

†Five representative *ro* mutants out of a total of 121 *ro* mutants that fail to complement more than one of the *ro* tester strains.

multiple processes, only some of which are cytoplasmic dynein-dependent. For example, actin and CapZ, a barbed-end actin-binding protein composed of α and β subunits, are required for many cellular processes, but they are also components of dynactin (11). One would predict that rare complementation groups may result from mutations in genes encoding CapZ subunits or actin that disrupt the activity of dynactin without adversely affecting dynactin-independent activities.

Interallelic complementation was observed for both *ro-1* and *ro-3*, which encode cytoplasmic dynein heavy chain and $p150^{Glued}$, respectively (Tables 1 and 2). Because cytoplasmic dynein and dynactin contain two molecules of cytoplasmic dynein heavy chain and $p150^{Glued}$, a possible interpretation of interallelic complementation is that a functional complex is made by combining two defective Ro1 or Ro3 polypeptides. If Ro1 and Ro3 consist of multiple, functionally independent domains, then two mutant polypeptides, each defective in a different domain, may combine to form a functional complex. An attractive extension of this hypothesis is that given a "sufficiently large" sample size of independent *ro-1* and *ro-3* mutants, a pair-wise complementation analysis of *ro-1* and *ro-3* mutants could allow the identification of all functionally independent domains within Ro1 and Ro3 polypeptides. We have initiated this study for *ro-1* by conducting pair-wise complementation tests using one *ro-1* mutant from each of the 24 screens. Our initial results indicate that six combinations of independent *ro-1* mutants are capable of interallelic complementation (Table 2). It is not clear how many potential functional domains are defined by these *ro-1* mutants; however, *ro-1* alleles A5, B10, and E11 and alleles C24, O39, and V22 appear to loosely define two groups. One complication of this analysis is that a mutation may disrupt more than one functional domain and a large sample size of *ro-1* mutants will be needed to control for this effect.

We observed 121 *ro* mutants showing noncomplementation with two or more *ro* mutants defining unlinked loci (Table 3). An interesting characteristic of these *ro* mutants is that some of them are allele-specific for noncomplementation with *ro-1* and *ro-3* mutants. *ro*(A40) does not complement the *ro-1*(B15) or *ro-3*(R2354) testers, but it does complement the *ro-1*(A59) and *ro-3*(C115) testers (Table 3). Three models for explaining unlinked noncomplementation are the dosage model, the poison-complex model, and the accumulated defects model (for a more complete discussion, see refs. 22–24). In the dosage model, formation of a diploid or a heterokaryon between an A^- and a B^- strain, where gene product A and B interact and are required for completion of a process, would result in noncomplementation if the fraction of active A^+/B^+ complex formed would be insufficient for completion of the process. In the poison-complex model, the A^-/B^- complex formed in the diploid/heterokaryotic strain is a "poison" that actively interferes with the activity of the A^+/B^+ complex. In the accumulated defects model, genes A and B define two independent pathways that impinge on a single process. Reduced dosage of functional A and B in an A^+/A^-; B^+/B^- diploid or heterokaryon results in a decrease in the efficiency of both independent pathways and the generation of a mutant phenotype. Some notable examples of unlinked, noncomplementing mutants are *S. cerevisiae* mutants defective in α- and β-tubulin (22), *S. cerevisiae* mutants defective in actin and actin-binding proteins (23), and *Chlamydomonas* mutants that are defective in genes encoding subunits of axonemal outer arm dynein (24, 25).

The dosage, poison-complex, and accumulated defects models of unlinked noncomplementation suggest that the respective gene products either interact directly or define independent pathways that are both required for successful completion of a process. All 23 mutants defining the 23 complementation groups are noncomplementing with one or more of the 121 mutants that show unlinked noncomplementation. Based on the dosage and poison-complex models and the observation that *ro-1*, *ro-3*, and *ro-4* encode either dynein or dynactin subunits, the genes defined by the 23 complementation groups may encode proteins that are subunits of dynein/dynactin or

proteins that physically interact with the dynein/dynactin complexes. The allele specificity of noncomplementation seen for *ro-1* and *ro-3* mutants (described above) is also consistent with a physical-interaction model. However, nuclear movement and nuclear distribution in filamentous fungi are likely to require not only proper assembly and activity of cytoplasmic dynein and dynactin complexes, but also the coordinated activities of cytoplasmic dynein motors and components of the cytoskeleton (5). As in the accumulated defects model, noncomplementation may in some cases result from reduced activity of both motor and cytoskeletal "pathways." Finally, it is important to recognize that complementation analysis in *N. crassa* is conducted by the formation of heterokaryons and variation in nuclear ratios and uneven mixing of *ro* gene products may alter dosage and be contributing factors to unlinked noncomplementation. Therefore, while allele-specific, unlinked noncomplementation often is an indicator of protein-protein interaction, caution is required in the interpretation of noncomplementation among *N. crassa ro* mutants.

We thank Mario Jimenez and Catherine Metzroth for assistance in the purification of *ro* mutants and complementation analysis. This work was supported by Grant GM-51217 from the National Institutes of Health.

1. Walker, R. A. & Sheetz, M. P. (1993) *Annu. Rev. Biochem.* **62,** 429–451.
2. Vaisberg, E. A., Koonce, M. P. & McIntosh, J. R. (1993) *J. Cell Biol.* **123,** 849–858.
3. Saunders, W. S., Koshland, D., Eschel, D., Gibbons, I. R. & Hoyt, M. A. (1995) *J. Cell Biol.* **128,** 617–624.
4. Xiang, X., Beckwith, S. M. & Morris, N. R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2100–2104.
5. Plamann, M., Minke, P. F., Tinsley, J. H. & Bruno, K. S. (1994) *J. Cell Biol.* **127,** 139–149.
6. Li, Y.-Y., Yeh, E., Hays, T. & Bloom, K. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10096–10100.
7. Eshel, D., Urrestarazu, L. A., Vissers, S., Jauniaux, J., van Vliet-Reedijk, J. C., Planta, R. J. & Gibbons, I. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11172–11176.
8. Holzbaur, E. L. F. & Vallee, R. B. (1994) *Annu. Rev. Cell Biol.* **10,** 339–372.
9. Gill, S. R., Schroer, T. A., Szilak, I., Steuer, E. R., Sheetz, M. P. & Cleveland, D. W. (1991) *J. Cell Biol.* **115,** 1639–1650.
10. Schroer, T. A. & Sheetz, M. P. (1991) *J. Cell Biol.* **115,** 1309–1318.
11. Schafer, D. A., Gill, S. R., Cooper, J. A., Heuser, J. E. & Schroer, T. A. (1994) *J. Cell Biol.* **126,** 403–412.
12. Paschal, B. M., Holzbaur, E. L. F., Pfister, K. K., Clark, S., Meyer, D. I. & Vallee, R. B. (1993) *J. Biol. Chem.* **268,** 15318–15323.
13. Garen, A., Miller, B. R. & Paco-Larson, M. L. (1984) *Genetics* **107,** 645–655.
14. Harte, P. J. & Kankel, D. R. (1982) *Genetics* **101,** 477–501.
15. Holzbaur, E. L. F., Hammarback, J. A., Paschal, B. M., Kravit, N. G., Pfister, K. K. & Vallee, R. B. (1991) *Nature (London)* **351,** 579–583.
16. Clark, S. W. & Meyer, D. I. (1994) *J. Cell Biol.* **127,** 129–138.
17. Muhua, L., Karpova, T. S. & Cooper, J. A. (1994) *Cell* **78,** 669–679.
18. Garnjobst, L. & Tatum, E. L. (1967) *Genetics* **57,** 579–604.
19. Yarden, O., Plamann, M., Ebbole, D. J. & Yanofsky, C. (1992) *EMBO J.* **11,** 2159–2166.
20. Davis, R. H. & de Serres, F. J. (1970) *Methods Enzymol.* **27A,** 79–143.
21. Perkins, D. D., Radford, A., Newmyer, D. & Björkman, M. (1982) *Microbiol. Rev.* **46,** 426–570.
22. Stearns, T. & Botstein, D. (1988) *Genetics* **119,** 249–260.
23. Vinh, D. B. N., Welch, M. D., Corci, A. K., Wertman, K. F. & Drubin, D. G. (1993) *Genetics* **135,** 275–286.
24. Dutcher, S. K. & Lux, F. G. (1989) *Cell Motil. Cytoskel.* **14,** 104–117.
25. Kamiya, R. (1988) *J. Cell Biol.* **107,** 2253–2258.