Dynamics of cytoplasmic dynein in living cells and the effect of a mutation in the dynactin complex actin-related protein Arp1

Xin Xiang*, Gongshe Han*, Donald A. Winkelmann†, Wenqi Zuo‡ and N. Ronald Morris‡

Cytoplasmic dynein is a minus-end-directed microtubule motor that participates in multiple cellular activities such as organelle transport and mitotic spindle assembly [1]. To study the dynamic behavior of cytoplasmic dynein in the filamentous fungus *Aspergillus nidulans*, we replaced the gene for the cytoplasmic dynein heavy chain, *nudA,* with a gene encoding a green fluorescent protein (GFP)-tagged chimera, *GFP–nudA*. The GFP–NUDA fusion protein is fully functional *in vivo*: strains expressing only the GFP-tagged *nudA* grow as well as wild-type strains. Fluorescence microscopy showed GFP–NUDA to be in comet-like structures that moved in the hyphae toward the growing tip. Retrograde movement of some GFP–NUDA comets after they arrived at the tip was also observed. These dynamics of GFP–NUDA were not observed in cells treated with a microtubule-destabilizing drug, benomyl, suggesting they are microtubule-dependent. The rate of GFP–NUDA tip-ward movement is similar to the rate of cytoplasmic microtubule polymerization toward the hyphal tip, suggesting that GFP–NUDA is associated and moving with the polymerizing ends of microtubules. A mutation in actin-related protein Arp1 of the dynactin complex abolishes the presence of these dynamic GFP–NUDA structures near the hyphal tip, suggesting a targeting role of the dynactin complex.

Addresses: *Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, USA. †Department of Pathology and Laboratory Medicine, UMDNJ – Robert Wood Johnson Medical School, Piscataway, New Jersey 08854, USA. ‡Department of Pharmacology, UMDNJ – Robert Wood Johnson Medical School, Piscataway, New Jersey 08854, USA.

Correspondence: Xin Xiang
E-mail: xxiang@usuhs.mil

Received: **26 August 1999**
Revised: **17 January 2000**
Accepted: **29 March 2000**

Published: **5 May 2000**

Current Biology 2000, 10:603–606

0960-9822/00/$ – see front matter

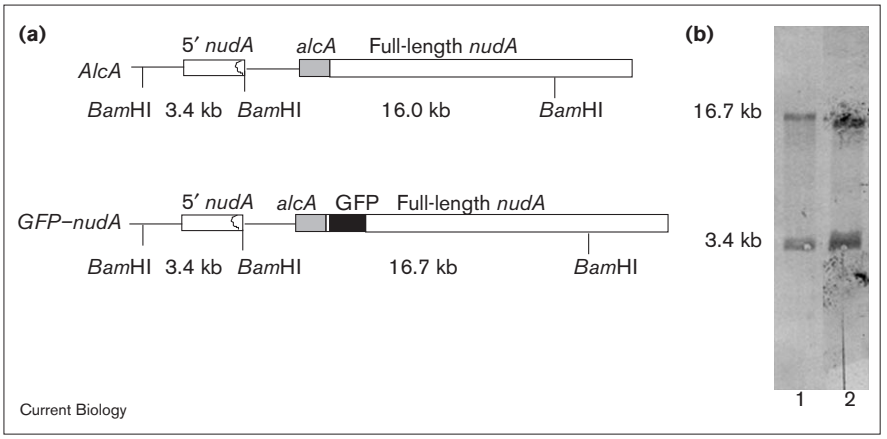
Results and discussion

GFP–NUDA moves to the hyphal tip

Cytoplasmic dynein is a multi-subunit microtubule motor protein whose functions require another multi-subunit complex, dynactin, which may serve as an organelle receptor for dynein [1] and may also be involved in regulating the dynein motor processivity [2]. To study the behavior of cytoplasmic dynein in living cells, we made a functional GFP–NUDA fusion in *A. nidulans* as illustrated in Figure 1 (see Materials and methods for details). Fluorescence time-lapse cinematography showed GFP–NUDA to be concentrated in moving comet-like structures that streamed rapidly toward the hyphal tip in the distal region of germlings (Figure 2 and see Supplementary material). After these comets arrived at the hyphal tip, some of them clearly underwent retrograde movement (see Supplementary material).

The tip-ward movement of GFP–NUDA exhibits a remarkable resemblance to the *in vivo* behavior of the mammalian CLIP-170 protein, which preferentially binds to the growing ends of microtubules and treadmills on the ends of microtubules in living cells [3,4]. CLIP-170 is implicated in dynein function, as shown by genetic data on a *Saccharomyces cerevisiae* CLIP-170 homolog, Bik1p [5]. Recently, dynactin has been shown to co-localize with CLIP-170 at the ends of microtubules in fixed mammalian cells [6,7]. Co-localization of cytoplasmic dynein with dynactin and CLIP-170 at the ends of microtubules has also been detected in fixed cells, but only when the cells are shifted from 37°C to room temperature, a condition that slows down vesicle transport [6]. Whether cytoplasmic dynein in mammalian cells also exhibits CLIP-170-like motile behavior is not clear. We observed tip-ward GFP–NUDA movement in *A. nidulans* at a variety of temperatures, such as room temperature, 25°C, 32°C, 37°C and 42°C. Based on the motile behavior of GFP–NUDA, it is likely that cytoplasmic dynein in *A. nidulans* might also be associated with growing ends of microtubules. To test this possibility, we first asked whether comet movement required microtubules. Treatment with 2.4 μg/ml of the microtubule destabilizing drug benomyl for 10 minutes at 32°C eliminated GFP–NUDA movement in living cells (see Figure 3 and Supplementary material). We also compared the rate of GFP–NUDA tip-ward movement and that of microtubule polymerization in *A. nidulans* using a strain in which a wild-type α tubulin gene *tubA* was replaced by a *GFP-tubA* fusion gene (X. Xiang, B. Liu and N. R. Morris, unpublished observations). At room temperature, microtubules elongated toward the hyphal tip at an average rate of 0.185 μm/second ($n = 9$, standard deviation 0.031 μm/second), whereas the GFP–NUDA comets move to the hyphal tip at 0.187 μm/second ($n = 8$, standard

Figure 1



Construction of the *GFP–nudA* strain. **(a)** The *nudA* locus in the *GFP–nudA* (*alcA(p)::GFP–nudA*) strain and in the *AlcA* (*alcA(p)::nudA*) control strain [19]. 5′ indicates the 5′ coding region. **(b)** Southern blot demonstrating that the gene replacement event in the *GFP–nudA* strain occurred as predicted. Genomic DNA from the control strain *AlcA* [19] and the *GFP–nudA* strain were extracted and digested with *Bam*HI. The 5′ end of the *nudA* gene was used as a probe as described [19]. As predicted, the *GFP–nudA* strain showed a 3.4 kb and a 16.7 kb signal (lane 1) whereas the *AlcA* strain showed a 3.4 kb and a 16.0 kb signal (lane 2) [19].

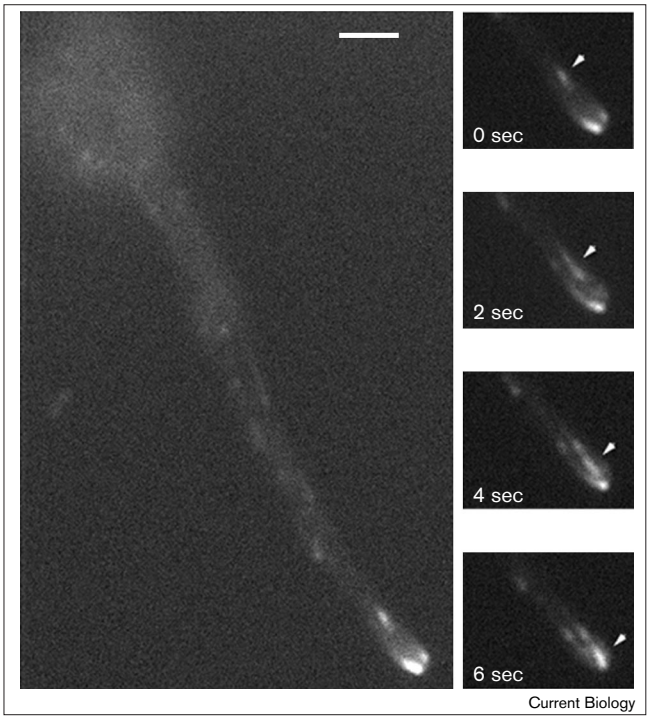
deviation 0.016 μm/second). Therefore, the rate of GFP–NUDA movement was essentially identical to that of microtubule polymerization. These data together with the fact that the GFP–NUDA movement requires intact microtubules, strongly suggest that the GFP–NUDA comets, like CLIP-170, move by virtue of their association with the plus ends of microtubules.

Movement of GFP–NUDA comets requires Arp1

Arp1 is a 45 kDa actin-related protein that is the major component of the multisubunit dynein accessory complex, dynactin [1]. To investigate whether the GFP–NUDA localization in *A. nidulans* requires a functional dynactin complex, we introduced the *GFP–nudA* fusion into the *nudK317* mutant, which has a temperature-sensitive mutation in the gene encoding Arp1 [8]. In the *nudK317* mutant at the restrictive temperature, the GFP–NUDA was seen as dispersed dots that did not exhibit the directed movement towards the hyphal tip observed in wild-type cells (Figure 4 and see Supplementary material). This alteration in NUDA localization is not due to a decrease in the NUDA protein level in the *nudK317* mutant, as evidenced by Western blot analysis (data not shown). It is also not due to any gross disruption of the microtubule network in the mutant as evidenced by staining with an anti-tubulin antibody (data not shown). Together, these results suggest that the Arp1 protein in *A. nidulans* is required for the targeting of the GFP–NUDA comet-like structures to the microtubule plus ends. However, loss of dynein targeting is not necessarily an Arp1 specific effect. Rather, it may be caused by the change in the integrity of the dynactin complex in the cell, as in *Neurospora crassa*, an Arp1 mutation makes the p150 dynactin subunit unstable [9]. In mammalian cells, overexpression of dynamitin, the 50 kDa subunit of dynactin, dissociates the Arp1 protein from the p150 subunit of the dynactin complex at the distal ends of microtubules without affecting the association of cytoplasmic dynein with the microtubule ends [6]. Thus, it is possible that the effect of the *nudK317* mutation could be mediated via the p150 dynactin subunit.

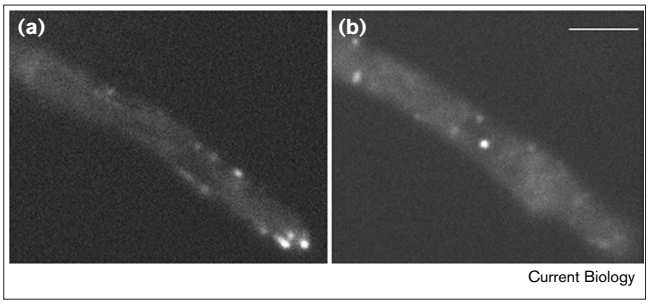
Experiments from the mammalian system suggested that the CLIP-170 protein recruits the dynactin complex to

Figure 2



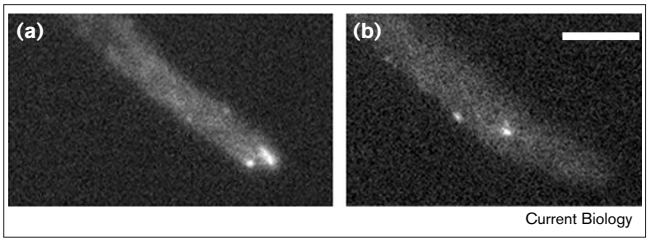
Images of GFP–NUDA in a living cell. Cells were incubated at 25°C for 18 h, and images were taken at room temperature. A *GFP–nudA* hypha is shown on the left, images of the distal region of the same hypha at different time points are shown on the right. Arrows indicate GFP–NUDA moving towards the tip of the hypha. The scale bar represents approximately 5 μm. A Quicktime movie of this sequence is in the Supplementary material.

Figure 3



GFP–NUDA in benomyl-treated cells. Cells were incubated at 32°C for 18 h and treated with 2.4 μg/ml benomyl at 32°C for 10 min before being observed on a heated stage at 32°C. **(a)** A control *GFP–nudA* cell without benomyl treatment; **(b)** a *GFP–nudA* cell with benomyl treatment. The scale bar represents approximately 5 μm. Quicktime movies of time-lapse sequences taken with these cells are in the Supplementary material.

Figure 4



GFP–NUDA in an Arp1 mutant *nudK317*. Cells were incubated at 42°C for 14 h and moved to a heated stage at 42°C before the images were taken. **(a)** A wild-type *GFP–nudA* cell. **(b)** A *GFP–nudA* cell with the *nudK317* background. The scale bar represents approximately 5 μm. Quicktime movies of time-lapse sequences taken with these cells are in the Supplementary material.

distal ends of microtubules near the cell periphery [7]. Our data suggested that the dynactin complex is also involved in recruiting cytoplasmic dynein to this site. The function of these localized proteins is most likely involved in centripetal transporting of endosomes and other membraneous vesicles that are initially located near the cell periphery [6,7]. However, exactly how interactions among these proteins and microtubules are regulated to achieve directional vesicle motility inside the cell requires future investigation. In another filamentous fungus, *N. crassa*, it has been shown that cytoplasmic dynein is required for retrograde vesicle transport from the hyphal tip to the cell body [10]. Possibly, the movement of GFP–NUDA towards the hyphal tip facilitates localized interactions between the motor and the vesicles need to be carried back to the cell body. It is interesting to note that some of the tip comets undergo retrograde movement. Whether such retrograde movement represents dynein-mediated minus-end motility on microtubules or an association with the ends of shortening microtubules remains to be determined.

In filamentous fungi, genes encoding proteins in cytoplasmic dynein were originally identified as genes required for nuclear distribution along the hyphae [11,12]. Nuclear migration may be mediated by interactions between the hyphal cortex and astral microtubules from the nuclear spindle pole body. In the budding yeast *S. cerevisiae*, cytoplasmic dynein involved in spindle orientation is found along the entire length of astral microtubules [13], and a dynein heavy chain mutant shows altered microtubule–cortex interactions and microtubule dynamics [14]. It is possible that the function of the proteins at the microtubule ends in *A. nidulans* is to interact with the cortex and/or affect microtubule dynamics. In mammalian cells, in addition to the CLIP-170–dynactin–dynein localization to the microtubule ends, the adenomatous polyposis coli tumor suppressor protein (APC) and its binding partner EB1 also locate to distal ends of microtubules [15,16]. EB1 has been shown to interact with components of the dynein and dynactin complexes [17], and a yeast homolog of EB1, Bim1p, also located at the distal ends of microtubules, promotes microtubule dynamics in G1 phase [18]. It will be interesting to see whether cytoplasmic dynein in *A. nidulans* is also involved in regulating microtubule dynamics for efficient retrograde transport and/or for other cellular processes such as nuclear migration.

Materials and methods

Construction of a strain with GFP-tagged cytoplasmic dynein heavy chain

A plasmid (pX11) was made in which the *Kpn*I site in pBluescript SK was removed by T4 DNA polymerase. The plasmid was then digested with *Sma*I and *Bam*HI and ligated with the 1.5 kb *Sma*I–*Bam*HI amino-terminal *nudA* fragment isolated from the pXX3 plasmid [19]. The resulting plasmid was named pXX13. For the construction of the *GFP–nudA* construct, the GFP2–5 gene [20] was amplified with two primers: GFPK5′ (5′-GGGGGTACCCATGAGTAAAGGAGAAGA-3′) and GFPK3′ (5′-CCCGGTACCTTGTATAGTTCATCCATG-3′), and the PCR product was digested with *Kpn*I and ligated into the KpnI site of pXX13. The resulting plasmid in which the *GFP* gene was inserted in the correct orientation between the eighth and ninth codons of the NUDA gene was named pXX14. pXX14 was digested with *Sma*I and *Bam*HI, and the 2.3 kb fragment containing *GFP* in the *Kpn*I site of the 5′ coding region of *nudA* was isolated and ligated into the *Sma*I–*Bam*HI sites of pAL3, in which genes can be inserted downstream of an *alcA* promoter [21]. This final plasmid was named pXX15 and transformed into the *A. nidulans* strain GR5. Homologous integration of the *nudA* sequence on the pXX15 plasmid into the genomic *nudA* sequence generated two copies of *nudA*, a truncated *nudA* gene with its own promoter and a full-length *nudA* gene with *GFP* under the control of the *alcA* promoter (Figure 1). This gene replacement strategy is essentially the same as that described for the construction of the *alcA(p)::nudA* (*AlcA*) strain [19], except that a 0.7 kb *GFP* tag was introduced at the amino terminus of *nudA* (Figure 1a). To verify the specific gene replacement event, Southern blot analysis was performed on *Bam*HI-digested genomic DNA of a putative *GFP–nudA* strain and on the *AlcA* strain as a control (Figure 1b). As predicted, the *AlcA* strain showed a 3.4 kb and a 16.0 kb signal [19], whereas the

GFP–nudA strain showed a 3.4 kb and a 16.7 kb (16.0 kb + 0.7 kb) signal. When grown on glycerol medium to induce the *alcA* promoter, the *GFP–nudA* strain grew and conidiated as well as a wild-type strain *R153* (*wA3; pyro4*) that had the same nutrient requirements (data not shown), indicating that the GFP–NUDA fusion protein was a functional substitute for NUDA *in vivo*.

Construction of GFP–nudA fusion in the nudK317 background

The *GFP-nudA* allele is genetically marked by the *Neurospora pyr4* gene that was integrated into the genome along with *GFP-nudA* in the same plasmid. This marker allows the cells to grow without pyrimidine. Therefore, a *nudK317* mutant carrying the *pyrG89* mutation was crossed to the *GFP-nudA* strain. Progeny carrying the *GFP-nudA* fusion gene and the *nudK317* mutation was identified by its ability to grow without pyrimidine supplementation and by their temperature sensitive nud phenotype at 42°C. The strain used in this study is XX94 (*GFP-nudA*, *nudK317*, *pyroA4*).

Acquisition of live cell images

MM + glycerol medium was used with pyridoxine as a supplement [19]. The red-shifted GFP fluorescence of the S65T variant was recorded with an Olympus IX70 inverted fluorescence microscope using a HiQ fluorescein filter set and 100 W Hg lamp. Cells were grown in liquid medium in a petri dish with a hole in its bottom covered with a coverglass sealed to the petri dish using an equal mixture of paraffin, lanolin and vaseline. A Bioptechs heated stage (the Delta TC3 culture dish system) was used to acquire images above ambient temperature. Images were collected and analyzed using a Princeton Instruments 5 MHz MicroMax cooled CCD camera, shutter and controller unit, and IPLab software (Scanalytics). Time-lapse sequences were converted to Quicktime movies using the IPLab software. The Quicktime movies have been speeded up tenfold.

Supplementary material

Supplementary material including Quicktime movies of time-lapse sequences is available at http://current-biology.com/supmat/supmatin.htm.

Acknowledgements

We thank John Doonan for sending us the plant-adapted GFP gene before publication and Bo Liu for sharing with us his modified GFP plasmid before publication. This work was supported by a Grant from the American Heart Association and a USUHS start-up grant (to X.X.) and NIH grants GM52309 (to N.R.M.) and AR38454 (to D.A.W.).

References

1. Karki S, Holzbaur EL: **Cytoplasmic dynein and dynactin in cell division and intracellular transport.** *Curr Opin Cell Biol* 1999, **11**:45-53.
2. King SJ, Schroer TA: **Dynactin increases the processivity of the cytoplasmic dynein motor.** *Nat Cell Biol* 2000, **2:**20-24.
3. Diamantopoulos GS, Perez F, Goodson HV, Batelier G, Melki R, Kreis TE, Rickard JE: **Dynamic localization of CLIP-170 to microtubule plus ends is coupled to microtubule assembly.** *J Cell Biol* 1999, **144:**99-112.
4. Perez F, Diamantopoulos GS, Stalder R, Kreis TE: **CLIP-170 highlights growing microtubule ends *in vivo*.** *Cell* 1999, **96:**517-527.
5. Geiser JR, Schott EJ, Kingsbury TJ, Cole NB, Totis LJ, Bhattacharyya G, *et al.*: ***Saccharomyces cerevisiae* genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways.** *Mol Biol Cell* 1997, **8:**1035-1050.
6. Vaughan KT, Tynan SH, Faulkner NE, Echeverri CJ, Vallee RB: **Colocalization of cytoplasmic dynein with dynactin and CLIP-170 at microtubule distal ends.** *J Cell Sci* 1999, **112:**1437-1447.
7. Valetti C, Wetzel DM, Schrader M, Hasbani MJ, Gill SR, Kreis TE, Schroer TA: **Role of dynactin in endocytic traffic: effects of dynamitin overexpression and colocalization with CLIP-170.** *Mol Biol Cell* 1999, **10:**4107-4120.
8. Xiang X, Zuo W, Efimov VP, Morris NR: **Isolation of a new set of *Aspergillus nidulans* mutants defective in nuclear migration.** *Curr Genet* 1999, **35:**626-630.
9. Minke PF, Lee IH, Tinsley JH, Bruno KS, Plamann M: ***Neurospora crassa ro-10* and *ro-11* genes encode novel proteins required for nuclear distribution.** *Mol Microbiol* 1999, **32:**1065-1076.
10. Seiler S, Plamann M, Schliwa M: **Kinesin and dynein mutants provide novel insights into the roles of vesicle traffic during cell morphogenesis in *Neurospora*.** *Curr Biol* 1999, **9:**779-785.
11. Plamann M, Minke PF, Tinsly JH, Bruno K: **Cytoplasmic dynein and centractin are required for normal nuclear distribution in filamentous fungi.** *J Cell Biol* 1994, **127:**139-149.
12. Xiang X, Beckwith SM, Morris NR: **Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*.** *Proc Natl Acad Sci USA* 1994, **91:**2100-2104.
13. Shaw SL, Yeh E, Maddox P, Salmon ED, Bloom K: **Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud.** *J Cell Biol* 1997, **139:**985-994.
14. Carminati JL, Stearns T: **Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex.** 1997, *J Cell Biol* **138:**629-641.
15. Nathke IS, Adams CL, Polakis P, Sellin JH, Nelson WJ: **The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration.** *J Cell Biol* 1996, **134:**165-179.
16. Morrison EE, Wardleworth BN, Askham JM, Markham AF, Meredith DM: **EB1, a protein which interacts with the APC tumour suppressor, is associated with the microtubule cytoskeleton throughout the cell cycle.** *Oncogene* 1998, **17:**3471-3477.
17. Berrueta L, Tirnauer JS, Schuyler SC, Pellman D, Bierer BE: **The APC-associated protein EB1 associates with components of the dynactin complex and cytoplasmic dynein intermediate chain.** *Curr Biol* 1999, **9:**425-428.
18. Tirnauer JS, O'Toole E, Berrueta L, Bierer BE, Pellman D: **Yeast Bim1p promotes the G1specific dynamics of microtubules.** *J Cell Biol* 1999, **145:**993-1007.
19. Xiang X, Roghi C, Morris NR: **Characterization and localization of the cytoplasmic dynein heavy chain in *Aspergillus nidulans*.** *Proc Natl Acad Sci USA* 1995, **92:**9890-9894.
20. Fernandez-Abalos JM, Fox H, Pitt C, Wells B, Doonan JH: **Plant-adapted green fluorescent protein is a versatile vital reporter for gene expression, protein localization and mitosis in the filamentous fungus, *Aspergillus nidulans*.** *Mol Microbiol* 1998, **27:**121-130.
21. Waring RB, May GS, Morris NR: **Characterization of an inducible expression system in *Aspergillus nidulans* using *AlcA* and tubulin-coding genes.** *Gene* 1989, **79:**119-130.