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Dynein motor regulation stabilizes interphase microtubule arrays and determines centrosome position

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Cytoplasmic dynein is a microtubule-based motor protein responsible for vesicle movement and spindle orientation in eukaryotic cells. We show here that dynein also supports microtubule architecture and determines centrosome position in interphase cells. Overexpression of the motor domain in *Dictyostelium* leads to a collapse of the interphase microtubule array, forming loose bundles that often enwrap the nucleus. Using green fluorescent protein (GFP)–α-tubulin to visualize microtubules in live cells, we show that the collapsed arrays remain associated with centrosomes and are highly motile, often circulating along the inner surface of the cell cortex. This is strikingly different from wild-type cells where centrosome movement is constrained by a balance of tension on the microtubule array. Centrosome motility involves force-generating microtubule interactions at the cortex, with the rate and direction consistent with a dynein-mediated mechanism. Mapping the overexpression effect to a C-terminal region of the heavy chain highlights a functional domain within the massive sequence important for regulating motor activity.

Keywords: centrosome/*Dictyostelium discoideum*/dynein/GFP–tubulin/microtubule

Introduction

Microtubule arrays of eukaryotic cells are important for stabilizing cell movement and establishing cell polarity. Most of their activities are mediated through directed trafficking of membrane-bound organelles or assembly into higher ordered structures with specialized function, such as axonemes or spindles in cell division (Kirschner and Mitchison, 1986; Hyman and Karsenti, 1996). The versatility in microtubule patterns is based on the dynamic properties of the tubulin subunits and the nucleation properties of their organizing centers (Kellogg *et al.*, 1994; Desai and Mitchison, 1997). However, the overall forms achieved by microtubule arrays are largely dictated by the selective interaction of a number of accessory and motor proteins that stabilize, cross-link, destabilize or anchor the tubulin polymer to other cellular structures (Mandelkow and Mandelkow, 1995; Wilson and Borisy, 1997; Perez *et al.*, 1999). Motor molecules of the kinesin and dynein superfamilies act not only in moving organelles (Vallee and Sheetz, 1996; Hirokawa, 1998), but also in anchoring distal microtubule ends to the cell cortex (Koonce, 1996; Heil-Chapdelaine *et al.*, 1999). In yeast and other fungi, dynein provides a cortical anchor for microtubules, serving to position spindles and nuclei and to regulate microtubule length (Eshel *et al.*, 1993; Li *et al.*, 1993; Plamann *et al.*, 1994; Xiang *et al.*, 1995; Carminati and Stearns, 1997; Inoue *et al.*, 1998; Yamamoto *et al.*, 1999). Higher ordered astral and spindle-like arrays can be reconstituted *in vitro*, but only if motor protein activity is present (Verde *et al.*, 1991; Walczak *et al.*, 1998). Similar activities are necessary *in vivo* to reorganize interphase microtubule arrays in cell fragments lacking organizing centers (Rodionov and Borisy, 1997), and for spindle assembly and function during cell division (Saunders and Hoyt, 1992; Gaglio *et al.*, 1996; Merdes *et al.*, 1996; Walczak and Mitchison, 1996).

We have shown previously that when the motor domain of a cytoplasmic dynein heavy chain (DHC) is overexpressed in *Dictyostelium* cells, the radial interphase microtubule array collapses around the nucleus (Koonce and Samsó, 1996). Tubulin staining patterns of fixed cells suggested that a cortical anchorage was perturbed and that the microtubule array was detached and 'free floating' in the cytoplasm. More recently, a green fluorescent protein (GFP)-tagged α-tubulin was expressed in *Dictyostelium* to visualize microtubule–cortex interactions during cytokinesis (Neujahr *et al.*, 1998). This revealed that in post-mitotic cells, the centrosome and microtubule array underwent periods of rapid, saltatory motility.

By combining overexpression of the dynein motor domain with GFP-labeled tubulin, we show here that the collapsed microtubule arrays are highly motile. Our data indicate that dynein serves as a cortical anchor for cytoplasmic microtubules in *Dictyostelium* and that it functions in a force-generating capacity to maintain the interphase radial pattern of microtubules. This activity helps to stabilize the centrosome position in *Dictyostelium* cells. We have mapped a site involved in this activity to a C-terminal fragment of the DHC.

Results

Biochemical characterization

Fixed *Dictyostelium* cells were immunolabeled for tubulin to correlate both a collapse of the radial interphase microtubule array and a displacement of the centrosome towards the cell periphery with the degree of overexpression of the cytoplasmic dynein motor domain (Figure 1). The phenotype is mild in clones expressing modest levels of the 380 kDa polypeptide (~1–5 times the native DHC), producing longer, more wavy microtubules and a noticeable reduction in the radial character of the array.

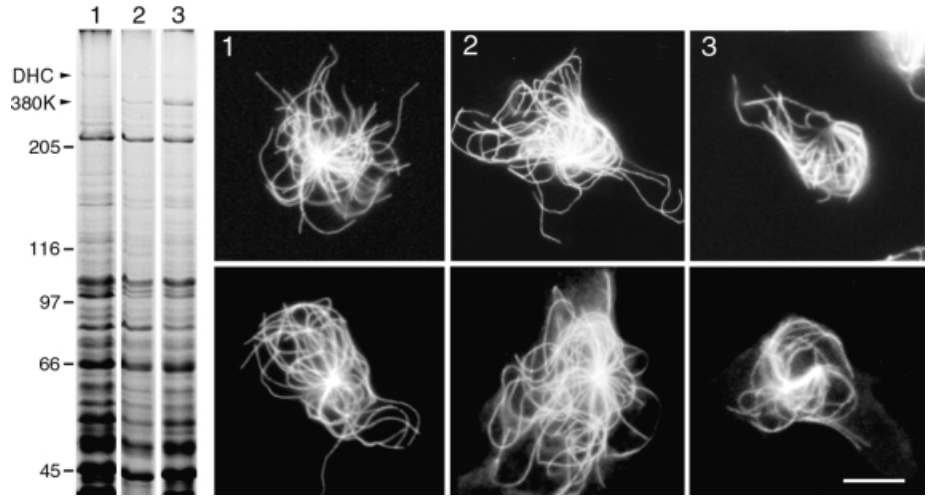

Fig. 1. Degree of motor domain overexpression correlates with phenotype severity. (Left panel) Coomassie Blue-stained gel showing high-speed supernatants from (1) wild-type cells, (2) a clone expressing a moderate amount of 380 kDa polypeptide and (3) a clone expressing a large amount of 380 kDa polypeptide. The positions of the native DHC and 380 kDa polypeptides are indicated by arrowheads. (Right panels) Representative cells from 1, 2 and 3 (left), fixed and stained with anti-tubulin antibody. In wild-type cells (1), the MTOC remains centrally located and is evenly surrounded by an array of microtubules. Panels 2 and 3 show moderate and extreme examples of the collapsed microtubule phenotype. Note that the microtubules in (2) are more curved than those in the wild-type examples. Bar, 5 μm.

For clones expressing larger amounts (~5–10 or more times the native DHC), most of the cells display dramatically altered microtubule arrays. Microtubule length is also perturbed. The average length of the longest microtubules that we could trace from their ends to the centrosome in wild-type AX2 cells is 14.6 ± 3.4 μm (n = 44, from seven cells). In cells overexpressing the 380 kDa fragment, the average is 21.6 ± 5.2 μm (n = 57, from eight cells), an ~50% increase.

GFP-labeled microtubule behavior in live 380K and wild-type AX2 cells

To monitor centrosome position and microtubule behavior in live cells, GFP–α-tubulin-tagged wild-type and mutant cells overexpressing the 380 kDa motor domain (380K) have been subjected to confocal scanning microscopy. The 380K cells differ dramatically from wild-type cells in the dislocation of the centrosome from its normal position in the central region of the cells. In wild-type cells, displacement of the centrosome by 1 or 2 μm in one direction is counteracted by displacement in another direction (Figure 2A and B). In contrast, the centrosome is circulating continuously in 380K cells as shown in Figure 2C and D, and only few corrections in directionality along its path are observed. This behavior correlates well with the microtubule organization seen in fixed cells strongly overexpressing the dynein motor domain (Figure 1). Wild-type microtubules typically emanate from the centrosome in a radial direction. The microtubules are slightly longer than the radius of a cell and therefore bent in their distal region (Figure 2B). The longer microtubules of the mutant cells are curled and often bundled close to the centrosome into a trailing, comet-like tail (Figure 2D).

The change in centrosome motility and microtubule organization caused by overexpression of the dominant-negative dynein fragment raises three questions: (i) are the forces responsible for this motility applied to the centrosome or to the nucleus linked to the centrosome; (ii) is the centrosome motility in 380K cells microtubule-dependent; and (iii) is there evidence for dynein molecules bound to the cell cortex to readjust centrosome position in wild-type cells?

A previous study of wild-type cells has shown that forces are applied directly on the centrosome rather than on the nucleus (Neujahr *et al*., 1998). Figure 3 provides two examples showing that this is also true for 380K cells. In *Dictyostelium*, lentiform nucleoli adhere to the inner side of the nuclear membrane, so that forces applied to the nucleus result in changes in their shape and position. In Figure 3A, the nucleus rotates to follow the movement of the centrosome. The nucleolus in front of the centrosome is compressed while the nucleolus behind is stretched, indicating that the nucleoli are elastically deformed by forces applied at the centrosome. In the example shown in Figure 3B, the nucleus is stretched and bent in the direction of movement, confirming that the centrosome remains as firmly attached to the nucleus in the mutant cells as it does in the wild-type cells.

As to the second question, we have compared the motility of centrosomes in 380K cells in the presence or absence of the microtubule polymerization blocker, nocodazole. The left panel of Figure 4 shows the variation in centrosome tracks of untreated 380K cells expressing variable amounts of the dynein motor domain. In the right panel, tracks of nocodazole-treated cells are shown, indicating that centrosome motility is suppressed when microtubules are shortened.

To address the third and most important question, we have searched for a minus-end-directed motor activity that corrects the centrosome position stepwise by one or a few micrometers, as shown in Figure 2A. For the control of centrosome position, it is crucial that the relevant motor activity is short lived. Engagement–disengagement cycles that meet this criterion are illustrated in Figure 5A and B. In both, smoothly bent microtubules (or small bundles) glide with their plus ends ahead along the cell cortex. At a fixed point on the cortex, the microtubules become anchored, their connection to the centrosome straightens

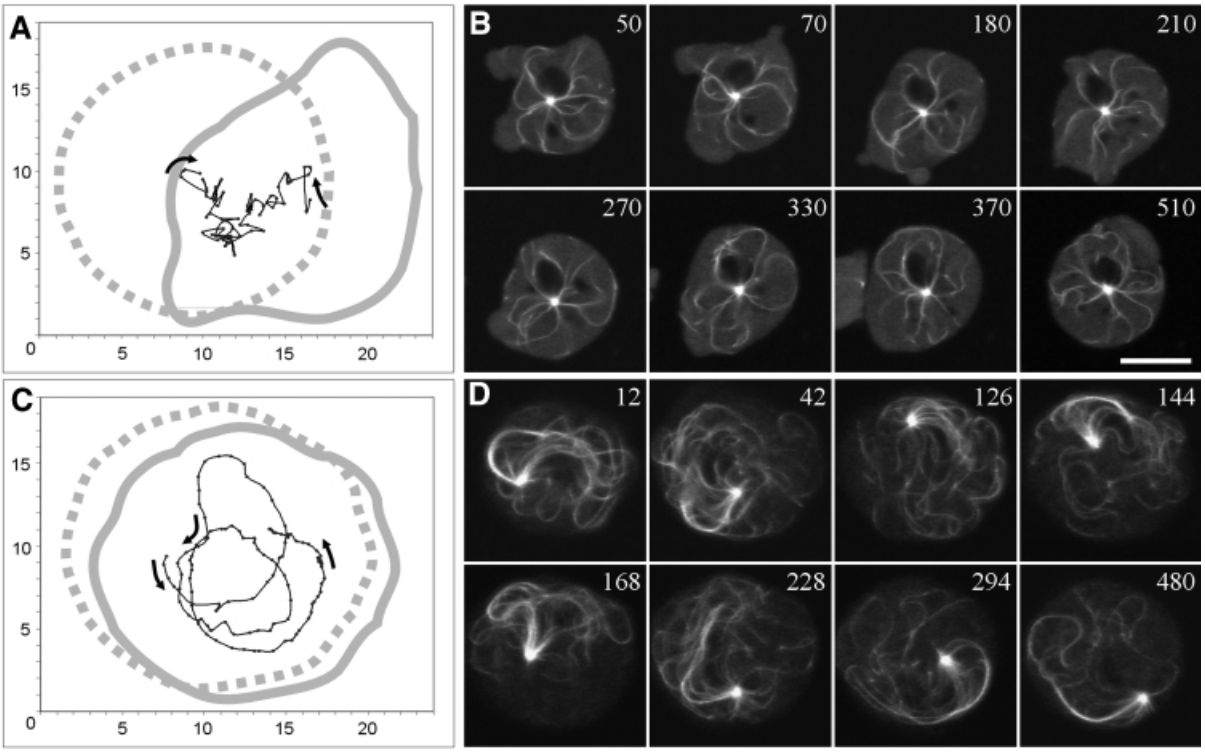

Fig. 2. Microtubule organization and centrosome movement in live cells labeled with GFP–α-tubulin. (A and B) Typical wild-type cell viewed over an 8.5 min period. The thick gray lines in (**A**) represent images of the cell borders at the beginning (solid line) and the end of the sequence (dotted line). The thin line records the centrosome position at 6 s intervals throughout the sequence. (**B**) Eight images of the cell during the observation period, showing that the centrosome remains near the cell center, surrounded by a radial pattern of microtubules. The dark area on top of the centrosome is the nucleus. (**C** and **D**) A representative 380K cell imaged under the same conditions as in (A) and (B), showing the excessive centrosome movement and long, bent microtubules that trail to form a comet-like array. Numbers are in micrometers for (A) and (C) and in seconds for (B) and (D). Bar, 10 μm.

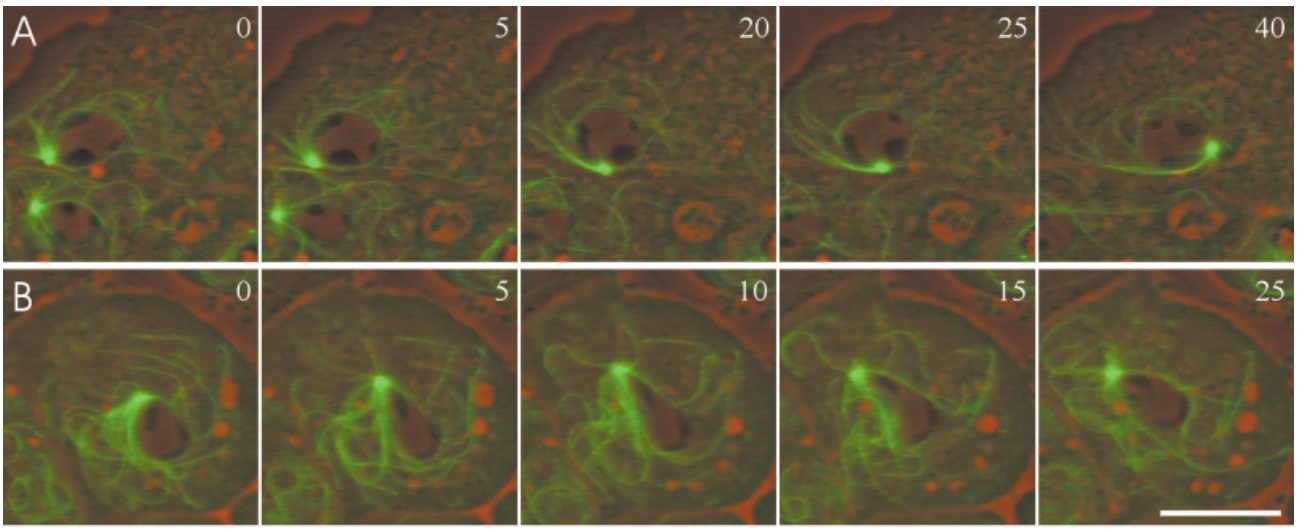

Fig. 3. Nuclear rotation and distortion in 380K cells. (**A**) Sequence showing a centrosome moving to the right, resulting in a counterclockwise rotation of the nucleus. By virtue of the distortions in the nucleoli (dark spots attached to the inside of the nuclear membrane), it is apparent that forces responsible for nuclear rotation are acting through the centrosome/microtubule array and not directly on the nucleus itself. Note that on the bottom of this image a second centrosome with an associated nucleus moves in the opposite direction. (**B**) A nucleus that is elongated, distorted into a bend, and finally relaxed in accord with the traction applied on the centrosome. In (A) and (B), the fluorescence of GFP–α-tubulin is shown in green and superimposed on phase contrast images in dark red. Numbers are seconds. Bar, 10 μm.

and the spanning microtubules are engaged in pulling the centrosome toward the site of their cortical connection. Engagement stops by detachment of the microtubules from these sites, so that the microtubules re-assume a smoothly bent shape.

Analysis of microtubule bending in 380K cells suggests that after suppression of dynein activity, plus-end-directed motor activity becomes more obvious. In Figure 5C and D, two sections of the run shown in Figure 2C and D have been selected. These sections represent periods of

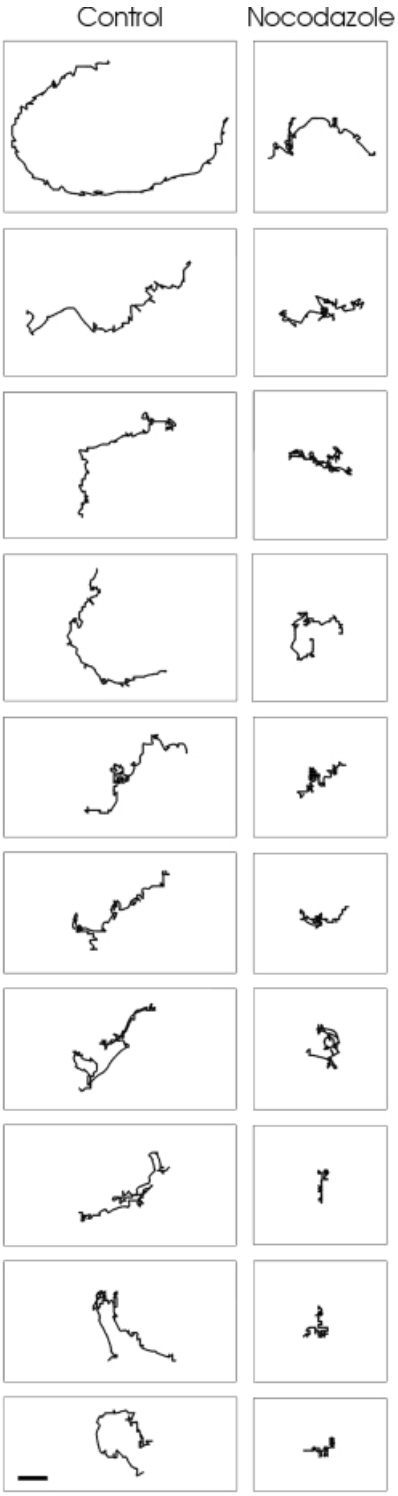

Fig. 4. Gallery of centrosome tracks in 380K cells in the absence and presence of nocodazole. In 10 μM nocodazole, centrosome movements are shown to be less persistent than in control cells. At this concentration of nocodazole, typically a few (<5), short (<1–2 μm) microtubules remain at the centrosome, and only some of them reach the cell cortex. The position of each centrosome has been determined at 0.5 s intervals for a period of 1 min. Ten control and 10 nocodazole-treated 380K cells that remained stationary during the experiment were chosen randomly, and the tracks ordered according to the maximal distance the centrosomes had traveled. Variability in the untreated cells is consistent with variations in expression level of the 380 kDa polypeptide. Bar, 10 μm.

slow net movement of the centrosome. Both sequences show microtubules turning under these circumstances into large loops as they would if either cortical or organelle-based motors act on them in a plus-end direction. These motors may contribute to the movement of the centrosome, assisting in pushing it ahead through the comet tail of bundled microtubules.

Our data show that overexpression of the dynein motor domain impairs the control of centrosome position. Because there may be fewer active dyneins on the cortex, the chance of microtubule engagement on the opposite side is reduced and thus the centrosome is free to move all the way to the cell cortex. However, we do not suggest that dynein activity is suppressed completely in 380K cells. Rather, guiding microtubules pointing in the direction of centrosome movement are observed occasionally in 380K cells, and these microtubules might be involved in centrosome dislocation by pulling the centrosome towards dynein anchored in the cell cortex.

A C-terminal fragment generates the mutant phenotype

A series of DHC polypeptide fragments was expressed to map further the domain responsible for the dominant-negative effect of the dynein motor domain (Figure 6A). A 70 kDa polypeptide (N3692–C4305) adjacent to the C-terminal end of the DHC is the smallest fragment characterized so far that alters the microtubule array. Several other, similarly expressed DHC fragments do not result in microtubule pattern changes, suggesting that this phenotype is not a general artifact of polypeptide overexpression. The 70 kDa fragment does not co-sediment with microtubules nor does it co-immunoprecipitate with the native dynein molecule (Figure 6B). Thus its effect is not mediated through competition for microtubule binding or by direct physical interaction with the native motor. The fragment may act to sequester a component important for regulating the activity or position of the native dynein motor.

Discussion

Using GFP to image microtubules in *Dictyostelium*, we can follow centrosome and microtubule dynamics in interphase cells and monitor the dramatic changes that result from overexpression of the motor domain of cytoplasmic dynein. Our results address a centering mechanism used in interphase cells to control the position of the centrosome and, indirectly, that of the nucleus. The questions are: what is the role of dynein in interphase cells; where is the force-generating motor located; and how is its activity controlled?

In wild-type cells, short-lived pulling forces dominate the movement of centrosomes, which are consistent with a minus-end-directed activity of a motor that is anchored to the cell cortex (Figure 5A and B). The rate of centrosome movement (0.4–2.5 μm/s) is consistent with, but does not prove, a cytoplasmic dynein-mediated mechanism. To single out the contribution of dynein from the actions of other motor proteins, we have overexpressed the 380 kDa dynein motor domain as a dominant-negative effector. In the transfected cells, phenotype severity correlates with the level of motor domain expression. In cells strongly

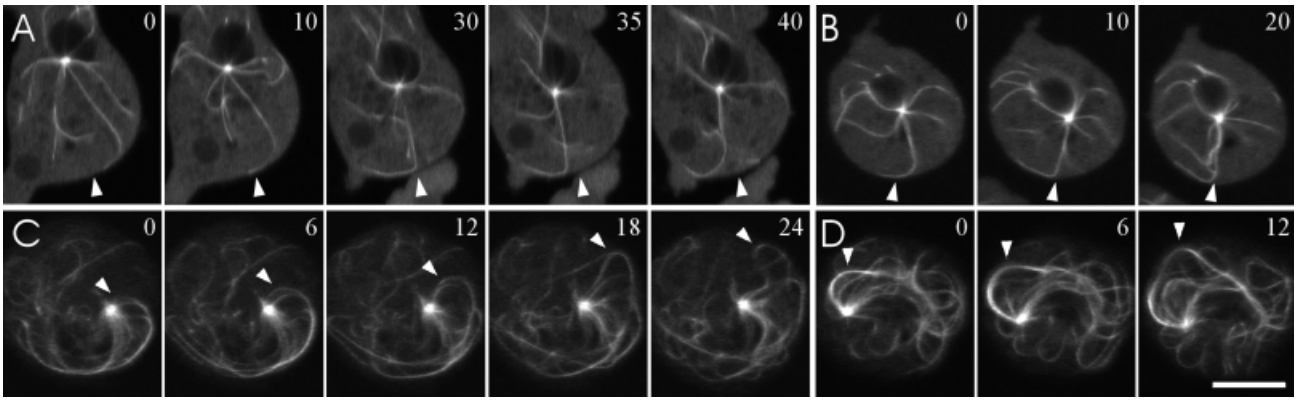

Fig. 5. Microtubule engagement in centrosome motility of wild-type and 380K cells. (**A**) Traction–disengagement cycle in a wild-type cell expressing GFP–α-tubulin. Here, a microtubule extends from the centrosome, bends left at ~90° and moves along the cortex. At 10 s, it reaches a point marked by the arrowhead, where it appears to engage a motor activity. By 30–35 s, the microtubule has been swept along the cortex, producing sufficient force to straighten and pull on its connection to the centrosome. Both the centrosome and attached nucleus move toward the arrowhead. At 40 s, the microtubule has moved past the cortical connection and the tension has been released. (**B**) A second example of the tension-generating engagement cycle in a wild-type cell. (**C** and **D**) Two short sequences from the 380K cell displayed in Figure 2C and D. Although during these periods the centrosome does not undergo significant net movement, the microtubules continue to move. Arrowheads in (C) point to a single or small bundle of microtubules that loop out from the main comet tail. The direction of motion is consistent with a plus-end-directed motor located on either an organelle or the cell cortex. (D) shows similar movement of a thicker group of microtubules, again from the same sequence as shown in Figure 2C and D.

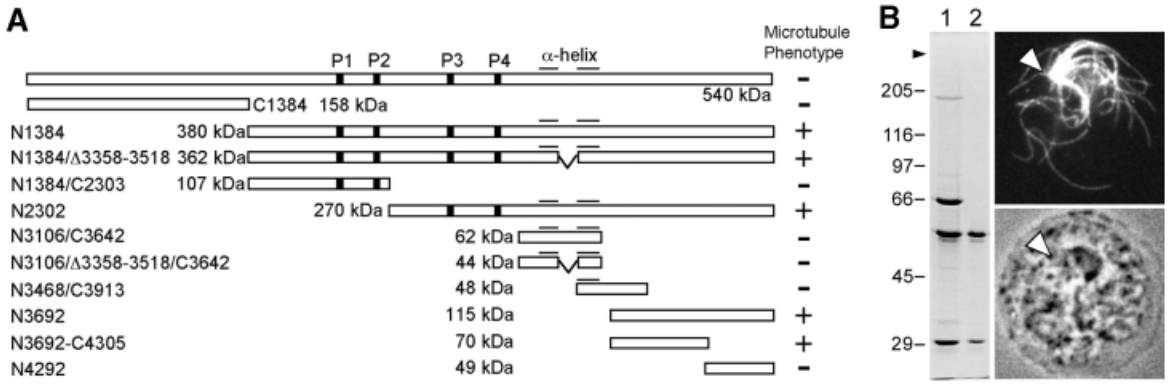

Fig. 6. Altered microtubule organization is linked to an activity located near the C-terminal end of the dynein heavy chain (DHC). (**A**) Scheme of DHC constructs expressed in *Dictyostelium* and analyzed for their ability to produce the collapsed microtubule phenotype. The top line represents the native DHC polypeptide, showing the positions of the four P-loops (P1–4) and the microtubule-binding domain (α-helix). The lines below indicate the relative size and position of 11 constructs tested for phenotypic effect. (–) represents a wild-type distribution of microtubules, (+) represents mutant microtubule organization. (**B**) Left panel: two lanes of a Coomassie Blue-stained gel. Lane 1 represents polypeptides immunoprecipitated from cells expressing the c-Myc-tagged N3692–C4305 fragment. The predicted 70 kDa polypeptide runs close to the 66 kDa marker; the antibody heavy and light chains (50 and 25 kDa) are also prominent. The identity of the other predominant bands is under investigation. Lane 2 shows an identical reaction performed on wild-type cells. The DHC does not co-purify with the 70 kDa fragment; its running position determined from an adjacent lane (not shown) is marked with an arrowhead. Right panel: a representative 70K-expressing cell that has been fixed and stained with anti-tubulin antibody. Corresponding fluorescence and phase contrast images are presented; the centrosome position near to the nucleus is marked with an arrowhead.

overexpressing the motor domain, the centrosome position becomes destabilized. The centrosome continuously circulates through the cell and often brushes the cell border with its comet-like tail of microtubules. Similar movement has been observed in unusually small cells occasionally produced in a myosin II-null mutant (Neujahr *et al.*, 1998), suggesting that overexpression of the dynein fragment accentuates a motility that also occurs under conditions of altered cell size.

In the mutant cells, a looping behavior of the trailing microtubules is seen in live and fixed cells (Figures 1, 5C and D), which indicates that a plus-end-directed motor activity is retained in the mutant and accentuated when the dynein activity is suppressed. By detaching microtubules from their cortical anchors they may become more sensitive to pushing forces mediated by plus-end-directed motors and thus lose their characteristic radial pattern. Alternatively, changes in polymerization dynamics leading to longer microtubules may also result in more wavy microtubules. Individual microtubules appear too flexible to support a sustained pushing force, but the bundles of the comet tail in the mutant cells may be rigid enough to transmit a motive effect. It remains to be determined how much this behavior contributes to motion of the centrosome in the mutant cells, in addition to dynein activity that may be retained.

There are several lines of evidence to indicate that in highly motile cells such as *Dictyostelium*, a distal anchorage maintains both the ordered array of microtubules and the centrosome position during movement (Sameshima *et al.*, 1988; Ueda *et al.*, 1997; Neujahr *et al.*, 1998). That dynein serves as a force-generating cortical motor has

been demonstrated in a number of mitotic cells, acting to position and orient the spindle apparatus (reviewed in Hyman and Karsenti, 1996; Karsenti *et al.*, 1996; Heil-Chapdelaine *et al.*, 1999) and to facilitate anaphase elongation (Aist *et al.*, 1991). Specifically, heavy chain knockouts in *Saccharomyces cerevisiae* eliminate cortical interactions that are important for pulling on the astral microtubules in order to position the spindle in the neck region between mother and daughter cells (Eshel *et al.*, 1993; Li *et al.*, 1993). Similar observations have been made during meiotic prophase in *Schizosaccharomyces pombe* (Yamamoto *et al.*, 1999). Dynein–dynactin complexes are also important for spindle positioning in *Caenorhabditis elegans* (Skop and White, 1998) and in MDCK cells (Busson *et al.*, 1998). Our work here addresses a similar, related function for dynein during interphase, to maintain the radial microtubule array and centrosome position.

We also show directly that the nucleus is rotated and bent in interphase cells of *Dictyostelium* through a tight link to the centrosome (Figure 3). Passive migration of the nucleus in connection with the centrosome is a general phenomenon in eukaryotic cells. Microtubule-mediated nuclear migrations have been characterized in *Drosophila* (Raff and Glover, 1989) and in *Xenopus* extracts (Reinsch and Karsenti, 1997). On the same line, dynein disruption affects microtubule anchorage at the growing tip of fungal hyphae, resulting in a failure to position nuclei evenly (Plamann *et al.*, 1994; Morris *et al.*, 1995; Xiang *et al.*, 1995; Inoue *et al.*, 1998). Thus dynein may play a general role in nuclear placement.

How does overexpression of heavy chain fragments inactivate a cortical dynein? The minimal 70 kDa fragment exerting a dominant-negative effect does not co-sediment with microtubules nor does it co-immunoprecipitate with the native dynein molecule. Thus it is unlikely that inactivation of dynein is mediated *in vivo* through competition of its overexpressed fragments for microtubule binding, or by their direct physical interaction with the full-length motor protein (Figure 6). These data suggest that the C-terminal region of the heavy chain comprises a motif important for binding the dynein motor domain to a factor that controls its activity. Overexpression of the dynein fragment would result in a cellular sink for this regulatory factor. There is evidence that the N-terminal end of the heavy chain and its associated anchorage complex, dynactin, can regulate the motor activity (Schroer, 1996; Iyadurai *et al.*, 1999; Karki and Holzbaur, 1999). If the putative binding factor is also a protein of the dynactin complex, dynein activity can be suppressed by two mechanisms: dynein may be displaced from its connection to cortical dynactin, or the dynein stays *in situ* but lacks an activating input from dynactin, an input that would be transmitted through the 70 kDa portion of the heavy chain. In support of a direct involvement of the C-terminal heavy chain region in protein interactions, Benashski *et al.* (1999) have shown that the 22 kDa light chain-1 of *Chlamydomonas* axonemal dynein chemically cross-links to a C-terminal UV cleavage fragment of the α-heavy chain. Furthermore, this fragment targets binding of an additional 45 kDa polypeptide to the motor domain. Cytoplasmic dyneins are associated with at least three light and two light intermediate chains (Vallee and Sheetz, 1996; Hirokawa, 1998), and these might have activities similar to the axonemal polypeptides.

In conclusion, the central position of a centrosome is determined in *Dictyostelium* interphase cells by counteracting forces that involve dynein and are transmitted through microtubules. These microtubules connect the centrosome in different directions with the cell cortex. It is essential for the stabilization of centrosome position that single cortical motors are prevented from dominating; this means no single motor should pull too long on the centrosome. There are different possibilities of control to be analyzed in future work. Drawing the centrosome in one direction will cause tension on microtubules pointing in the opposite direction. This tension might activate cortical motors, much like the regulation proposed for the kinetochore during cell division (Nicklas *et al.*, 1998). In addition, a microtubule moved on a cortex-attached dynein motor will be twisted, and the torsion may terminate engagement of the motor molecule. Also, timing of dynein activity might be imposed on the motor protein by its association with the dynactin complex.

Materials and methods

Molecular genetics

Dynein heavy chain. Plasmids designed to express portions of the DHC were assembled as described (Koonce and Samsó, 1996; Koonce, 1997). Briefly, DNA fragments were subcloned between the native DHC promoter and an actin 8 transcription termination sequence. Constructs also contained a G418-selectable marker. AX2 cells were transformed using $Ca^{2+}PO_4$ and cloned as previously described (Koonce and Samsó, 1996). Cells were maintained in HL-5 medium containing 10 μg/ml geneticin.

For biochemical characterization, high speed supernatants (HSS) of cell lysates, SDS–PAGE and immunoblotting were performed as described by Koonce and McIntosh (1990). For immunofluoresence labeling, cells were plated onto acid-washed coverslips, fixed with 2.5% formaldehyde in 15 mM PIPES pH 7.0, 1 mM EGTA, permeabilized in MeOH containing 1% formaldehyde, and stained with tubulin antibody (Koonce and McIntosh, 1990). For immunoprecipitation, an 11 amino acid c-Myc epitope tag was appended to the C-terminus of the N3692–C4305 construct of DHC (details on request). c-Myc-tagged polypeptide was purified from HSS by incubating 3 ml of supernatant with 50 μl of anti c-Myc monoclonal antibody (Evan *et al.*, 1985) for 1 h at 4°C. A 25 μl aliquot of protein A–Sepharose (Pharmacia) was then added to bind the antibody. The resin was washed three times and resuspended in 50 μl of PHEM buffer (Schliwa and van Blerkom, 1981). Bound polypeptides were resolved by SDS–PAGE and Coomassie Blue staining.

GFP–tubulin. The S65T variant of GFP was placed under the control of the actin 15 promoter and fused to the N-terminal end of *D.discoideum* α-tubulin (for construct details, see Neujahr *et al.*, 1998). The plasmid backbone (pDBsr) contained a blasticidin resistance cassette, permitting dual selection of GFP–tubulin and dynein motor domain transformants. Wild-type AX2 cells and AX2 cells expressing the 380 kDa fragment of the DHC (380K cells) were transformed by electroporation, and selected for growth in 20 μg/ml blasticidin S.

Light microscopy

Live cell recording. Cells were plated onto acid-washed glass coverslips; once attached, the nutrient medium was replaced with 17 mM K/Na phosphate buffer, pH 6.0, to reduce background fluorescence. Cells were overlaid and flattened by compression with 0.2 mm thick sheets of agarose (Yumura *et al.*, 1984) just before observation, and maintained in humid chambers during microscopy. The agar overlay dramatically improved the ease with which individual microtubules could be followed. Cells were imaged at 24 ± 1°C in either a Zeiss LSM 410 confocal or a Zeiss Axiovert microscope using a 100× 1.4 NA Plan-Neofluar objective. Imaging system details were as described by Neujahr *et al.* (1998). Both phase contrast and GFP fluorescence images were recorded in parallel and stored in TIFF format on a computer hard drive. Figures were assembled in Adobe Photoshop and images of live cells processed

using the AVS software (Advanced Visual Systems, Waltham, MA 02154). Centrosome positions were determined by recording their pixel coordinates on successive images using custom-developed software; changes in position were plotted over a 1 min time frame.

Digital deconvolution. To obtain average length distributions of the wild-type and 380K cell microtubules, agarose-compressed cells were fixed with formaldehyde and stained with anti-tubulin antibody as described above. Through-focus Z series (step size 200 nm) were collected using a Photometrics PXL camera (Photometrics Ltd., Tuscon, AZ) and a 60× 1.4 NA objective mounted on a custom-modified Nikon Optiphot microscope. Isee Software (Inovision Corp., Durham, NC) was used to acquire the image stacks; these were subsequently imported into DeltaVision software (Applied Precision Inc., Issaquah, WA) for digital deconvolution. Microtubule lengths were measured in true three-dimensional space using subroutines included in the DeltaVision software.

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