Pushing Forces Drive the Comet-like Motility of Microtubule Arrays in *Dictyostelium*

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Overexpression of dynein fragments in *Dictyostelium* induces the movement of the entire interphase microtubule array. Centrosomes in these cells circulate through the cytoplasm at rates between 0.4 and 2.5 $\mu\text{m/s}$ and are trailed by a comet-tail like arrangement of the microtubule array. Previous work suggested that these cells use a dynein-mediated pulling mechanism to generate this dramatic movement and that similar forces are at work to maintain the interphase MTOC position in wild-type cells. In the present study, we address the nature of the forces used to produce microtubule movement. We have used a laser microbeam to sever the connection between the motile centrosomes and trailing microtubules, demonstrating that the major force for such motility results from a pushing on the microtubules. We eliminate the possibility that microtubule assembly/disassembly reactions are significant contributors to this motility and suggest that the cell cortex figures prominently in locating force-producing molecules. Our findings indicate that interphase microtubules in *Dictyostelium* are subject to both dynein- and kinesin-like forces and that these act in concert to maintain centrosome position in the cell and to support the radial character of the microtubule network.

INTRODUCTION

Microtubules are dynamic polymers that are essential for eukaryotic cell viability. They provide general support for overall cell shape and can be modeled into a number of functional configurations for use in cell-motility events. For example, microtubules form the structural core of cilia and flagella and the spindle apparatus during cell division. Microtubules also form extended arrays that support highly asymmetric cell shapes, such as those commonly seen in neurons. These distinctive architectures are actively built and maintained; that is, individual microtubules are acted upon by accessory proteins to alter their dynamics, to cross-link with adjacent microtubules or other structures, and to produce various movements. In most interphase cells, the “default” microtubule arrangement consists of a clustering of minus ends at or near the cell center, with the plus ends extended in a radial manner out to, or near the cell periphery. This arrangement, too, is actively maintained, and it can be altered depending on cell-cycle progression or cell stimulation.

In *Dictyostelium*, overexpression of the dynein motor domain produces a phenotype in which the microtubule array loses its radial character and becomes motile through the cytoplasm (Koonce *et al.*, 1999). Microtubules in these cells (380K cells) remain firmly attached to the centrosome and, in

large part, are dramatically rearranged to form a trailing “comet-tail”. The same phenotype can be generated in multiple ways: by expressing small fragments of the dynein motor that do not have catalytic or microtubule-binding activity (Koonce *et al.*, 1999), by expression of the *Dictyostelium* Lis-1 protein (Rehberg *et al.*, 2005), by expression of an N-terminal fragment of the CP-224 MAP (Hestermann and Gräf, 2004), or expression of an N-terminal fragment of the *Dictyostelium* NudE-like homolog (Koonce and Tikhonenko, unpublished results). Thus, the effect appears to involve both the Lis-1/Ndel-1/dynein regulatory pathway (Smith *et al.*, 2000, Dujardin *et al.*, 2003, Liang *et al.*, 2004, Shu *et al.*, 2004) and linkages to the cell cortex. Disruption of dynein-cargo linkages by dominant-negative expression of dynein intermediate-chain fragments also produces such a phenotype (Ma *et al.*, 1999), but with far greater effects on the microtubule array and on cell division. This more severe dynein disruption is lethal, arguing that the Lis-1/Ndel-1 governs only a subset of dynein activities. We are actively pursuing the connections between this regulatory pathway and dynein motor activity.

In addition to the loss of cortical attachments, this novel centrosome motility must be based on a pulling or pushing force applied directly to the centrosome or along the microtubules. A priori, this force can be generated by microtubule polymerization or by a motor protein(s). In this respect, a number of dynein perturbations in other organisms have similar consequences (Smith *et al.*, 2000, Palazzo *et al.*, 2001, Burakov *et al.*, 2003, Dujardin *et al.*, 2003). Taken together, these results strongly indicate a general role for dynein in stabilizing and/or positioning interphase microtubule arrays. Our previous work supported a model in which cortically anchored dynein motors exert transient pulling forces on microtubules. A balance of these force transients over the entire cortical surface would thus provide a means by which

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the cell continually reinforces the central position of the centrosome and radial character of the microtubule array, whether the cell is stationary or actively moving. One possibility for the aberrant movement in the mutant cells is that this balance of dynein activity is disturbed (activity on some motors is either reduced or stimulated), such that a cortical dynein-microtubule engagement becomes dominant and provides an extended pulling force to displace the centrosome. In the current work, we directly test this idea by using a laser microbeam to sever the connection between the centrosome and the trailing microtubule array. We present evidence that the bulk of the centrosome movement in the mutant cells results from kinesin-like pushing forces acting on the trailing microtubules. The work suggests that both kinesin and dynein-like motor activities can displace interphase microtubules and that a balance between these activities is important for maintaining the character of the microtubule array.

MATERIALS AND METHODS

Cell Culture

Dictyostelium AX-2 cell lines expressing α -tubulin GFP alone (control cells; Neujahr *et al.*, 1998) or with the 380-kDa motor domain of cytoplasmic dynein (380K cells; Koonce and Samsó, 1996) were prepared and maintained as described (Koonce *et al.*, 1999).

For use, cells were plated on glass coverslips and allowed to adhere for 1–2 h in 17 mM K/Na phosphate buffer, pH 6.0, to reduce background fluorescence; they were then overlaid and flattened with small agarose sheets (Yumura *et al.*, 1984). These coverslips were either inverted over slides and sealed with VALAP or assembled into Rose chambers, containing a small piece of moistened Kimwipe to maintain humidity. Latrunculin A was purchased from Sigma Chemical Co. (St. Louis, MO) and used at a final concentration of 5 μ M in both phosphate buffer and the agarose sheets.

Laser Microsurgery and Imaging

Laser microsurgery was conducted on a custom-assembled workstation centered around an inverted fluorescence microscope (model TE2000E; Nikon Instruments, Melville, NY). We used an independent second (lower) epi-port that is available on this model, to direct a collimated laser beam into the back aperture of a 60 $\times$ 1.4-NA PlanApo lens. A 532-nm beam was used for laser cutting (Nd:YAG laser; 7-ns pulses, 10 Hz). This system is described in more detail in Khodjakov *et al.* (2004). The same optical system was also used to record nonlaser sequences.

All light sources in our system were shuttered with fast UniBlitz shutters (Vincent Associates, Rochester, NY), so that each cell was exposed to light only during laser operations and/or image acquisition. Wide-field images were collected under low light level conditions with a Coolsnap HQ camera (Photometrics, Tucson, AZ). The whole system was driven by IP Lab software (Scanalytics, Billerica, MA) run on a PC. Image sequences were collected at 3-s intervals, and were analyzed using Image J (v1.31, NIH). The panels shown in the figures were compiled using Photoshop 6.0 (Adobe Systems, San Jose, CA). For the latrunculin work, centrosome position was followed automatically using the cross-correlation tracking algorithm in ISEE Software (ISEE Imaging, Durham, NC).

RESULTS

Individual interphase microtubules in control *Dictyostelium* cells are exceptionally motile, displaying a variety of rapid movements that appear to be driven by both plus end- and minus end-directed motors (Figure 1; Supplementary Movie 1; Kimble *et al.*, 2000; Koonce and Khodjakov, 2002). Such movements include simple bending and straightening, flailing back and forth in a whip-like manner, and arcing along the cell cortex. Forces acting on the microtubules appear able to originate at any point along the entire microtubule length. Overexpression of the dynein motor domain introduces an additional motile component: the entire microtubule array appears to circulate through the cytoplasm and along the inner surface of the cell cortex, at rates up to 2.5 μ m/s (Koonce *et al.*, 1999; see also Supplementary Movies 3a and 5c). We used a laser microbeam to sever the

Figure 1. Interphase microtubule movements in a control cell. An arrowhead marks the plus end of a single GFP-labeled microtubule in *Dictyostelium*. In the first four frames (0–30 s), the plus end follows a whip-like bending trajectory through the cytoplasm, in a direction consistent with the application of kinesin-like forces to the microtubule. In the last four frames (33–45 s), the same microtubule end now leads, in a direction consistent with the application of dynein force. The microtubule itself maintains a constant length (13.3 μ m) throughout the sequence. The position of the centrosome is marked with an asterisk in the first panel ($t = 0$). Time indicates seconds of observation; scale bar, 2.5 μ m.

connection between the centrosome and the trailing comet tail of microtubules in these 380K cells (Figure 2; Supplementary Movies 2a–d). In 15 of 18 cells, centrosomes immediately cease their movement, suggesting that much of the motility is driven by force exerted on the trailing microtubules. The microtubule arrays in the three cells whose centrosomes continued moving were less focused, and we could not be certain that most of the microtubules had actually been severed. Control irradiations in the cytoplasm, adjacent to or in front of the centrosome had no effect on this movement. In some instances after cutting (Figure 2D), microtubules were reorganized on the other side of the centrosome and began to drive motion in the direction opposite the preirradiation movement.

In nearly every case, the laser-mediated liberation of minus microtubule ends resulted in the disassembly of the trailing arrays. However, in a few cells (e.g., Figure 3), the microtubule array appeared tightly bundled and did not immediately disassemble. In these cells, we found that the microtubule bundles themselves were capable of motion, independent of the centrosome. This result indicates that the centrosome itself is not required; instead, the force responsible for the motility acts along the microtubule bundle (Supplementary Movie 3b).

To distinguish whether the force is due to molecular motors or microtubule-end dynamics, we measured assembly/disassembly rates for *Dictyostelium* microtubules. The goal was to determine whether this organism's tubulins have any unusual kinetic properties that could contribute both to the lateral movements in control cells and to the centrosome movements in the 380K cells. We restricted our analysis to those microtubules whose entire length remained in focus throughout the observation period. Our records revealed that while transitions between growing and shrinking are extremely rare, *Dictyostelium* microtubules do undergo dynamic instability (Figure 4A). Using the laser microbeam, we also created free plus and minus ends of single microtubules. Except for those in tightly bundled microtubule arrangements (as described above), free minus ends of microtubules in *Dictyostelium* are unstable (Figure 4B). In a number of cases, we could readily follow this depolymerization and thus generate rate measurements for minus end

Figure 2. Laser-mediated cutting of motile microtubule comet tails. (A–D). Four examples of 380K cells in which the trailing GFP-labeled microtubule bundle is separated from the leading centrosome. The first panel in the A–C sequence shows a “before” view; the second panel shows the cell immediately after (or during as in C) laser cutting of the microtubule tail. The two rows in D show an extended sequence of a binucleate cell, containing two microtubule arrays. The arrowhead marks the site of laser ablation. In all cases shown here, the centrosome stopped movement upon severing of the microtubule bundle. Note in D that the second microtubule array movement is unaffected by the ablation and that in the last few panels (153 s and beyond), the unaffected microtubules on the side opposite to the cut have begun to organize and push the centrosome toward the bottom of the frame. Time is displayed in seconds of observation; scale bar, 5 μ m.

depolymerization (Figure 4C). We saw no evidence for tubulin assembly at free minus ends of microtubules. Plus ends were more stable: only half of the newly created plus ends depolymerized to varying degrees, whereas the other half remained stable throughout the observation period (Figure 4D).

Our measurements of microtubule end dynamics (assembly and disassembly) are shown in Table 1; these averages are comparable to kinetics seen in GFP-labeled mammalian microtubules (also included in Table 1; Rusan *et al.*, 2001). In contrast, the rapid movements of individual microtubules in *Dictyostelium* occur at rates averaging 1.1 μ m/s, approximately four times faster than could be explained solely on the basis of assembly or disassembly. There were no rate differences between what we interpreted as plus end- or minus end-directed force production, a finding consistent with other measurements of organelle trafficking in *Dictyostelium* (Table 1). These results strongly imply that the rapid microtubule and centrosome movements in control and 380K cells are largely due to molecular motor activities, rather than to unusual kinetics of assembly.

Finally, to address potential contributions of the actin-rich cell cortex to the microtubule motions, we examined the effects of latrunculin in both control and 380K cells. Cells treated with 5 μ M latrunculin showed the rapid, characteristic effects of actin depolymerization: cells halted their motility, rounded up, and detached from the coverslip. We applied an agar overlay (presoaked in latrunculin) to hold cells in place so as to enable their live observation, and we also examined fixed cells to confirm the efficacy of the drug in destabilizing the actin arrays (unpublished data). The

individual bending motions of microtubules occurred in control, latrunculin-treated control, and latrunculin-treated 380K cells (Supplementary Movies 5a–c). However, there were differences in the overall microtubule patterns in control cells and in the motility of the comet-tail arrangements in the 380K cells. In control cells, latrunculin-treated microtubules appeared to be more curved and fewer microtubules appeared to engage in tension-generating events on cell cortex (Figure 5). Although these differences are readily seen in the Supplementary Movies, we also include a plot of centrosome position over time (Figure 5). Note that the lateral back and forth motions of the centrosome are much reduced in the latrunculin treatment. A significant difference was also seen in the 380K cells. Comet tail-like microtubule arrangements could be found (Figure 5), and occasional short centrosome movements (1–2 μ m) were observed. However, none of the latrunculin-treated 380K cells displayed the persistent directional motions that were seen in untreated 380K cells. Thus, actin filament disruption does not appear to affect individual microtubule motions, but it does alter the overall architecture in control cells and significantly inhibits movement of the entire array in the 380K cells.

Together, these results support the idea that a balance of plus end- and minus end-directed forces acting on the microtubules are involved with maintaining the organization of the radial interphase microtubule array in eukaryotic cells. In addition, they also suggest that an intact actin-filament cortex is instrumental in supporting tension-generating microtubule interactions. These ideas are summarized in Figure 6 and are further elaborated in the Discussion.

Figure 3. Movements of the microtubule bundle. These panels show examples of tightly packed bundles of microtubules in 380K cells that exhibit movement independent of the centrosome. In the uncut example shown in A, the centrosome leads the movement over a 3.5-min time period. In frames 72–87, the centrosome pauses briefly, but the trailing microtubule bundle continues its movement (marked by arrowheads). The comet tail shown in B required two attempts to fully sever with the laser microbeam. A partial cut is shown after 129 s, and the fully cut bundle is shown in 159 s. The centrosome is marked with an asterisk. On complete cutting, the bundle itself began moving, independent of the centrosome, with the new minus ends leading. The peak rate of movement here is 0.5 $\mu\text{m/s}$. Time is displayed in seconds of observation; scale bar, 5 μm .

DISCUSSION

In the studies described, we sought to address the general question of how centrosome position is determined and maintained in eukaryotic cells. The dramatic motility of the microtubule array in the 380K cells, combined with laser-microbeam ablation, provides an opportunity to address the nature of the force-generating machinery that can actively displace both microtubules and centrosomes. There are two types of microtubule motility described in this study: the bending, straightening, sweeping motions of individual microtubules, and a larger scale motion of the entire microtubule array. The individual microtubule motions occur in both control and 380K cells, whether or not the actin filament meshwork has been disrupted by latrunculin. The larger scale comet-like movements of the entire array are induced by dominant-negative expression of dynein motor fragments, and ceases if actin filaments are disrupted.

Single Microtubule Motility

The individual microtubule movements appear to result from direct interactions with motor proteins. Because each microtubule behaves independently, the motions are clearly not due to any bulk cytoplasmic rearrangements or membrane flow (e.g., Kaverina *et al.*, 1998, Ligon *et al.*, 2001, Salmon *et al.*, 2002). The rate of movement is much faster than could be accounted for solely by polymerization or depolymerization and is consistent with the microtubule

Figure 4. Microtubule end dynamics in *Dictyostelium* control cells. (A) Example of dynamic instability. Arrowhead marks the growing and shrinking plus end of a single GFP-microtubule; the minus end is anchored at the centrosome at the bottom of the field (asterisk in first panel). (B) Binucleate cell with two independent microtubule arrays. A laser microbeam was used to ablate the centrosome anchoring the right-hand network ($t = 0$ before, $t = 15$ just after irradiation), resulting in the generation of free minus ends and depolymerization of the right-hand microtubule network. As a control, the left-hand network is unaffected. (C) Panel follows minus-end depolymerization of a single microtubule. (D) Panel shows laser-mediated cutting of a single microtubule ($t = 9$ before, $t = 12$ just after). Note here that the newly created minus end (–) is unstable, but the new plus end (+) remains a constant length (9.5 μm). Time in seconds of observation; scale bar: (A, C, and D) 2.5 μm ; (B) 10 μm .

motor-based activity seen in this organism. Microtubule bending and translocation would occur when the resistance encountered by a motor-driven cargo exceeds the stiffness provided by its microtubule track. Because of their inherent polarity, dyneins would pull on a microtubule and thus straighten the microtubule between the motor and the minus-end anchorage at the centrosome. In the other direction, kinesins would push between the motor attachment and the centrosome, causing a bending of the polymer. As revealed in Figure 1, microtubules frequently undergo both types of movement, indicating that both kinesin- and dynein-like motor activities affect microtubule position in *Dictyostelium*, under normal interphase conditions.

Microtubule Array Motility

The comet-tail phenotype of the 380K cells can be generated by multiple perturbations that target dynein motor regulation or anchorage of microtubules at their plus ends. (Koonce *et al.*, 1999, Rehberg *et al.*, 2005; Hestermann and Gräf, 2004). Measured *in vitro*, dynein motor domain overexpression in the mutant 380K cells causes a ~40% decrease in the frequency of minus end organelle movements, but has no effect on the plus end-directed motility (Pollock *et al.*,

Table 1. Average rates and comparisons of microtubule assembly, disassembly, and motor-driven movements in control cells

Microtubule end kinetics	<i>Dictyostelium</i>	Mammalian ^a
Plus end polymerization	14.4 ± 1.5 μm/min (n = 6)	11.5 ± 7 μm/min
Plus end depolymerization	16.8 ± 5.1 μm/min (n = 12)	13.1 ± 8 μm/min
Minus end depolymerization	18.1 ± 3.3 μm/min (n = 12)	
Microtubule end movements	1.14 ± 0.1 μm/s (69.0 ± 6.5 μm/min; n = 12)	
Organelle movements		
Centrosomes	0.4–2.5 μm/s ^b	
Minus end-directed GFP-dynein	1.84 ± 0.34 μm/s ^c	
Plus end-directed GFP-dynein	1.77 ± 0.40 μm/s ^c	
Aggregate organelles	1.4–2.8 μm/s ^d	

^a Data from Rusan *et al.* (2001).
^b Data from Koonce *et al.* (1999).
^c Data from Ma and Chisholm (2002).
^d Data from Roos *et al.* (1987).

1998). The distribution of organelles in these cells appears no different from in controls. Therefore, the remaining ~60% of minus end-directed organelle movements must be sufficient for most trafficking requirements and could be used to produce the straightening motions of individual microtubules in the 380K cells. A striking difference between control and 380K cells lies in the rapid comet like movements of the microtubule array. If pushing and pulling forces acting on the microtubule array are balanced in normal cells, then a 40% decrease in dynein activity may be significant to allow a pushing force to dominate. The laser work presented here is consistent with this idea, indicating pushing-type forces act on and move the centrosomes and microtubules in the mutant 380K cells. The obvious candidate for such a pushing action is a plus end-directed kinesin-type motor. It remains unclear how a pushing force directed to one or perhaps only a few flexible microtubules can support movement of the entire array (and the attached nucleus), but this mechanism has been shown to work in yeast (Tran *et al.*, 2001).

Subpopulations of Motor Activity

Although motor activity bound to organelles could be sufficient to move individual microtubules, moving the entire

array would require a far more sturdy anchorage. The most obvious location that has such structural rigidity is the peripheral actin-rich cortical meshwork. There are a number of examples that support both the targeting of motors to the cortex (Holleran *et al.*, 1996, Iwai *et al.*, 2004) and force production at this site (reviewed in Allan and Näthke, 2001, Bloom, 2001, Dujardin and Vallee, 2002). Thus, we surmise that there are two distinct populations of motors that engage microtubules: one on actively moving organelles and the other enmeshed into the actin-rich cortex. Our observation that latrunculin stops the comet-like microtubule movements is particularly important since it directly tests this idea. Disrupting the cortical meshwork eliminates motor anchorage, and also the force contributions of both pushing and pulling motors at this site. In control cells, eliminating contributions of both motors only mildly affects the radial character of the array. In the mutant 380K cells, comet tails

Figure 5. Effects of latrunculin on centrosome movement. (A–D) Still frames of GFP-labeled microtubules in a control cell (A), a control cell treated with 5 μM latrunculin (B), a 380K cell (C), and a 380K cell treated with 5 μM latrunculin (D). Below each panel is a tracing of that centrosome’s position throughout the 5-min recording period. Note that for both control and 380K cells, latrunculin treatment significantly decreases the range of centrosome movement. Latrunculin had no obvious effect on individual microtubule bending movements in control or 380K cells (see Supplementary Movies for the entire sequences). Scale bar for light micrographs, 5 μm; Tracings are outlined with 10-μm² box.

Figure 6. Summary of motor function in interphase cells. In this model, motor molecules exert two types of forces along microtubules. Free motors in the cytoplasm, or ones bound to simple organelles, will move in the direction specified by their mechanochemistry (toward minus ends for dynein, toward plus ends for kinesin, directions indicated here by black arrows). However, if the motors are anchored at more elaborate sites, for example, enmeshed in the cortex (red), attached to a complex cargo with significant resistance, or anchored at adhesion sites, these motors appear able to exert enough force to move the microtubule substrate. Thus, anchored dyneins will pull on a microtubule and anchored kinesins will push microtubules (green arrows). The combined activity of both anchored motors is likely to be instrumental in maintaining centrosome position and supporting the radial character of the interphase microtubule array.

no longer move after latrunculin treatment, a result consistent with displacement of the now dominant plus end-directed forces. Given that there are no differences in vesicle motion or in the bending movements of single microtubules after latrunculin treatment (Figure 5), we infer that motor-organellar linkages and motor activities per se are not sensitive to actin-filament disruption.

A Role For The Cortex

There are numerous reports that describe a general integration of actin filament and microtubule polymer systems, including effects on organelle motility (Rogers and Gelfand, 1998, Rodionov *et al.*, 1998), centrosome position (Euteneuer and Schliwa, 1985, Whitehead *et al.*, 1996, Piel *et al.*, 2000, Burakov *et al.*, 2003), and actin-microtubule based motor interactions (Huang *et al.*, 1999, Weber *et al.*, 2004). To couple these two filament systems in the comet-tail motion reported here, either myosin motors are stably attached to the microtubules and pull along actin filaments, or a set of microtubule-based motors must be anchored in the actin-rich cortex that push on microtubules. Given rates that are consistent with the microtubule-based motility in *Dictyostelium* and contrasting the relatively short lengths of actin filaments (avg. 200 nm; Podolski and Steck, 1990) with persistence of the directional comet-like movements (Figure 3), the results presented in this study are most consistent with the latter mechanism. Though we cannot conclusively rule out a myosin role, we favor the idea that the actin-rich cortical meshwork serves as a platform to anchor microtubule-based motors (or linker proteins).

Summary

Our data are summarized in the model presented in Figure 6. Here, both dynein and kinesin function in mediating organelle transport and provide support for the interphase microtubule array. Indeed, we believe that a balance of multiple forces and nonforce linkers (push, pull, and static) engage microtubules at or near their ends, so as to maintain the radial character of the interphase array. The cell cortex figures prominently in this model, by offering a relatively firm platform on which to push or pull against. Cortically linked dynein has long been implicated in the positioning of mitotic and meiotic spindles (e.g., Carminati and Stearns, 1997, Bloom, 2001, Gönczy, 2002), and in the engagement of cytoplasmic microtubules (Allan and Näthke, 2001, Schuyler and Pellman, 2001, Dujardin and Vallee, 2002, Hestermann *et al.*, 2002). Recently, a role for a kinesin-like pushing force has been described in orienting microtubules along the cortex of *Xenopus* embryos (Marrari *et al.*, 2004). Our work further indicates that along with dynein, kinesin-like motors may play much more active roles in organizing microtubule-based structures than previously considered, especially in interphase cells.

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