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Cytoplasmic dynein: tension generation on microtubules and the nucleus

Nandini Shekhar[1,#], **Jun Wu**[1,#], **Richard B. Dickinson**[1], and **Tanmay P. Lele**[1,*]
[1]Department of Chemical Engineering, University of Florida, Gainesville, FL 32611

Abstract

Cytoplasmic dynein is a microtubule dependent motor protein that is central to vesicle transport, cell division and organelle positioning. Recent studies suggest that dynein can generate significant pulling forces on intracellular structures as it motors along microtubules. In this review, we discuss how dynein-generated pulling forces position the nucleus and the centrosome.

Key Terms

Cytoplasmic Dynein; Force Generation; Nuclear Positioning; Centrosome Centering

Introduction

Cytoplasmic dynein (hereafter referred to as dynein) is a large multiprotein microtubule motor (1.2 MDa) that plays important functions in cell division[1], motility[2], cargo transport[3] and organelle positioning[4]. Disruption of dynein activity can result in serious human disorders[5]. Transport cargoes for dynein can include Golgi vesicles[6], peroxisomes[7], mitochondria[8], endosomes [9] and lysosomes[6]; transcription factors[10], proteins[11], RNPs (messenger ribonucleoproteins)[12] and viruses[13]. Recent studies suggest that dynein is also capable of producing significant tensile forces on intracellular scaffolds like the nucleus and the centrosomal array of microtubules. In this review, we discuss force generation by dynein and its implications in cellular function in the context of two specific examples: nuclear motion and centrosome centering.

Dynein structure and motor activity

Dynein structure (Fig. 1) consists of two identical heavy chains (~ 530 kDa), two intermediate chains (74 kDa), four light intermediate chains (52–61 kDa) and several light chains (10–25 kDa)[14]. Structurally the heavy chain has three distinct units: a globular head that contains ATPase units, a cargo-binding tail and a stalk with a microtubule binding domain at its tip. The carboxy terminal of the heavy chain forms the motor domain consisting of six AAA+ (ATPases associated with various cellular activities) units arranged in a ring[15, 16]. Four of these domains have the capability to bind ATP[16, 17]. While hydrolysis of ATP in AAA1 is critical for motility[18], the other domains are thought to play a regulatory role[19].

Extending from the motor domain between AAA4 and AAA5 is a 15 nm anti parallel coiled coil stalk with a small domain at its tip that binds to microtubules in an ATP dependent

*Address correspondence to: Tanmay P. Lele (tlele@che.ufl.edu), Department of Chemical Engineering, Bldg 723, University of Florida, Gainesville, FL 32611 Ph: 352-392-0317.
#These authors contributed equally to the manuscript

fashion (called the microtubule binding domain or MTBD)[20, 21]. Extending from the NH2 terminal of the heavy chain is a tail that has sites for binding to cargo and accessory subunits[22]. The accessory subunits include the intermediate and light intermediate chains. The light chains of dynein – light chain 8 (LC8), LC7 and T-complex testis-specific protein 1 (TCTEX1)- mediate interactions with several other proteins that are required for proper motor function[23]. Some of the proteins include dynactin, bicaudal D, LIS1, nuclear distribution protein E (NUDE or NDE) and NUDE-like (NUDEL or NDEL)[23]. Inhibition of accessory proteins like dynactin and LIS1 results in loss of dynein function[1, 24].

Dynein is a processive motor due to dimerization of the heavy chains that ensure persistent binding to the microtubule during motoring[25]. The processivity of dynein is on the order of microns, making it an ideal candidate to transport cargo over significant distances in the cell[25, 26]. The large and complex structure of dynein and its associated proteins complicates investigations of the force generating and stepping mechanism of the motor[27]. There is some uncertainty with respect to its step size and stall force. Under no load bovine brain dynein has been shown to walk with 24–32 nm steps and decrease its step size to 8 nm near a stall force of 1.1 pN[28]. In contrast, an optical trap study with porcine brain dynein reported that dynein takes 8 nm steps invariant to load and ATP concentration and stalls at 6–8 pN[29]. Using single molecule fluorescent assays, Reck-Peterson *et al.*[25] and Gennerich *et al.*[30] observed that the molecule predominantly takes 8 nm steps with occasional longer (12–24 nm) and backward steps under loads up to 7 pN. Different models have been proposed to explain the mechanism of dynein motility [25, 28, 29]. In the cell, dynein works as part of a large macromolecular complex with dynactin which may affect its processivity[26, 31].

Force generation by dynein on the nucleus

There is mounting evidence that dynein localizes to the nuclear envelope and moves the nucleus in a variety of cell types[32–36]. In mammalian cells, dynein is recruited to the nuclear envelope through interactions with the cytoplasmic domain of the Nesprin protein families consisting of the Syne/Nesprin-1/2[35, 36]. KASH domain proteins localize to the nuclear envelope through association with the nuclear lamina, which can allow a continuous path for force transfer between dynein and the nucleus [34, 37, 38].

In *Drosophila melanogaster*, it has been shown that microtubule-based movement of the photoreceptor (R cell) nuclei during eye development requires dynein [33, 39]. In *Caenorhabditis elegans*, the KASH domain protein ZYG-12 may interact with the dynein light intermediate chain (DLI-1) to recruit the dynein heavy chain[40]. Another nuclear envelope protein UNC-83 was also found to interact with dynein to mediate nuclear movement in *C. elegans*[41]. Consistent with this study, the bidirectional movements of nuclei in dorsal hypodermis of the precomma stage embryo of *C. elegans* were found to be driven by dynein and the motion was disrupted by UNC-83 mutant[32]. Studies with the fission yeast *Schizosaccharomyces Pombe* have shown that the dynein complex is recruited to the nuclear envelope by Kms2, an outer nuclear envelope protein; the resulting nuclear associated dynein causes nuclear migration during meiosis [42, 43]. During meiotic prophase in fission yeast *S. Pombe*, the nucleus undergoes oscillatory motion between cell poles in a dynein dependent manner[44]. A similar dynein-driven mechanism has been proposed for spindle body positioning in yeast cells[45].

While dynein pulls the nucleus toward the minus-ends (i.e. away from the cell periphery), kinesin-1 (a plus-ended motor) is also known to cause nuclear translation. For example, in U2OS cells, dynein inhibition causes rapid movement of the nucleus and centrosome towards opposite corners of the cell, while the simultaneous inhibition of kinesin-1 and dynein eliminates the separation of the nucleus and centrosome[46]. This observation suggests

that kinesin-1 pulls the nucleus away from the centrosome, consistent with its plus-end directed processivity. In *C. elegans* embryos, UNC-83 recruits kinesin-1 and dynein at the nuclear envelope and helps mediate nuclear migration[41]. In this system, kinesin-1 appears to provide the predominant forces that translate the nucleus while small dynein-mediated backward movements help the nucleus bypass roadblocks in the cell[32]. Similarly, in developing C2C12 myotubes kinesin-1 bound to the nuclear envelope has been shown to influence nuclear dynamics by exerting forces on its surface. Inhibition of kinesin-1 reduces nuclear rotation and translation resulting in abnormal accumulation of nuclei in the center of the cell[47].

How might nuclear attached dynein exert forces on the nucleus that can move it in the cell? To answer this question, a mechanistic model for dynein dependent nuclear rotation in NIH 3T3 fibroblasts was recently proposed by Wu *et al.*[48]. In this model, individual dynein molecules walking on microtubules in the vicinity of the nucleus are assumed to transiently bind and pull the nucleus toward the microtubule minus ends (Fig. 2A and 2B). The pulling forces are generated when the cargo end of dynein is anchored in the nucleus and the head tries to move toward the minus end. Pulling forces generated by an ensemble of dynein molecules can exert a torque on the nucleus. By accounting for the statistics of dynein binding and unbinding, effective interaction range between dynein molecules and perinuclear microtubules, and dynein's force-velocity relation, Wu *et al.* calculated the net mean force on the nucleus per unit length of the microtubule. Solving the torque balances for a random configuration of microtubules allowed the calculation of the rotation speed of the nucleus.

A key insight from the model was that the experimentally observed fluctuations and persistence in nuclear rotation are due to the dynamic instability of microtubules. The model predicted that nuclear rotation should decrease with decreasing distance between the nucleus and the centrosome. This prediction was experimentally demonstrated to be true. Thus force generation by dynein on microtubules undergoing dynamic instability appears sufficient to explain the key features of nuclear rotation in living cells.

Dynein-mediated centering of the centrosome

The centrosome serves as the microtubule organizing center (MTOC) in mammalian cells. During interphase, the centrosome normally stays at the center of a cell and close to the nucleus. Centrosomal centrality is important because microtubules originating from the centrosome are responsible for the intracellular transportation of different organelles[49]. Centrosome positioning involves force generation by microtubules. Microtubules have a large bending stiffness, with persistence length on millimeter scales[50]. However, they are nearly always bent or buckled in cells, implying that they are being subjected to substantial lateral forces along their lengths[51, 52] or compressive forces at their tips[53].

Pushing forces by compressed microtubules have been proposed to center the centrosome and spindle bodies (Fig. 3A, [44, 54, 55]). In interphase *S. pombe*, upon growing to the periphery of cells, microtubules are thought to exert transient forces produced by plus end polymerization that push the MTOC[44]. *In vitro* experiments[56] with reconstituted microtubule asters have shown that the MTOC can indeed be centered by elongating microtubules pushing on the boundaries of a micro fabricated chamber. For an off-center centrosome, shorter microtubules being stiffer bear higher compressive forces than longer microtubules and therefore generate net forces that tend to always center the centrosome.

While the pushing mechanism of centrosome centering appears to be a valid explanation for *in vitro* experiments, in cells inhibiting cytoplasmic dynein results in mislocalized centrosomes[4, 57, 58]. How might dynein participate in centrosome centering? *In vitro* studies

with reconstituted microtubules[59] that consist of 'cortical' interactions between dynein adsorbed on micro fabricated barriers and dynamic microtubule ends argue that pulling forces are more stable in centering the asters than pushing forces alone. The pulling forces are however not generated by dynein activity, but rather due to shortening of dynein bound microtubule ends. In this view, dynein is important only due to its ability to bind to growing ends and modulate their dynamics. One problem with this mechanism is that the cortical pulling forces tend to de-center the centrosome rather than center it, as more microtubules may reach the cortex on the side closer to the cell boundary[60]. Grill and Heyman[61] tried to resolve this issue by proposing a model in which the pulling force is dependent on the density of dynein binding sites rather than the microtubule number. There are limited dynein binding sites on the cortex, and the side further from the de-centered centrosome will contain more total binding sites resulting in larger forces. This model however still has the weakness that the force generation is due to cortical dynein binding to shortening microtubules, which would be small compared to active motor forces generated by dynein.

Conversely, other studies suggest that length-wise pulling forces are exerted by dynein on microtubules in living cells (Fig. 3B). In a recent study in fission yeast, it has been proposed that the oscillatory movement of the spindle pole body (SPB) is a collective behavior caused by dynein motor forces and dynein redistribution along the lengths of microtubules[45]. In this model, the attachment of dynein to the microtubule is assumed to depend on the length of the microtubule, while the detachment of dynein from the microtubule is in response to the increasing load. This results in the redistribution of dynein molecules on the microtubule in response to the load, generating asymmetric pulling forces to move the SPB in an oscillatory manner. A recent computational study by Zhu *et al.*[62] based on the observations and measurements reported in Burakov *et al.*[57] argued that dyneins are anchored to the cortex throughout the cell and pull on microtubules along their lengths thereby centering the centrosome. Length-wise pulling is also supported by the observation that the centrosome centers prior to the microtubules reaching the cell boundary in large fertilized Xenopus eggs[60]. The microtubules can take over tens of minutes to reach the cell boundary due to the size of the cell (~1200 μm), yet the centrosome is observed to center. This study ruled out the possibility of both the pushing and cortical pulling mechanism leaving the length dependent force model by dynein as the only feasible mechanism[4, 60, 62].

More recently, direct evidence of length-wise pulling forces in mammalian cells was provided by Wu *et al.*[4]. The authors measured shape changes of individual microtubules following laser severing in bovine capillary endothelial (BCE) cells. Surprisingly, regions near newly created minus ends increased in curvature following severing, while regions near new microtubule plus ends depolymerized without any observable change in shape (Fig. 4A). With dynein inhibited, regions near severed minus ends straightened rapidly following severing. These observations suggest that dynein exerts length-wise pulling forces on microtubules. Importantly, an increase in curvature on microtubule severing could be observed independent of the location of the cut, i.e. throughout the cell. Moreover, the lack of any observable straightening suggests that dynein prevents lateral motion of microtubules.

Wu *et al.* proposed a mechanistic model for dynein force generation on microtubules that accounted for length-wise stochastic binding and unbinding of dynein motors from the microtubules (Fig. 4B). An ensemble of these motors generates a steady force in the direction tangent to the microtubule and a frictional resistance transverse to the microtubule. A centrosomal array of microtubules subjected to dynein pulling forces and resisted by dynein friction is predicted to center on the experimentally observed timescale, with or without the pushing forces derived from microtubule buckling at the cell periphery. The model predicts that the relaxation time of centrosome centering is on the scale of tens of

minutes, depending on the cell shape and molecular parameters such as the average force per dynein linkage and friction co-efficient for lateral motion per dynein linkage.

Wu *et al.* simulated centrosome centering in cells in the presence of dynein motors[4]. As shown in Fig. 5A, dynamic microtubules grow out of an off-center centrosome in a square cell with side length of 40 μm. The distribution of dynein along the microtubule length is uniform, thus the longer microtubules on the side that is further away from the cell boundary exert larger pulling forces on the centrosome and pull it towards the center. Based on parameters determined from the laser ablation experiments on single microtubules, the centrosome is predicted to center and fluctuate near the center on the order of tens of minutes, consistent with experimental observations. Without pulling forces (Fig. 5B), the centrosome remains essentially in place for the duration of the simulation, suggesting that pushing forces are not sufficient to center the centrosome in the highly viscous cellular environment (the viscosity was deduced from bend straightening in laser ablation experiments in dynein inhibited cells). Interestingly, on reducing cell size (to 12 μm) and the viscosity of the background fluid (10^{-3} of the *in vivo* value), pushing forces alone are predicted to center MT asters consistent with observations by Holy *et al.*[56]. Pushing forces thus cannot center the centrosome in the cell where motor interactions impose a large friction opposing microtubule motion. In addition, in this model, the energy for centrosome centering is from ATP hydrolysis, which is absent in the Lann model[59].

Summary

A combination of live-cell engineering methods like laser ablation, quantitative sub-cellular imaging and mechanistic computational models of force generation can offer significant insight into the function of intracellular motors like dynein in the complex environment of the living cell. Such studies highlight how force generation in a living cell is significantly different from what may be suggested by *in vitro* experimentation. For example, pushing forces could center MT asters *in vitro*, but they are too weak for centering the centrosome or rotating the nucleus in living mammalian cells. Dynein motors act not only to pull on microtubules and the nuclear surface, but also provide significant frictional resistance that determines characteristic time scales for nuclear rotation and centering of microtubule arrays. More quantitative measurements of the mechanical properties of the dynein complex in the living cell such as stiffness, stall force, length of the dynein ‘spring’ and force-velocity relations can significantly help in developing more reliable quantitative models of dynein function *in vivo*.

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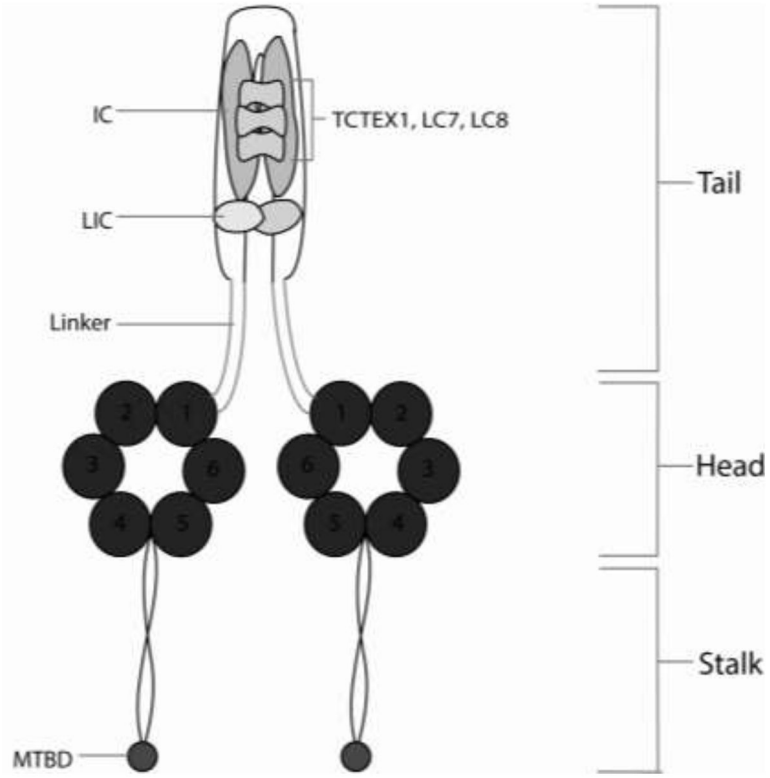

FIGURE 1.

Dynein Structure. The cytoplasmic dynein heavy chain consists of a C-terminal head region and an N-terminal tail. The motor domain has six AAA domains concatenated into a ring. Arising from AAA4 and AAA5 is an antiparallel coiled coil stalk with a Microtubule Binding Domain (MTBD) at its tip. The linker element is known to play a role in the force generation of the motor domain. The dynein tail mediates homodimerization of the heavy chains and recruits the Intermediate Chains (IC) and Light Intermediate Chains (LIC). The IC recruit the smaller light chains- light chain 8 (LC8), LC7 and T-complex testis-specific protein 1 (TCTEX1).

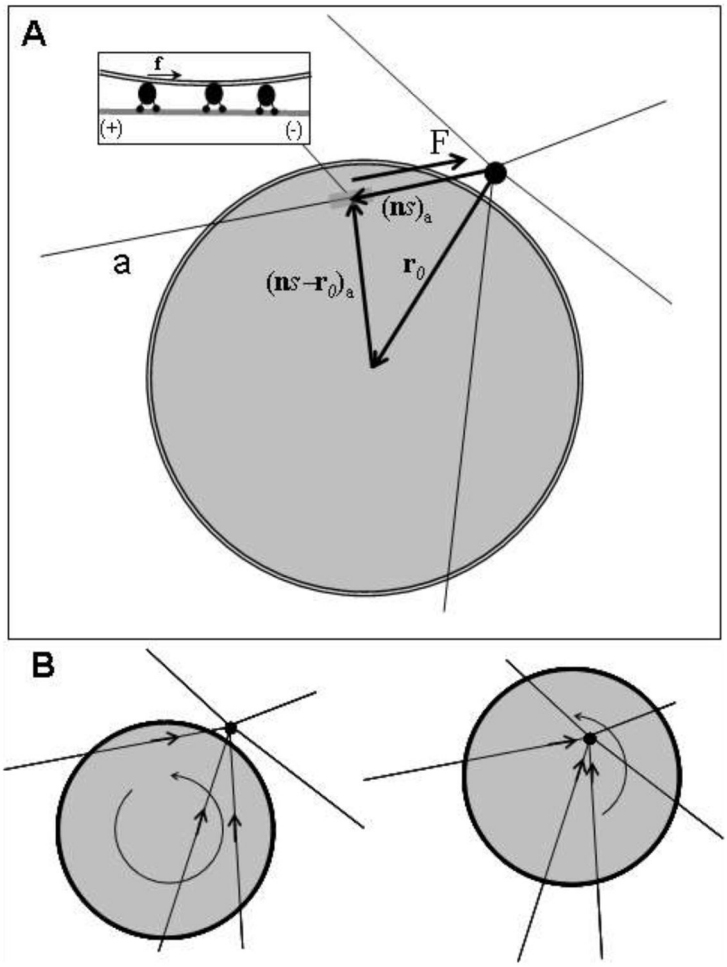

FIGURE 2.

Schematic of the nuclear rotation model. (A) Dynein molecules walking on microtubules (straight lines) generate forces (**f**) on the nuclear surface directed toward the centrosome (intersection of straight lines). The resulting mean net force **F** from the microtubule and the lever arm (vector $\mathbf{n}s - r_0$ where s is the position on the contour, **n** is a unit vector directed towards the MT plus-end, and r_0 is a unit vector directed from the centrosome to the center of the nucleus) create a torque on the nucleus. (B) The magnitude of the torque depends on the centrosome position, because the lever arm length is smaller when the centrosome is closer to the nucleus centroid. Reprinted from Wu *et al.*[48] with permission from John Wiley & Sons.

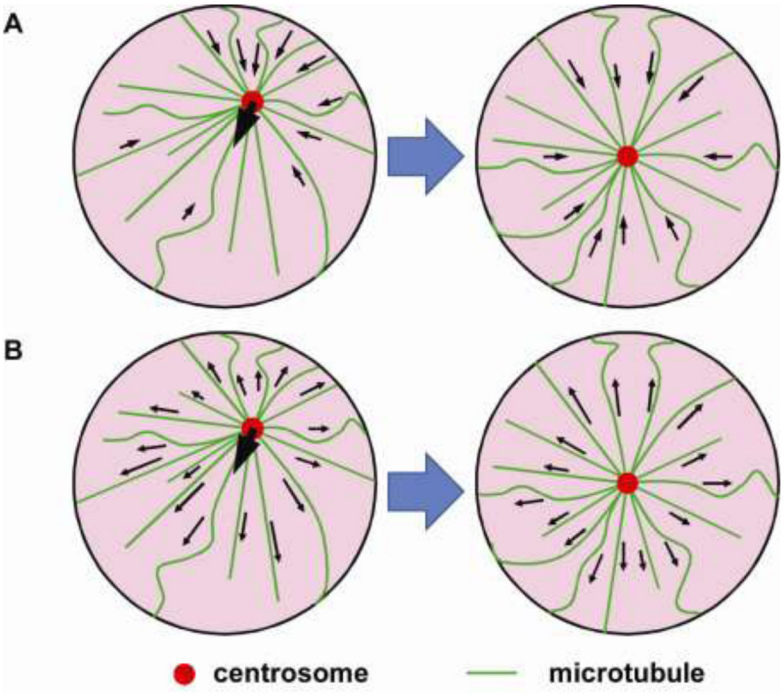

FIGURE 3.

Centrosome centering by microtubules. (A) Centering by pushing microtubules elongating and impinging on the cell periphery. Buckling forces are larger and contacts more frequent leading to a greater pushing force on the side of the centrosome closest to the cell edge. (B) Centering by pulling microtubules. Motors pull on the sides of the microtubules; hence longer microtubules exert greater force on the centrosome. Microtubules are on average longer and pull with greater force on the side of the centrosome furthest from the cell periphery.

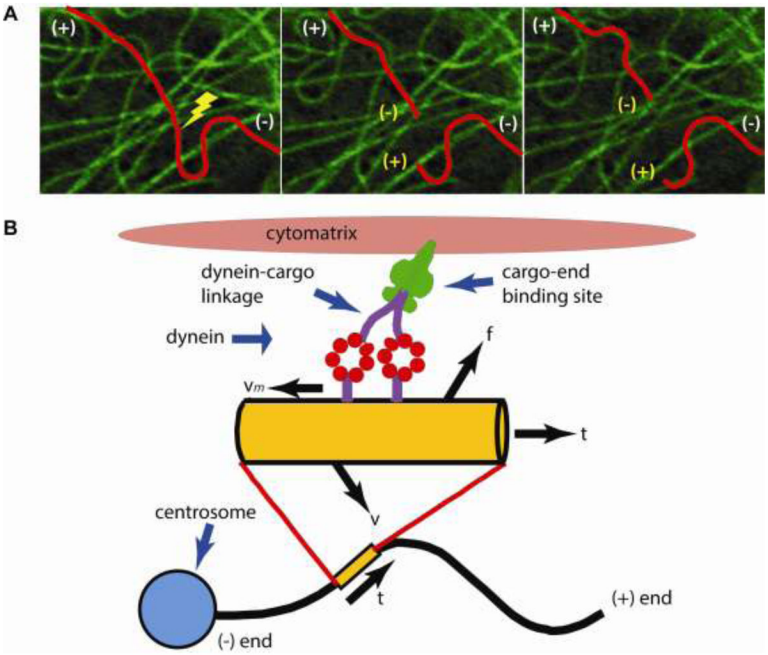

FIGURE 4.

Schematic of dynein walking along microtubules. (A) Images highlighting changes in shape after laser-based severing of a single microtubule in a living cell. The newly generated minus end of the microtubule increases in bending after laser severing. The position of the cut is indicated by the flash and the severed microtubule is highlighted in red. New plus and minus ends created upon severing are indicated by the yellow symbols; the white symbols indicate the ends of the original microtubule. (B) Cartoon of a dynein motor indicating how the minus-directed motor bound to the cytomatrix exerts a force towards the microtubule plus end. Individual dynein molecules walk towards the microtubule minus end at a speed $\boldsymbol{v_m}$ (along the local tangent direction, $\boldsymbol{t}$) that depends on the opposing force $\boldsymbol{f}$. Each segment of the microtubule moves relative to the cytoskeleton with a velocity $\boldsymbol{v}$. Adapted from Wu *et al.*[63] with permission from the Royal Society of Chemistry.

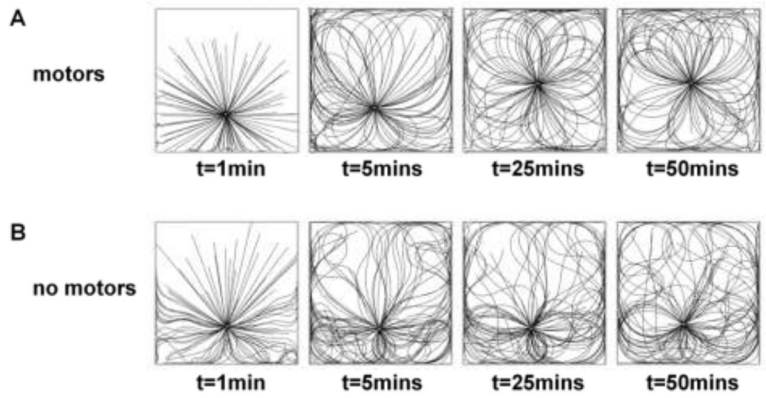

FIGURE 5.
Simulations of centrosome centering in square cells with and without dynein motor activity. (A) The motor-driven microtubules can center the centrosome in tens of minutes. (B) Without motor activity, the buckling is of Euler type. The centrosome does not center in the duration of simulation. Reprinted from Wu *et al.*[4] with permission from American Society for Cell Biology