Microtubule flux: drivers wanted
Benjamin H Kwok and Tarun M Kapoor

While the metaphase spindle maintains a constant shape and size during cell division, its major component microtubules are continuously being polymerized, depolymerized and transported towards the two spindle poles in a process called microtubule poleward flux. This process has been observed in all metazoan cells. Recent studies have indicated that Kinesin-5s, which can drive the relative sliding of microtubules, and kinesin-13s, which regulate microtubule polymerization, are directly involved in microtubule poleward flux. The availability of molecular and chemical tools to perturb protein functions together with improvements in imaging and analytical methods have allowed the examination of these two kinesins' roles in poleward flux at high temporal and spatial resolution. These advances have shed some light on the molecular mechanisms that drive microtubule poleward flux.

Addresses
Laboratory of Chemistry and Cell Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Corresponding author: Kapoor, Tarun M (kapoor@mail.rockefeller.edu)

Introduction
The stable propagation of our genomes requires accurate partitioning of replicated DNA into two daughter cells [1]. A bipolar mitotic spindle is assembled to segregate chromosomes during each cell division. Microtubules — polymers of the cytoskeletal protein tubulin — provide the mechanical framework for chromosome segregation. In humans, cell division is completed within a small fraction of the cell’s life, with mitotic spindle assembly occurring within minutes, and chromosome segregation being completed within an hour [2]. Therefore, error-free cell division requires precise control over tubulin polymerization and transport dynamics.

Intense research over the past half century has provided insight into the properties of spindle microtubules [3]. In every cell type examined, tubulin has very fast and complex polymerization dynamics. For example, in mammalian cells, tubulin in the spindle turns over with a half-life that can be less than one minute [4,5], indicating that tubulin constantly exchanges between microtubule polymers in the spindle and soluble dimers in the cytosol. This exchange results from the superposition of at least two phenomena: dynamic instability and poleward flux. Dynamic instability refers to a special property of microtubules [6] whereby individual filaments can undergo several transitions between growing and shrinking states, with growing and shrinking filaments being able to co-exist within a population of microtubules [7]. Poleward flux describes a property of spindle microtubules that can be nicely visualized using a method in which photo-activation of fluorescent tubulin is used to put a fiduciary mark on spindle microtubules (Figure 1) [8]. If the mark is in the shape of a rectangular bar that spans the width of the spindle and is positioned at the spindle center, this bar splits into two bars that move towards the opposite spindle poles at \( \sim 2 \) \( \mu \text{m/min} \) (or slower, depending on the cell types), while the spindle itself does not change shape or size. Possibly the most straightforward interpretation of this observation is that the marked microtubules move poleward and that this transport is somehow coupled to polymer assembly near chromosomes and disassembly near spindle poles. However, the precise details of the polymer dynamics underlying poleward flux and how it is linked to dynamic instability remain poorly understood.

A measure of our understanding of a cellular process is our ability to reconstitute it from purified components. In a recent study, some of the key aspects of dynamic instability have been reconstituted with pure tubulin and recombinant microtubule-associated proteins [9]. Poleward flux has not been reconstituted in vitro but key advances have been made recently, and some of these are highlighted in this review.

Spindle microtubule organization
A detailed and precise understanding of the organization of microtubules within the spindle is needed for a complete description of spindle microtubule dynamics. Currently, good estimates for the lengths of all spindle microtubules, their numbers, and the positions of the two ends of each filament, known as plus- and minus-ends, are lacking for most model systems or cell types studied.

For the purpose of this review, and in most current qualitative models, spindle microtubules are divided into two types, kinetochore and non-kinetochore (Figure 1). Kinetochore microtubules mediate chromosome–spindle attachment, and bundles of these microtubules are typically more stable to perturbations that depolymerize...
microtubules, such as temperatures below physiological conditions [10]. Therefore, electron microscopy analysis has been possible for these microtubules. In mammalian cells, kinetochore microtubules consist of parallel bundles of long microtubules [11]. The number of kinetochore microtubules needed for the attachment of one chromosome to the spindle varies among different organisms, being typically 20–30 in mammals and only one in budding yeast [12]. Even these relatively stable microtubules turn over on a time-scale of minutes and the half-lives increase from 3 min in prometaphase to 7 min in metaphase [13], corresponding to the stabilization that is associated with proper attachments of chromosomes to opposite poles of the spindle. Importantly, heterogeneity of dynamics within a single kinetochore bundle has been shown, with polymerizing and depolymerizing plus-ends simultaneously interacting with one kinetochore [14]. The non-kinetochore microtubules, which can represent ~70% of the spindle microtubules in mammalian cells, are much more dynamic than the kinetochore microtubules (with a half-life of ~20s) [4,5,13]. Because of these fast dynamics, the precise organization of these spindle filaments is less well understood.

Different mechanisms contribute to microtubule formation, including centrosomal and non-centrosomal pathways, requiring the constant incorporation and alignment of newly formed microtubules within the spindle [15]. It is generally accepted that a subset of microtubules must originate from one spindle pole and extend towards the opposite pole. The interaction of these microtubules, in either a parallel or an antiparallel manner, contributes to the maintenance of bipolar spindle size and shape. The differences between the dynamics of the different types of spindle microtubules and the heterogeneity within each type have only recently been observed using high-resolution microscopy [4,16,17]. The discussion below takes into account only some of these properties and focuses more on some recent findings using molecular perturbations and recombinant proteins.

Can antiparallel microtubule sliding drive flux?

One prominent aspect of flux is the antiparallel movement of microtubules in the middle of the spindle [18]. It is possible that a motor protein slides these microtubules apart, in a similar manner to myosin in the muscle, and that this process can power poleward flux.

Kinesin-5s form a widely conserved family of slow plus-end-directed microtubule-based motor proteins [19,20]. For at least two reasons, these kinesins have been favored to be the molecules driving antiparallel microtubule sliding in the spindle. First, loss of kinesin-5 function in all cell types examined leads to monopolar spindles in which antiparallel microtubule overlap is absent [21]. Second, these motor proteins form tetramers with pairs of motor domains at each end of a dumb-bell-shaped rod [22]. This unique organization would allow the motor to bind two microtubules simultaneously.
Recently, kinesin-5’s motor function has been examined in controlled *in vitro* experiments [23**]. In this study, one set of microtubules was immobilized on a coverslip surface. The chemistry of the surface was then modified to prevent the binding of other microtubules, which were left free-floating or tethered to optical traps for micromanipulation. Recombinant full-length tetrameric Eg5, the vertebrate kinesin-5, could crosslink surface-immobilized and suspended microtubules. The Eg5-driven movement depended on the relative orientations of the crosslinked microtubules (Figure 2). Parallel microtubules were aligned into bundles. Antiparallel microtubules moved apart with an average speed twice that observed for orientations in which the motor could only walk on one microtubule, for example when the motor interacts with the end of one filament and the second filament is bound along its length. For microtubules that interacted at different angles, the observed movements could be accounted for by vector sums of Eg5’s motility along each filament. These findings indicate that Eg5 has the properties needed to drive the antiparallel sliding of spindle microtubules.

Another motor protein known to drive the relative motion of two cytoskeletal filaments is myosin in the muscle. This motor is non-processive, dissociating from actin filaments after each enzymatic cycle [24,25]. To transmit force between two filaments, muscle myosin forms thick filaments made up of multiple motors such that when one motor dissociates, other motors interact with the actin tracks, keeping them crosslinked. Similar to myosin, it may be possible that kinesin-5s are also non-processive motors that must function in higher-order assemblies rather than as single tetramers. Alternatively, like conventional kinesin, which can undergo many ATP-hydrolysis cycles to walk as many as 100 successive steps along microtubules [26], each pair of motor domains in the Eg5 molecules could be processive. This would allow individual Eg5 molecules to crosslink microtubules in the spindle and drive their relative sliding. This ‘double processivity’ in the tetrameric kinesin-5 could be a specialization that is be better suited to the dynamic spindle microtubules than to actin in the muscle. Recently, single molecule studies with Eg5 have shown that this motor is processive [27,28**]. Imaging the movements of single full-length tetramers on microtubules revealed that, in addition to its directional motility, Eg5 can also stay bound to microtubules and diffuse [28**]. Such a mode of motion on microtubules has been observed for other kinesins and is currently a poorly understood property that may be common to many motor proteins. How this additional mode of motion contributes to Eg5’s crosslinking function and whether it generates any friction during the relative motion of two microtubules is not known. Additional studies of the micromechanical properties of kinesin-5 will be needed to fully explain this motor protein’s role in driving poleward flux and to

**Figure 2**

Illustration of the microtubule sliding activity of Eg5 *in vitro*. Three scenarios involving microtubule crosslinking by Eg5 are shown. (a) In the parallel scenario, the suspended microtubule does not move relative to the surface-bound microtubule (velocity v = 0) while Eg5 moves at its own speed (v) towards the plus ends (+) of both microtubules. (b) In the antiparallel case, Eg5 walks on both microtubules at the speed v to slide the crosslinked microtubules apart. The movement of the suspended microtubule relative to the surface immobilized microtubule will be at the speed (2v) twice Eg5’s speed on a single microtubule. (c) When the crosslinked microtubules are at an angle (orthogonal), the velocity of the suspended microtubule’s movement will be equal to the vector sum of those of Eg5 on both microtubules.
interpret the consequences of perturbing its function in the context of the whole spindle.

While the first studies examining kinesin-5’s function in the spindle suggested that this motor did not contribute to poleward flux [29], recent studies involving chemical inhibitors for this protein and improved methods for imaging and analysis have provided a different answer [30]. One factor that has complicated analysis of kinesin-5’s role in driving antiparallel microtubule sliding is the fact that, in the absence of kinesin-5 function, monopolar spindles form. To generate bipolar spindles without Eg5 activity, additional molecular or mechanical perturbations are needed. In a recent study, Miyamoto et al. examined the role of Eg5 in driving poleward flux in bipolar spindles assembled in meiotic Xenopus egg extracts. In this study, poleward flux was examined using fluorescent speckle microscopy, a method in which heterogeneity in the fluorescence of spindle microtubules allows tubulin transport and polymerization dynamics to be tracked. An automated analytical method based on a cross-correlation algorithm was used to track fluorescently-labeled tubulins and rapidly determine the average flux rate from many spindles. They took advantage of an observation that spindle collapse associated with Eg5 inhibition can be inhibited by ‘pinning down’ the spindles between coverslips used for imaging [31]. How this mechanical perturbation works is unclear, but it yielded bipolar spindles that were morphologically similar to unperturbed spindles. Chemical inhibition of Eg5 in the ‘pinned’ bipolar spindles suppressed poleward flux in a dose-dependent manner. These data are consistent with Eg5 driving antiparallel microtubule sliding in the spindle and indicate that this process is required for poleward flux of microtubules.

The above findings predict that Eg5-dependent sliding would not be possible without antiparallel microtubule overlap, and therefore that monopolar spindles, which have only parallel microtubules, would not flux. Consistent with this hypothesis, poleward flux was not observed in the monopolar spindles that form during the early stages of spindle assembly around demembraned sperm nuclei in the Xenopus egg extract [32]. In addition, monopolar spindles assembled in these cell-free extracts immuno-depleted of Eg5 did not flux either [30].

However, analysis of monopolar spindles in cultured mammalian cells indicates that antiparallel microtubule sliding may not be an absolute requirement for poleward flux. Cameron et al. used cell-permeable Eg5 inhibitors to assemble monopolar spindles in kangaroo rat PtK1 cells [33]. Quantitative fluorescent speckle microscopy (qFSM) and photoactivation of fluorescence were used to track microtubule dynamics. qFSM involves computer-based methods, different from the cross-correlation method described above, to detect and track fluorescently labeled tubulin speckles automatically [34]. Poleward microtubule flux was observed in these monopolar spindles, although at a slightly reduced rate (~0.5 μm/min) relative to the slow flux observed in untreated bipolar spindles (~0.6 μm/min). It is known that bipolar metaphase spindles do not collapse in mammalian cells when Eg5 inhibitors are added, unlike spindles assembled in Xenopus egg extracts [35]. This allowed Cameron et al. to examine Eg5’s contribution to poleward flux in bipolar spindles. As in monopolar spindles, a reduction in flux rate was observed in the bipolar spindles when Eg5 was inhibited. These data suggest that mechanisms other than Eg5-dependent antiparallel sliding can also contribute to poleward flux. The relative contributions of these different molecular mechanisms may vary between cell types or between mitosis and meiosis. In addition, these differences may also be due to the different types of analysis used to examine flux in cells and in cell-free extracts. The study by Cameron et al. focuses on kinetochore microtubules while Miyamoto et al. characterize mainly non-kinetochore microtubule dynamics. However, both studies are consistent with a model in which Eg5-dependent antiparallel sliding of microtubules plays a significant, although not necessarily essential, role in driving poleward flux in spindles.

Other mechanisms that may drive flux
In addition to kinesin-5-dependent microtubule sliding, the disassembly of microtubules at their minus ends, near spindle poles, may ‘reel in’ microtubules to drive flux. The identification of kinesin-13 motors that can depolymerize microtubules from both ends in vitro raised the possibility that poleward flux can be driven by these motor proteins [36,37]. To examine this, Rogers et al. used inhibitory antibodies to block the function of kinesin-13s in living Drosophila syncytial blastoderm-stage embryos [38]. Fluorescent speckle microscopy has revealed that spindles in these embryos flux at ~2 μm/min [39,40], similar to the rates observed in spindles assembled in Xenopus egg extracts [30,32]. Inactivation of the spindle pole-associated kinesin-13, KLP10A, resulted in increase in spindle length at about twice the rate of flux. Persistent fluorescent tubulin speckles were observed at spindle poles, consistent with the inhibition of microtubule disassembly at both ends of the spindle.

Other recent studies examining kinesin-13 function in human cells also support a role for these motor proteins in driving flux. Knockdown of Kif2a, a kinesin-13 that is mainly concentrated at spindle poles, yields monopolar spindles [41]. Surprisingly, simultaneous depletion of Kif2a and another kinesin-13, MCAK, results in normal size bipolar spindles [42]. Photoactivation of fluorescence revealed that microtubules in these spindles did not flux poleward.
However, compelling evidence that microtubule minus-end disassembly drives poleward flux is lacking. To ‘reel in’ microtubules, kinesin-13s would have to be processive and somehow anchored at spindle poles. Treatment of *Xenopus* spindles with inhibitors of dynein/dynacitin suppresses microtubule disassembly near spindle poles, as spindles elongate [43*]. Kif2a targeting to spindle poles is also disrupted by these inhibitors, consistent with a model in which this kinesin-13 disassembles microtubules at spindle poles to drive poleward flux. However, the micromechanical properties of this kinesin and the basis of its spindle pole targeting remain poorly characterized. Currently, a marker to track the positions of microtubule minus-ends in spindles is not available and it therefore remains unknown whether Kif2a disassembles microtubule from minus-ends. In addition, it is possible that the observed effects on poleward flux resulting from kinesin-13 inhibition are indirect effects of suppressing plus-end microtubule dynamics.

There is yet another mechanism that could potentially drive poleward flux: the generation of a pushing force by microtubule polymerization at plus ends. This hypothesis is supported by a recent study examining the function of a family of microtubule plus-end tracking proteins, named CLASPs [44]. In this study, Maiato *et al.* took advantage of the fact that *Drosophila* has only one CLASP and used RNA-interference to knock down this protein efficiently in S2 cells. Photobleaching of fluorescent tubulin was used to mark kinetochore microtubule bundles. These marks translocated polewards in untreated cells at 0.7 micron/min. In the absence of CLASP, this poleward translocation was inhibited. Laser microsurgery was used to cut kinetochore microtubule bundles. In control cells, these microtubule bundles regrew, and without CLASP, this recovery was blocked. Together, these data indicate that kinetochore microtubule poleward flux may require CLASP to modulate microtubule polymerization at kinetochores. It is also possible that plus-end-directed motor proteins, such as CENP-E (kinesin-7) and chromokinesins (kinesin-4) [45], also contribute to the poleward translocation of kinetochore and non-kinetochore microtubules. In addition, it is known that tubulin polymerization can do mechanical work and generate force. The direct contribution of these other mechanisms to poleward flux is likely to be explored in coming years.

**Conclusions**

It is now evident that kinesin-5s, which can drive the relative sliding of microtubules, and kinesin-13s, which regulate microtubule polymerization, contribute to poleward flux. The coordination of these two mechanisms and their relative contributions to flux remain mysterious.

The variations in the contributions of these mechanisms may represent specializations important for different types of cell divisions, such as fast embryonic division cycles or slower somatic divisions, in which checkpoints can arrest cell division for hours. Coupling between these mechanisms may rely on mechanical feedback — for example, microtubule pushing through sliding can activate polymerization, and in the absence of proper polymerization dynamics, sliding can be blocked, as microtubule focusing at spindle poles may constrain elongation. Consistent with this hypothesis, when Eg5 is inhibited in *Xenopus* extract spindles, depolymerization of microtubules near the poles is also suppressed [30*]. When the function of two kinesin-1.3s is blocked, the spindle length does not change and therefore microtubule sliding activity must be suppressed [41*,42**]. Additionally, when spindle pole organization is disrupted, microtubule sliding continues and spindles elongate without proper microtubule disassembly [43*,46*]. To understand the interdependency between microtubule sliding activity and microtubule polymerization activity, it will be important in future experiments to measure the forces generated by kinesin-5-driven antiparallel microtubule sliding and kinesin-13-driven microtubule depolymerization, both *in vitro* with purified components and, subsequently, in bipolar spindles.

**Acknowledgements**

We thank members of the Kapoor laboratory for their helpful discussions and comments during the preparation of this manuscript. T.M.K. and B.H.K. are grateful to the NIH/NIGMS (GM65933) for support. B.H.K. is a Merck postdoctoral fellow.

**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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activity and antiparallel microtubule sliding activity, leading to spindle expansion at twice the flux rate.


Similar to [43], spindle elongation is observed when microtubule depolymerizing activity was inhibited via disruption of the spindle poles. Antiparallel sliding activity driven by Eg5 is shown to be the major contributing factor to the observed spindle expansion.