Mitotic motors and chromosome segregation: the mechanism of anaphase B

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Abstract
Anaphase B spindle elongation plays an important role in chromosome segregation. In the present paper, we discuss our model for anaphase B in Drosophila syncytial embryos, in which spindle elongation depends on an ip (interpolar) MT (microtubule) sliding filament mechanism generated by homotetrameric kinesin-5 motors acting in concert with poleward ipMT flux, which acts as an ‘on/off’ switch. Specifically, the pre-anaphase B spindle is maintained at a steady-state length by the balance between ipMT sliding and ipMT depolymerization at spindle poles, producing poleward flux. Cyclin B degradation at anaphase B onset triggers: (i) an MT catastrophe gradient causing ipMT plus ends to invade the overlap zone where ipMT sliding forces are generated; and (ii) the inhibition of ipMT minus-end depolymerization so flux is turned ‘off’, tipping the balance of forces to allow outward ipMT sliding to push apart the spindle poles. We briefly comment on the relationship of this model to anaphase B in other systems.

Introduction
Mitosis is the process by which identical copies of replicated genetic instructions, packaged into chromosomes, are distributed to the daughter products of every cell division. This process is thought to occur in virtually all cells on Earth, prokaryotes as well as eukaryotes, using the mitotic spindle, a subcellular protein machine whose moving parts, at least in eukaryotic cells, consist of dynamic MTs (microtubules) plus ensembles of kinesin and dynein motors, which cooperate to orchestrate the separation of duplicated DNA [1–3]. Chromosomes are segregated by two complementary processes, chromosome-to-pole motility (anaphase A) and spindle elongation (anaphase B). The relative timing of anaphase A and B and the extent to which each is utilized varies in different systems, for example, mitotic spindles in Drosophila embryos and S2 cells utilize a combination of both processes [4–6], whereas in Saccharomyces cerevisiae and Caenorhabditis elegans embryos [7,8], spindle elongation is the major mechanism of chromosome segregation. In the present paper, we review current research on anaphase B spindle elongation in Drosophila. A review of early anaphase B work can be found in [9], whereas [10] is a recent review on cell cycle control of anaphase B.

Spindle elongation during anaphase is accompanied by the sliding apart of AP (antiparallel) ipMT (interpolar) MT bundles [11–13]. Depending upon the cell type under consideration, this can reflect the generation of compressive forces within these bundles which push apart the spindle poles, the generation of tensile forces on astral MTs at the cell cortex which pull apart the spindle poles, or a combination of both. Kinesin-5, a proposed bipolar homotetrameric motor that can cross-link and slide AP ipMTs apart, has emerged as an essential motor for mitosis in most systems studied, presumably by serving to slide apart ipMT bundles and exert pushing forces on the poles [14–17]. Exceptions include C. elegans embryos, where kinesin-5 serves as a brake that controls the rate of pole–pole separation driven by cortical pulling forces [18,19], and Schizosaccharomyces pombe, where another motor, kinesin-6, is proposed to push apart the spindle poles [20].

Kinesin-5 cross-links and slides antiparallel ipMTs apart
The native kinesin-5 holoenzyme was first purified from Drosophila embryos as a slow plus-end-directed bipolar homotetrameric complex capable of cross-linking MTs into bundles [21–23]. Inhibition experiments revealed that this kinesin-5 (also known as KLP61F) is necessary for spindle maintenance and elongation [16,24,25]. The injection of anti-KLP61F antibody into these embryos led to spindle collapse at the end of prometaphase, driven by the minus-end-directed kinesin-14 Ncd, which opposes the action of KLP61F [16,24], suggesting that the bipolar spindle is maintained by a balance of forces [26].

In vitro motility assays revealed that purified Xenopus and Drosophila kinesin-5 can cross-link MTs and move along both of them simultaneously [27,28], so that it pushes AP MTs apart and bundles parallel MTs. It has a 3-fold preference for cross-linking AP MTs [28] and its directional motion is activated by MT–MT cross-linking [29]. Thus kinesin-5 can diffuse along single MTs and use its mechanochemical cycle to generate force upon cross-linking adjacent AP MTs. Studies in yeast in which engineered tetrameric or dimeric constructs

Key words: anaphase B, mitotic motor, poleward flux, spindle elongation.

Abbreviations used: AP, antiparallel; FRAP, fluorescence recovery after photobleaching; FSM, fluorescent speckle microscopy; ip, interpolar; MT, microtubule.

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Figure 1 | Microtubule dynamics during the anaphase B switch

(A) Kymograph of time-lapse FSM movies of Drosophila embryo injected with a low concentration of rhodamine–tubulin reveals that, during pre-anaphase B (i.e. metaphase (Meta) and anaphase A (ana A)), the spindle maintains a constant length, speckles of fluorescent tubulin flux towards the spindle poles at 0.05 μm/s, but during anaphase B, speckles move away from the equator at the same rate as the poles, as shown in the cartoon where blue lines are ipMTs with orange speckles. Thus ipMTs persistently slide apart, but before anaphase B (AnaB), ipMTs depolymerize at spindle poles creating flux; then at anaphase B onset, depolymerization ceases and the poles are pushed apart. (B) During anaphase B, the rates of poleward flux and spindle elongation are inversely related, showing that poleward flux needs to be inhibited to allow efficient spindle elongation. Grey dots are experimental data from control and KLP3A-inhibited embryos, orange dots are from mathematical modelling. (C) Molecular model: AP ipMTs are constantly slid apart by KLP61F (green). Before anaphase B, MTs are depolymerized at the poles by KLP10A (purple), thus keeping the spindle length constant. At anaphase B onset, depolymerization stops and KLP61F-driven sliding of AP ipMT bundles leads to spindle elongation. (D) The onset of anaphase B requires cyclin B degradation and is accompanied by two important changes: MT depolymerization at the poles is inhibited (see C), and MT plus ends invade the spindle equator forming a robust midzone. These changes allow KLP61F to drive spindle elongation. Adapted from [4,14,35,37] with permission.

were the only functional forms of kinesin-5 showed that, even though dimeric forms can bind MTs, only homotetrameric forms are fully functional in vivo [30]. Kinesin-5 thus has biochemical properties allowing it to act via a sliding filament mechanism, in which it cross-links MTs, organizes parallel MTs into bundles and slides apart AP ipMTs to push spindle poles outward [15].

MT depolymerization at the poles maintains the spindle at constant length and is turned off to allow spindle elongation

Poleward flux, the movement of tubulin subunits within MTs from the equator towards the poles [31], has been observed in all systems examined to date except yeast. FSM (fluorescent speckle microscopy) [32,33] of Drosophila spindles revealed that tubulin speckles move away from the equator at a constant rate throughout metaphase and anaphase (Figure 1A). However, whereas tubulin subunits moved poleward and pole–pole spacing remained constant throughout pre-anaphase B (metaphase and anaphase A), during anaphase B, tubulin subunits moved away from the equator at the same rate as the poles. This observation led to the hypothesis that AP ipMTs are constantly slid apart, but this constant sliding is converted into poleward flux by depolymerization at the poles before anaphase B onset and that turning off this depolymerization allows spindle elongation [4] (Figure 1A). Interestingly, the inhibition of the kinesin-4 KLP3A, which organizes ipMTs into robust bundles [34], caused poleward flux to persist and anaphase spindle elongation to fail. A plot of spindle elongation rate as a function of poleward flux during anaphase B showed that the rate of elongation is inversely proportional to the rate of flux [35] (Figure 1B), consistent with our model that depolymerization at the poles can control both the rate and onset of spindle elongation and that it must be inhibited for robust spindle elongation.
This depolymerization at the poles is likely to be due to the activity of the kinesin-13 KLP10A, whose inhibition leads to a persistent spindle elongation with no poleward flux [36]. This suggests that kinesin-5 KLP61F continuously slides AP ipMTs apart and that the kinesin-13 KLP10A depolymerizes MTs at the poles leading to poleward flux and maintaining the spindle at a constant length. At anaphase B onset, this depolymerization is turned off, allowing kinesin-5-driven ipMT sliding to exert forces on the spindle poles, thus driving spindle elongation (Figure 1C). A quantitative mathematical model based on this qualitative model showed that this mechanism is consistent with the experimental data [35]. This model assumes that KLP61F is responsible for outward sliding throughout and thus is necessary for poleward flux. To test this, we used partial inhibition of KLP61F: antibody microinjection leads to a spatial gradient of inhibition within the syncytium, with maximal inhibition causing spindle collapse, as expected, and partial inhibition producing short spindles that exhibit no poleward flux [14], supporting the idea that KLP61F is necessary for flux.

MT plus end dynamics change at anaphase B onset
At anaphase B onset, a change in MT organization occurs. FRAP (fluorescence recovery after photobleaching) experiments revealed that tubulin turns over rapidly in the mitotic spindle. In Drosophila syncytial embryos, bleached regions anywhere in the spindle recovered with a half-time of \( \sim 5 \text{ s} \) [37]. Before anaphase B, this fluorescence recovery was complete; however, after anaphase B onset, it was incomplete near the poles. This suggests that, before anaphase B, MT plus ends are distributed throughout the spindle, but that they concentrate at the equator at anaphase B onset [37]. Imaging of the MT plus end tip tracker Eb1 confirmed this: Eb1 is uniformly distributed along spindle MTs until anaphase B onset, at which point it abruptly redistributes to the spindle equator [37]. This reorganization is dependent on cyclin B degradation because, in the presence of non-degradable cyclin B, MT plus ends do not redistribute to the equator, poleward flux persists (D. Cheranbamthur, I. Brust-Mascher and J.M. Scholey, unpublished work), and the spindle does not elongate [37]. We hypothesize that the reorganization of spindle MT plus ends leads to a more robust overlap zone, which allows KLP61F motors to sustain a steady linear rate of spindle elongation. Our quantitative model predicts that this MT reorganization depends on a spatial gradient of MT plus end catastrophe events that evolves in response to cyclin B degradation [37].

Model for anaphase B in Drosophila embryos
The following model has emerged from our research: kinesin-5 motors cross-link and slide apart AP ipMTs in a sliding filament mechanism. This continuous sliding apart is converted into poleward flux by kinesin-13 at the poles, which depolymerizes MTs, thus keeping the spindle poles at a constant distance. This is accompanied by net polymerization at the plus ends. After cyclin B degradation at least two changes occur to allow spindle elongation: (i) depolymerization at the poles is ‘turned off’, thus allowing the sliding apart of MTs to push the spindle poles apart, and (ii) MT plus ends concentrate at the equator, thus stabilizing the overlap zone and allowing a robust sliding by kinesin-5. These two changes lead to a robust spindle elongation (Figure 1D). It is worth emphasizing that, in Drosophila syncytial embryos, experiments using mutants suggest that cortical forces do not contribute to anaphase B [14].

Drosophila embryos expressing a functional KLP61F-GFP (green fluorescent protein) transgene were used to study the dynamics of this motor. FRAP experiments revealed that KLP61F turns over rapidly and displays the same change in recovery as tubulin, i.e. a lower extent of recovery is seen at the poles after anaphase B onset [38]. FSM revealed that a fraction of the KLP61F motors remains stationary at the equator, consistent with kinesin-5 cross-linking and pushing AP ipMTs apart while staying in place relative to the laboratory frame of reference [38]. These dynamic properties conform to a reaction–diffusion model in which KLP61F partitions between MT-bound and freely diffusing states [38].

Anaphase B in other systems
Given the recognized diversity in spindle design [39] how widespread is this mechanism of anaphase B? Kinesin-5 plays a critical role in mitosis in almost all systems (for a review, see [40]). The budding yeast S. cerevisiae contains two kinesin-5 motors that overlap in function, with neither being essential on its own, whereas at least one of them is required for viability. In these cells, kinesin-5 is essential for spindle assembly and maintenance and is responsible for most of the spindle elongation seen during anaphase [17,41,42]. It is also clear that the proper organization of the midzone is required for continuous spindle elongation [43]. In fission yeast S. pombe, there is no poleward flux and photobleached marks move at the same rates as the poles during anaphase spindle elongation [44]. This is consistent with either pulling from the cortex or outward sliding within the spindle. However, in experiments in which the spindle pole bodies were cut off, the remaining spindle fragment was able to elongate [45], indicating that the force for anaphase B is generated from within the spindle itself, although kinesin-6 is proposed to generate this force [20]. In contrast with Drosophila embryos, kinesin-5 is not essential in C. elegans embryos. Laser ablation revealed that the midzone is dispensable, and after ablation, the poles moved rapidly towards the cortex, suggesting that elongation forces are generated at the cortex [18]. More recently, it was shown that, in kinesin-5 mutants, spindle elongation occurs at a higher rate, suggesting that kinesin-5 is in fact acting as a brake [19].
Poleward flux has been observed in most organisms studied, and kinesin-5 is necessary for flux in some [40]. Evidence suggests that the suppression of MT depolymerization at spindle poles allows spindle elongation in other systems [46,47]. The decrease of poleward flux at anaphase onset has been observed in Drosophila S2 cells [48,49], although to a smaller extent than in Drosophila embryos. Xenopus extract spindles elongated in a kinesin-5-dependent manner when depolymerization at the poles was inhibited [46]. It should be interesting to measure the rates of poleward flux before and during spindle elongation in other systems.

Finally, it is interesting to comment on the situation in prokaryotes. In their classic repicon model, Jacob, Brenner and Cuzin [50] proposed that bacterial ‘sister chromatids’ attach to the cell membrane and are segregated as a result of membrane growth between the membrane attachment sites. However, growing evidence suggests that, as in eukaryotes, bacterial cells also utilize polymer-motor-based spindle-like systems to segregate their DNA. For example, the bacterial Par M system uses an elongating filament acting as a polymer ratchet to exert compressive forces that push chromosomes apart [51], whereas the Par A system uses a depolymerizing polymer ratchet mechanism to pull the chromosomes apart [52]. Although the latter system is remarkably reminiscent of the ‘pacman-flux’ mechanism proposed for anaphase A in Drosophila [36], the former system may be a primitive form of anaphase spindle elongation.

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References


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