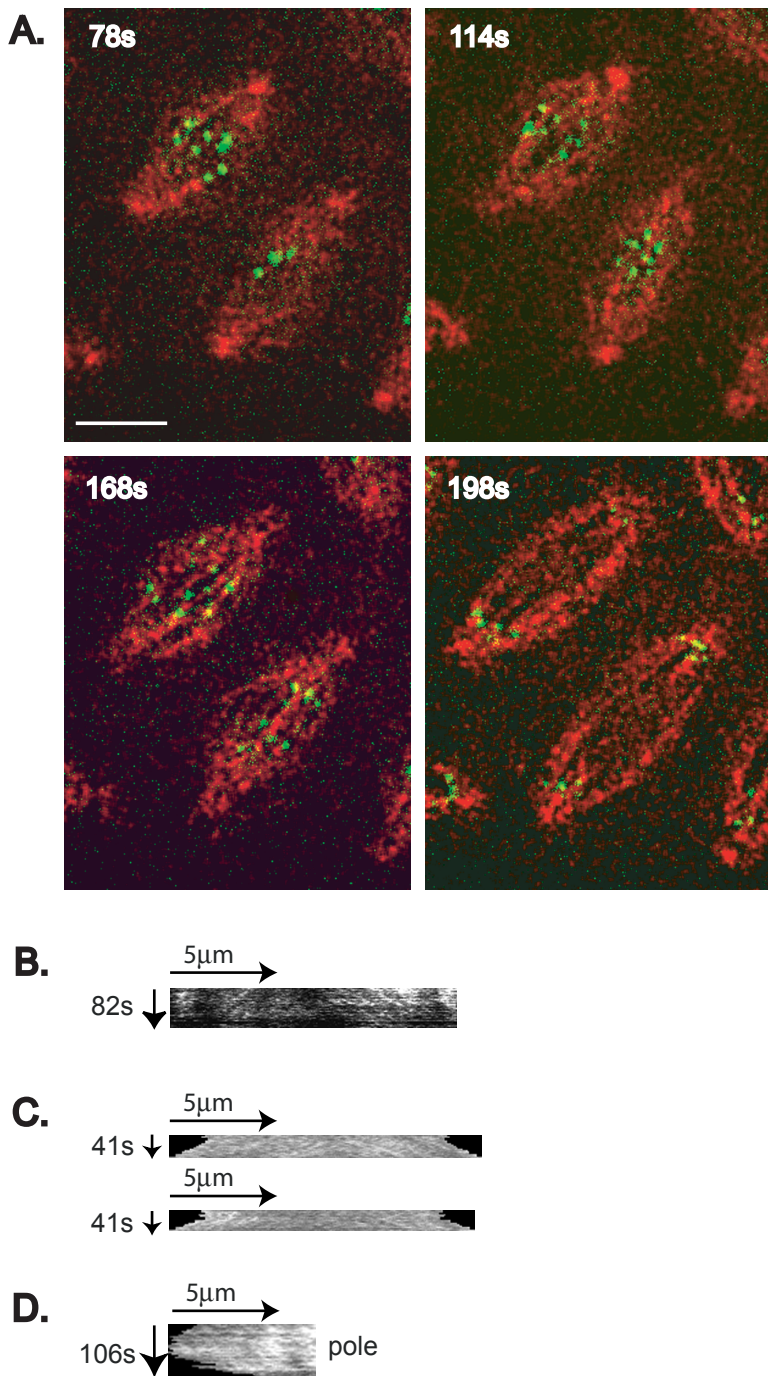


Microtubule Flux and Sliding in mitotic spindles of *Drosophila* Embryos.

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We previously proposed that spindle morphogenesis in *Drosophila* embryos involves progression through four transient isometric structures in which a constant spacing of the spindle poles is maintained by a balance of outward and inward forces generated by multiple microtubule (MT) motors and that tipping this balance drives pole-pole separation (Sharp et al., 2000, *Nature*. 407:41-47). In this study we used Fluorescent Speckle Microscopy (Waterman-Storer et al. 1999, *Meth Cell Biol* **61**:155-173) to directly visualize MT flux and MT sliding and evaluate the influence of MT dynamics on the isometric state that persists through metaphase and anaphase A and on pole-pole separation in anaphase B. We injected low concentrations of rhodamine labeled tubulin into transgenic *Drosophila* embryos expressing GFP::tubulin or GFP::CID, and followed fluorescent speckles and pole or kinetochore position. During metaphase and anaphase A, fluorescent punctae flux towards the poles along interpolar and kinetochore MTs at 0.03 μ m/s, too slow to drive chromatid-to-pole motion at 0.11 μ m/s, and during anaphase B, fluorescent punctae move on interpolar MTs away from the spindle equator at the same rate as the poles, consistent with MT-MT sliding. Loss of the minus-end directed kinesin Ncd, a candidate flux motor or brake, did not affect flux in the metaphase/anaphase A isometric state or MT sliding in anaphase B, but decreased the duration of the isometric state. We propose that flux, involving MT-polymerization at the equator and MT-depolymerization at the poles, balances motor-generated forces during the metaphase/anaphase A isometric state, and that a suppression of MT depolymerization at the poles tips the balance of forces, allowing MT polymerization at the equator and motor-generated sliding forces to drive spindle elongation during anaphase B.



Kinetochore to pole motility and microtubule flux and sliding.

A. Time-lapse confocal images of a *Drosophila* embryo expressing GFP::CID, a kinetochore marker, injected with a low concentration of rhodamine tubulin. Kinetochore to pole motility was obtained by tracking the positions of the kinetochores and the poles over time. Speckle movement was measured using kymographs of individual microtubule bundles (**B,C,D**), which could be identified as kinetochore or interpolar microtubule bundles depending on the presence or absence of GFP::CID. Time is given in seconds from Nuclear Envelope Breakdown, the scale bar is 5 μm . **B.** Kymograph of a microtubule bundle during the metaphase and anaphase A isometric state. **C.** Kymographs of interpolar bundles during anaphase B. **D.** Kymograph of a kinetochore microtubule bundle during metaphase and anaphase A, with the pole on the right and the kinetochore on the left, the bundle shortens during anaphase A, as the kinetochore moves towards the pole.