Current Biology 19, 163–168, January 27, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2008.12.017

Report

Mature *Drosophila* Meiosis I Spindles Comprise Microtubules of Mixed Polarity

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Summary

New information has been obtained recently regarding microtubule organization in *Xenopus* extract spindles. These spindles assemble in vitro by chromatin-mediated microtubule nucleation [1] and consist of randomly interspersed long and short microtubules [2] with minus ends distributed throughout the spindle [3]. Fluorescence speckle microscopy has led to the proposal that the *Xenopus* steady-state spindles contain two overlapping arrays of parallel or antiparallel microtubules with differing poleward-flux velocities [4]. Although some of these features have also been reported for *C. elegans* female meiotic spindles [5], it is not clear whether they are representative of microtubule organization and dynamics in oocyte meiotic spindles. Here we examine anastral meiosis I spindles of live *Drosophila* oocytes expressing the microtubule plus end-tracking protein, EB1, fused to GFP, and find fluorescent particles throughout the spindle and movement toward both the poles and the equator. EB1 particle velocities, corresponding to microtubule growth rates, are similar in both directions, but slower than growth from the poles in mitotic spindles of early embryos. Meiosis I spindles yielded data from photobleaching analysis showing similar microtubule growth rates and dynamics at the poles and the equator, consistent with spindle microtubules of mixed polarity, differing from early-embryo mitotic spindles.

Results and Discussion

EB1-GFP Particle Tracking in the MI Spindle

The end-binding protein, EB1, targets to polymerizing microtubule ends [6], where it has been used as a marker to identify growing microtubules and track microtubule growth [7]. We used an EB1-GFP fusion protein that labels particles in *Drosophila* early-embryo mitotic spindles (Movie S1, available online), as reported previously [8], to analyze microtubule growth in anastral-oocyte meiosis I (MI) spindles. Mature MI spindles of late stage 13 or stage 14 oocytes expressing EB1-GFP showed fluorescent puncta throughout the spindle (Figure 1A). The smallest of these were the same intensity (41 ± 1 arbitrary units [a.u.], mean ± SEM, n = 94) as the smallest discrete particles in the cytoplasm (40 ± 1 a.u., n = 83) after correction for background, which was higher in the spindle than in the cytoplasm (Figure 1B). On the basis of this analysis, the smallest particles in the spindle probably correspond to single microtubule ends. Kymographs showed particle displacement in the spindle, correlated with movement in opposite directions in time-lapse sequences, toward either the pole or the equator (Figure 1C and Movie S2). Velocities of single particles tracked manually in the image sequences (spindles, n = 5) were the same toward the pole (v = −0.19 ± 0.03 μm/s, n = 22) as toward the equator (v = 0.17 ± 0.03 μm/s, n = 17) (Figure 1D) and did not differ significantly from velocities determined from slopes of lines formed by particles in kymographs (poleward, v = −0.16 ± 0.01 μm/s, n = 135; equatorward, v = 0.18 ± 0.01 μm/s, n = 147; spindles, n = 6).

EB1 has been reported to bind to growing, but not shrinking, microtubule ends [6]; thus, the particle movement in oocyte MI spindles may, unexpectedly, correspond to microtubule growth from both the chromosomes and the poles. EB1 particle velocity in MI spindles was faster by ~3-fold than the reported growth rates (0.063 μm/s) in interphase *Drosophila* S2 cells and approximately the same as the reported shrinkage rates (0.145 μm/s) [8]. EB1 particle velocity in cycle-10 mitotic spindles of syncytial blastoderm embryos was 0.30 ± 0.02 μm/s (particles, n = 55; spindles, n = 7; embryos, n = 4), faster by ~1.8-fold than MI spindles. EB1-GFP appeared as fluorescent particles and streaks in MI spindles, but usually did not appear as comets, as reported for polymerizing microtubule ends in other cells [6, 7], although comets were observed in mitotic spindles (Movie S1).

EB1 thus tracks microtubule ends in anastral-oocyte MI spindles, appearing as fluorescent puncta or streaks that, unexpectedly, move toward either the pole or the equator. The velocities of poleward and equatorward EB1 particle movement were the same, but slower than EB1 particle motion away from the poles in mitotic spindles.

Fluorescence Flow Analysis of EB1-GFP in the MI Spindle

Given that oocyte MI spindle assembly involves chromatin-mediated microtubule nucleation [9, 10] and the unexpected finding of microtubule growth both poleward and equatorward, inferred from EB1 particle movement, we assayed the MI spindles for net microtubule movement by fluorescence flow analysis to determine the dynamic state of the spindles. We analyzed net movement of EB1-GFP in the spindle by calculating the fluorescence median position for each MI spindle half —the position on the spindle axis with equal amounts of fluorescence toward the equator and pole. We performed the calculation over time (~230 s) to determine the velocity of the median position, as a measure of net microtubule movement. The mean net velocity for the MI half spindles (n = 16) was 0.0007 ± 0.0016 μm/s (mean ± SD) toward the equator (Figure 2A). The near-zero velocity and large standard deviation indicate that the distribution of EB1 and, thus, of microtubule plus ends does not change significantly over time. The data demonstrate that there is little net poleward or equatorward EB1 motion in the MI spindle. Five spindles showed slow net poleward movement in one half and slow net equatorward movement in the other, and the remaining three spindles showed slow net equatorward motion in both halves, a distribution that is not significantly different from random (χ^2 = 2.75, 1 df, p = 0.10). Thus, fluorescence flow

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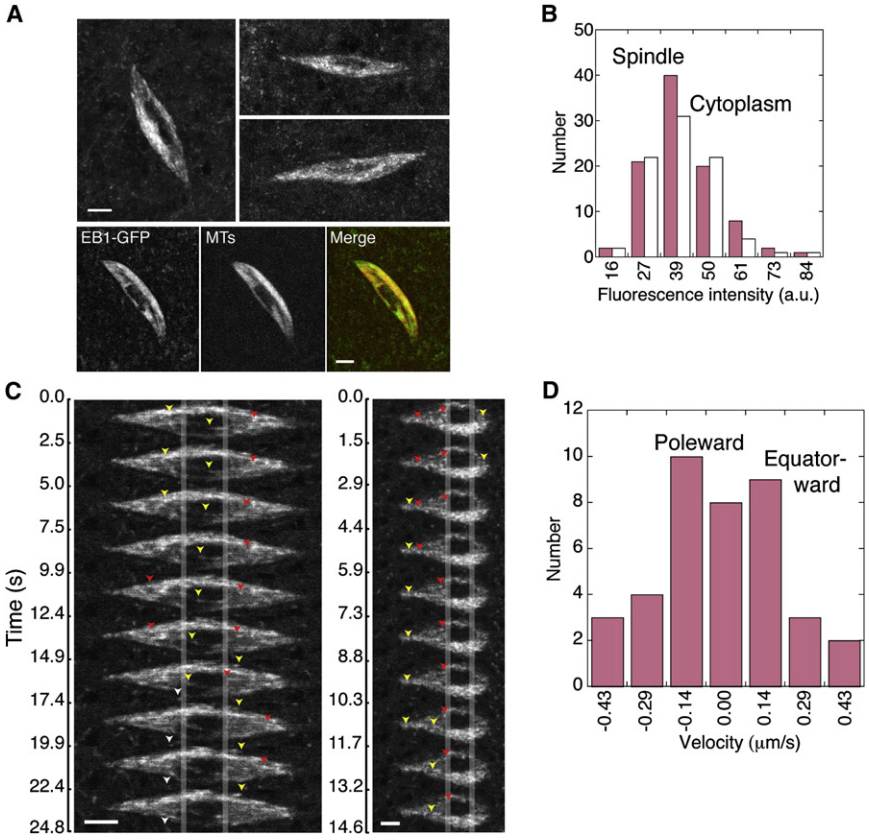

Figure 1. EB1-GFP in the MI Spindle

(A) Anastral-oocyte MI spindles from flies expressing EB1-GFP, visible as small fluorescent particles in the spindle and cytoplasm. Bottom: MI spindle from flies coexpressing EB1-GFP (left) and Ncd-mRFP, a kinesin-14 motor that specifically labels MI spindle microtubules (MTs) [23] (middle). EB1-GFP is indicated with a green color, Ncd-mRFP with red. A merged image is shown on the right; colocalized EB1 and Ncd appears yellow. The dark region at the spindle center corresponds to the meiotic chromosomes, which exclude EB1. Projections are from z-series images. Scale bars represent 3 μm.

(B) Fluorescence intensity of EB1-GFP particles ($\leq 5 \times 5$ pixels) in the MI spindle (pink) and cytoplasm (white). a.u. denotes arbitrary units.

(C) MI-spindle images showing poleward (yellow or white arrowheads) and equatorward (red arrowheads) EB1 particle movement over time. Vertical lines indicate the alignment. Scale bars represent 3 μm (left) and 2 μm (right).

(D) Particle velocity from manual tracking of particles in spindle images. "−" indicates poleward movement, "+" indicates equatorward movement.

analysis shows essentially no net change over time in EB1-GFP distribution in the MI spindle, consistent with a steady-state spindle.

By contrast, assembling cycle-10 mitotic spindles showed progressive movement of the fluorescence median from a position near the pole toward the equator, with a velocity of 0.016 ± 0.001 μm/s (mean ± SEM, n = 16) (Figure 2B). The movement of the fluorescence median indicates that plus ends are initially concentrated at the poles and become distributed across the spindle as assembly proceeds. The peak of movement toward the equator is approximately mid-metaphase; the fluorescence median then moved back toward the pole as the spindle elongated in anaphase. The velocity of movement of the fluorescence median toward the equator is ~20 times slower than the velocity of microtubule growth in the mitotic spindle, 0.30 ± 0.02 μm/s, estimated from EB1 particle tracking. The slow movement of the fluorescence median is consistent with slow structural changes in the spindle caused by changes in microtubule stability, slow poleward flux, and microtubule translocation or sliding, resulting in net microtubule flow from the poles toward the equator during spindle assembly in early metaphase.

Fluorescence flow analysis is thus consistent with the conclusion that the MI spindle is at steady state. The EB1-GFP particle tracking shows movement in the MI spindle toward both the poles and the equator, indicating that microtubules in the steady-state MI spindle grow both poleward and equatorward at the same velocity. This differs from early mitotic spindles where EB1 particles move away from the poles toward the equator and fluorescence flow is equatorward, demonstrating that net microtubule movement occurs

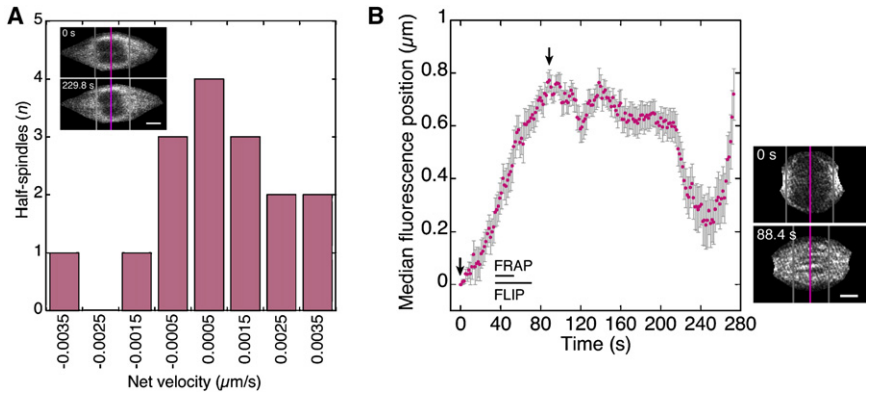

Figure 2. Fluorescence Flow Analysis of EB1 in the MI and Mitotic Spindle

(A) Velocity of EB1-GFP fluorescence median in MI half spindles measured from a fixed point outside the spindle pole. "−" indicates poleward movement, "+" indicates equatorward movement. Inset: MI spindle at beginning (top) and end (bottom) of analysis; pink line, equator; gray lines, fluorescence median position in half spindles. Scale bar represents 1.5 μm.

(B) Normalized fluorescence median position over time in mitotic half spindles. Error bars represent SEM. Velocity between the arrows is 0.016 ± 0.002 μm/s (mean ± SEM, n = 16). The mitotic spindle (right) is shown at times corresponding to those indicated by the arrows. Scale bar represents 3 μm. FRAP and FLIP assays (Figures S2 and S4) were performed at the indicated times.