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Organization of cellulose synthase trafficking and motility in the plasma membrane by the cortical microtubule array.

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Plant cell morphogenesis relies on the organization and function of two polymer arrays on either side of the plasma membrane - the a-centrosomal cortical microtubule array in the cytosol and the cellulose microfibril array in the cell wall. A principal function of the cortical microtubule array has long been hypothesized to be guidance of oriented cellulose deposition, thus creating the material anisotropy responsible for directed expansion of the cell wall during turgor-driven growth. [1].

An important limitation in testing the microtubule guidance hypothesis and in exploring the functional relationship between cortical microtubules and cellulose synthase has been the ability to observe both macromolecular complexes as dynamic systems in living cells. We have addressed this limitation by creating and expressing a functional fluorescent protein fusion to CESA6, a cellulose synthase isoform required to support rapid axial tissue growth. Combined with spinning disk confocal microscopy, this tool has permitted visualization of active cellulose synthase complexes, revealing their patterns of organization and dynamic behavior as they move through the plasma membrane while creating microfibrils. Co-visualization with labeled microtubules [2] reveals that CESA6 complexes track in a bidirectional fashion along individual microtubule bundles of cortical array (Fig. 1), and that these trajectories reorient as the cortical array reorients. In the absence of cortical microtubules, CESA6 complexes are still able to achieve an organized pattern of trajectories, but the pattern of organization differs from that in the presence of cortical array function [3].

The ability to image and track individual cellulose synthase complexes has now also allowed us to observe discreet protein delivery events at the plasma membrane (Figure 2). Mapping and analysis of these delivery events is facilitated by photo-bleaching existing protein in the plasma membrane. These experiments have revealed that cortical microtubules not only guide the trajectories of cellulose synthase complexes but also target the delivery of CESA protein to the plasma membrane. Further, cortical microtubules interact with CESA trafficking compartments by a novel mechanism that permits membrane-bound organelles to track shrinking polymer ends (Figure 3). This tip-tracking capable mechanism is not dependent on microtubule polarity and may permit efficient tethering of cargo to treadmilling microtubules [2].

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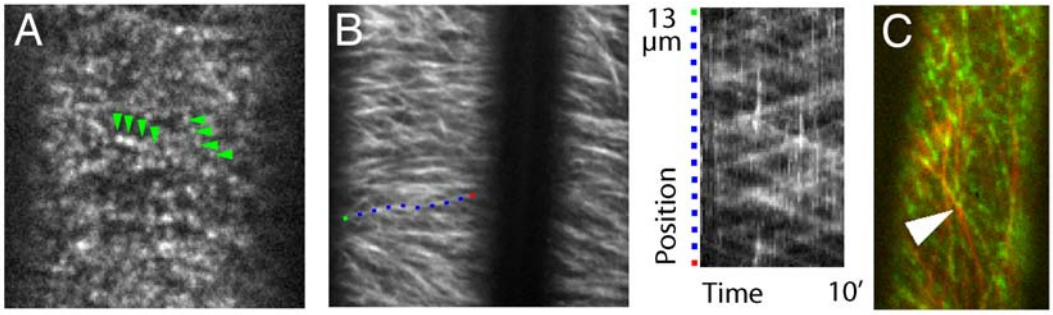

Figure 1. Imaging of dynamic CESA complexes at the plasma membrane and co-localization with cortical microtubules. (A) YFP::CesA at the plane of the plasma membrane. (B) Kymograph analysis of bidirectional complex movement. (C) YFP:CESA6 (green) co-localization with CFP:aTubulin (red).

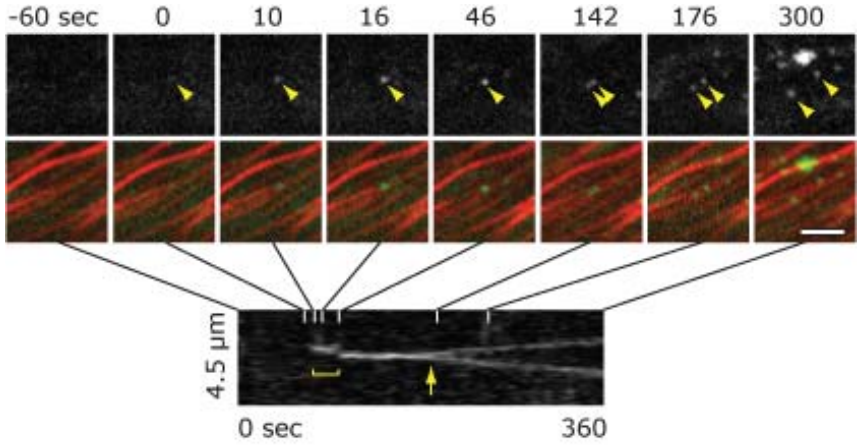

Figure 2. Delivery of two CESA complexes to the plasma membrane in association with cortical microtubules. Time series showing YFP:CESA signal (upper series, green in lower series) and CFP:aTubulin (red in lower series). The bottom panel is a kymograph taken along the axis of particle motility. At the arrow, two particles are seen to initiate movement at steady velocity in opposed trajectories, consistent with their identify as CESA complexes being displaced by the force of cellulose polymerization.

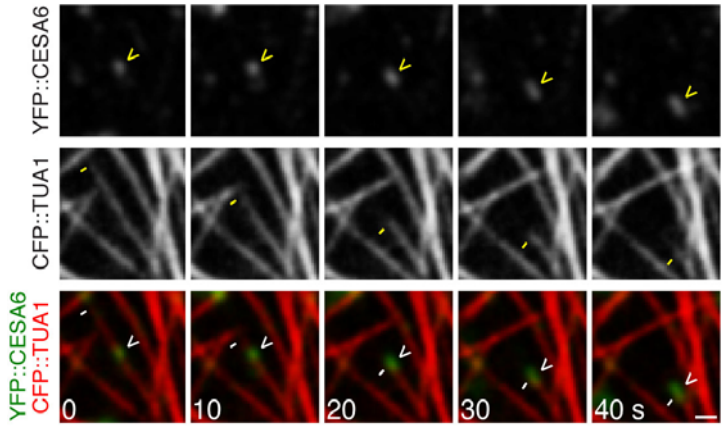

Figure 3. Small CESA trafficking compartments track depolymerizing ends of microtubules. A microtubule-tethered compartment (arrowhead) is initially stationary and then moves with the shrinking polymer end (dash). Scale bar, 1 µm.

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