AUGMIN Subunit 8 Participates in Microtubule Reorientation in Arabidopsis Hypocotyls

Microtubules have important roles in intracellular trafficking. The targeted transport of cellulose synthase along microtubule tracks determines the orientation of cellulose microfibrils and hence influences cell shape and elongation (Paredez et al., 2006; Lloyd, 2006). The ability of microtubules to alter their cellular layout in response to various stimuli is central to their function. In the rapidly elongating cells of the hypocotyl, cortical microtubules are arranged in parallel arrays transverse to the direction of maximal growth and reorient to become parallel to the growth axis when growth declines (Ehrhardt and Shaw, 2006). While the transverse arrays have been shown to arise from radial microtubule-star arrays, the formation of which heralds the onset of rapid cell growth (Sambade et al., 2012), little is known about the molecules that direct microtubule reorientation.

Now, Cao et al. (2013) have identified AUGMIN Subunit 8 (AUG8) as a plus-end binding protein that facilitates microtubule reorientation in Arabidopsis thaliana hypocotyls. Originally identified in a screen for T-DNA mutations that accentuate the ROP1 overexpression phenotype in pollen tubes, AUG8 was found to belong to the QWRF protein family. Based on a report by Pignocchi et al. (2009) that another member of this family, EDE1 (ENDOSPERM DEFECTIVE1), is a microtubule-associated protein (MAP), the authors hypothesized that AUG8 interacts with microtubules. In support of this notion, the hypocotyls of dark-grown aug8 knockdown mutants were found to be longer than those of the wild type, whereas those of AUG8 overexpression lines (AUG8-OX) were shorter. Closer inspection using immunofluorescence microscopy revealed that AUG8 has a striking effect on the orientation of microtubules in the epidermal cells of hypocotyls of five-day-old seedlings. Microtubules were transversely arranged in a greater proportion of aug8 cells (90%) than in those of the wild type (59%) and AUG8-OX (20%). Thus, AUG8 appears to negatively regulate hypocotyl elongation by altering microtubule organization.

Next, the authors confirmed that AUG8 is indeed a MAP by demonstrating that His-tagged AUG8 co-sediments with taxol-stabilized microtubules and that AUG8 promotes microtubule polymerization in vitro. To track the in vivo localization of AUG8, the authors examined aug8 hypocotyl epidermal cells transformed with both AUG8-GFP and the microtubule label, mCherry-TUA5, using time-lapse fluorescence microscopy (see figure) and kymographic analysis. AUG8 associated specifically with the plus-ends of rapidly growing microtubules. Furthermore, AUG8 localized to microtubule branch sites immediately before branching, suggesting that AUG8 promotes microtubule reorientation. An analysis of microtubule dynamics showed that aug8 cells had higher catastrophe and rescue rates than those of the wild type and that microtubules in the mutant cells spent less time in the growth phase. Thus, AUG8 appears to regulate microtubule dynamics.

Based on the observation that AUG8 binds to the plus-ends of microtubules regardless of their direction of growth, the authors predict that other factors acting upstream of AUG8 initiate microtubule reorientation. It will be interesting to identify these factors and to decipher the mechanisms underlying microtubule reorientation in response to various signals.

REFERENCES


