From Eye to Insight

PLANT CELL DEVELOPMENT AND MORPHOGENESIS

Clear Imaging of Plant Tubulin with TIRF

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Abstract

The article discusses how tubulin molecules, which make up microtubules, in plant cells can be studied with total internal reflection fluorescence (TIRF) microscopy to better understand tubulin dynamics and cell development and morphogenesis. To visualize tubulin molecules in microtubules, an imaging solution is needed that allows them to be easily resolved near the plant cell surface. Conventional widefield microscopy detects too much out-of-focus fluorescence signal leading to reduced resolution. TIRF microscopy minimizes out-of-focus fluorescence and enables the tubulin to be clearly resolved, making TIRF a useful tool for studying molecular dynamics.

Introduction

Live cell imaging techniques and molecular genetics are often utilized for the study of plant cell development and morphogenesis in terms of visualization and investigation of the organization and dynamic behavior of molecules and organelles. Of special interest is how cells generate asymmetries and specific shapes. The focus of this article is on the organization of the cortical microtubule cytoskeleton and how it functions to organize the plasma membrane and cell wall to guide patterns of cell growth and division.

Challenges

For the study of microtubules in plant cells, a fluorescence imaging solution is needed that allows microtubules near the surface of the plant cell specimen to be easily resolved. Conventional widefield fluorescence microscopy is unable to provide the high signal-to-background ratio necessary to resolve plant microtubules, due to a large amount of out-of-focus fluorescence signals.

Methods

Microtubule cytoskeleton reorganization dynamics were studied with fluorescently labeled tubulin molecules at the surface of plant cells, like *Arabidopsis thaliana* (thale cress plant), using a DMi8 S microscope with an Infinity TIRF (Total Internal Reflection Fluorescence) module. In addition, epi-fluorescence microscopy using a 100x Plan Apo objective was performed. The DMi8 S with Infinity TIRF was also used in HILO (Highly Inclined and Laminated Optical) mode. TIRF microscopy allows scientists to image only the tubulin molecules close to the coverslip and the bottom of the plant cell specimen.

Results

The images in figure 1 below show fluorescent microtubule structures in hypocotyl cells of a live Arabidopsis thaliana plant. Figure 1A shows an epi-fluorescence image of the plant. The microtubule structures are obscured by out-of-focus fluorescence, making the study of these structures impossible. Figure 1B shows the same structures imaged with HILO mode which offers a slight improvement over epifluorescence. Figure 1C shows a TIRF image with the tubulin molecules clearly visible. The results indicate that the DMi8 S microscope with an Infinity TIRF module is a great tool for imaging tubulin dynamics in plant cells. HILO mode was used to scan through the surface of the plant to find the most suitable cells for live cell imaging. This mode clears a lot of the signal-obscuring, out-of-focus light and allows for higher depth of focus than TIRF which makes finding structures of interest easier. TIRF microscopy can then be used to track tubulin dynamics in plant cells over time to study how cytoskeleton reorganization contributes to plant growth.

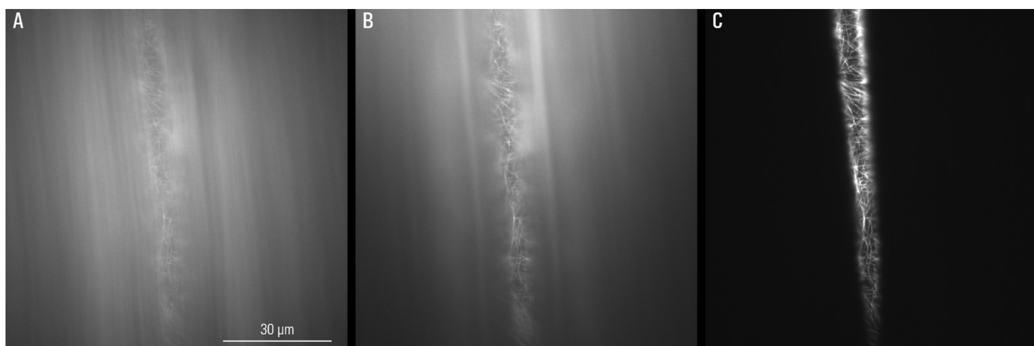

Fig. 1: Images of hypocotyl cells in Arabidopsis thaliana expressing mCherry-TUA5 to label the microtubule cytoskeleton: A) Epifluorescence; B) HILO; and C) TIRF. Images courtesy of Dr. Heather Cartwright, Dr. Jelmer Lindeboom, and Dr. David Ehrhardt of the Carnegie Institution for Science, Stanford, CA.

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