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Characterizing Microtubule Organization in the *Arabidopsis Thaliana* Root Apical Meristem via Correlative Microscopy

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Growth in plants occurs at shoot and root apices, with new tissues arising from stem cell centres known as meristems, areas analogous to animal stem cell niches (Figure 1A). Meristem size depends on a balance between the rate of uncommitted stem cell proliferation and cell differentiation, determined by different hormone levels and their cross-talk. Recent discoveries indicate that the cytoskeleton plays an important part in this process, with microtubules (MTs) regulating the hormone auxin and its transport via complex feedback mechanisms [1]. In addition, MT organization in dividing cells has also been implicated in meristem maintenance, with the *Arabidopsis thaliana* MT-associated CLASP protein playing a critical role [2, 3].

CLASP accumulates at specific cell edges, enabling MT growth around these edges and promoting the formation of MT bundles that span adjacent cell faces [4]. Unlike the transverse conformation MTs typically adopt in elongating cells (Figure 1B), CLASP promotes transfacial MT bundle formation (Figure 1C). These transfacial bundles are strongly associated with maintaining the capacity for division, an important stem cell feature.

To date, imaging of plant MTs has mainly employed immunofluorescence on fixed samples [5] or the use of fluorescent fusions to study MT dynamics using confocal laser scanning microscopy [6]. Though informative, these methods do not allow for resolving of individual MTs within bundles and the precise progression of MT configurations (or bundle association with CLASP) during stem cell differentiation is as yet not understood. To characterize the developmental switches that lead to changes in MT organization a correlative approach is necessary. High pressure freezing, freeze substitution and imaging with fluorescence light microscopy (fLM) followed by transmission electron microscopy (TEM) or tomography will enable the structural analysis of MTs at *Arabidopsis thaliana* cell edges at high resolution and in three dimensions.

Preliminary analysis has been done on an *Arabidopsis thaliana* sample containing the CLASP protein tagged with a histidine tag and GFP. An fLM image of a high pressure frozen, freeze substituted and thick-sectioned root apical meristem indicates fluorescent signal at cell edges (Figure 2). Further immunolocalization studies on this same sample (using Ni-NTA-Nanogold labelling) will confirm whether the GFP signal corresponds to the CLASP protein. Tomographic analyses can provide information about the 3-dimensional organization of microtubules at these locations. Study of multiple cells in different stages of development will provide a picture of MT progression from transfacial to transverse orientation, and the role of CLASP in these transitions.

Figures

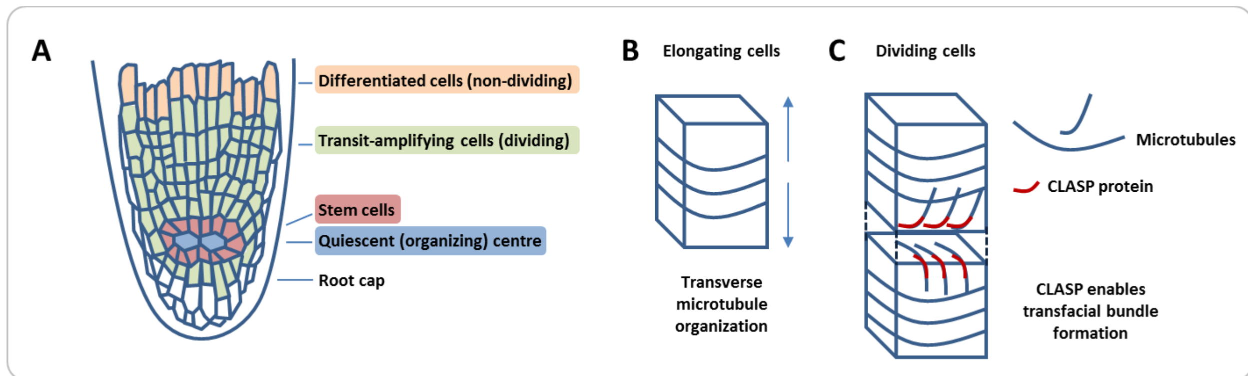

Figure 1. (A) Schematic of the plant root apical meristem and associated cell types. The quiescent (organizing) centre sends signals to the stem cells to prevent them from differentiating. Stem cells divide asymmetrically to produce daughter cells that divide a number of times in the meristematic zone before they stop dividing and terminally differentiate. (B-C) Spatial microtubule organization in cells: in elongating cells, MTs adopt a transverse organization (B), whereas in dividing cells (stem cells and dividing daughter cells), MT-associated protein CLASP enables microtubule growth around sharp cell edges (C).

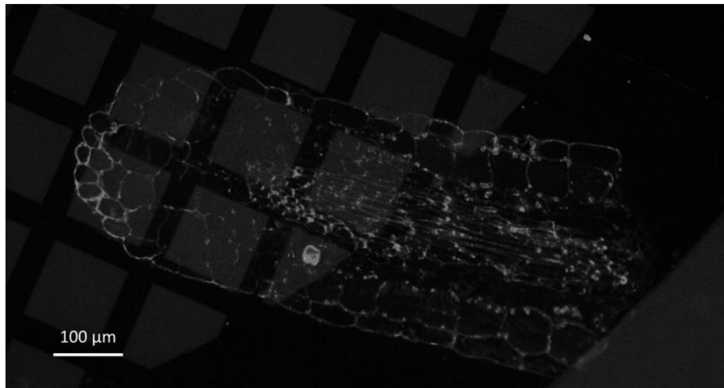

Figure 2. Fluorescent light micrograph of *Arabidopsis thaliana* containing CLASP-His-GFP. The image shows a high pressure frozen, freeze substituted and thick-sectioned root apical meristem. Green fluorescent signal is present at cell edges.

References

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