

Analyzing Gibberellin Patterning in *Arabidopsis thaliana* with the GPS1 Biosensor

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Introduction

Plant hormones are chemicals that regulate growth in response to internal and external stimuli. Gibberellins (GAs) regulate germination, cell elongation, and various other developmental programs. By studying GA patterning in developing plant tissues, we hope to identify sites of biosynthesis and transport, characterize organ-specific expression, and uncover novel GA functions in plant development. This line of research has applications in developing new high-yielding crop varieties.

Methods

The GPS1 Biosensor: Imaging of GA relies on the nGPS1 transgenic line, in which fluorescent proteins bind to the Gibberellin receptor in the nucleus. We excite the blue protein with a confocal microscope, and measure the resulting emission of the yellow protein. When Gibberellin binds to its receptor, the conformation changes, bringing the blue and yellow proteins closer and causing more emission in the yellow spectrum (fig. 1). Thus, cells that express a higher ratio of yellow to blue emission are higher in Gibberellin.

Image Analysis: Our confocal microscopes give three channels: a cell wall staining, blue emission, and transfer emission. We want to obtain ratios of intensities between the energy transfer (magenta) and blue channels. To nullify the effects of pixel noise, we segmented nuclei using the IMARIS image processing suite and developed an extension in mathematica for computing the ratio of mean intensities within each nucleus surfaces. Afterwards, we manually segmented our cell wall images (fig. 3) and plotted the computed ratio against other cell properties such as area, length, and distance from the shoot apical meristem.

Key Results

We observed GA gradients in root and shoot tissues consistent with the notion that GA causes cell elongation. GA was expressed strongly in elongating cell tissues and very weakly in dividing or immobile tissues. No direct correlation between cell area and GA expression was observed. Finally, we observed higher GA expression in guard cells, which could indicate a previously unknown role in stomatal functions.

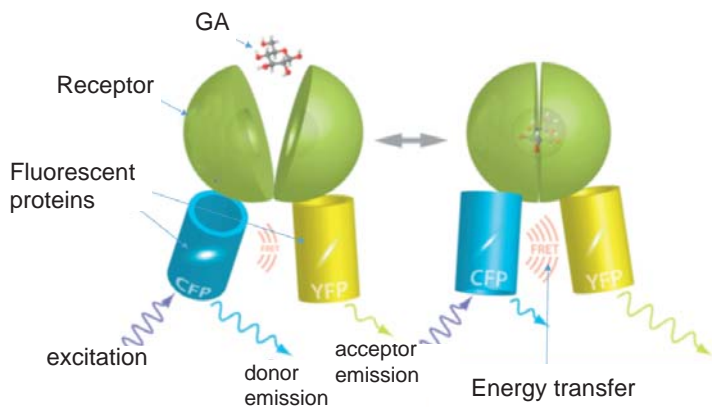


fig. 1

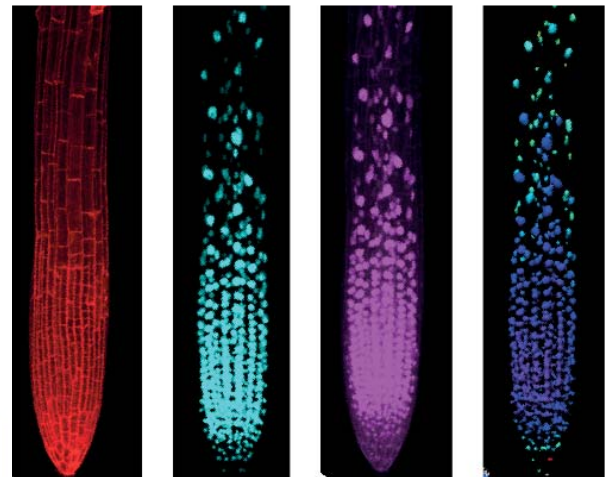


fig. 2

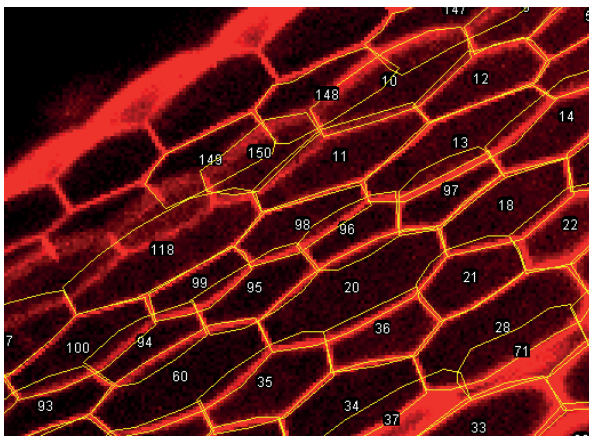


fig. 3

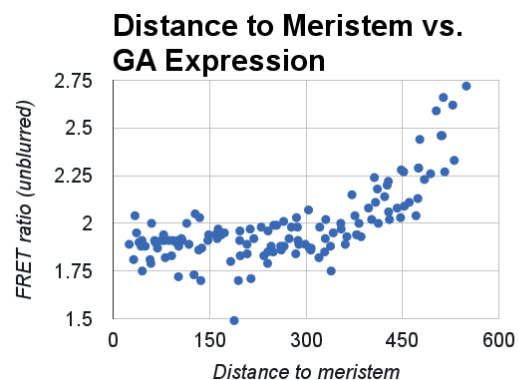


fig. 4