INTERNSHIP PROPOSAL M2 RESEARCH 2024-2025

Integrating geometry and mechanics during seed growth

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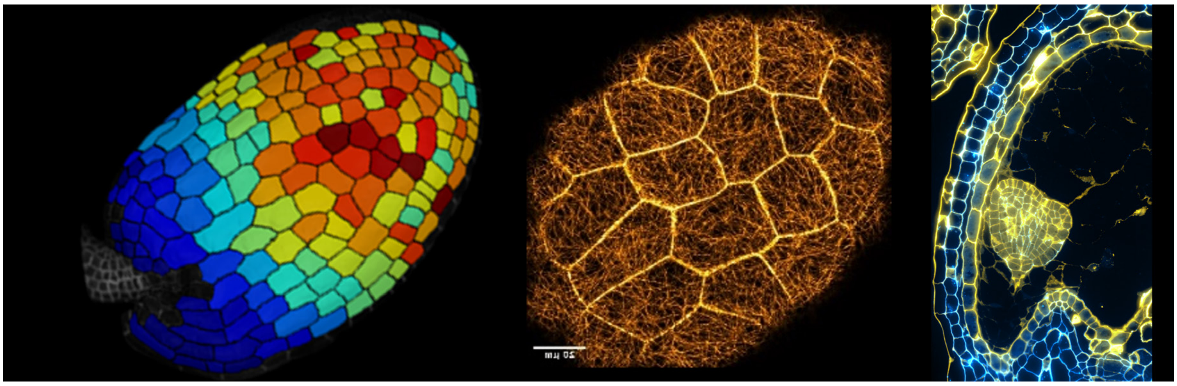

SCIENTIFIC CONTEXT:

How do multicellular organisms develop their shapes? The underlying process of morphogenesis is complex and involves the integration of mechanical, biochemical, and genetic factors across different scales, from the molecular to the tissue scale. In plants, tissue topology is fixed as cells are locked in place by their shared cell walls, necessitating precise coordination of cellular growth rates and directions to avoid mechanical conflict between cells. In recent years, it has become apparent that mechanical stresses arising at the tissue-and organ scale during growth are integrated into growth control at the cellular scale to promote robust morphogenesis. Seeds have emerged as an excellent model to study the integration of tissue mechanics into morphogenesis, as we have recently shown that spatiotemporally distinct responses to mechanical stresses at different stages of seed development contribute to both the control of seed size (Creff et al., 2023) and seed shape (Bauer et al., 2024). In developing seeds, cells initially grow preferentially along the longitudinal axis of the seed, a pattern that could be functionally linked to shape-driven mechanical stresses at the organ surface in computational models (Bauer et al., 2024). At later developmental stages, cell growth becomes isotropic (non-directional), and their growth rates are controlled through two antagonistic effects of internal pressure within the seeds, which both drives growth directly and inhibits it indirectly through promoting cell wall stiffening in the seed coat. However, it is still unclear (1) how mechanical stresses are perceived by seed coat cells and translated into growth, and (2) how cells change their growth pattern although shape-driven stresses at the organ scale do not change?

RESEARCH PROPOSAL:

Based on our latest data, we now propose that seeds use a geometry-based mechanosensing system to fine-tune the integration mechanical stress into seed growth over time. This hypothesis is based on our findings in roots, where cell edges (where two faces of a cell meet) translate mechanical signals into directional growth through the receptor-like protein RLP4 (Elliott et al. 2024). RLP4 can promote growth through recruiting the small GTPase RAB-A5c, which mediates a trafficking pathway required for directional growth (Kirchhelle et al. 2016), and its localisation at cell edges is sensitive to mechanical stresses as well as cell geometry.

We found that components of edge-based growth controls are expressed at the seed surface, and are upregulated in response to mechanical stimulation of seeds. We now propose that RLP4 acts as a mechanosensor in developing seeds to integrate local geometry and tissue-scale shape-driven stress: Initially, RLP4 patterning in polyhedral cells will primarily depend of tissue-scale shape driven stress. As cells grow, their geometry changes towards more rounded shapes, which will change RLP4 patterning independently of shape-driven stress, and thus reduces the sensitivity of cells to tissue-scale signals. This model provides a molecular basis for mechanosensing in seeds, and can explain how seeds fine-tune their sensitivity to tissue-scale mechanics over time. To test this hypothesis, we will develop a 4D quantitative imaging system for seed development, which will allow us to functionally relate seed shape, cellular growth patterns, and molecular candidates during seed growth. With this system we will be able to:

1. Generate quantitative maps of candidate edge-pattered proteins and molecular probes during seed development

2. Perturb cellular geometry, pressure, and growth patterns with pharmacological, mechanical, and genetic approaches

Collectively, these results will allow us to formulate mechanistic, multiscale models for the integration of mechanical signals into seed development.

METHODOLOGIES:

We will adapt an existing microfluidics system for 4D life imaging of developing seeds at submicron resolution, which will be based at the team's microscopy platform at the RDP. This system will allow us to simultaneously analyse cellular growth patterns and subcellular protein localisations in cells with changing growth patterns. We will also use state-of-the-art molecular probes to analyse micromechanical properties of the cell wall and membrane using FLIM (fluorescent lifetime imaging, Michels et al 2019). We will also use bespoke image analysis tools developed in the team to identify correlations between growth, cell wall mechanics, and polarity patterning in the seeds, which will allow the design of experiments that will disrupt these patterns.

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