

High-throughput imaging of nuclear glutathione compartmentalization in Arabidopsis thaliana protoplasts

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Cellular proliferation must be coordinated with overall health and metabolic status in multicellular organisms. Glutathione (GSH), a highly abundant tripeptide, is the primary antioxidant of many Eukaryotic organisms. Further, its abundance, redox status, and subcellular localization are highly influenced by plant stress, metabolism, and cell cycle phase. Crucially, fluctuations in GSH nuclear content are implicated in promoting plant cell cycle regulation. As such, GSH is well-positioned to be an integrator of stress and metabolic information in cell cycle control. The molecular basis of how GSH achieves this function is not known. A central aspect of this question is understanding how GSH subcellular localization is regulated. Previous observations show that GSH is redistributed to the nucleus following injury to the *Arabidopsis* root apical meristem, which then promotes cell cycle progression during regeneration of that organ. We hypothesize that a transient increase in nuclear GSH content triggers rapid cell divisions. Yet, how GSH is concentrated in the nucleus is not currently understood in plants. Here, I leverage imaging cytometry to investigate the nuclear redistribution of GSH during cell cycle progression in isolated root protoplasts. GSH-specific live cell staining in protoplasts from *Arabidopsis* nuclear marker lines were used to identify cell populations with distinctly nuclear and cytoplasmic distribution. Further, through cytometric measurement of ploidy, GSH localization was categorized by cell cycle phase, recapitulating concentration of GSH in the *Arabidopsis* nucleus. Using this technique, I demonstrate the utility of imaging-based cytometry for high-throughput analysis of subcellular co-localization. Future work will couple imaging-based cell sorting with pooled CRISPR mutagenesis to identify regulators of GSH subcellular localization.