Gene Regulatory Networks controlling Arabidopsis Root Stem Cells

Identifying the transcription factors (TFs) and associated regulatory processes involved in stem cell regulation is key for understanding the initiation and growth of tissues and organs. Although many TFs have been described in the *Arabidopsis* root stem cells, a comprehensive view of the transcriptional signature of the stem cells is lacking. We used a systems biology approach to predict interactions among the genes involved in stem cell identity and maintenance.

We first transcriptionally profiled four stem cell populations and developed a gene regulatory network (GRN) inference algorithm, GENIST, which combines spatial and temporal transcriptomic datasets to identify important TFs and infer gene-to-gene interactions. Our approach resulted in a map of gene interactions that orchestrates the transcriptional regulation of stem cells. In addition to linking known stem cell factors, our resulting GRNs predicted additional TFs involved in stem cell identity and maintenance. We mathematically modeled and experimentally validated some of our predicted transcription factors, which confirmed the robustness of our algorithm and our resulting networks. Our approach resulted in the finding of a factor, *PERIANTHIA* (*PAN),* which may play an important role in stem cell maintenance and QC function.

We then developed an imaging system to perform *in vivo*, long-term imaging experiments that will be used to understand the dynamics of the regulatory interactions between PAN and its downstream TFs in a cell-specific manner. For this, we designed and 3-D printed a Multi-sample *Arabidopsis* Growth and Imaging Chamber (MAGIC) that provides near-physiological imaging conditions and allows high-throughput time-course imaging experiments in the ZEISS Lightsheet Z.1. We showed MAGIC's imaging capabilities by following cell divisions, as an indicator of plant growth and development, over prolonged time periods, and demonstrated that plants imaged with our chamber undergo cell divisions for >16 times longer than those with the glass capillary system supplied by the ZEISS Z1. Future *in vivo* observations of the expression of PAN and its predicted downstream factors will be key to refine our model predictions and obtain information about the dynamics of the regulatory processes.

Our systems biology approach illustrates the strength of integrating computational and technological tools into the experimental approaches to solve key biological questions. We anticipate that our algorithm and our approach can be applied to solve similar problems in a diverse number of systems, which can result in unsupervised predictions of gene functions and gene candidates.