Cell type specific BIRD Factor Interactions in the *Arabidopsis* Root

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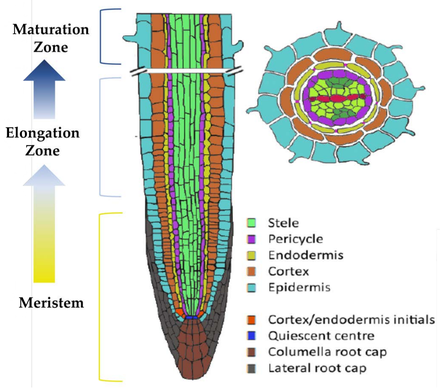
Background and Significance

Transcriptional regulation is a key factor in resolving the puzzle of how identical nuclear DNA sequences specify diverse cellular identities. While transcription factors (TFs) make up a relatively small proportion of genes – less than 10% of protein coding genes across eukaryotes[1–3], combinatorial regulation by TF complexes allows for a greater number of specific TF-DNA interactions than can be accomplished by individual TFs alone. A single TF can have multiple DNA targets depending on its interaction partners. Studying these TF complexes requires great spatial specificity - a protein may take part in different complexes in different cells with resultant different roles. For this reason, a model organism with stable spatial organization and known temporal progression is a valuable resource. This project will test for location-specific interactions within a set of TFs important to root development, as well as for these interactions' location-specific regulatory roles.

Figure 1. *Arabidopsis* cell types and developmental stages have clear spatial organization: Longitudinal (left) and radial (right) cross-section schematics of an *Arabidopsis* root are shown. The developmental stage of root cells increases away from the root tip. Endodermis (yellow). Cortex (orange). Epidermis (light blue) – differentiates into hair and non-hair cells. Stele (light green). Columella (brown). Lateral Root Cap (dark brown). Figure modified from De Smet et al (2015).



Arabidopsis thaliana is a well characterized model system for development. *Arabidopsis* roots have a clearly organized stem cell niche and developmental stages are organized spatially rather than temporally (Fig. 1). Cell differentiation is exhibited in the fate decision process by which epidermal cells are assigned hair or non-hair cell identity. *Arabidopsis* roots also demonstrate asymmetric cell division in the division of Cortex/Endodermal Initial cells into cortex and endodermal cells. Key regulators in this asymmetric process have been identified and diverse mutants are readily available.

The BIRD family of TFs, also known as the Indeterminate Domain (IDD) family, has known roles in the Cortex/Endodermis asymmetric cell division process. Additionally, interactions between BIRDs have been shown by yeast two-hybrid and in transfected *Arabidopsis* cell cultures. Bimolecular fluorescence complementation (BiFC) showed that BALD IBIS and JACKDAW interact when expressed in Arabidopsis protoplasts[5] and that MGP and JKD interact when expressed in onion epidermal cells[6]. The *in situ* formation and localization of these BIRD dimers, however, has not been probed

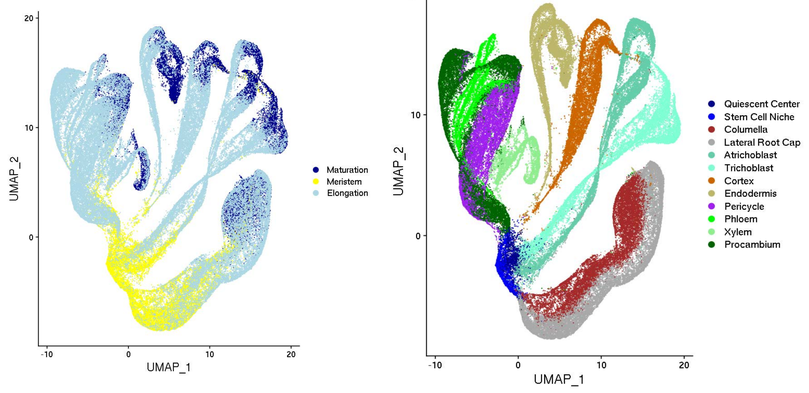
Specific Aims

1. Map protein-protein interactions among the BIRD family of transcription factors
2. Assess the role of BIRD TF interaction complexes in root development

Profile BIRD overlap using scRNA Atlas

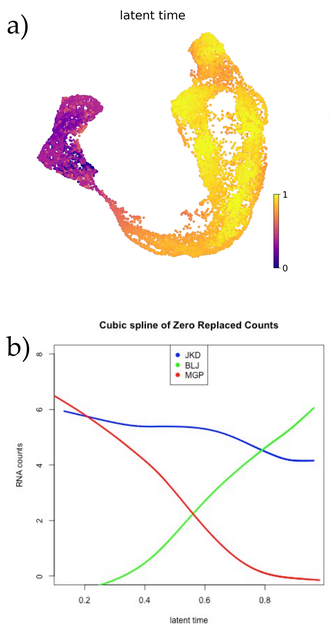
The Benfey lab and collaborators have published a preprint of an atlas of the *Arabidopsis* root[7]. Cell type annotation of the atlas shows that all major tissue types were distinctly captured by scRNA profiling. The UMAP representation of the data recapitulates the biological structure of the root – meristematic cell types branch out into files of mature tissue (Fig. 2). This annotated atlas is a valuable resource for examining co-expression of transcripts without the time or spectrum limitations of microscopy.

Figure 2: scRNA Atlas captures cell type and developmental stage: On the right, cells are annotated by cell type; on the left, cells are annotated by developmental stage. Cell-by-cell annotation was performed to assign cell identity. Correlation-based annotation, Index of Cell Identity (ICI)[8], and marker-based annotation were all used to annotate cell identity. The dataset includes over 110,000 cells.



While transcript and protein localization do not always agree, no BIRD proteins are yet known to be mobile. The scRNA atlas is therefore a useful estimator of protein expression overlap for BIRD TFs for which dual-marker lines are not readily available. The scRNA data will inform prioritization when constructing such lines for further study. So far, the transcriptional patterns observed over latent time correspond to known protein expression, and provide additional information on the extent of BIRD expression overlap (Fig. 3).

Figure 3: BIRD expression varies over developmental time: The latent time (a) of 13,481 endodermal cells was calculated using the RNA velocity-based package scVelo[9]. The latent time estimation was used to plot BIRD expression over developmental time (b) for three BIRDs of interest. Translational and transcriptional fusions of the three BIRDs (recJKD::JKD:YPET, recBLJ::3xYPET, and recMGP::MGP:YPET) published in Moreno-Risueno et. al, 2015, are shown in (c).



Identify BIRD interactions *in planta*

In order to determine if BIRD proteins interact in their native environment, I will use the Fluorescence Correlation Spectroscopy (FCS) method Number & Brightness (N&B) which allows for cell type resolution. By calculating both individual N&B as well as the correlation between two proteins tagged with different fluorophores, it is possible to calculate the proportions of complex stoichiometries, as shown in Figure 4c where Clark et al, 2020 examine SHORTROOT (SHR) and SCARECROW (SCR) TF complexes in quiescent center (QC) cells of the *Arabidopsis* root.

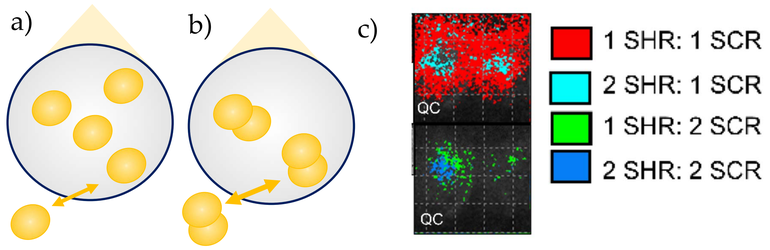
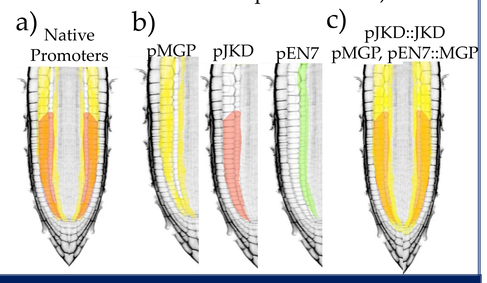


Figure 4. Variance in Pixel Intensity depends on Stoichiometry: a) and b) show diagrams of monomers (a) and dimers (b) as captured in a single image pixel. While the measured intensities will be the same for both pixels as they each capture four pixels of the same brightness, over time, the variance in intensity will be larger for the monomer. (c) is from Clark et al, 2020. Pixels have been colored based on the detected stoichiometry.

Probe functionality of BIRD interactions

After investigating BIRD TF protein-protein interactions and their locations using N&B analysis, I will attempt to perturb the domain of these interactions by changing BIRD expression domains. The N&B results will inform in which cell types expression range will be expanded or constricted. An example of a potential mutant is outlined in Figure 5. Single-cell analysis will then be used to evaluate transcriptional changes due to loss or expansion of the interaction.

Figure 5. Expand potential interaction range: a) shows the MGP (yellow) and JKD (orange) proteins expressed under their native promoters. b) shows the expression ranges of the JKD and MGP promoters as well as the endodermis specific EN7 promoter. c) shows the increased endodermis overlap when MGP is expressed under the EN7 promoter as well as its native promoter.



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