What is a plant cell type in the age of single cell biology? It's complicated.

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Abstract

One of the fundamental questions in developmental biology is how a cell is specified to differentiate as a specialized cell type. Traditionally plant cell types were defined based on their function, location, morphology, and lineage. Currently, in the age of single cell biology, researchers typically attempt to assign plant cells to cell types by clustering them based on their transcriptomes. However, the transcriptome also reflects the dynamic state of the cell, such as its phase in the cell cycle and its response to signals, raising questions about how to define a cell type. We suggest that this complexity and dynamics of the cell states is of interest and further consider the roles signaling, stochasticity, cell cycle, and mechanical forces play in plant cell fate specification. Once established cell identity must also be maintained. With the wealth of single cell data coming out, the field is poised to elucidate both the complexity and dynamics of cell states.

Introduction: Classical definitions of plant cell types

Researchers traditionally categorized cells into different cell "types" based on their function, location, morphology, and lineage (Carter et al., 1986). For instance, stomatal guard cells are defined by their function (to open and close regulating gas exchange), location (a pair of guard cells surrounding the pore, spaced at least once cell apart from other guard cell pairs in the

epidermis), morphology (distinctive crescent or dumbbell shape surrounding one half of the pore), and lineage (derived from the progressive asymmetric divisions of meristemoid mother cell, meristemoid, and symmetric division of the guard mother cell) (Figure 1A) (Bergmann & Sack, 2007). Likewise, plant anatomy studies generally classify plant cells in the ground tissue (tissue between the epidermis and the vasculature) by their cell wall morphology into three cell types: parenchyma (thin primary cell wall), collenchyma (thick cell wall), and sclerenchyma (thick secondary cell walls containing lignin) (Figure 1B) (Crang et al., 2019). Although the cell wall is a useful characteristic of the cell morphology for defining cell type, these cell types are often subclassified further by their other characteristics. For instance, in the leaf there are two major types of parenchyma - elongated tightly packed palisade mesophyll and rounder loosely packed spongy mesophyll cells.

These simple cell type definitions appeared clear and easy to use. However, the widespread use of single cell approaches, particularly single cell or single nucleus RNA-seq and ATAC-seq, has revealed more complexities of defining cell types. For example, when a cell receives a signal from the surrounding tissue environment which causes a change in its transcriptome, does this cell become a different cell type? Sometimes yes; the signal causes the cell to irreversibly transition to a new cell type. Sometimes no; when the signal is removed the cell transitions back to the original state (Fleck JS, 2023). This raises the concept of the cell "state" at a specific point in time. Likewise, is a cell in a different phase of the cell cycle a different cell type? Cells in different phases of the cell cycle have significantly different transcriptomes and often cluster as separate from other cell types just based on their cell cycle phase. Typically, researchers computational remove these competing signatures of the cell cycle to reveal the underlying cell "type" (Barron & Li, 2016). Thus, there is still no consensus agreement on the definition of a cell type despite much debate and philosophical pondering (Doyle, 2022; Efroni, 2018; Fleck JS, 2023; Clevers, 2017).

The concept of cell identity is inexorably linked to the question of how a cell is specified to become a specific cell type. Cell fate describes the commitment of a cell that has begun its journey on the path to some final type (Casey et al., 2020). Differentiation describes the process through which the cell acquires the unique functions, morphologies, and features of that cell type. For the purpose of this work, cell type will refer to a cell's identity at any given snapshot in time while cell fate will refer to a cell's trajectory through differentiation and ultimate destiny. Plant cells typically have high levels of totipotency and ability to de-differentiate and take on a new identity, so often cell fates are malleable.

Classically researchers asked whether cell fate was specified by the lineage of the cell or its position (Scheres, 2001). Specification by position can be interpreted as intercellular signaling playing a key role specifying cell fate. Specification by lineage, also referred to as ontogeny, suggests that a cell passes the fate decision on to its descendants through the stable expression of transcription factors or other lineage determinants. In the 1970s and 1980s researchers attempted to distinguish between position versus lineage using sector analysis or careful observation of

cellular and organ growth patterns throughout development. Such efforts have verified that cells generally divide to maintain clonal layers in the shoot apical meristem, generally maintaining their lineage (Jr, 1933; Stewart, 1970). In the shoot apical meristem, the outer L1 clonal layer of cells divide almost exclusively anticlinally (perpendicular to the surface of the meristem) such that daughter cells remain in the same cell layer, which gives rise to the epidermis, while the L2 second layer of cells generally gives rise to the ground tissues, and the L3 to the stem and vasculature (Figure 1C) (Reeve, 1942). However, Stewart and Burk observed that about 1 in 3100 epidermal cells divided periclinally (parallel to the surface of the meristem) to make a new internal cell (Stewart, 1970). The internal cell lost its epidermal identity, adopted an internal fate, and differentiated as a mesophyll cell (Stewart, 1970). Thus, it was concluded that plant cell fate is specified by position (Scheres, 2001). Clearly, plant cells are prone to de-differentiation and re-differentiation. These patterns of de/re-differentiation have prompted researchers to probe deeper into how to define cell lineages in plants (Efroni, 2018). Subsequently, decades of in depth research of many different cell types has revealed the combined importance of both intercellular signaling and cell lineage in determining cell identity.

The trajectory a cell undergoes in the process of becoming a particular cell type was famously envisioned by Waddington (1957) as a ball rolling down a hill into a series of valleys where the top of a hill represents a totipotent cell and the bottom of each valley a final mature cell type. The valleys represent trajectories, and a cell entering one valley is restricted to that particular cell fate, unless the hill between valleys is low and fluctuations can push the ball over or signals change the underlying epigenetic tethers which alters the landscape (Jaeger & Monk, 2014). Which “valley” or trajectory a cell begins to travel has been hypothesized to be influenced by the initial state of the cell, the state of its gene regulatory network, and external conditions (Fishell & Kepecs, 2020; Jaeger & Monk, 2014; Kauffman, 1993). While Waddington’s landscape is metaphorical, dynamical systems have been used to model gene regulatory networks and analyze the trajectories of the cells over time as they are attracted to stable states representing cell identities in the phase space (Jaeger & Monk, 2014). For instance, Huang and Tindall (2007) modeled a simple cell fate system using 2 genes that repress each other but enhance their own expression, creating an unstable stem cell (gene A = gene B) and two stable differentiated states (gene A > gene B or gene B > gene A) (Huang et al., 2007). Although this model works well for short lived stem cell lineages, it raises questions for how to model plant cell lineages, which have much more stable meristem cells.

Meristem cells have been divided by location into different cell fates: organizing center, central zone stem cells, peripheral zone, and rib zones (Steeves, 1989). For instance, the size of the central zone full of stem cells in the shoot apical meristem is maintained by WUSCHEL (WUS) which activates (as opposed to represses) its own repressor (Figure 1D), CLAVATA3 (CLV3), creating a much more stable version of Huang and Tindall’s gene A = gene B stem cell scenario (Schoof et al., 2000). A reduction in WUS or increase in CLV3 reduces stem cell number and consequently meristem size. In wild type meristems, this reduction in WUS initiates

the feedback loop by also reducing CLV3 giving rise to homeostasis in the size of the stem cell pool. When CLV3 expression is reduced, the number of stem cells increases through respecification of peripheral zone cells as stem cells (Reddy & Meyerowitz, 2005). Further diverging from the A B model, WUS does not specify organizing center identity and CLV3 (an extracellular small peptide ligand, not a transcription factor) does not specify stem cell identity. Instead, the WUS transcription factor physically moves through plasmodesmata to overlying cell layers where it specifies stem cell identity and activates expression of CLV3 (Yadav et al., 2011; Zhou et al., 2018). The biological complexity of this example, which combines two different modes of intercellular signaling in a negative feedback loop, relative to the simple model of cell fate specification illustrates the challenge plant developmental biology researchers face in elucidating the specification of cell identity.

Here, we focus on reviewing the dynamic nature of cell identity and fate in plants. We first look at single cell omics and some of the questions it raises about cell identity. This review will not be an exhaustive overview on single cell methods for plants, as there are already many excellent reviews on this topic (Trapnell, 2015; Zhu et al., 2022). Nor will we attempt to define what a cell type is because we think the complexity, transient states, and dynamics are biologically important. We consider the impact of dynamic processes such as intercellular signaling, stochasticity, cell cycle, and mechanical stress on cell fate. We conclude by discussing plasticity in plant cell fate. While we recognize epigenetics is crucial in cell fate specification and maintenance, we refer the reader to other reviews on this topic (Bieluszewski et al., 2023; Bieluszewski et al., 2021; Birnbaum & Roudier, 2017). We apologize to those whose work could not be covered due to space limitations and point the reader to more exhaustive reviews on each of these individual topics.

Single Cell omics: Interpreting cell types as clusters based on gene expression

In the past few decades, the classification of cell types has expanded to use molecular information, especially marker genes and transcriptomic data (Amini et al., 2023). The introduction of single cell and single nucleus RNA sequencing (scRNA-seq) has catapulted this trend into the forefront of studies of cell fate, bringing new exciting findings as well as complexity to the concept of cell fate. Since transcription factor networks specify cell identity, we assume that cells of the same type will have the same or closely related transcriptomes (Almeida et al., 2021; D'Alessio et al., 2015; Strader et al., 2022; Zaret & Mango, 2016). This assumption is based in part on a multitude of mutant screens for the loss of a particular cell identity that have identified transcription factors as key factors specifying cell fate. Thus, in scRNA-seq results researchers cluster the cells based on the similarities of their transcriptomes and assume that different clusters are different cell types. One of the best characterized examples of understanding cell types in plants with scRNA-seq come from the *Arabidopsis* primary root. Before scRNA-seq, researchers used microarray gene expression data and fluorescently tagged and sorted cells to identify 15 cell types and corresponding marker genes in the *Arabidopsis* root (Birnbaum et al., 2003; Brady et al., 2007; Li et al., 2016). By careful selection of fluorescent

markers that were specifically expressed in known cell types, these initial datasets linked the definitions of cell types based on function, location and morphology to their transcriptomes. scRNA-seq studies of Arabidopsis roots have identified anywhere between 8 and 24 clusters of cells using unsupervised clustering algorithms (Denyer et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Zhang et al., 2019). Many scRNA-seq clusters can be cleanly matched to previously established cell types with the help of the previously identified marker genes, but often known cell type markers will map to multiple clusters or, conversely, some clusters can't be identified as any known cell type (Denyer et al., 2019; Zhang et al., 2019). Nevertheless, analysis of these clusters revealed cell type specific hormone biosynthesis and response patterns, revealing the role of cytokinin in lateral root cap development (Zhang et al., 2019).

Other tissues have also been characterized with scRNA-seq. For example, scRNA-seq has been used to enhance the floral meristem atlas developed by Refahi et al. (2021). The original atlas used 28 expression patterns of key marker genes to annotate 11 domains in the L1 layer of early-stage floral meristems (Refahi et al., 2021). Neumann et al. (2022) mapped scRNA-seq data onto the atlas to identify 15 total transcriptomic clusters. Using this dataset, they were able to predict where in the meristem vascular cells would differentiate before they can be distinguished anatomically (Neumann et al., 2022). In the maize shoot apical meristem (SAM), scRNA-seq shed light on the differentiation trajectory of cells from the meristem tip to primordia and epidermal cell fates and helped validate the role of key genes such as *KNOTTED1* in promoting differentiation of sheath (Satterlee et al., 2020).

Despite the fact that most researchers use scRNA-seq clusters to identify cell types, the fact that not all cell types map to a single cluster and not all clusters can be mapped to a known cell type raises questions about how we define cell types. For instance, are some cell types heterogeneous enough that they should really be considered multiple cell types? Are clusters that cannot be mapped to a known cell type signs that there are cryptic cell types not previously identified by markers and morphology? Or are unmapped clusters simply due to technical issues such as noise, high dropout rates, batch effects, doublets, etc. (Kiselev et al., 2019)? Is transcriptomic data alone insufficient to identify some cell types? There is evidence from subclustering that some cell types can be separated on the basis of which organ they are from, such as guard cells and companion cells from siliques subclustering separately from the same cell type in other organs (Lee et al., 2023). In many cases clusters that do not correspond to a known cell type are presumed to represent stressed, dying, or actively dividing cells (Conde et al., 2022). Parameters for unsupervised clustering can be readily changed to create more or fewer clusters from the same set of scRNA-seq data, making manual adjustment of clustering still necessary to create biologically meaningful clusters, though some bioinformatics tools do exist to aid this decision (Crow et al., 2018; Kiselev et al., 2019). In much the same way that trying to define a cell type is a complex question with no easy answer, trying to cluster cells from scRNA-seq to understand cell types is complex, requiring user input to decide how many clusters to make and to interpret clusters with known genetic markers of cell identity. Nevertheless, scRNA-

seq has expanded our ability to identify more cell types, including the breadth and heterogeneity of transcriptomic cell states within a given cell type.

Researchers have hypothesized that cell types can be defined by core regulatory complexes comprised of a unique set of terminal selectors (transcription factors and other proteins) that determine cell fate, often approximated in single cell datasets via co-expressed transcription factors (Arendt, 2016). Interestingly, animal somatic cells can be coerced into de-differentiating back into stem cells (iPSCs) through expression of four transcription factors (Yamanaka, 2008). Findings like this lend credence to the use of transcriptomic data and, in particular, the core regulatory complex concept to define cell types.

Another opportunity for expanding knowledge on cell types is the ability for scRNA-seq to capture cells during transitions from one cell type to another. Researchers use pseudotime analysis to study transitions by ordering cells at different stages of development/differentiation according to either transcriptomic similarity (Trapnell et al., 2014) or RNA velocity, which uses the ratio of spliced and unspliced transcripts to model the rate of change in expression for genes varying during differentiation (Bergen et al., 2020). This has been achieved to great effect in certain cell differentiation pathways, especially cell files in primary root tips (Denyer et al., 2019; Otero et al., 2022) and guard cells (Lopez-Anido et al., 2021; Sun et al., 2022; Xia et al., 2022), allowing researchers to reconstruct the transcriptomic changes and underlying differentiation of immature epidermal cells into guard cells and mature pavement cells (Figure 2B). Pseudotime trajectories are particularly promising avenues for studying de- and re-differentiating tissues. One of the best studied examples of this is lateral root initiation, where scRNA-seq has highlighted the importance of chromatin remodeling, cell wall modification, cell cycle, and stem cell related genes as xylem pole pericycle cells re-differentiate into lateral root meristems (Gala et al., 2021; Serrano-Ron et al., 2021). Similar trends have been noted in regenerating root tips that have been removed (Efroni et al., 2016). Pseudotime analyses of single cells during shoot-borne root development in tomato was instrumental in identifying phloem parenchyma as the progenitors of shoot-borne roots and identifying a new ephemeral and transitional cell type during re-differentiation (Omary et al., 2022). These results led to the discovery of a conserved superlocus involved in both lateral and shoot-borne root ontogeny across flowering plants (Omary et al., 2022). When accompanied by robust biological validation, single cell trajectory analyses show much promise for future studies of cell fate in plants, particularly in understudied systems such as somatic embryogenesis and the transition from mesophyll to epidermis after epidermal wounding.

Although there are a few primary differences between single cell and single nucleus RNA-seq, though both types of datasets yield comparable results for clustering and identifying cell types (Guillotin et al., 2023). Single nucleus RNA-seq captures only mRNA from the nucleus as opposed to the entire cytoplasm, resulting in lower yields of RNA but benefits from not requiring protoplasting, which leads to transcriptomic changes and fails to capture cell types

that are difficult to protoplast such as giant cells and trichomes (Guillotin et al., 2023; Lee et al., 2023; Yadav et al., 2009; Zhang et al., 2021).

One primary drawback of scRNA-seq or snRNA-seq is the loss of morphological and spatial information that classical biologists used to classify cells. Spatial transcriptomics shows promise for alleviating these issues. The list of commercial spatial transcriptomic technologies continues to grow, though most fall into one of two categories. One group is a largely untargeted, sequencing based approach that involves permeabilizing mRNA from tissues onto polydT probed spots or beads on a slide (e.g. 10x Genomics Visium, Stereo-seq, slide-seq) (Chen et al., 2022; Rodriques et al., 2019; Stahl et al., 2016). The other group uses in-situ hybridization-based methods to target a few dozen to ~100s of genes of interest (e.g. MERFISH, smFISH, *in situ* sequencing) (Chen et al., 2015; Ke et al., 2013; Moffitt et al., 2016) but typically offers higher resolution (even single molecule resolution) than the untargeted technologies. To date a few studies have used spatial transcriptomics in plants (Chen et al., 2023). One study used spatial transcriptomics to identify clusters of cells and genetic markers involved in the transition from vegetative to female cones in *Picea abies* (Orozco, 2020). Another group developed a technique termed Spatial metaTranscriptomics to simultaneously profile *Arabidopsis* mRNA and microbial rRNAs and were able to correlate microbial community hotspot composition on *Arabidopsis* leaves with host gene expression patterns (Saarenpää et al., 2022). The resolution of new spatial techniques is rapidly improving, creating new opportunities for understanding differences between cell types based on their location. For instance, stereo-seq has enabled one group to detect transcriptomic differences between cells of the upper and lower epidermis of the Arabidopsis leaf at single cell spatial resolution that were not detectable without spatial information (Figure 2A and 2C) (Xia et al., 2022). Spatial technologies continue to improve with the development of new techniques such as PHYTOMap, which uses sequence-by-hybridization to visualize gene expression at the single cell level in whole-mount tissues such as the Arabidopsis primary root tip in 3-D (Nobori et al., 2023).

Additional single cell methods have and will continue to improve our understanding of cell fate. Single cell ATAC-seq for chromatin accessibility recapitulates expression patterns seen in scRNA-seq and can complement scRNA-seq datasets (Farmer et al., 2021). Another promising advance in the field of single cell biology will be single cell proteomics, which will allow for a more accurate view of activity from genes that are post-transcriptionally regulated or cell-cell mobile, which is not possible with transcriptome-based methods. Perhaps someday single cell metabolomics will be possible, which could integrate function into our understanding of cell fate.

Analysis of scRNA-seq has revealed the dynamics and complexity of cell states. While it is clear that transcription factors in gene regulatory networks specify cell fate, we now turn to examining some of the other factors contributing to the complex and dynamic transcriptomes of cells. In the following sections, we consider how intercellular signaling, stochasticity, cell cycle, and mechanical forces impact plant cell fate specification.

Cell-cell signaling is important for cell specification

Plant cell type specification relies heavily on location, to a greater extent than cell lineage (Poethig, 1989). It is therefore unsurprising that cell-cell signaling is a major regulator of cell identity in plants. As a plant develops, multiple layers of tissues undergo differentiation at the same time. For instance, during leaf development, the epidermis and mesophyll undergo patterning simultaneously. It is important that different tissue types signal to one another during development to ensure that cell types are properly spaced relative to one another. Signaling over distances occurs in a variety of ways, including through small RNAs, peptides, hormones, and even mobile transcription factors (Figure 3A). Over the past couple of decades, a tremendous body of research has explored the roles of numerous signals that impact cell fate decisions in plants, which we cannot begin to cover here. The following are a few illustrative examples of how some of these different types of cell-cell signals lead to cell fate specification.

Small RNAs such miRNAs move from cell to cell through plasmodesmata and through phloem (Kehr & Kragler, 2018; Vaten et al., 2011). In Arabidopsis, miRNA miR165/166 is produced in the endodermis of the developing root (Carlsbecker et al., 2010). miR165/166 moves into the inner layers of the root where it cleaves and causes the degradation of HD-ZIP class III transcription factor mRNAs (Carlsbecker et al., 2010). Stronger degradation of HD-ZIP class III transcription factor mRNA leads to protoxylem specification and weaker degradation of HD-ZIP class III transcription factor mRNA leads to metaxylem specification (Figure 3B; Carlsbecker et al., 2010). The result is protoxylem differentiation closer to the endodermis where miR165/166 concentration is presumably higher and metaxylem differentiation a bit farther from the endodermis where miR165/166 concentration is presumably slightly lower (Carlsbecker et al., 2010). It was additionally found that miR165/166 also patterns ground tissue and the pericycle in the root as well (Miyashima et al., 2011).

CLE peptide signaling is a well-known method of cell communication that can specify cell fate. There are at least 26 CLE peptides in Arabidopsis (Cock, 2001; Jun et al., 2010). The CLE peptide CLV3 is expressed in and secreted from the central zone cells of the SAM, then travels down through the apoplast (the fluid filled cell wall space outside of the cell) to cells of the organizing center where it binds transmembrane receptors including CLAVATA1 (CLV1) (Figure 1D and 3A), leading to the downregulation of the transcription factor WUSHEL (Figure 1D) (Brand et al., 2000). As described above, WUSCHEL moves through plasmodesmata and confers stem cell fate to the cells above the organizing center (Mayer et al., 1998; Yadav et al., 2011). When CLV3 is unable to downregulate WUSHEL, like in the case of *clv3* mutants, there is an increase of stem cells in the SAM (Clark SE, 1995). Eleven other *CLE* genes partially compensate for a mutation in *clv3* as shown by mutating these other *cle* genes in the *clv3* mutant background (*dodeca-cle*), which strongly increases meristem size (Rodriguez-Leal et al., 2019). Another CLE peptide, CLE40, is similarly involved in stem cell specification in the RAM (Stahl et al., 2009).

Another example of signaling through small peptides is communication from the cells of the mesophyll to the cells of the epidermis by way of the small peptide Stomagen (Sugano et al., 2010). The mesophyll cells of Arabidopsis secrete the peptide Stomagen, which travels to the epidermis and promotes specification of stomatal precursor cells (Lee et al., 2015; Sugano et al., 2010). Stomagen has been found to be important for light-dependent stomatal development downstream of the bZIP transcription factor HY5 (Wang et al., 2021). Although HY5 is located in both the epidermis and the mesophyll, mesophyll HY5 alone is capable of increasing stomatal development in a light-dependent manner (Wang et al., 2021). This suggests that light perceived by the photosynthesizing mesophyll cells is influencing epidermal stomatal patterning through the cell signal Stomagen. Such communication between two layers can fine-tune photosynthesis according to environmental conditions. Stomagen is only one of several signals controlling stomatal development (Herrmann & Torii, 2021).

A classic example of cell signaling through transcription factor movement leading to cell specification is signaling in the root between the stele (vascular tissue) and endodermal/cortex initial cells (Figure 3C). The GRAS transcription factor SHORT-ROOT (SHR) is expressed only in the stele (Helariutta et al., 2000) but then moves one cell layer outside of the stele, which includes the endodermal/cortex initial cells (Nakajima et al., 2001). SHR promotes the expression of SCR, another GRAS transcription factor, that is necessary for the asymmetric division of endodermal/cortex initial giving rise to an inner layer of endodermal cells and an outer layer of cortex cells (Di Laurenzio et al., 1996; Pysh et al., 1999). In the absence of SHR's cross-tissue signaling, the endodermal/cortex initials fail to divide into an endodermal layer and a cortex layer but instead remain a single tissue layer that has identity markers of cortex (Helariutta et al., 2000) but not of endodermis (Benfey et al., 1993; van den Berg et al., 1995). In summary, intercellular signaling through a wide range of mechanisms is instrumental in cell fate specification in plants.

Stochasticity is used by the plant to initiate patterning of specialized cell types

Often cell fate decisions are deterministic, dictated by signaling and lineage as we have been discussing; however, for some cells the fate decision appears to be stochastic, in other words chosen from a random probability distribution (Losick & Desplan, 2008; Meyer & Roeder, 2014; Roeder, 2018). Gene expression is fundamentally stochastic and has been shown to be influenced by both intrinsic and extrinsic noise in Arabidopsis as in other organisms (Araujo et al., 2017; Elowitz et al., 2002). Extrinsic noise is caused by cell state differences between cells caused by cell-to-cell variability in the amount of a transcription factor, RNA polymerase, cell environment, etc. that changes the expression of both alleles of the gene in the same way (Figure 4A). Intrinsic noise, on the other hand, originates from random variability, such as transcriptional bursting, in the expression of each allele of the same gene independently (Figure 4A). Araújo et al. measured the contribution of intrinsic and extrinsic noise to gene expression by creating plants containing two transgenes expressing two different fluorescent proteins under identical promoters: p35S:2xNLS-YFP and p35S:2xNLS-CFP (Araujo et al.,

2017). Extrinsic noise was evident when both reporters were expressed in equal proportions, but to varying intensities between cells, whereas skew of fluorescence more toward yellow or more toward cyan within a given cell indicated intrinsic noise. Araújo et al. found that extrinsic noise in gene expression was more prevalent than intrinsic noise in gene expression in rosette leaves. The extrinsic noise was slightly correlated between neighboring cells of developing leaves, while this correlation disappeared in mature leaves (Araujo et al., 2017). The fact that many elements making up cell state are often inherited equally between two daughter cells arising from the same parental cell likely contributes to some of this correlation within developing leaves where cell division is still actively occurring, as the authors point out. However, the authors find that inheritance of proteins and mRNA equally is not enough to explain all the correlation. One could imagine that cell cycle synchronization of adjacent cells may also contribute to this correlation, as well as communication between adjacent cells. It is possible that spatial correlation of extrinsic noise during leaf development could play a role in pattern formation.

Stochasticity has been strongly implicated in several cell type patterning systems in Arabidopsis (Meyer & Roeder, 2014; Roeder, 2018). One such patterning system is trichome formation during leaf development. Trichomes do not touch one another but rather are regularly ordered across the surface of the leaf (Hulskamp, 2004). The mechanism used for trichome patterning has long been thought to occur through reaction diffusion patterning, which relies on initial stochastic differences between otherwise identical progenitor cells to initiate patterning. Genetic and subcellular observations of trichome development have been consistent with reaction diffusion. For instance, the positive regulators of trichome formation GLABRA1 and GLABRA3 have been shown to promote expression of the trichome inhibitor TRIPTYCHON and TRIPTYCHON has been shown to move between cells (Figure 5; Digiuni et al., 2008). Mathematically, reaction diffusion has also been shown to fit trichome patterning (Digiuni et al., 2008; Kondo S, 2010; Torii, 2012). However, there is also evidence for an activator depletion model, which can explain the spacing of trichomes (Pesch & Hulskamp, 2009). The trichome activator TRANSPARENT TESTA GLABRA1 (TTG1) protein moves cell to cell and is sequestered in trichome initials by binding to GLABRA3. Consequently, TTG is depleted from the surrounding epidermal cells, preventing them from developing as trichomes (Figure 5) (Balkunde et al., 2011; Bouyer et al., 2008). In depth modeling and analysis of a weak *ttg1-9* mutant phenotype revealed that only a combined activator inhibitor and activator depletion model fully explains the trichome spacing data (Balkunde et al., 2020). Although trichome spacing appears more random in *ttg1-9*, stochasticity underlying the trichome patterning process is not increased, and the pattern can be best explained by the combined activator inhibitor and activator depletion model. While these combined activator inhibitor and activator depletion patterning processes do space trichomes across the leaf surface there is still about 44% noise in trichome spacing relative to a hexagonal pattern (Greese et al., 2014), indicating the initial role for stochasticity underlying the patterning.

Stochasticity is important for giant cell patterning in the *Arabidopsis* sepal. The sepal has epidermal pavement cells of a variety of sizes. Some pavement cells are much larger, highly endoreduplicated, and elongated than surrounding cells and these cells have been named giant cells (Roeder et al., 2010). The HD-ZIP Class IV transcription factor ATML1 promotes giant cell formation (Meyer et al., 2017; Roeder et al., 2012). To determine how ATML1 does this, Meyer et al. live imaged developing sepal buds with mCitrine-tagged ATML1 under the ATML1 promoter during the developmental time window of giant cell specification (Meyer et al., 2017). They found that mCitrine-ATML1 concentration fluctuated within each cell nucleus over time and that peak ATML1 concentration reached during the G2 phase of the cell cycle is strongly correlated with giant cell specification (Figure 4B) (Meyer et al., 2017). Simulations from a computational model in which these fluctuations were made to be stochastic produced patterns of giant cells similar to what is found on wild-type sepals (Meyer et al., 2017). Further, the fact that giant cell spatial position varies from sepal to sepal is consistent with ATML1 fluctuations being stochastic. Thus, randomness in the accumulation of a protein can cause cell size patterning to arise from a field of protodermal cells.

Cell cycle is tightly linked to cell specification

Cell fate specification often occurs simultaneously with cell growth and division, so the question of whether cell cycle plays a role in cell fate is an old one. Recently, many examples of Cyclin-Dependent Kinase (CDK) inhibitors playing a role in cell fate specification have come to light. Plants have a large number of CDK inhibitors. One reason for this may be that plants need to modulate their cell division and development according to environmental signals (Kumar & Larkin, 2017).

Recently, Han et al. showed how the CDK inhibitor SMR4 affects stomatal differentiation (Han et al., 2022). During stomatal development, an undifferentiated protodermal cell differentiates into a meristemoid mother cell, which can then divide asymmetrically to give rise to a meristemoid (Dong & Bergmann, 2010). The meristemoid undergoes asymmetric divisions, each time renewing itself (as the smaller cell) and giving rise to a stomatal lineage ground cell (SLGC as the larger cell). The meristemoid eventually differentiates to a guard mother cell and divides symmetrically to produce two guard cells of equal size (Dong & Bergmann, 2010). Han et al. show that the asymmetric divisions have a much faster cell cycle than the symmetric divisions (Han et al., 2022). They find that this difference in cell cycle speed is due to a CDK inhibitor SMR4 that functions to prolong the G1 phase in symmetric divisions. The stomatal development regulatory transcription factor MUTE that functions to confer guard mother cell identity directly upregulates the CDK inhibitor SMR4. Importantly, forced over-expression of SMR4 in all cells of the stomatal lineage (*proPOLAR::SMR4*) results in fewer asymmetric meristemoid divisions and in premature differentiation of stomata. This results in enlarged pavement cell like skewed stomata that express multiple markers of differentiated guard cells. Thus, stomatal lineage over-expression of a cell cycle regulator SMR4 is able to drive premature exit from proliferative meristemoid asymmetric divisions to stomatal differentiation

(Han et al., 2022). Thus, developmental regulator transcription factor (MUTE) uses a cell cycle regulator to promote stomatal differentiation (Figure 6).

Some CDK inhibitors have a profound effect on gene expression. The HD-ZIP class IV transcription factor ATML1 is a transcription factor that both specifies epidermal identity and patterns giant cells in the sepal in Arabidopsis (Abe et al., 2003; Roeder et al., 2010). ATML1 is a master regulator of epidermal identity; mis-expressing ATML1 in subepidermal layers results in differentiation of mesophyll cells into different epidermal cell types, including stomata (Takada et al., 2013). ATML1 specifies giant cells in a dose-dependent manner during the G2 phase of the cell cycle (Meyer et al., 2017). In this case, specification of cell fate is restricted to the G2 phase of the cell cycle and ATMl1 concentration in G1 does not affect the cell fate decision. The CDK inhibitor LGO acts downstream of ATML1 during giant cell differentiation (Meyer et al., 2017). LGO inhibits mitosis and promotes endoreduplication. Notably, overexpressing LGO results in many transcriptional changes in addition to increasing the number of large, highly endoreduplicated cells (Schwarz & Roeder, 2016). Most of these transcriptional changes occur in an ATML1-independent manner. For instance, in an RNA-seq experiment, 292 genes were expressed differently in *LGO-OX;atml1-3* versus *atml1-3* alone, whereas only 30 genes were expressed differently between *LGO-OX;atml1-3* and *LGO-OX* alone (Schwarz & Roeder, 2016).

The effect of some CDK inhibitors on gene expression may even occur through direct association with DNA. A subset of KRPs (another family of plant CDK inhibitors) have been found to localize in a punctate pattern within the nucleus, despite the fact that these proteins do not have a DNA-binding domain (Bird et al., 2007). KRP5 associates mostly with chromocenters and heterochromatin but has also been shown to associate with protein-coding genes (Jegu et al., 2013). These protein-coding genes are enriched for cell wall organization (Jegu et al., 2013). Several of these genes were tested for transcriptional changes in response to KRP5 and were found to be upregulated in KRP5-OE as compared to wild type (Jegu et al., 2013). However, KRPs have also been shown to bind to DNA for reasons other than gene transcriptional control. For instance, KRP4, a CDK that inhibits the start of S phase, has been shown to bind DNA during mitosis (Boruc et al., 2010). D'Ario et al. found that a method of equal inheritance of KRP4 between daughter cells by binding to DNA allows for KRP4 to act as a cell size sensor (D'Ario, 2021). They found that cell size homeostasis of the shoot apical meristem occurred because smaller cells resulting from cell division took longer to progress to S phase than larger cells (D'Ario, 2021). KRP4 protein does not contain a DNA binding domain, so how KRP4 binds to DNA remains a mystery, but is likely to be indirect.

The Retinoblastoma-related cell cycle proteins (RBR) are homologues of the human retinoblastoma protein, a known tumor suppressor gene. They are found throughout plant lineages and are ancient in eukaryotes (Desvoyes et al., 2014; Desvoyes & Gutierrez, 2020). RBR proteins regulate the transition between G1 to S phase of the cell cycle (Desvoyes & Gutierrez, 2020). RBRs are also intricately linked with cell specification in addition to their roles

in cell cycle. In Arabidopsis, RBR has been found to be necessary for maintenance of stomatal guard cell terminal differentiation through its direct interaction with the transcription factor FAMA (Matos et al., 2014). Mutating FAMA so that it is functional but can no longer interact with RBR results in guard cells with extra cell divisions, sometimes leading to guard cells within other guard cells (Matos et al., 2014). Importantly, these guard cells with ectopic divisions express markers of early stomatal lineage cells, indicating a kind of reversion from terminal guard cell fate when FAMA cannot interact with RBR (Matos et al., 2014). Similarly, in the Arabidopsis root, mutating another RBR-interacting transcription factor, SCARECROW (SCR), to abolish its RBR interaction domain leads to an extra asymmetric cell division that results in an additional layer of ground tissue (Cruz-Ramirez et al., 2012).

Contributions of mechanical forces to cell identity specification

Since the development of molecular techniques such as RNA sequencing and fluorescent reporters, much of the work in understanding cell fate has focused on molecular pathways. However, the role of mechanical forces on cell growth and plant development have been gaining more attention from researchers in recent years, though the concept is by no means new (Arber 1950). Interest in mechanics was renewed in part after finding that microtubules respond dynamically to mechanical stress (Hamant et al., 2008) and guide growth orientation through cellulose deposition (Paredez et al., 2006). Trends in microtubule alignment have helped biologists to model the complex mechanical landscape of plant tissues (Hamant et al., 2008; Robinson et al., 2013; Verger et al., 2018). In particular, mechanical stress influences not only microtubule orientation (and therefore cellular growth orientation), but also the orientation of the auxin efflux transporter PIN-FORMED1 and therefore the distribution of auxin (Heisler et al., 2010; Li et al., 2019; Nakayama et al., 2012; Reinhardt et al., 2003).

Mechanical feedback and microtubule orientations have helped researchers to model organogenesis. In sepals, regional differences in growth rate and microtubule orientation helped demonstrate that mechanical feedback between the slow growing tip and fast-growing base guides sepal shape (Hervieux et al., 2016). Disrupting these regional mechanical conflicts by increasing transcriptional noise and local growth heterogeneity disrupts normal sepal shape development (Trinh et al., 2023). In roots, wounding (Omary et al., 2022) and bending (Ditengou et al., 2008; Richter et al., 2009) have long been known as cues that can initiate lateral root formation, which involves the re-specification of pericycle cells into de-novo meristems. In shoots, Arber (1950) hypothesized that mechanical pulling of the epidermis on mesophyll helps to shape the former and create sponginess in the latter (Arber, 1950). More recent work with brassinosteroid and cell adhesion mutants has found that indeed, the pulling of the epidermis on internal layers is responsible for the shaping of the plant body (Kelly-Bellow et al., 2023; Marcotrigiano, 2010; Verger et al., 2018).

Despite the growing body of work relating mechanical stresses to overall organ growth patterns, their impacts on an individual cell's fate are less well known (Roeder et al., 2022).

Landrein et al. (2015) found that the high levels of mechanical stress in the boundaries between floral primordia and the shoot apical meristem contribute to *SHOOT MERISTEMLESS* (*STM*) expression using a unique boundary-specific *STM* reporter. Furthermore, *STM* is induced by cell ablation and micromechanical perturbations (Figure 7A) while regulation of auxin signaling and some boundary identity genes (such as *CUC1*) can be decoupled from these perturbations, showing that mechanical forces can have very specific effects on certain genetic pathways contributing to cell identity (Landrein et al., 2015). Mechanical conflicts at the boundary of new organ formation is also critical during shoot regeneration from callus, allowing nearby progenitor cells to begin the trajectory from callus to de-novo meristem cells (Figure 7B) (Varapparambath et al., 2022). In particular, CUC2 mediated activation of XTH9, which loosens the cell walls around the incipient meristem, is necessary to generate mechanical conflict and subsequent polarization of PIN1 (Varapparambath et al., 2022). This demonstrates the ability of mechanical signals to influence cell fate in developing meristems.

Outside of meristematic regions, epidermal cell fate, which is specified by the transcription factor ATML1, is repressed in mesophyll cells due in part due to mechanical pressure exerted by the epidermis (Iida et al., 2023). When the compressive force is released through removal of the epidermis, *ATML1* expression is activated, allowing mesophyll cells to switch to an epidermal cell fate (Figure 7C) (Iida et al., 2023). Alternatively, applying pressure to the epidermis was sufficient to repress *ATML1* expression in the epidermis (Figure 7D) (Iida et al., 2023). Little is known about how plant cells sense mechanical forces and convert them into physiological and developmental signals, though calcium signaling is at least one important mediator for rapid mechanical responses (Bakshi et al., 2023; Li et al., 2019; Toyota et al., 2018). As interest in mechanical forces in plant development grows, our understanding of the mechanisms underlying mechanoperception may shed more light on how plants perceive mechanical stresses and how it informs cell fate decisions.

Plasticity in differentiated cell types.

Intuitively, it is convenient to think of cell differentiation as irreversible. Cells lose their plasticity as they differentiate. However, increasing evidence suggests that differentiated cells can regain plasticity under certain special circumstances.

One of the highly differentiated and conspicuous cell types in plants is trichomes (hairs). In Arabidopsis, trichomes are highly endoreduplicated cells, typically 32-64C, in the epidermis. Once a trichome cell is formed, it is hard to imagine that it could undergo division and revert back into epidermal cells. However, studies from Bramsiepe et al., have shown just that. If trichome cells cannot maintain their endoreduplication, they revert to epidermal cells by undergoing cell divisions (Bramsiepe et al., 2010). First the authors observed that in *glabra3* (*gl3)* mutants, weak *cyclin dependent kinase a;1* (*cdka;1)* mutants, and *pGL2::ICK1/KRP1* lines expressing the CDK inhibitor in trichomes, endoreduplication is reduced and the leaves have fewer trichomes than wild type. Even though in the young leaf the number of trichomes initiated is not different between wild

type and *cdka;1* mutants, the mature leaf shows a significant reduction in trichomes. Based on these observations, the authors hypothesized that trichomes might be aborting in the weak *cdka;1* mutant as well as the *pGL2::ICK1/KRP1* transgenic plant (Figure 8). To obtain a more sensitive background where they could observe the aborting trichomes, the authors further reduced endoreduplication levels by creating double mutant combinations of the weak *cdka;1* or *pGL2::ICK1/KRP1* with the *gl3* mutant and observed a dramatic reduction in number of trichomes compared to single mutants. Live imaging of the double mutant lines showed that some of the trichomes undergo cell division to form the epidermal cells, which was never observed in wild type. Based on these observations, the authors concluded that if endoreduplication is reduced in trichomes, the trichome cells are unable to maintain their differentiation status.

Plasticity of differentiated cells is not only observed in a single layer of cells but also between the layers in Arabidopsis leaves. Results from Iida et al., 2023 showed that when the leaf epidermal tissue was damaged, the uppermost mesophyll cells helped in restoring the epidermal tissue (Iida et al., 2023). The mesophyll cells below the damaged epidermis started expressing the master regulator of epidermis, ATML1 which helps in forming the epidermis due to mechanical sensing as described in the mechanics section of this review.

Plasticity of the differentiated cells might be more prevalent than recognized so far; we were only able to discover and test these ideas with recent advancements in microscopy. It will be intriguing to investigate if this plasticity plays a role in injury or in any other stress conditions. Further, it will be interesting to look at the chromatin dynamics and molecular signatures of this process using the next generation sequencing technologies like ATAC-seq, single cell and spatial transcriptomics.

Thus, differentiated plant cells must maintain their cell identity. This finding becomes even more evident with the observation that a single fully differentiated mesophyll cell can de-differentiate to a totipotent state from which an entire new plant can be regenerated (Xu et al., 2021). Regeneration can be defined as the ability to produce a partial/complete organ or organism from a differentiated cell through de-differentiation (fate change). Plants exhibit incredible capability of regeneration (Sugimoto et al., 2019). In fact, plants are commonly propagated using regeneration of plant cuttings (stem, leaves, roots). Both wounding and hormone cues together trigger cut tissue explants or even single cells to form a callus (Sugimoto et al., 2019), which was thought to be a de-differentiated, disorganized mass of tissue. Surprisingly, far from a disorganized de-differentiated mass, instead callus has a similar structure and cell types to an enlarged root meristem (Sugimoto et al., 2010). Regeneration involves silencing of genes to erase the original cell identities and allow new cell fates to be adopted. During callus formation specifically H3K4me2 marks are eliminated from the genome, which leads to a primed state ready for formation of new cell types and tissues (Ishihara et al., 2019). Studies from the isolated mesophyll cells showed that two transcription factors WUSCHEL (WUS) and DORNRÖSCHEN (DRN)/ENHANCER OF SHOOT REGENERATION1 (ESR1) are required for successful regeneration (Xu et al., 2021). Inducible overexpression of either *WUS* or *DRN* induced more

successful plantlets from the calli compared to mock. Loss of function of *wus-101* and *drn-1* resulted in zero regenerated plantlets emphasizing the significance of these genes in regeneration. Regeneration thus highlights the dynamic and changeable nature of the plant cell type.

Conclusions and Outlook

What is a cell type? As we have discussed, this seemingly simple question has come to prominence in the current age of single cell biology. For decades researchers have defined and studied plant cell types based on their function, location, morphology, and lineage. These classic definitions are useful! These familiar cell types often can be associated with individual clusters of cells derived from scRNA-seq based on the expression of known marker genes. But what happens when a cluster does not map to any known cell type or when a known cell type maps to more than one cluster? Furthermore, the number of clusters formed is a bit arbitrary and each cluster can be sub-clustered into more clusters. The advent of spatial transcriptomics will be important for bridging classic definitions of cell type with transcriptomic definitions. Some of the mysterious clusters appear to represent transient cell fates, which may be a powerful way to elucidate the dynamic transitions in cell identity. Thus, the question of cell type is tied to the fundamental question of how cell fate is specified.

Many of the factors adding complexity to the transcriptome of a cell, making it harder to classify it as a strict cell type, may be exactly the same factors that are involved in specifying cell fate. Classically it has been thought that plant cell fate is specified based on position instead of lineage, and over the past decades a multitude of signals have been elucidated that specify cell identity. Signals include small secreted signaling peptides traveling through the cell wall to a receptor on the surface of the cell, small molecule plant hormones that travel both through the cell wall and through the plasmodesmata connecting one cell to its neighbor, microRNAs and other RNAs that move from one cell to another through plasmodesmata, and even transcription factors directly moving between cells through plasmodesmata. These signals undoubtedly influence the transcriptome of the cell, and some of them push the cell into a new fate. Likewise, stochastic gene expression undoubtedly affects the transcriptome of the cell. This stochastic gene expression can also be used to break symmetry between cells, causing one cell to become specified as a different cell type. One of the big challenges for the future will be to devise new strategies to separate the noise of scRNA-seq that results from missing data due to read depth from the true stochasticity in gene expression so that stochasticity can be accurately analyzed in these datasets. Third, the cell cycle is often computationally removed from single cell data in an attempt to reveal the underlying cell type, but evidence is emerging that the cell cycle can play several roles in cell fate specification. The speed of the cell cycle appears to be associated with certain cell fates, and other cell fates can only be specified at one stage of the cell cycle, not another. One challenge for the future will be to use the single cell datasets to identify more of these relationships between cell cycle and cell fate specification. Finally, mechanical forces also are known to be important in shaping morphogenesis, but evidence is just starting to emerge that they may also be able to influence cell identities, through unknown mechanisms. Future

challenges include determining to what extent mechanical forces can alter gene expression and the mechanism through which gene expression is changed. Also, it is worth determining whether these changes in gene expression are sufficient to trigger changes in cell fate. If so, is this a commonly used mechanism or specific to a few situations? Finally, cell identities are dynamic and must be maintained. Plants, in particular, have tremendous regenerative ability and whole plants can be regenerated from single somatic cells. Endoreduplication appears to be a major mechanism promoting the maintenance of cell fate. The dynamics of cell identity changes should be interesting to further explore in single cell data. In summary, the same factors that now often seem like they are noise obscuring the true cell type may be the most powerful factors to investigate in the future to reveal the complex and dynamic mechanisms through which cell fate is specified. It is in the complexity of the single cell data that the biggest challenges and most innovative new insights may be found.

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Figure legends:

Figure 1: **Classical definitions of cell types**. (A) Illustrations of the 4 main characteristics classically used to define cell types using stomata as an example. Stomata are defined by their location and patterning on the epidermis, by their function is gas exchange, by their lineage via asymmetric cell divisions followed by a final symmetric cell division, and their morphology as unique crescent shaped cells. (B) Classic literature classified cells by cell wall morphology into parenchyma (thin primary cell walls), collenchyma (thick primary cell walls), and sclerenchyma (thick, lignified secondary cell walls). (C) Cells of the shoot apical meristem have been defined by layers into L1, L2, and L3 based on cell division plane and cell fate. (D) Feedback loop between WUSCHEL and CLAVATA3 (mediated by CLAVATA1) in meristem cell identity homeostasis.

Figure 2: **Single cell and spatial RNA-seq and cell fate**. (A) Section of an Arabidopsis leaf with cell types annotated based on Stereo-seq spatial RNA sequencing. (B) Pseudotime trajectory of immature epidermal cells (“pre-branch”) to one of two fates, one of which encompasses most guard cells. (C) Single cell Uniform Manifold Approximation and Projection (UMAP) of upper and lower epidermal cells based on transcriptome alone (i) or transcriptome and spatial information (ii). Adapted from Xia et al. (2022) under the Creative Commons Attribution License.

Figure 3: **Cell to cell signaling**. (A) Types of signaling molecules and their routes of travel between cells. (B) Cell fate of protoxylem and metaxylem elements in the Arabidopsis root driven by the miR165/166 signaling. MIR165/166 travels through plasmodesmata from the endodermis to the inner cell files, establishing a gradient of miR165/166 concentration gradient. The transcripts of HD-ZIP class III transcription factors are cleaved by miR165/166. (C) SHORTROOT (SHR) and SCARECROW (SCR) signaling in the Arabidopsis root. SHR travels one cell layer from the stele into the cortex/endodermis initial, where it induces SCR expression, which in turn results in an asymmetric cell division of the initial to yield an endodermis cell and a cortex cell.

Figure 4: **Stochasticity in cell fate**. (A) Hypothetical models of intrinsic vs extrinsic noise where two fluorescent reporters are being driven by the same promoter. With intrinsic noise, the levels of each reporter varies stochastically, leading to different ratios of magenta and green signals. With extrinsic noise, both reporters are expressed in equal proportions, but to varying intensities between cells. (B) Stochastic fluctuations of ATML1 lead to giant cell fate when ATML1 crosses a concentration threshold in G2 of the cell cycle, but not when crossed only during G1 phase.

Figure 5: **Trichome patterning**. Two main models for trichome patterning involve the cell to cell movement of key regulators - the trichome inhibitor TRY and the trichome activator TTG1. Modeling suggests the pattern formation process involves both an activator inhibitor component and an activator depletion component.

Figure 6: **The effects of cell cycle on cell fate**. Model depicting how MUTE induces SMR4 to slow down cell cycle progression, leading to a switch from proliferation and continued asymmetric cell divisions to differentiation and symmetric cell division.

Figure 7: **The effects of mechanical forces on cell identity**. (A) Schematic of the expression levels of STM under normal mechanical conditions, compression, and around ablated cells, illustrating induction by mechanical stresses. In this experiment, the SAMs were recovering from NPA treatment, so just beginning to reinitiate the formation of primordia. (B) Mechanical conflict between shoot meristem progenitor cells and surrounding non-progenitor cells expression CUC2-induced XTH9. This conflict helps polarize PIN1 in the progenitor cells and contributes to shoot meristem cell fate. (C) Removal of epidermal cells leads to the derepression of ATML1 in the underlying mesophyll a day after removal, due to loss of mechanical compression. (D) Similarly, applying mechanical pressure on mesophyll represses ATML1 expression, which becomes derepressed after removing the pressure.

Figure 8: **Plasticity of differentiating trichomes**. Wild-type rosette leaf surface (A) and budding trichome (B). Aborting trichome in a *pGL2::ICK1/KRP1* expressing rosette leaf (C) and an aborting trichome beginning to divide (D). (E) potential remnants of an aborted trichome that has divided several times, with putative divisions marked in (E'). Scale bars: (A) 30 μm; (B–D)

10 μm. Reprinted under the Creative Commons Attribution License from Bramsiepe et al. (2010).

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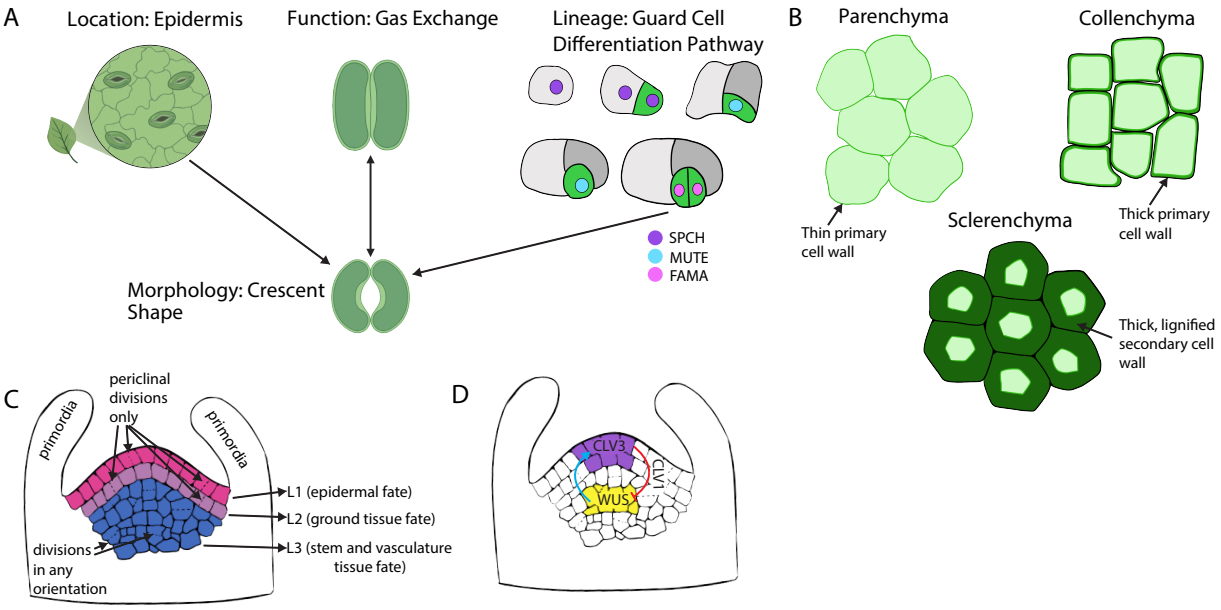
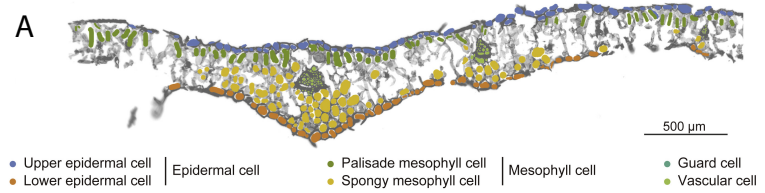
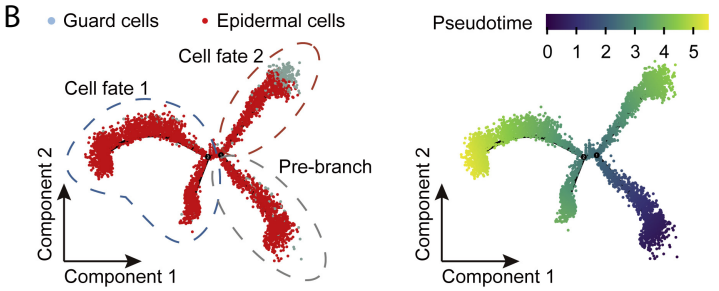

A
Location: Epidermis
Function: Gas Exchange
Lineage: Guard Cell Differentiation Pathway
SPCH
MUTE
FAMA
Morphology: Crescent Shape
B
Parenchyma
Collenchyma
Thin primary cell wall
Thick primary cell wall
Sclerenchyma
Thick, lignified secondary cell wall
C
primordia
periclinal divisions only
primordia
L1 (epidermal fate)
L2 (ground tissue fate)
L3 (stem and vasculature tissue fate)
divisions in any orientation
D
CLV3
CLV1
WUS

Figure 1


A
500 µm
Upper epidermal cell
Lower epidermal cell
Epidermal cell
Palisade mesophyll cell
Spongy mesophyll cell
Mesophyll cell
Guard cell
Vascular cell


B
Guard cells
Epidermal cells
Cell fate 2
Cell fate 1
Pre-branch
Component 2
Component 1
Pseudotime
0 1 2 3 4 5
Component 2
Component 1

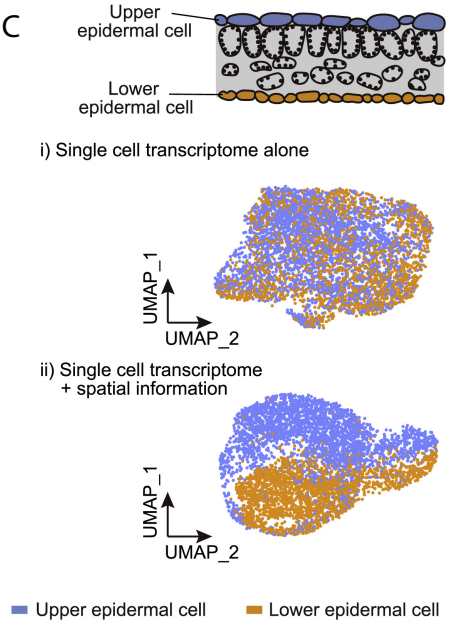

C
Upper epidermal cell
Lower epidermal cell
i) Single cell transcriptome alone
UMAP_1
UMAP_2
ii) Single cell transcriptome + spatial information
UMAP_1
UMAP_2
Upper epidermal cell
Lower epidermal cell

Figure 2

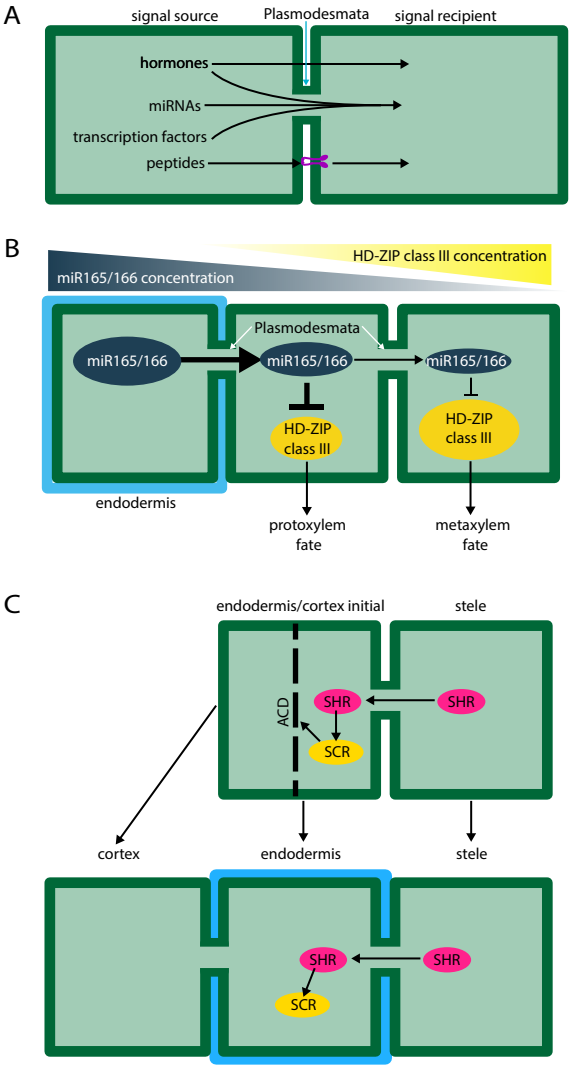

A
signal source
Plasmodesmata
signal recipient
hormones
miRNAs
transcription factors
peptides
B
HD-ZIP class III concentration
miR165/166 concentration
Plasmodesmata
miR165/166
miR165/166
miR165/166
HD-ZIP class III
HD-ZIP class III
endodermis
protoxylem fate
metaxylem fate
C
endodermis/cortex initial
stele
ACD
SHR
SHR
SCR
cortex
endodermis
stele
SHR
SHR
SCR

Figure 3

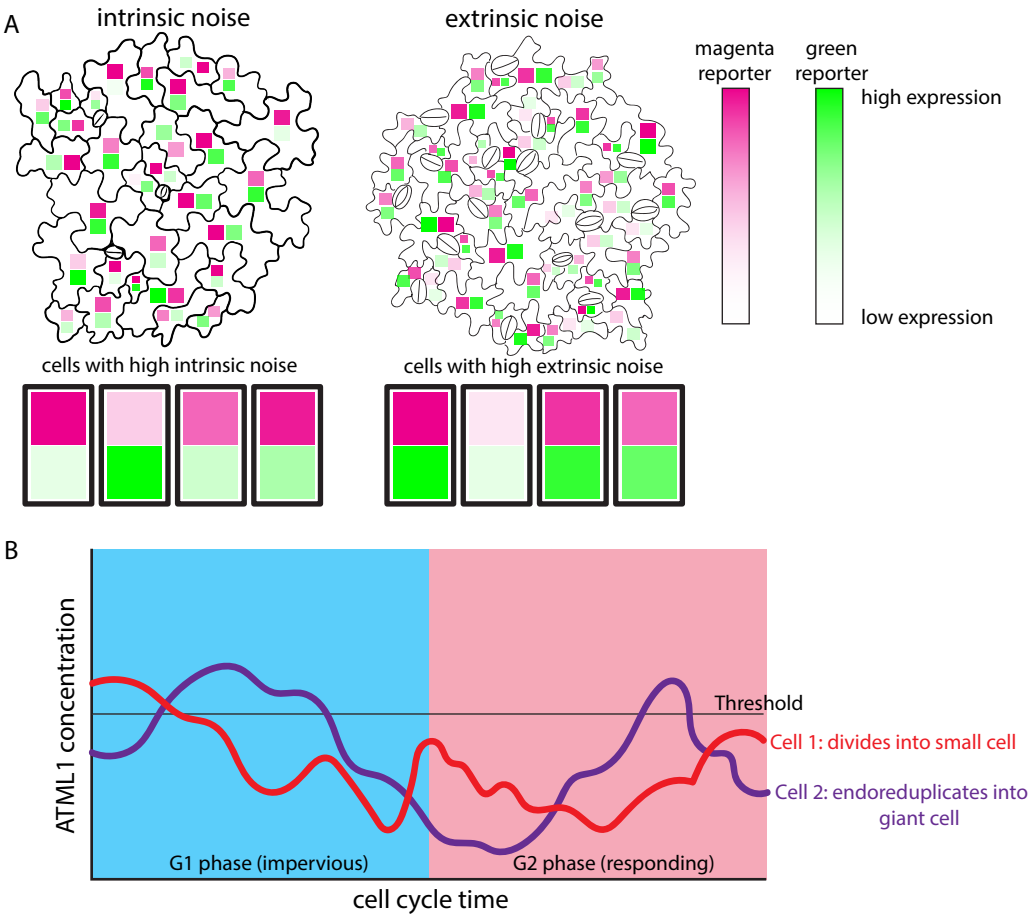

A
intrinsic noise
extrinsic noise
magenta reporter
green reporter
high expression
low expression
cells with high intrinsic noise
cells with high extrinsic noise
B
ATML1 concentration
Threshold
Cell 1: divides into small cell
Cell 2: endoreduplicates into giant cell
G1 phase (impervious)
G2 phase (responding)
cell cycle time

Figure 4

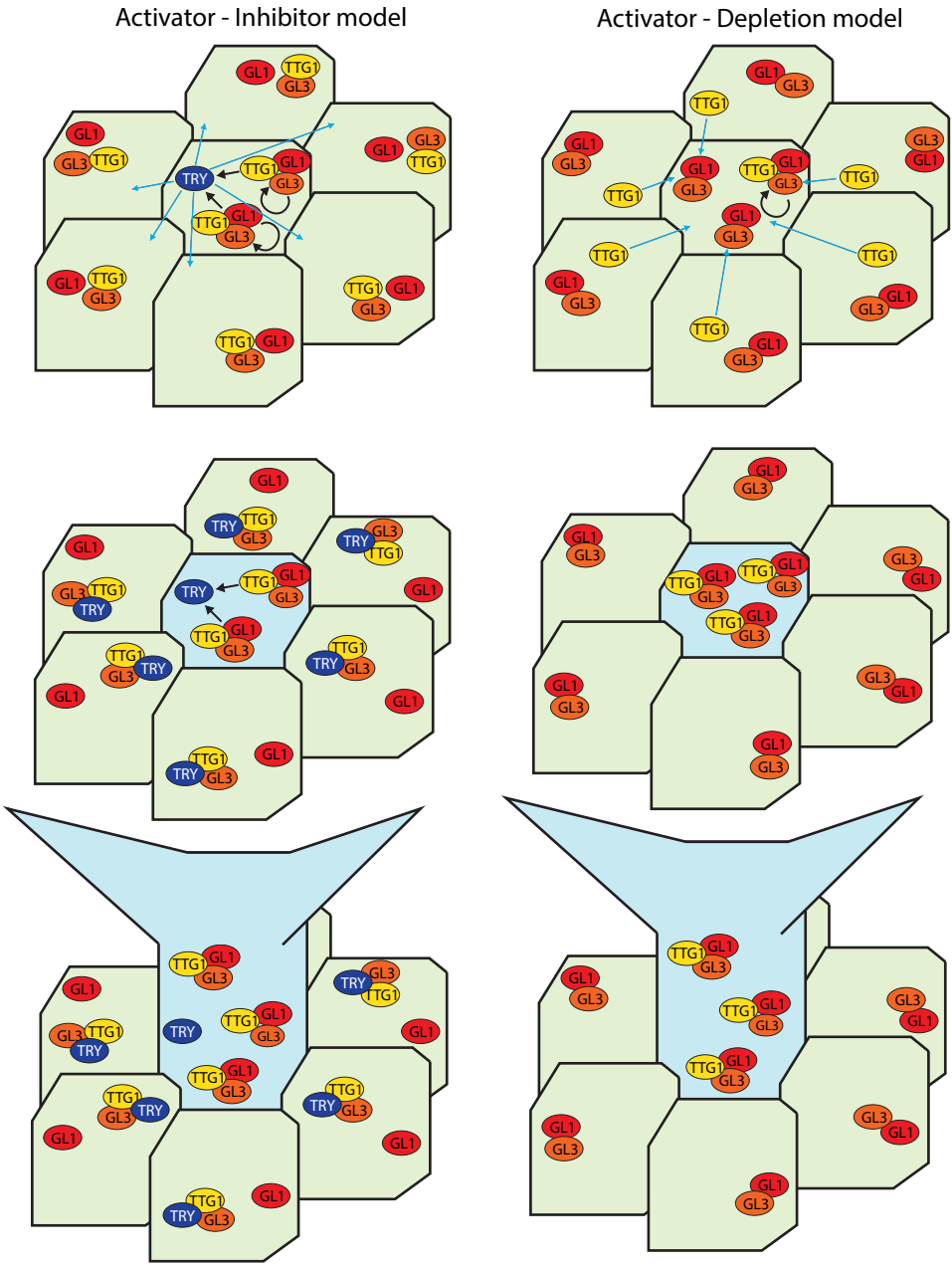

Activator - Inhibitor model
Activator - Depletion model
GL1
GL3
TTG1
TRY

Figure 5

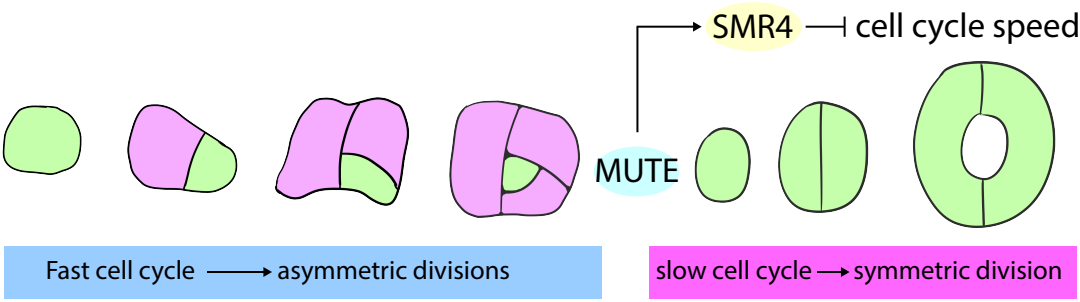

SMR4
cell cycle speed
MUTE
Fast cell cycle
asymmetric divisions
slow cell cycle
symmetric division

Figure 6

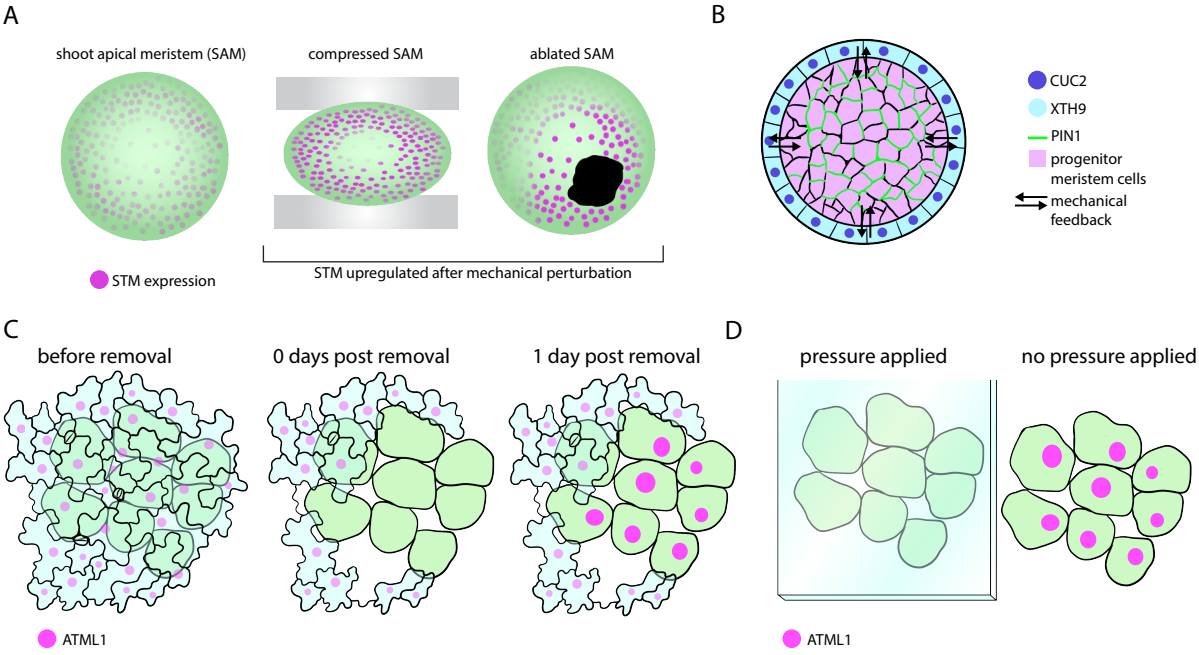

A
shoot apical meristem (SAM)
compressed SAM
ablated SAM
STM expression
STM upregulated after mechanical perturbation
B
CUC2
XTH9
PIN1
progenitor meristem cells
mechanical feedback
C
before removal
0 days post removal
1 day post removal
ATML1
D
pressure applied
no pressure applied
ATML1

Figure 7

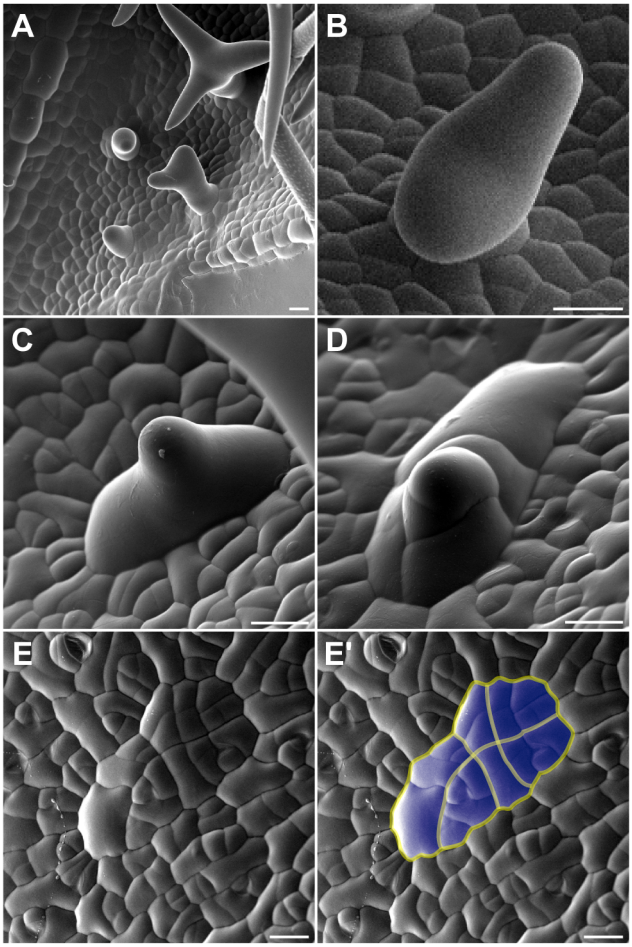


Figure 8