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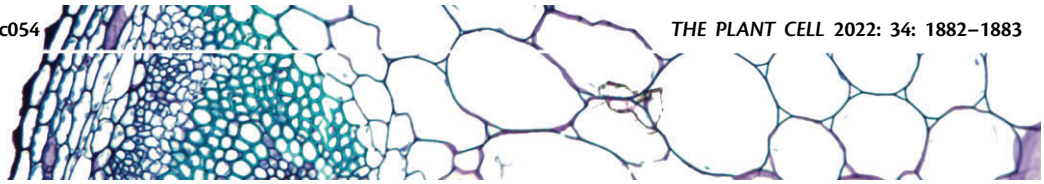

Single-nucleus transcriptomics for an integrative view of grass stomatal processes

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In Brief

In Poaceae species such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and maize (*Zea mays*), stomata form a specific structure composed of two subsidiary cells (SCs) and two guard cells (GCs), which intimately communicate (see Figure A). This unique morphology allows grass stomata to move faster than eudicot stomata. However, how SCs and GCs coordinate stomatal movement is poorly understood (Nunes et al., 2020).

Single-cell RNA sequencing (scRNA-seq) is a powerful approach to decipher the complexity of genetic programs underlying cell fate and inter-cellular communication. However, it often involves the generation of cell wall-free protoplasts by enzymatic digestion, which induces transcriptional effects that are challenging to control (Denyer and Timmermans, 2022). By contrast, single-nucleus RNA-seq (snRNA-seq) has the advantage of avoiding protoplast preparation but has yet to be applied to better understand grass stomatal cell coordination. In this issue, **Guiling Sun, Mingzhang Xia, Jieping Li, Wen Ma, and co-workers** (Sun et al., 2022) applied snRNA-seq to maize mature leaf peels and leaf bases. Their method yielded well-resolved SC and GC clusters (see Figure B) and enabled a system view of SC- and GC-specific processes. In addition, this study identified new candidate genes involved in grass stomatal responses.

First, the authors characterized SC- and GC-specific transcriptomes by applying RNA-seq to fluorescence-sorted, propidium iodide-stained single nuclei from mature leaf peels. They demonstrated the high reproducibility of their method by showing strong superimposition of replicate cell clustering graphs. Second, they identified the SC and GC clusters using known marker genes and validated cluster assignment by expressing a reporter gene under the control of SC- and GC-specific cis-regulatory regions. Starch metabolism and chloroplast activity modulate stomatal movement by turgor pressure regulation. The authors observed that photosynthesis- and starch-related transcripts are expressed at low and high levels in GCs, respectively, when compared with other cell types in peels. This suggests that GCs rely on a distant glucose source to regulate turgor pressure and stimulates the debate about whether GCs are photosynthetically active.

Signaling pathways controlling stomatal movement include CO_2 and abscisic acid (ABA) signaling. The authors observed that an ABA-degrading enzyme, CYP707A4, is highly expressed in SCs. Consistent with this observation, laser microdissection capture of GCs and SCs showed a lower

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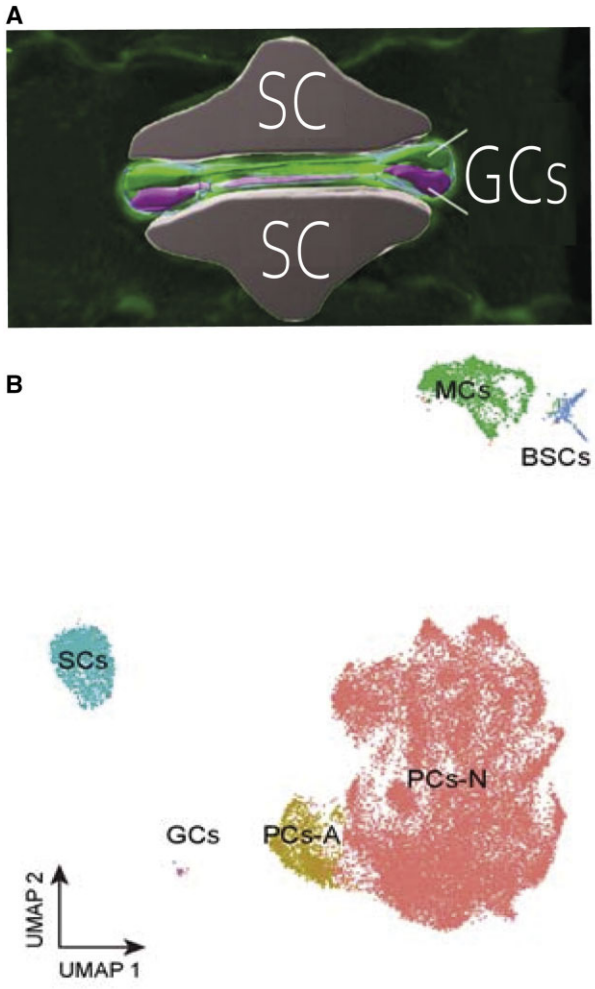

Figure SnRNA-seq approach for the characterization of grass stomatal cells signature genes. A, The graminoid stomatal complex. SCs in gray and GCs in green and magenta. B, snRNA-seq-based clustering of cell types in mature leaf peels. MCs, mesophyll cells; BSCs, bundle sheath-related cells; and PCs, pavement cells. Adapted from Sun et al. (2022), Figure 1A and Supplemental Figure S7A.

ABA content in SCs than in GCs. They also showed that the expression of genes encoding ABA transporters is higher in SCs and their results suggest a novel role for ZmNRT1.2 in ABA export outside SCs. Together, these results suggest that the ABA flux is targeted to GCs rather than SCs. Sun and colleagues provide an additional candidate gene by showing that CO_2-mediated stomatal movement is impaired in an *mpk12* maize mutant. The authors also applied the snRNA-seq method to leaf bases to track SC and GC developmental trajectories, which were consistent with those described in a previous report (Marand et al., 2021). Indeed, the cell lineage clusters are equally identified with their own SC and GC signature genes than with similar scRNA-seq data (Satterlee et al., 2020), further validating the snRNA-seq approach.

Together with other reports, this study validates the utility of snRNA-seq in acquiring signature genes of specific cell types. However, as GC nuclei were captured at a lower frequency than SC nuclei owing to their particular morphology, further optimization is needed to avoid or reduce such biases. Nonetheless, snRNA-seq surely paves the way toward a deeper understanding of the speedy grass stomatal complex.

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