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IN BRIEF

Guard Cell Proteome Reveals Signals and Surprises

Guard cell (GC) pairs regulate plant gas exchange and water loss by controlling the size of the stomate, the opening between them. Not an open and shut case, the regulation of stomatal size responds to a complex interplay of signals that transduce multiple environmental cues, including humidity, CO_2 levels, abiotic stresses, pathogens, and light. These signals are integrated through the action of ion pumps and channels to produce a change in the size of the stomatal aperture; for example, in GC opening, the action of proton pumps hyperpolarizes the cell membrane and activates voltage-gated K^+ ion channels. Ions flow in, and water follows, thereby increasing GC volume. The cell walls of the GC are structured such that this increase in cell volume causes the stomate to open (reviewed in Pandey et al., 2007). Abscisic acid (ABA) signaling of plant water status is particularly important in GC function: ABA both inhibits stomatal opening and induces stomatal closure.

GCs are a model system for signal transduction and have been examined using a plethora of techniques. **Zhao et al. (pages 3210–3226)** have now brought proteomics tools to bear on the examination of GC function, isolating GCs and determining their protein composition. Upon digestion of isolated epidermal pieces with cell wall–degrading enzymes, GCs are the last cell type to be released as protoplasts due to their thick cell walls. This differential susceptibility to enzymatic digestion can be exploited to obtain strikingly pure preparations of GC protoplasts (see figure, left) appropriate for single-cell-type proteome analysis.

The authors applied this method to 100 preparations from an amazing 22,000 *Arabidopsis* plants to isolate 3×10^8 GC protoplasts. They then used multiple proteomics methods, including 2D gels and the gel-free method of 2D liquid chromatography–matrix assisted laser desorption/ionization multidimensional protein identification technology, to identify 1734 unique proteins. This extensive single-cell proteome includes many proteins not previously identified from analysis of the GC transcriptome. Comparisons with the whole predicted *Arabidopsis* proteome, with the reported proteomes of leaves and other organs and with the set of genes known to function in GCs, yielded many intriguing candidates for future research.

One protein of remarkably high abundance in GCs (figure, center) was identified as THIOGLUCOSIDE GLUCOHYDROLASE1 (TGG1), an enzyme that hydrolyses glucosinolates, resulting (after subsequent steps) in the formation of isothiocyanates and other compounds toxic to microbes and herbivores (Barth and Jander, 2006). In this study, the authors found that *tgg1* mutants have decreased sensitivity to ABA inhibition of K^+ uptake channels and stomatal opening (figure, right); thus, the authors complete the scientific circle from protoplasts, through proteins, to phenotype. Given the usefulness of GCs as a model system and the importance of stomatal regulation in agriculture, the proteins identified in this study are likely to be important targets for future research.

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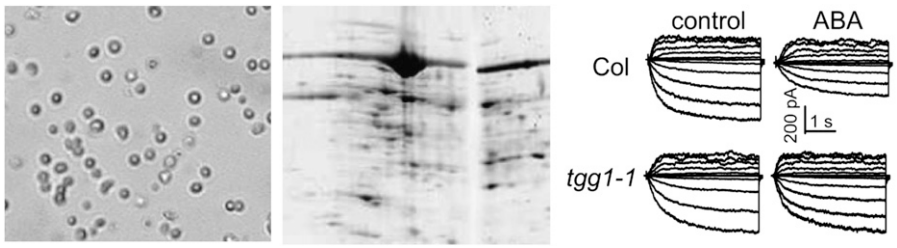

Purified guard cell protoplasts (left) yield a pattern of protein spots on a 2D gel (center). Mutant analysis of GCs lacking TGG1, the protein present in the strongest spot, reveals a decreased sensitivity to ABA (right, comparing current flow in the wild type at the top and in the mutant at the bottom).

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