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### Direct Measurement of Electron Transport in Plant Vacuoles: Functional Insights into Cytochrome B561a

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Ascorbate (Asc) is a key antioxidant in plant cells, and its redox function depends on its ratio with the one-electron oxidation product, monodehydroascorbate (MDHA). In this work, we demonstrate (doi: 10.1111/nph.18823) that a transmembrane electron transporter, localized to the tonoplast of *Arabidopsis* mesophyll vacuoles, functions as a reversible, Asc-dependent MDHA oxidoreductase.

Nearly four decades after the first patch-clamp recordings in plant cells, we report electron currents measured in isolated vacuoles, dependent on Asc (electron donor) and either ferricyanide or MDHA (electron acceptors) applied on opposite sides of the membrane. These currents were mediated by the tonoplast-localized cytochrome b561 isoform A (CYB561A). In CYB561A-deficient mutants, electron transport across the tonoplast was abolished, and under high-light stress, these mutants accumulated excessive leaf anthocyanins suggesting a role for CYB561A in modulating the redox response during photooxidative stress.

The kinetic behavior of CYB561A was described using an extended Michaelis–Menten model derived from a simplified kinetic scheme, offering insights into the interplay between donor and acceptor availability in regulating transmembrane electron transport.

### 10 Single-cell Biophysics: Techniques and Findings in Single-cell Analysis and Its Implications for Understanding Cellular Heterogeneity