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DIRECT RECORDINGS OF TRANSMEMBRANE ELECTRON CURRENTS IN PLANTS

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Electron transfer across biological membranes is achieved by specialized proteins, including b-type cytochromes, and is a fundamental step in physiological processes such as mitochondrial respiration, photosynthesis and iron uptake. Despite their importance, only few direct recordings of trans-membrane electron currents have been performed and no examples are known in plants. By using a classical electrophysiological approach, we provide robust evidence of trans-membrane electron flow mediated by a soybean CYBDOM, a member of the cytochrome b561 (CYB561) family. After expression of CYBDOM in Xenopus laevis oocytes and applying the two-electrode voltage-clamp technique we show that CYBDOM-mediated currents were activated by the presence of an extracellular electron acceptor, which acted in a concentration- and type-specific manner. Current amplitudes were strongly potentiated in oocytes pre-injected with ascorbate, the canonical electron donor for CYB561 proteins. Then we asked if it would be feasible to directly measure trans-membrane electron currents in native plant membranes. To answer this question, we performed patch-clamp recordings on large vacuoles isolated from Arabidopsis thaliana mesophyll cells. Tonoplast membranes are predicted to contain CYB561 proteins that may contribute to vacuolar iron mobilization. Current recordings in the whole-vacuole configuration showed that bath application of ascorbate (to the cytosolic side) elicit small inward currents in the absence of ferricyanide in the pipette solution (luminal side), allowing dose-response analyses that were found in line with the functional properties of the CYDOM expressed in Xenopus oocytes. Importantly, these measurements represent, about thirty-three years after the first report on plant ion channel activity, the first electron current recordings in a native plant membrane system.