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DIRECT RECORDING OF TRANS-PLASMA MEMBRANE ELECTRON CURRENTS MEDIATED BY A SOYBEAN MEMBER OF THE CYTOCHROME *b*561 FAMILY

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Cells and sealed plasma membrane (PM) vesicles isolated from different plant species and organs constitutively reduce external electron acceptors like ferricyanide or other suitable artificial dyes. Reduction of the external electron acceptors is typically associated with PM depolarization, supporting the view that the electrons required for the redox activity are provided by a cytoplasmic reductant via a trans-membrane electron transport system whose molecular identity remained elusive. By their activity, these electron transport systems provide a connection between cytoplasmic and apoplastic redox couples that are physically separated by the plasma membrane. Despite their importance in different physiological processes, including redox signalling and iron uptake, no direct recordings of trans-plasma-membrane electron currents have been described in plants.

Cytochromes b561 (CYB561) and related proteins containing a CYB561-core domain are membrane-spanning proteins encoded in angiosperms by gene families of about 15 members. The CYB561 core domain is constituted by a bundle of four trans-membrane alpha-helices with two hemes b facing opposite sides of the membrane. The simplest members of the CYB561 family consist of a single CYB561-core domain plus two additional trans-membrane helices with no obvious catalytic role. The CYB561 protein family includes CYB561-related proteins in which an additional domain, known as DOMON and sometimes present in two copies, extends towards the extracytoplasmic space. These proteins have been named CYBDOMs. The hydrophilic DOMON domain binds an additional heme b group such that all CYBDOMs contain two hemes in the CYB561 core plus one heme for each extracellular DOMON. Very little is known on the physiological role of CYBDOMs, for which angiosperms often contain around 10 encoding genes.

In this work, we provide robust electrophysiological evidence of trans-plasma-membrane electron flow mediated by a soybean CYBDOM, a member of the cytochrome b561 family, which localizes to the plasma membrane in transgenic Arabidopsis plants and CYBDOM cRNA-injected Xenopus oocytes. In oocytes, two-electrode voltage-clamp experiments showed that CYBDOM-mediated currents were activated by extracellular electron acceptors in a concentration- and type-specific manner. Current amplitudes were voltage-dependent, strongly potentiated in oocytes pre-injected with ascorbate, the canonical electron donor for cytochromes b561, and abolished by mutating a highly conserved histidine residue (H292L) predicted to coordinate the cytoplasmic

heme b group. We believe that this novel approach opens new perspectives in plant trans-membrane electron transport and beyond.