Plasma Membrane Purification - All protein extraction and purification steps were carried out keeping samples on ice, in a centrifuge held at 4°C, or in a 4°C cold room. A portion (20 g) of root and hypocotyl from 3-day-old soybean seedlings was collected. Roots and hypocotyls were ground using a mortar and pestle with 140 mL of grinding buffer containing 400 mM sucrose, 75 mM MOPS, 5 mM EDTA, 0.5 mM EGTA, 10 mM potassium fluoride, 1 mM dithiothreitol and 2% polyvinylpyrrolidone 40 on ice. The homogenate was filtered with four layers of Miracloth (Calbiochem, San Diego, CA, USA) and centrifuged at 10,000 x g for 15 min to pellet insoluble material. The supernatant was adjusted to a volume of 180 mL with grinding buffer, then transferred to ultracentrifuge tubes and centrifuged at 200,000 x g for 30 min. After discarding the supernatant, the precipitate, which included the proteins, was collected by dissolving it into 3 mL of grinding buffer and diluting with 6 mL of buffer containing 330 mM sucrose, 5 mM potassium phosphate buffer (pH 7.8) and 3 mM potassium chloride. The solution was mixed with 27 mL of buffer containing 330 mM sucrose, 5 mM K_3PO_4 (pH 7.8), 3 mM KCl, 6.2% polyethylene glycol (MW: 3350, Sigma-Aldrich, St. Louis, MO, USA) and 6.2% dextran (Sigma-Aldrich). The plasma membrane fraction of the mixture was purified by two-phase partitioning. The mixture was centrifuged at 2,450 x g for 4 min and the upper phase of the solution was collected. Partitioning was carried out three more times and the final plasma membrane-enriched solution was diluted four times with 20 mM Tris-HCl (pH 7.5). This solution was then centrifuged at 200,000 x g for 30 min and the precipitated plasma membrane fraction was dissolved in sample buffer containing 250 mM sucrose and 20 mM Tris-HCl (pH 7.5). The solution was again centrifuged at 50,000 x g for 1 h and the pellet dissolved in 100 µL of sample buffer containing 250 mM sucrose and 20 mM Tris-HCl (pH 7.5) (Kawamura and Uemura, 2003).

Plasma Membrane Purity Measurement - Purity of the plasma membrane fraction in solution was determined by measurement of plasma membrane-specific H^+-ATPase activity compared to total ATPase activity. The assay was based on quantification of liberated phosphate from ATP using the molybdenum blue method (Sze, 1985). Vanadate (Na_3VO_4), nitrate (KNO_3) and azide (NaN_3) were used as inhibitors of ATPases that are associated with a specific site, respectively plasma membrane, vacuolar membrane and mitochondrial membrane. The reaction solution was composed of 30 mM MES-Tris (pH 6.5), 50 mM KCl, 3 mM $MgSO_4$, 3 mM ATP and the respective inhibitors, 0.1 mM Na_3VO_4, 50 mM KNO_3 or 10 mM NaN_3. Water was added instead of inhibitor as a control. Purified protein (1 µg) was aliquoted to the

reaction solutions and incubated for 15 min at 30°C. The reaction was terminated by adding two times the volume of a stop solution containing 0.8 N H_2SO_4, 0.5% ammonium molybdate and 1% SDS. Ascorbate was then added at a final concentration of 0.3% and the mixture was placed at room temperature for 30 min. The concentration of the reaction product, ammonium phosphomolybdate chelate, was measured by absorbance at 750 nm using an Ultramark microplate imaging system (Bio-Rad, Hercules, CA, USA). To generate a standard curve, K_2HPO_4 was used at a range of 0.1 to 0.5 mM (Zhong et al., 1997). Please see the publication (Komatsu et al., 2009) for the purity used in this database.

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