Calcium regulation in Tradescantia virginiana: Roles for involvement of inositol 1,4,5 - trisphosphate and cyclic ADP -ribose

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Abstract

Fluorescent ratiometric imaging and spectrofluorometric analysis was used to study two signal transduction mechanisms in the stamen hair cells of *Tradescantia virginiana*. The first study determined the metabolic pathway necessary for the inactivation of Inositol 1,4,5-trisphosphate mediated calcium release from intracellular stores in the living plant cell. *Tradescantia* stamen hair cells, preloaded with the calcium sensitive ratiometric dye fura-2-dextran, were injected with analogs of Inositol 1,4,5-trisphosphate and cytosolic calcium levels monitored by ratiometric imaging. The injected analogs were selected due to their insensitivity to various kinases and phosphatases for which Inositol 1,4,5-trisphosphate is a substrate. We determined that the 5-phosphatase is the preferred pathway for inactivation of Inositol 1,4,5-trisphosphate in the living plant cell.^ The second study investigated cyclic ADP-ribose mediated calcium release in the intact plant cell and determined the presence of the metabolic machinery necessary to synthesize cyclic ADP-ribose from its precursor NAD^+. Cyclic ADP-ribose was observed to cause calcium release in the stamen hair cells of *Tradescantia* that were preloaded with the calcium sensitive dye fura-2-dextran. Evidence of cyclic ADP-ribose synthesis was determined using two experimental techniques. Homogenates of the sea urchin *Lytechnicus piclus* were used as bioassays to detect cyclic ADP-ribose in extracts of *Tradescantia* stamen hair cells that were incubated with b NAD^+. Cyclic ADP-ribose synthesis was detected from fluorimetric analysis of the homogenate as the calcium sensitive dye fluo-3 was present in the homogenate to detect cyclic ADP-ribose mediated calcium release from sea urchin egg microsomes. We also determined cyclic ADP-ribose synthesis by injection of fura-2-dextran loaded stamen hair cells with b NAD^+ and observing a delayed calcium increase in the cytosol. ^

These results establish the metabolic fate of inositol 1,4,5-trisphosphate in plant cells and demonstrate the biochemical capability for plant cells to synthesize cyclic ADP-ribose to mediate calcium release in plants. ^

Subject Area

Biology, Molecular|Biology, Cell|Biology, Plant Physiology

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