A Ca2+-dependent protein kinase that phosphorylates PI4P 5-kinases involved in pollen tube growth

Franziska Hempel

The regulatory phospholipid, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), is involved in regulating multiple physiological processes in eukaryotic cells. A prominent function for PtdIns(4,5)P_2 has been described in the control of polar tip growth of pollen tubes. In plants PtdIns(4,5)P_2 is synthesized by PI4P 5-kinases. A key enzyme of PtdIns(4,5)P_2 formation in pollen tubes of *Arabidopsis thaliana* is the PI4P 5-kinase PIP5K6. Based on the working hypothesis that PI4P 5-kinase activity can be modulated by reversible phosphorylation, it was found that recombinant PIP5K6 was phosphorylated *in vitro* by pollen tube extracts harboring protein kinase activity. Using combined in-gel kinase assays and mass-spectrometry, the calcium-dependent protein kinase CPK11 was identified as a candidate protein kinase phosphorylating PIP5K6. Interaction between CPK11 and PIP5K6 was confirmed by yeast-two-hybrid analyses. *In vitro* coincubation of the two purified recombinant enzymes enabled strictly calcium-dependent phosphorylation of PIP5K6 by CPK11. Effects of the coincubation on PI4P 5-kinase activity of PIP5K6 were tested in the presence and absence of CPK11 and calcium, revealing calcium-dependent inhibition of PIP5K6 *in vitro*. In line with these results, effects of transient expression of PIP5K6 on growth and morphology of tobacco pollen tubes were attenuated upon coexpression of CPK11 *in vivo*. Thus, our results show that PIP5K6 is negatively regulated by CPK11 *in vitro* and *in vivo*. To our knowledge, this is the first reported link between calcium-signaling and phoshoinositide metabolism in plants. Current work is aimed at identifying and characterizing relevant phosphorylation sites to better understand the molecular mechanism by which CPK11 regulates PIP5K6. This work was supported by the German Research Foundation (DFG, grant He3424-3 to IH and the RTG1026).