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Mini-Review

Roles of phosphoinositides in regulation of stomatal movements

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Guard cells sense various environmental and internal stimuli and, in response, modulate the stomatal aperture to a size optimal for growth and adaptation. Among the many factors involved in the fine regulation of stomata, we have focused our studies on the role of phosphoinositides. Our recent study published in the *Plant Journal* (52:803–16) provides evidence for an important role for phosphatidylinositol 4,5-bis-phosphate (PtdIns(4,5)P_2) in inducing stomatal opening. Light induces translocation of a PtdIns(4,5)P_2-binding protein from the cytosol to the plasma membrane and treatments that increase the intracellular PtdIns(4,5)P_2 level induce stomatal opening in the absence of light irradiation. Inhibition of anion channel activity, a negative regulator for stomatal opening, was suggested as a mechanism of PtdIns(4,5)P_2-induced stomatal opening. We also reported that phosphatidylinositol 3-phosphate (PtdIns(3)P) and phosphatidylinositol 4-phosphate (PtdIns(4)P) regulate actin dynamics in guard cells. The effects of the phosphoinositides were specific, and were not induced by other lipids with similar structures. The roles of different interacting partners are likely to be important for these lipids to produce specific changes in guard cell activity.

Introduction

Phosphoinositides are a family of inositol-containing phospholipids found in all eukaryotic cells and they play many important roles in signal transduction pathways. Their roles in regulation of stomatal movements have been reported previously.[1-5] The level of phosphoinositides changes rapidly in response to ABA treatment, while that of other membrane lipids remains similar,[1] suggesting that phosphoinositides are likely to be involved in ABA signaling. Various phosphoinositides use distinct mechanisms to induce distinct effects in regulating the stomatal aperture. Phosphatidylinositol 4,5-bis-phosphate (PtdIns(4,5)P_2) is necessary for both stomatal opening and closing. PtdIns(4,5)P_2 serves as a substrate of phospholipase C (PLC), which cleaves PtdIns(4,5)P_2 to produce inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3), an important second messenger in stomatal closing. However, it remains less clear how PtdIns(4,5)P_2 modulates stomatal opening. We recently identified a mechanism by which PtdIns(4,5)P_2 enhances stomatal opening.[6] Phosphatidylinositol 3-phosphate (PtdIns(3)P) functions as a positive effector of stomatal closing by activating ROS formation[5] and Ca^{2+} oscillations.[4] Phosphatidylinositol 4-phosphate (PtdIns(4)P) similarly enhances stomatal closing. Our recent data identify an additional target of (PtdIns(3)P) and (PtdIns(4)P): the actin cytoskeleton.[7]

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The Role of PtdIns(4,5)P_2 in Stomatal Opening

We observed that exogenously applied PtdIns(4,5)P_2 induced stomatal opening without light irradiation, and GFP:$PH_{PLC\delta1}$, a biosensor widely used to detect PtdIns(4,5)P_2 in plant cells,[8,9] translocated from the cytosol to the plasma membrane in response to white light irradiation. These results suggest a light-dependent increase in PtdIns(4,5)P_2 levels at the plasma membrane of guard cells. As a mechanism of action, we proposed inhibition of anion channel activity by PtdIns(4,5)P_2. Activated anion channels allow the efflux of anions, and, thus, membrane depolarization in plant cells. The channels retain a significant open probability at strongly hyperpolarized potentials, as low as -200 mV,[10-12] and act as a leak pathway that inhibits further hyperpolarization of the membrane and consequent K^+ uptake through voltage-dependent K^+ channels. Therefore, suppression of the anion leak should enhance stomatal opening. This hypothesis is supported by the observation that stomata open in response to treatment with anion channel inhibitors.[12-14] Based on these findings, we suggested that light elevates the level of PtdIns(4,5)P_2, which inhibits anion channel activity, and, consequently, enhances stomatal opening. Recently, blue light is shown to inactivate anion current from guard cells of intact Arabidopsis and *V. faba* plants, supporting this possibility.[15]

The importance of PtdIns(4,5)P_2 in stomatal opening is further supported by our observations that mutant plants which do not express PIP5K4, an enzyme that generates PtdIns(4,5)P_2, are compromised in light-induced stomatal opening. However, PIP5K4 is not likely to be the only enzyme responsible for synthesis of PtdIns(4,5)P_2 in guard cells, since eight different PIP5Ks are expressed in guard cells. The different enzymes may be expressed and activated under different conditions. Further characterization of these enzymes will be necessary to understand how PtdIns(4,5)P_2 synthesis is regulated under different conditions.

Roles of PtdIns(4,5)P_2 Metabolites in Stomatal Movement

Intracellular levels of PtdIns(4,5)P_2 are modulated by the activity of kinases, phosphatases, and lipases, and many PtdIns(4,5)P_2

precursors and metabolites have effects opposite to that of PtdIns(4,5)P_2 in stomatal movements. The PtdIns(4)P-binding protein, which presumably reduces the level of free PtdIns(4)P, enhances stomatal opening, suggesting that PtdIns(4)P itself exerts the opposite effect and enhances stomatal closing.[4] In addition, wortmannin, which inhibits PI4K as well as PI3K, inhibits ABA-induced stomatal closing.[7] The opposite effects of these two lipids suggest that the transition between PtdIns(4)P and PtdIns(4,5)P_2 is precisely regulated in guard cells. Consistent with this hypothesis, Im et al., provided evidence suggesting that PIP5K, not PI4K, is the rate-limiting enzyme in PtdIns(4,5)P_2 biosynthesis.[16] They found that the ratio of PtdIns(4)P to PtdIns(4,5)P_2 in wild-type tobacco cells was ≥ 10:1, and in tobacco cells expressing human *PIPKI*α, a 100-fold increase in plasma membrane PtdIns(4,5)P_2 was observed without any change in the PtdIns(4)P level.

Hydrolysis of PtdIns(4,5)P_2 by phospholipase C produces a second messenger, Ins(1,4,5)P_3, which induces stomatal closing.[17-19] The level of Ins(1,4,5)P_3 increases rapidly and transiently after ABA treatment of guard cells.[1] Photolysis of microinjected caged Ins(1,4,5)P_3 leads to Ca^{2+} release, inactivates guard cell K^+ channels, and induces stomatal closing.[17,18] Conversely, microinjection of the Ins(1,4,5)P_3 antagonist heparin[20] or reduction of Ins(1,4,5)P_3 production by inhibiting the activity or expression of PI-PLCs inhibits ABA-mediated signal transduction in guard cells.[21,22] Among the nine putative *PI-PLC* isoforms found in the Arabidopsis genome,[23,24] only *PLC1* has been studied in depth, but not in guard cells. *PLC1* is highly induced under dehydration and high salinity conditions[25] and is required for secondary responses to ABA signals.[26] It remains to be determined which of the many PI-PLCs are involved in production of Ins(1,4,5)P_3 in guard cells.

PtdIns(4,5)P_2 may be dephosphorylated by enzymes called inositol polyphosphate 5-phosphatases (5PTases), which dephosphorylate inositol phosphates and/or phosphoinositides.[27,28] Dephosphorylation of inositol phosphates would shorten the duration of the ABA response mediated by Ins(1,4,5)P_3, while dephosphorylation of PtdIns(4,5)P_2 may reduce the initial production of Ins(1,4,5)P_3 by reducing the substrate for PLC. Therefore, regardless of which substrate is acted upon, the 5PTases are likely to reduce ABA responses of plants. We predict that further analysis of the 5PTases may provide additional information on the role of this group of enzymes in guard cell signaling, based on our previous observation that when lithium, an inhibitor of inositol mono- and bis-phosphate phosphatases, was present in the guard cell protoplast medium, the level of Ins(1,4,5)P_3 increased approximately two fold after ABA treatment of guard cells.[1] The 5PTases may also inhibit stomatal opening by reducing the level of PtdIns(4,5)P_2 in the guard cells. Defective stomatal opening in plants that overexpress *5PTase 1*[29] may be due to a decreased level of PtdIns(4,5)P_2, but this possibility was not investigated.

Actin Cytoskeleton—Downstream Target of PtdIns(3)P and PtdIns(4)P in Stomatal Closing

We recently demonstrated that PtdIns(3)P and PtdIns(4)P are involved in ABA-induced stomatal closing via regulation of actin dynamics.[7] Actin filaments transversely arranged in open guard cells undergo fragmentation and become randomly oriented upon exposure to ABA.[30] These ABA-induced changes in actin filaments are inhibited by wortmannin, an inhibitor of PtdIns(3)P and PtdIns(4)P synthesis. The inhibitor effect was reversed by addition of exogenous PtdIns(3)P or PtdIns(4)P (our unpublished results). Expression of the binding domains of PtdIns(3)P or PtdIns(4)-binding proteins in guard cells, using the biolistic bombardment method of transformation, also inhibited ABA-induced actin reorganization in a manner similar to wortmannin. These results suggest that maintenance of normal levels of PtdIns(3)P and/or PtdIns(4)P is required for ABA-induced actin dynamics in guard cells and for stomatal closing. Actin binding proteins such as fimbrin or villin, which bind to and stabilize actin filaments, are potential candidates that mediate lipid regulation of the actin cytoskeleton in guard cells.

We also proposed that ROS are necessary intermediates for PtdIns(3)P-mediated actin reorganization. ROS reduced the proportion of cells that maintain transverse actin filaments, and, conversely, agents that reduce ROS levels increased the proportion of cells with transverse actin filaments following ABA treatment. Intracellular Ca^{2+} levels, which are elevated by ROS, may mediate the ROS induced changes in the actin cytoskeleton, since some actin-binding proteins such as villin and profilin bind actin in a Ca^{2+}-dependent manner. Alternatively, the elevated levels of ROS generated by ABA treatment may induce actin depolymerization by directly weakening inter-monomer bonds.

Conclusions and Perspectives

To adjust the stomatal aperture for optimal growth and adaptation, guard cells need to integrate signals of differing amplitudes from multiple pathways. The complex metabolism of phosphoinositides, which produce metabolites with different functions and with distinct interacting partner proteins, may be necessary to integrate these complex signals and produce the appropriate responses. Identification of downstream targets and interacting partners of each phosphoinositide and elucidation of the regulatory mechanisms of each enzyme participating in phosphoinositide metabolism, such as PIKs, PIP5Ks, PI-PLCs, and phosphoinositide phosphatases, will improve our understanding of the fine control of stomatal movements. A difficult problem in phosphoinositide research is that many of these lipids are present only in minute quantities in the cell, which makes it exceedingly difficult to detect signal-dependent changes in their levels in guard cells using biochemical methods. Genetic analyses are useful for identifying molecules participating in the signaling pathway, but are not sufficient to identify the rate-limiting steps in vivo. More sensitive methods of analysis of these lipids will greatly accelerate this field of research.

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