Mini-Review

Towards understanding the function of stress-inducible PtdIns(4,5)P₂ in plants

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The phosphoinositide (PI) system is a central regulatory network between all eukaryotic kingdoms. Studies on mammalian and yeast cells indicate that cellular functions regulated by PIs include the production of soluble inositolpolyphosphates with signaling functions as well as recruitment of proteins required for endo- or exocytosis. In contrast to other models, knowledge on PI functions in plants is limited and, despite of reports of transient PI-increases upon stress-treatments, plant cellular processes involving changes in PI-levels have remained unclear. In previous studies various groups have proposed that PI-increases upon hyperosmotic stress support the generation of soluble second messengers with possible roles in stress adaptation. Based on a combination of biochemical analysis and imaging of fluorescent reporters we have now demonstrated that intact phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) associates with clathrin-coated vesicles (CCVs) in a stress-inducible manner in plant cells. In analogy to previous studies on other models, association with CCVs suggests a role for PtdIns(4,5)P₂ in the recruitment of vesicle coat proteins and in membrane internalization that is alternative to functions in second messenger production. The determination of subcellular sites of PtdIns(4,5)P₂ increases, thus, opens new avenues of investigation in the plant PI-field and allows development of testable hypotheses to delineate PI-functions in plants.

Stress-Induced Formation of Phosphoinositide-Derived Second Messengers in Plants

The phosphoinositide (PI) system is a central regulatory network of all eukaryotic cells, and physiological functions of PIs in mammalian and yeast cells have been extensively reviewed.1,2 Whereas all major components of a PI-system have been detected in plant cells,3,4 only recently the identification and characterization of Arabidopsis mutants deficient in defined steps of PI biosynthesis has yielded insights into what cellular processes are regulated by PIs in plants.5-9 As a first step in characterizing the plant PI system, mutant characterization studies have focused on the constitutive roles of PIs in plant function or development. An interesting aspect of earlier reports, however, was the observation of transient increases in PtdIns(4,5)P₂ and derived metabolites with so far unclear physiological roles that are formed upon application of stress conditions.10-13

It has previously been proposed that PtdIns(4,5)P₂ formed transiently upon challenge of plant cells with hyperosmotic stress is hydrolyzed by phospholipase C (PLC) to form inositol-1,4,5-trisphosphate (InsP₃) which may be involved in the release of Ca²⁺ from internal stores.10 In this context it must be noted that despite of substantial efforts so far no “IP₃-receptor” has been identified in any plant system, and that the mode of a proposed InsP₃-mediated Ca²⁺-release from intracellular stores is not understood. While PLC-mediated hydrolysis of PtdIns(4,5)P₂ is clearly an early event in the response of plant cells to hyperosmotic stress, it remains to be solved whether InsP₃ represents a relevant signal by itself or whether its conversion to other derived metabolites is important. For instance, another role of stress-induced InsP₃ may be that of a precursor of inositolpolyphosphates acting as cofactors to receptor proteins for auxin or jasmonic acid, as previously discussed,14-17 a concept that so far has not received particular experimental attention.

Additional Roles of PIs in Stress-Adaptation of Plants

In previous studies we have demonstrated that the fatty acid composition of PIs transiently formed upon hyperosmotic stress treatments differs from that of PIs that are constitutively present, and that stress-induced species contained significantly higher proportions of the polyunsaturated fatty acids (PUFAs) linoleic acid and linolenic acid than constitutive species.18 These data immediately indicate that stress-induced and constitutive PIs must be derived from different pools of precursors,18,19 a distinction possibly made at the level of phosphatidylinositol synthesis based on a preference of PI-synthase isoforms for cytidinediphospho-diacylglycerol precursors differing in their associated fatty acids.20 Importantly, dynamic changes in the levels of downstream metabolites of PtdIns(4,5)P₂, diacylglycerol (DAG) and phosphatidic acid (PtdOH), formed in Arabidopsis plants subjected to hyperosmotic stress and their polyunsaturated fatty acid composition were consistent with PLC-mediated hydrolysis of PtdIns(4,5)P₂18 as had been proposed before.10 Slight differences in the fatty acid compositions of stress-induced PtdIns(4,5)P₂ and of DAG or PtdOH detected18 were initially ignored in light of fatty...
acid analyses performed at the limit of detection. Besides PUFAs, however, stress-induced PtdIns(4,5)P₂ species reproducibly also contained saturated and monounsaturated fatty acids, i.e., increased levels of stearic and oleic acids, that were not found to be increased in DAG or PtdOH.¹⁸

Reexamination of the incongruence in fatty acid patterns suggests that not only a polyunsaturated hydrolyzable pool of PtdIns(4,5)P₂ was increasing with hyperosmotic stress but also a more saturated pool that was not hydrolyzed and may serve other, alternative regulatory functions. In our most recent contribution²¹ we attempted to define the subcellular sites at which such intact PtdIns(4,5)P₂ increased upon hyperosmotic challenge in order to create new hypotheses regarding roles of intact PtdIns(4,5)P₂ not hydrolyzed by PLC. In time course experiments after hyperosmotic stress various enriched subcellular fractions were tested for the levels and fatty acid composition of associated PtdIns(4,5)P₂. This experimental setup allowed a limited degree of spatio-temporal resolution of PtdIns(4,5)P₂ formed upon hyperosmotic stress. PtdIns(4,5)P₂ was found to be increased first in fractions enriched for plasma membranes, whereas at later time points PtdIns(4,5)P₂-levels were increased mostly in endomembrane fractions subsequently identified as CCVs.²¹ By synchronous visualization of fluorescence-tagged clathrin light chain with a fluorescence-tagged reporter for PtdIns(4,5)P₂,²²⁻²⁴ stress-induced colocalization of clathrin light chain with PtdIns(4,5)P₂ was confirmed using an independent approach.²¹ A major response to hyperosmotic stress is membrane internalization following water efflux and plasmolysis in order to prevent membrane leakage and maintain cellular integrity. One possible route of plasma membrane internalization is by the formation of CCVs.²⁷⁻²⁹ Our results suggest a role for salt-stress-induced PtdIns(4,5)P₂ in the formation and/or internalization of CCVs at the plasma membrane that now invites further study. Note that the results reported²¹ are only correlative and do not at this point answer whether increased PtdIns(4,5)P₂ levels observed are cause or effect of increased CCV formation. Nonetheless, our data represent an advance mainly because new hypotheses can be formulated, opening the field of plant PI-functions to new experimental approaches. An involvement of PtdIns(4,5)P₂ in the recruitment of clathrin to endocytotic vesicles has previously been reported for yeast cells.²⁶ As the recycling of plasma membrane vesicles is a key element of exocytosis and insertion of integral membrane proteins, PtdIns(4,5)P₂ may be involved in fundamental aspects of membrane trafficking of plant cells in a similar fashion to mammalian or yeast cells. Future experiments will be directed to test whether and how clathrin recruitment and CCV formation are affected in plants with perturbed PI-metabolism; whether and what other vesicular coat proteins may depend on PtdIns(4,5)P₂ for recruitment; or what phosphatidylinositol-4-phosphate 5-kinases are responsible for PtdIns(4,5)P₂ production at the plasma membrane and/or the vesicle surface.

**Functional Pools of Stress-Induced PtdIns(4,5)P₂**

Together with previous data it is clear that stress-induced PtdIns(4,5)P₂ represents at least two functional pools involved (i) in second messenger production and (ii) in CCV formation, respectively (Fig. 1). In contrast to PtdIns(4,5)P₂ serving as a substrate for PLC and containing PUFAs,¹⁸ intact PtdIns(4,5)P₂ associated with CCVs contained mainly stearic and oleic acids and was less unsaturated.²¹

-The reported differences in fatty acid composition define molecular species of PIs that will exhibit different biophysical properties with regard to their lateral mobility within membranes²⁷⁻²⁹ and, thus, may associate with different protein partners, as previously proposed.¹⁹ In the absence of sensitive means of detection of molecular species of PIs in living cells, it remains to be seen whether different physiological functions of the proposed pools are a consequence of their origin from different sets of biosynthetic enzymes, a consequence of distinct lateral diffusion within the membrane according to the associated fatty acids, or both. It must be noted that CCV-associated structural phospholipids, such as phosphatidycholine (PtdCho) or phosphatidylethanolamine (PtdEtn) contained a large proportion of PUFAs,²¹ an observation consistent with a recent report for endocytotic vesicles in Caenorhabditis elegans.³⁰ In C. elegans, the presence of PUFAs was required for vesicle recycling, however, it is not clear whether required PUFAs were associated with structural lipids or with PIs.

**Perspective**

The evolutionary conservation of the PI system between eukaryotic kingdoms is highlighted by the presence of genes for all essential components of the endocytotic machinery as well as for factors required for other key processes in plant genomes analyzed so far. While it is tempting to speculate that key cellular processes regulated by PIs, such as endo- or exocytosis, may be fundamentally similar in mammalian, fungal and plant cells, differences will emerge when the physiological consequences are considered; secretion of neurotransmitters at the synaptic cleft clearly will have different consequences than secretion of cell wall material by a plant cell. Future experiments will reveal relevant details of the PI-system's

![Diagram of Functional Pools of Stress-Induced PtdIns(4,5)P₂](image-url)
involvement in plant function, development and adaptive responses to environmental stresses.

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