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Yu Mei

Functional Characterization of *Arabidopsis* Phosphatidylinositol Monophosphate 5-kinase 2 in Lateral Root Development, Gravitropism and Salt Tolerance

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Xue, H.W., Chen, X., and Mei, Y. (2009). Function and regulation of phospholipid signalling in plants. Biochem. J. **421:** 145–156.

I would like to dedicate this thesis to my parents and to my husband for supporting me all the way.

Supervisor's Foreword

Plant phosphatidylinositol (PI) signaling pathway is an important signal transduction pathway that involves in multiple developmental processes, including growth control, defense, hormone function, light response, ion regulation, seed germination, cytoskeleton regulation, and flowering control, etc. Phosphatidylinositol monophosphate 5-kinase (PIP5K) catalyzes the synthesis of PI-4,5-bisphosphate (PtdIns(4,5)P_2) and is a key enzyme in PI signaling pathway. In the dicot model plant *Arabidopsis*, 15 genes encoding putative PIP5Ks have been identified. How these genes are expressed in vivo, what functions they have and how they are regulated are questions of great interest and importance. Studies have revealed the physiological functions of some PIP5Ks; however, the functions and molecular mechanisms of PIP5K2 are not reported before. This thesis focuses on the functional characterization of *Arabidopsis* PIP5K2. Through genetic, physiological, molecular, and cell biological studies, the detailed expression pattern and functions of PIP5K2 were systemically analyzed, and the underlying mechanism was investigated and discussed.

The expression pattern of *PIP5K2* is analyzed first with RT-PCR and promoter-GUS fusion studies. Since *Arabidopsis* has multiple members of PIP5Ks, the information of expression pattern is of great importance in order to better understand the physiological functions. It is found that *PIP5K2* is expressed in various tissues and its expression is enhanced by exogenous auxin and salt treatment, indicating a role of PIP5K2 in auxin- and/or salt-related processes. By employing a knockout mutant of *PIP5K2*, *pip5k2*, the physiological functions of PIP5K2 are then investigated. It is found that *pip5k2* shows reduced lateral root formation, which could be recovered with exogenous auxin, and interestingly, delayed root gravity response that could not be recovered with exogenous auxin. Reduced auxin accumulation is confirmed in *pip5k2* which is responsible for reduced lateral root formation. On the other hand, vesicle trafficking and PIN protein cycling is suppressed under *PIP5K2* deficiency, which is well recovered by transformation rescue of *PIP5K2* or treatment of exogenous PtdIns(4,5)P_2. In addition, *pip5k2* is found to be hypersensitive to salt treatment, including Na^+ and K^+, but not to Li^+ or Cs^+. Salt

stress-induced bulk-flow endocytosis is also found to be suppressed under *PIP5K2* deficiency.

It is demonstrated in this thesis that *Arabidopsis* PIP5K2 is involved in regulating lateral root formation and root gravity response through regulation of auxin accumulation and polar auxin transport. A critical role of PIP5K2/PtdIns(4,5)P_2 in root development through regulation of PIN protein cycling is revealed. It is also proven that PIP5K2 gets involved in salt tolerance in a way independent of SOS pathway or cytoskeleton regulation, but probably through regulation of salt stress-induced bulk-flow endocytosis. These results provide direct evidence of crosstalk between the PI signaling pathway and auxin response, and new insights into the control of polar auxin transport and salt stress responses.

Shanghai, June 2014 Prof. Hong-Wei Xue

Abstract

Phophatidylinositol monophosphate 5-kinase (PIP5K), which catalyzes the synthesis of PtdIns-4, 5 bisphosphate [PtdIns(4,5)P2] by phosphorylation of PtdIns-4-phosphate at the 5 position of the inositol ring, is a key enzyme in phosphatidylinositol signaling pathway. In *Arabidopsis thaliana*, 15 genes encoding putative PIP5Ks have been identified. In this study, we isolated and characterized the expression pattern, physiological functions, and the underlying mechanism of *Arabidopsis* PIP5K2 through molecular and genetic approaches.

Expression pattern studies using RT-PCR and promoter-GUS methods reveal that *PIP5K2* is expressed in various tissues including cotyledon, hypocotyl, root, leaf, flower, and silique, especially during lateral root initiation and elongation. Subcellular localization study reveals that the majority of PIP5K2 is localized on the plasma membrane, which is obvious after plasmolysis.

Knockout mutant of *PIP5K2, pip5k2* shows reduced lateral root formation, which could be rescued by exogenous auxin, and suppressed root gravity response that could not be rescued by exogenous auxin. Transformation rescue of *PIP5K2* in *pip5k2* background rescues all the phenotype, indicating the defects are caused by *PIP5K2* deficiency. Statistical analysis of the number of lateral root primordia and lateral roots at different developmental stages shows that lateral root initiation rather than lateral root emergence is significantly affected in *pip5k2*, which leads to the reduced numbers of lateral roots. Reduced auxin accumulation in *pip5k2* is confirmed by GUS activity detection in cross progenies with DR5-GUS marker line. Further analysis revealed the suppressed expression of auxin biosynthesis-related genes and stimulation of metabolism-related genes that convert auxin into inactive conjugates, which is consistent with the result of decreased free auxin accumulation in the mutant. These results indicate that *PIP5K2* deficiency caused decreased auxin accumulation, and the decrease of auxin into lateral root primordia and emerged lateral roots may cause the reduction of lateral root formation in *pip5k2*.

The result that suppressed root gravity response could not be rescued by exogenous auxin suggests that the regulation of PIP5K2 in gravity response is achieved by other potential routes, very likely by affecting polar auxin transport. This is supported by the result that *pip5k2* is hypersensitive to auxin transport