LABORATORY OF STRESS RESPONSE

Associate Professor: *MIKAMI, Koji*

As their sessile nature, growth and development of plants are largely influenced by environmental factors; thus, plants must have highly sophisticated systems to sense and respond to these factors. Much effort has been put into the analysis of stress-signal transduction in plants, which led us to the identification of stress-responsive genes and transcription factors responsible for the stress-dependent transcription. However, it is still unknown how plants sense various kinds of environmental stresses separately and how environmental stresses are converted into intracellular signals. Based on our knowledge in mammalian cells, it is possible that, in plants, the transmission of the signals via cell surface receptors or sensors may result in the production of intracellular second messengers, such as inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG), both of which initiate cascades of intracellular signal transduction in response to environmental stresses. Moreover, since phosphatidylinositols, some of which are precursors of the second messengers or are second messengers themselves, are produced by sequential phosphorylation and dephosphorylation via lipid kinases and lipid phosphatases in the PI pathway (Figure 1), these reactions could be activated by environmental stimuli. Indeed, stress-dependent production of PI 3,4-bisphosphate [$PI(3,4)P_2$] and IP_3 has already been reported in various kinds of plant species. It is, therefore, important to analyze the PI pathway to understand the molecular mechanisms of sensing of and response to extracellular signals in plants.

Since phosphoinositide-specific phospholipase C (PI-PLC) plays an essential role in the PI pathway through generation of IP_3 and DG by the hydrolysis of $PI(3,4)P_2$ (Figure 1), I have investigated the functions of PI-PLCs, PpPLC1 and PpPLC2, isolated from the moss *Physcomitrella patens*, because *Physcomitrella* is now recognized as a model system for plants with easy application of molecular genetic approaches such as gene-targeted mutagenesis via the homologous recombination. However, there is no information about PI pathway-regulating enzymes other than PI-PLCs in *Physcomitrella*, thus it is necessary to analyze the lipid kinases and phosphatases to confirm the similarity of the PI pathways between *Physcomitrella* and higher plants. Accordingly, the structural characterization of *Physcomitrella* lipid kinases and PI-PLCs were performed.

I. PI 4-kinase

PI 4-kinase (PI4K) phosphorylates the D4 position of PI to yield PI 4-phosphate [PI(4)P]. Two major types of PI4Ks, type II and type III, have been identified in animals and yeasts. The type II PI4Ks are membrane-bound 55 kD enzymes, which consists of the conserved catalytic domain and small N-terminal extension. There are two forms, a and b, for type III PI4Ks; the former consists of the PH domain and catalytic domain with a long N-terminal extension and 200 to 230 kD in size, whereas the latter is 110 kD protein composed with LKU, NH and catalytic domains. Plants also have all forms of PI4K.

Until to date, I have obtained only one full-length cDNA encoding a β form of type III PI4K, PpPI4Kβ1, whose structure is very close to those of the β form of type III PI4Ks from other species. Interestingly, although the repetitive sequence domain between LKU and NH domains was reported as a unique domain in *Arabidopsis* AtPI4Kβ1, the same domain was found in PpPI4Kβ1 (Figure 2), indicating that this domain is specific in plants. Thus, PpPI4Kβ1 is useful to investigate the plant-specific function of the type III PI4Kβ.

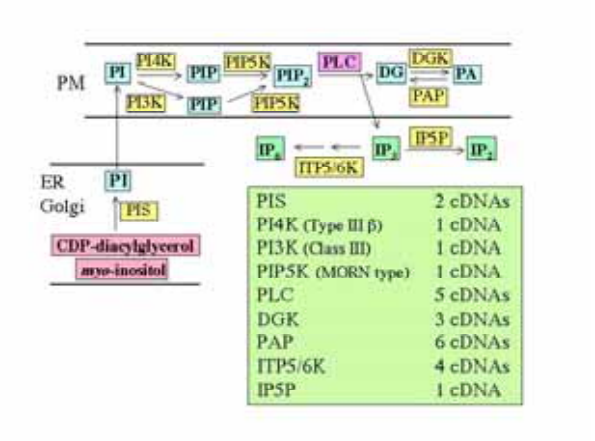

Figure 1. Schematic representation of the PI pathway. Light blue and yellow boxes indicate phosphoinositides and enzymes (PI synthase, lipid kinases or lipid phosphatases), respectively. Current situation of the cloning of *Physcomitrella* full-length cDNAs encoding enzymes involved in the PI pathway was indicated in the large box. PM, plasma membrane.

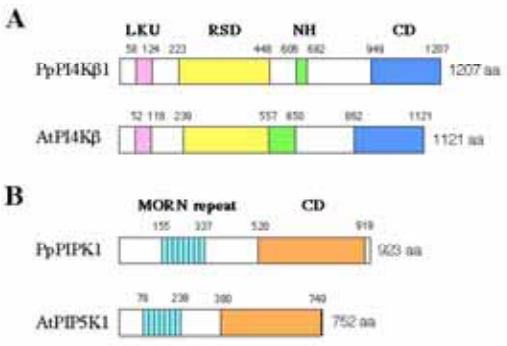

Figure 2. Structural characteristics of plant β form of type III PI4Ks and PIPKs. Protein domains represent in PpPI4Kβ1 (A) and PpPIPK1 (B), in comparison with *Arabidopsis* homologues. Numbers indicate amino acid positions. Accession nos. are as follows; AtPI4Kβ, AJ002685; AtPI5K1, Q56YP2. RSD, repetitive sequence domain; CD, catalytic domain.

II. PI-phosphate kinase

PI-phosphate kinase (PIPK) catalyzes the synthesis of PI(5)P, PI(3,5)P2 and PI(3,4)P2 by phosphorylation of PI and PI-phosphates. In animals, PIP5Ks are classified into three subfamilies, type I to III, according to their substrate specificity. Type I PIPKs are PI 5-kinases that phosphorylate PI and PI(3)P to generate PI(5)P and $PI(3,5)P_2$, while type II enzymes produce $PI(3,4)P_2$ from PI(3)P by their PI 4-kinase activity. Type III enzymes catalyze phosphorylation of the D5 position of PI as PI 5-kinase. The first plant PIPK was *Arabidopsis* AtPIP5K1 (Mikami *et al*., Plant J. *15*, 563-568, 1998) whose catalytic domain is similar to both animal type I and II enzymes, thus AtPIP5K1 was classified as type I/II. Indeed, it has been reported that AtPIP5K1 has both the PI 4-kinase and PI 5-kinase activities. Interestingly, AtPIP5K1 had a long N-terminal extension containing an unidentified repetitive sequence that was named recently a MORN repeat; however, the function of this repeat is still unknown. Taken together, the structure of type I/II PIPKs is plant-specific and, thus, the activation mode of them may also be plant-specific. Plants have the type III PIPKs as is in animals.

I obtained a *Physcomitrella* full-length cDNA encoding PIPK, designated PpPIPK1. As shown in Figure 2, PpPIPK1 consists of a N-terminal extension containing the MORN repeat and the catalytic domain classified as the type I/II enzyme. As the conservation of plant-specific structure between *Physcomitrella* and *Arabidopsis*, PpPIPK1 is useful to analyze the plant-specific mode of activation and functions of plant type I/II PIPKs.

III. PI-PLC

As mentioned above, PLC catalyzes the hydrolysis of $PI(4,5)P_2$ to generate two second messengers in a Ca^{2+}-dependent manner. Although animal PI-PLCs were classified into 5 isozymes, named β, γ, δ, ε and ζ types. All of them contain the X and Y domains to compose their catalytic domain and C2 domain as Ca^{2+}-dependent membrane-interacting module, although isozyme specific-domain structures are composed by the combination with other protein motifs in relation to their activation modes. It is well known that β and γ isozymes are activated by G protein-coupled receptors and receptor tyrosine kinases, respectively, whereas the activation of ε isozymes is regulated by heteromeric G proteins and Ras. In contrast to the variety of isozymes with isozyme-specific activation modes in animal PI-PLCs, plant PI-PLCs reported showed the same structure that resembles closely those of the ζ isozymes, which are sperm-specific enzymes composed by the two EF-hand repeats and X, Y and C2 domains. In plant PI-PLCs, there is one EF-hand repeat called the N domain. Until to date, it is unclear how plant PI-PLCs are activated.

It has been reported that two *Physcomitrella* PI-PLC, PpPLC1 and PpPLC2, are structurally close to plant PLCs reported, however PpPLC2 containing point mutations in the Y domain and an insertion in the N domain is inactive

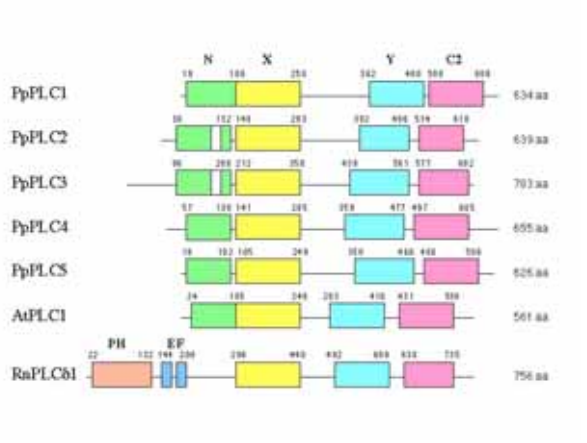

Figure 3. Structural characteristics of five *Physcomitrella* PI-PLCs. Protein domains represent in five *Physcomitrella* PI-PLCs, PpPLC1 to PpPLC5, in comparison with AtPLC1 from *Arabidopsis* and RnPLCδ1 from *Rattus norvegicus*. All PI-PLCs indicated have the X, Y and C2 domains, although the PH (pleckstrin homology) domain, which regulates membrane localization of animal PI-PLCs, is not found in any plant PI-PLCs. Numbers indicate amino acid positions. Accession nos. are as follows; PpPLC1, BAD02919; PpPLC2, BAD02918; AtPLC1, BAA07547; RnPLCδ1, NP_058731.

form of PI-PLC (Mikami *et al*., J. Exp. Bot. *55*,1437-1439, 2004). In addition, I have cloned other 3 full-length cDNAs encoding PpPLC3, PpPLC4 and PpPLC5. As shown in Figure 3, PpPLC4 and PpPLC5 have structures similar to that of PpPLC1, although PpPLC3 has mutations in the Y domain and an insertion in the N domain, which is similar to PpPLC2. Thus, *Physcomitrella* seems to have three PI-PLC isozymes whose structural organization is identical to those of higher plant PI-PLCs, although the inactivated forms such as PpPLC2 and PpPLC3 are found in only *Physcomitrella*. These findings indicate that *Physcomitrella* is useful to investigate the activation mode of plant PI-PLCs and the functional diversity among PI-PLC isoforms.

IV. Conclusion

Structures of lipid kinases and PI-PLCs involved in the PI pathway in *Physcomitrella* are basically similar to those in higher plants, suggesting the conservation of the activation modes and, probably, functions between lower and higher land plants. As gene-targeted disruption by homologous recombination is available in *Physcomitrella*, the functions of enzymes presented above could be analyzed directly. In fact, since I already produced gene-targeted mutants of PpPLC5 and PpPIPK1 in addition to PpPLC1, analyses of these mutants may provide new insight into the activation modes and functions of PIPK and PI-PLC in stress responses and development.