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Short Conceptual Overview

The intracellular phospholipase A_1 protein family

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Abstract

Phospholipase A_1 is an enzyme that hydrolyzes phospholipids, producing 2-acyl-lysophospholipids and fatty acids. The intracellular phospholipase A_1 ($iPLA_1$) protein family is a relatively recently discovered lipid-metabolizing enzyme family. Lower eukaryotes, such as yeasts and nematodes, and plants have only one $iPLA_1$ protein, whereas mammals have three $iPLA_1$ family proteins (PA-PLA_1/DDHD1/$iPLA_1\alpha$, p125/Sec23IP/$iPLA_1\beta$ and KIAA0725p/DDHD2/$iPLA_1\gamma$). Mammalian $iPLA_1$ proteins are localized in different cellular compartments, and two of them, p125 and KIAA0725p, have been implicated in membrane trafficking events. Recent gene targeting studies on several organisms showed that $iPLA_1$ family proteins are involved in various physiological functions, including plant shoot gravitropism, epithelial stem cell differentiation and spermiogenesis. In this review, we describe the features of $iPLA_1$ family proteins and recent progress regarding our understanding of their physiological functions.

Keywords: membrane traffic; phosphatidic acid; phospholipase A.

Introduction

Phospholipids are a major component of biological membranes. They are metabolized by phospholipases, which can be grouped into four classes (phospholipases A, B, C and D). Phospholipases play a variety of functions, such as in membrane synthesis and turnover, production of signaling molecules and intracellular membrane trafficking. Phospholipase A_1 (PLA_1) is an enzyme that hydrolyzes phospholipids, producing 2-acyl-lysophospholipids and fatty acids (Figure 1A). Several PLA_1 enzymes have been identified, and their structures and functions have been studied. PLA_1 enzymes can be divided into two groups based on their cellular localization: group 1 consists of extracellular enzymes and group 2 of intracellular enzymes (1, 2).

The group 1 enzymes, extracellular PLA_1 enzymes, belong to the pancreatic lipase gene family, which is conserved in a wide range of organisms (3). Currently, six extracellular PLA_1 molecules are known in mammals. Among them, phosphatidylserine-specific PLA_1 (PS-PLA_1) and membrane-associated phosphatidic acid-selective $PLA_1\alpha$ and β (mPA-$PLA_1\alpha$ and β) exhibit strict substrate specificities, acting almost exclusively on phosphatidylserine (PS) and phosphatidic acid (PA), respectively. These extracellular PLA_1s have been suggested to be involved in signal transduction pathways by producing lipid mediators, lysophosphatidylserine (LPS) and lysophosphatidic acid (LPA) (4, 5). A recent gene targeting study revealed the physiological significance of mPA-$PLA_1\alpha$. mPA-$PLA_1\alpha$-deficient mice exhibit wavy hairs due to aberrant formation of the inner root sheath in hair follicles, indicating its involvement in hair follicle development (6). Analysis of signaling pathways has suggested that LPA produced by mPA-$PLA_1\alpha$ binds to membrane receptor P2Y5, leading to activation of the $G_{12/13}$-RhoA-Rock pathway. The other three extracellular PLA_1s have broad substrate specificities and hydrolyze not only phospholipids, but also triacylglycerols and galactolipids (4).

The group 2 enzymes, intracellular PLA_1 ($iPLA_1$) family enzymes, were discovered relatively recently. In the mid-1990s, the John Glomset group purified an $iPLA_1$ protein named phosphatidic acid-preferring phospholiase A_1 (PA-PLA_1) according to its substrate specificity and cloned its cDNA (7–9). Now it is clear that $iPLA_1$ family proteins are highly conserved in a wide range of eukaryotic organisms, from yeasts through plants to mammals. Lower eukaryotes and plants possess one $iPLA_1$ protein, whereas mammals have three of them, each originally named PA-PLA_1, p125 and KIAA0725p (7, 10, 11) (Figure 1B). In the human genome, the genes of PA-PLA_1, p125 and KIAA0725p are located on chromosomes 14, 10 and 8, respectively. Phylogenetic analysis revealed that gene duplication occurred two times, with the first duplication likely occurring in a very early stage of evolution to generate PA-PLA_1 and an ancestral protein of p125 and KIAA0725p (Figure 1C). Interestingly, the three proteins show different subcellular localizations (9, 11, 12). Except for the presence of a short consensus sequence of lipases, the overall primary structures of $iPLA_1$ proteins are remarkably different from those of other phospholipases and lipases. Thus, $iPLA_1$ proteins appear to constitute a novel protein family. One common structural feature of iPLA proteins is that they share a domain named DDHD, which is ~180 amino acids long and characterized by four conserved amino acid residues (three Asp and one His, hence it is named DDHD). Moreover, iPLA proteins form homooligomers (8).

Although analysis of the physiological functions of $iPLA_1$ lags behind that of extracellular PLA_1, recent genetic studies

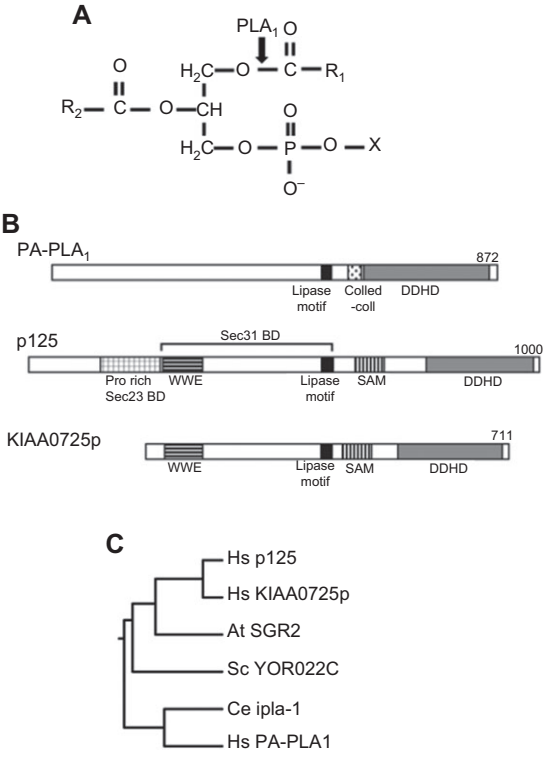

Figure 1 iPLA$_1$ family proteins.
(A) Hydrolytic site of PLA$_1$. (B) Schematic representation of the domain structure of iPLA$_1$ family proteins in human. The numbers at the upper right indicate the numbers of amino acid residues in the respective proteins. (C) Phylogenetic tree for iPLA$_1$ family proteins. The rooted phylogenetic tree was generated and displayed by the CLUSTALW program at GenomeNet of Kyoto University. The following sequences were used: *H. sapiens* p125 (NCBI Reference Sequence NP_009121), *H. sapiens* KIAA0725p (NCBI Reference Sequence NP_056029.2), *H. sapiens* PA-PLA$_1$ (NCBI Reference Sequence NP_085140.2), *S. cerevisiae* YOR022C (NCBI Reference Sequence NP_014665.1), *A. thaliana* SGR2 (NCBI Reference Sequence NP_174433.2) and *C. elegans* ipla-1 (NCBI Reference Sequence NP_001122623.1).

have revealed their physiological functions. In *Arabidopsis thaliana* and *Caenorhabditis elegans*, iPLA$_1$ gene mutation disrupts shoot gravitropism (13) and the formation of the vulva due to a defect in the asymmetrical division of stem cells (14), respectively. Moreover, gene targeting in mice showed that p125 is involved in spermiogenesis (15). In the present review, we summarize studies on mammalian iPLA$_1$ proteins, including biochemical, cell biological and genetic ones. In addition, we refer to iPLA$_1$s in *A. thaliana* and *C. elegans*, in particular their physiological functions.

Mammalian iPLA$_1$ family proteins

PA-PLA$_1$ (also known as DDHD1 and iPLA$_1\alpha$)

In 1994, the Glomset group detected cytosolic PLA$_1$ activity in bovine testis (7). The enzyme, named PA-PLA$_1$, exhibited sigmoidal kinetics toward PA in a Triton X-100 mixed micelle assay system. Similar activity was observed in bovine brain, but not in liver, spleen, heart or serum, which suggests that PA-PLA$_1$ functions in a process particular to the testis and brain and not in general lipid metabolism common to all cells. Interestingly, mature testis were found to contain 10-fold more activity than newborn calf testis, which raises the possibility that PA-PLA$_1$ is involved in spermatogenesis or sperm function. Purification (8) and subsequent cDNA cloning (9) revealed that bovine PA-PLA$_1$ is an 875 amino acid protein (872 amino acids in the case of human PA-PLA$_1$) and possesses a sequence, SHSLG, that resembles a lipase consensus sequence, GXSXG (X represents any amino acid). Indeed, the Ser residue at the third position of the consensus sequence is essential for enzymatic activity (9). Except for the lipase consensus sequence, the overall primary structure of PA-PLA$_1$ is remarkably different from those of conventional phospholipases and lipases. PA-PLA$_1$ has no signal sequence, and ectopically expressed PA-PLA$_1$ is localized to the cytosol (9), consistent with the idea that PA-PLA$_1$ is an intracellular enzyme. Although its name is derived from the observation that the enzyme prefers PA *in vitro*, the *in vivo* substrate(s) of PA-PLA$_1$ has not yet been identified. The substrate preference of this enzyme varies depending on the assay conditions (7, 8, 16). The activity of PA-PLA$_1$ toward PA is 4–10-fold greater than those toward phosphatidylinositol (PI), PS, phosphatidylethanolamine (PE) and phosphatidylcholines (PC) in a Triton X-100-mixed micelle system, whereas in the absence of Triton X-100, PE as well as PA is a good substrate.

Yamashita et al. (17) recently presented the hypothesis that PA-PLA$_1$ plays a role in the activation of GPR55, a G-protein-coupled putative cannabinoid receptor (18), by producing its agonist, 2-arachidonoyl-lyso phosphatidylinositol (LPI) (19). Upon treatment of cells ectopically expressing PA-PLA$_1$ with a calcium ionophore, ionomycin, PI was cleaved to produce LPI, some of which was secreted into the medium. Several lines of evidence suggest the involvement of phospholipase D (PLD) in this process. First, inhibition of PLD by n-butanol and the overexpression of an inactive PLD1 mutant blocked the production of LPI. Second, PA, a product of the PLD reaction, augmented the PLA$_1$ activity toward PI.

PA-PLA$_1$ activity may be regulated by phosphorylation and dephosphorylation (20). *In vitro* experiments showed that protein kinase CK2 (CK2) phosphorylates Ser-93, -105 and -716 of bovine PA-PLA$_1$, and that extracellular signal-regulated kinase 2 (ERK2) phosphorylates Ser-730. Ser-716 and -730 are dephosphorylated by protein phosphatase 2A (PP2A). Phosphorylation of PA-PLA$_1$ by CK2 reduced phospholipase activity by 50%, whereas phosphorylation by ERK2 did not significantly affect the activity (20). As the catalytic subunits of CK2 (CK2α and α') directly bind to PA-PLA$_1$ in the presence of Mg^{2+}-ATP, it is not clear whether the reduced phospholipase activity is a consequence of the phosphorylation of PA-PLA$_1$, its association with CK2 or both (20). Phosphorylation of Ser-727 in human PA-PLA$_1$ (corresponding to Ser-730 in bovine PA-PLA$_1$) has been shown by a quantitative phosphoproteomics study (21). It should be noted that CK2α' is one of the proteins involved in spermiogenesis (22). CK2α' knockout male

mice are infertile, having oligospermia and globozoospermia ('round-headed' spermatozoa lacking the acrosome) (23). It is possible that in male germ cells, CK2 phosphorylates PA-PLA_1 as well as candidate proteins in the nucleus (24). This possibility and the involvement of PA-PLA_1 in spermiogenesis remain to be examined in future studies.

p125 [also known as Sec23IP (Sec23-interacting protein), p125A, and $iPLA_1\beta$]

The second member of the $iPLA_1$ family, p125, was discovered not as an enzyme but as a component implicated in vesicular transport from the endoplasmic reticulum (ER). The export of newly synthesized secretory and membrane proteins from the ER is mediated by COPII-coated vesicles (25, 26), which are produced in a specialized ER subdomain known as ER exit sites or the transitional ER in mammalian and certain cells (27, 28). COPII consists of two heterodimeric complexes, Sec23-Sec24 and Sec13-Sec31, and a low molecular weight GTP binding protein, Sar1. Activation of Sar1 on the ER membrane results in sequential recruitment of the Sec23-Sec24 complex and the Sec13-Sec31 complex, which leads to vesicle budding. Thus, Sec23-Sec24 and Sec13-Sec31 constitute the inner and outer layers of the COPII coat, respectively (29). Concomitant with this budding process, cargo proteins are selectively incorporated into forming vesicles through interaction with Sec24 (30). Sec23 exhibits GAP activity toward Sar1 (31), and Sec31 further accelerates Sar1 GTP hydrolysis (32). Hydrolysis of GTP on Sar1 results in disassembly of the COPII coat, thereby leading to uncoating. Recent studies indicated that Sar1 GTP hydrolysis is necessary for vesicle fission (33) and bound cargo proteins delay the uncoating process (34).

p125 was isolated as a mammalian Sec23-interacting protein by affinity chromatography on GST-tagged mouse Sec23-coupled resin (10). p125 is a 1000 amino acid protein and comprises an N-terminal proline-rich region responsible for the interaction with Sec23 and central and C-terminal regions, which exhibit high homology to PA-PLA_1 (21% identical at the amino acid level). Differing from PA-PLA_1, p125 is expressed ubiquitously in many tissues and cells. In cultured cells, p125 is localized at ER exit sites as well as in the cytosol. Knockdown of p125 by RNA interference caused the redistribution of ER exit sites, and its overexpression caused the coalescence of ER exit sites with post-ER compartments, including the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and perhaps also the *cis*-Golgi, suggesting that p125 is involved in the architecture of ER exit sites (12). Although p125 exhibits high sequence homology to PA-PLA_1 and possesses a lipase consensus sequence, GHSLG, phospholipase activity has not been detected for p125 (11). There is no clear-cut explanation for this lack of enzymatic activity. It is possible that the phospholipase activity of p125 was lost during the course of evolution, and instead it may have acquired some specific functions, including a role in the organization of ER exit sites. Consistent with this idea, p125 exists only in metazoans, whereas PA-PLA_1 is conserved throughout eukaryotic cells from yeasts to mammals.

It should be noted that PA-PLA_1 is present almost exclusively in the cytosol, whereas some fractions of p125 and KIAA0725p (third member of the mammalian $iPLA_1$ family) are associated with membranes. Analyses of truncation and chimeric proteins revealed that the central and C-terminal regions of p125 are the primary determinants for membrane attachment, and the N-terminal Sec23-interacting region coordinates membrane specificity, i.e., it directs localization to ER exit sites (12, 35). Very recently, Inoue et al. (36) found that p125 and KIAA0725p, but not PA-PLA_1, bind to phosphatidylinositol 4-phosphate [PI(4)P], which is an abundant phosphoinositide in ER exit sites (37) and the Golgi [for a review, see (38)]. The PI(4)P-binding of the two proteins may contribute to their membrane association.

Recent work by Ong et al. (39) showed that p125 is not only a Sec23IP but also a 'Sec31IP.' They searched for Sec31-interacting proteins using the C-terminal 180-residue region of human Sec31A, which was shown to inhibit the transport of vesicular stomatitis virus G protein (VSV-G) from the ER to the Golgi (40), and identified p125 as a Sec31 partner. The Sec31-interacting domain of p125 lies between residues 260–600 and differs from the region responsible for the binding to Sec23, which indicates that p125 interacts with Sec23 and Sec31 using different regions. They proposed a model in which p125 bridges the inner and outer layers of the COPII coat.

Whereas it is certain that p125 plays a role in the organization of ER exit sites, its role in protein transport from the ER is somewhat controversial. Shimoi et al. (12) reported that siRNA-mediated knockdown of p125 did not significantly affect the transport of VSV-G from the ER, whereas Ong et al. (39) detected a delay in VSV-G export from the ER in p125-depleted cells. One possible explanation for these different observations is that the efficiencies of p125 knockdown were different (39). However, no delay in VSV-G transport from the ER was observed in embryonic fibroblasts prepared from p125 knockout (KO) mice (T. Kogure and K. Tani, unpublished observation). Therefore, this issue remains unresolved.

Our recent gene KO study disclosed the physiological function of p125 in mammals (15). Male p125 KO mice, but not female ones, are subfertile. Histological analysis of testis showed that the layer structure of the seminiferous tubules, which consists of germ cells, spermatogonia, spermatocytes and spermatids organized in strict order of maturation toward the lumen (41), is largely preserved, but spermatids near the lumen of the tubules are not elongated in p125-deficient mice. Many sperm from p125-deficient mice were found to have a round-shaped head and an abnormal mitochondrial sheath and to lack the acrosome. This phenotype is quite similar to that observed in males with globozoospermia, a rare disorder in male infertility. The acrosome is a specialized secretory organelle responsible for fertilization and is localized in the head of mammalian sperm. In the late spermatocyte stage, acrosomal components are first expressed, and the acrosome is formed in the spermatid stage through the fusion of proacrosomal vesicles derived from the *trans*-Golgi network (42). The expression stage of p125 is related to the formation

of the acrosome; it is expressed in spermatocytes and round spermatids, but not in sperm. In spermatocytes, Sec31A (an ER exit site marker) is localized in a ring-shaped structure with some large dots and numerous small dots. p125 is co-localized with the ring-shaped structure and large dots, but not with the small dot-like structures. Possibly, p125 is localized in a certain population of ER exit sites in spermatocytes. p125 may facilitate the secretion of certain proteins that are critically required for acrosome formation. A globozoospermia-like phenotype has been observed in mice lacking HIV-1 Rev binding protein (Hrb) (43), Golgi-associated PDZ and coiled-coil motif containing protein (GOPC) (44) and protein interacting with C kinase 1 (PICK1) (45), and these proteins are supposed to function at the Golgi-acrosome interface. In addition, as described above, deletion of CK2α′, a catalytic subunit of CK2 that is able to phosphorylate PA-PLA$_1$, abrogates acrosome formation, which leads to male infertility (23). Three Ser residues of PA-PLA$_1$ that are reported to be phoshorylated by CK2 (20) are not conserved in p125. However, the interaction between CK2 and p125 and also CK2-mediated phosphorylation of p125 might be worth examining.

Orthologous phenotype analysis combined with morpholino-mediated gene knockdown in *Xenopus* embryos predicted that the p125 gene is a candidate responsible for Waardenburg syndrome, a disorder characterized by craniofacial defects and pigment abnormalities (46). However, apparent craniofacial symptoms were not observed in p125 KO mice, which suggests that the *Xenopus* and mammalian p125s may have somewhat different physiological functions. Mircoarray analysis has suggested a correlation of the expression level of p125 and femur strength in rats (47). This possibility should be examined in future studies.

Our KO mice study showed that p125 is important for spermiogenesis, but no other notable defects were observed in p125 KO mice. Why are cells other than testicular cells not affected by the depletion of p125, even with its ubiquitous expression? It is unlikely that two other iPLA$_1$ proteins, PA-PLA$_1$ and KIAA0725p, can compensate for the loss of p125 function because they do not interact with Sec23. One possible explanation is that the loss of p125 function is compensated for by factor(s) that have a similar function to that of p125. One such candidate is Sec16A (the mammalian orthologue of yeast Sec16p), which exists in many cells and tissues. Like p125, Sec16A interacts with both inner and outer COPII coat components (Sec23 and Sec13, respectively) and is involved in the organization of ER exit sites (48–51). The abundance of Sec16A may be sufficient for the loss of p125 function in most cells, but not in testicular cells because sperm-specific proteins are produced in large numbers and exported from the ER in the limited stage(s) of spermiogenesis. In addition to linking the inner and outer layers of the COPII coat, p125 and Sec16A may have a similar function. A recent study suggested that Sec16p, in cooperation with Sec24p, reduces the GTPase activity of the COPII coat to prevent premature vesicle scission in yeast (52). Interestingly, overexpression of p125 induces the coalescence of ER exit sites with post-ER compartments, which is reminiscent of the phenotype in which the GTPase activity of Sar1 is blocked (12).

KIAA0725p (also known as DDHD2 and iPLA$_1$γ)

KIAA0725p was discovered through a database search using the amino acid sequence of p125 (11). It is a 711 amino acid protein that exhibits more sequence similarity to p125 (55% identical at the amino acid level) than to PA-PLA$_1$ (22% identical), but lacks an N-terminal proline-rich, Sec23-interacting region. Indeed, KIAA0725p does not bind to Sec23. KIAA0725p has a lipase consensus sequence, GHSLG, and shows PLA$_1$ activity *in vitro*. However, its specific activity is much lower than that of PA-PLA$_1$ (36). Like that of PA-PLA$_1$, the substrate specificity of KIAA0725p varies depending on the assay conditions. KIAA0725p expressed in cells showed high hydrolytic activities toward PE and PA and low activities toward PS and PC in the absence of Triton X-100. In the presence of Triton X-100, KIAA0725p showed lipase activity toward PA but only weak activity toward PE.

KIAA0725p was found to be expressed in all tissues tested and to be localized on the *cis*-Golgi and possibly the ERGIC, as well as the cytosol (53). FRAP analysis indicated that KIAA0725p is rapidly cycled between the Golgi and cytosolic pools (54). The targeting of KIAA0725p to the Golgi may be regulated by two factors. One is its own catalytic activity (54). Whereas wild-type KIAA0725p and the S351A mutant, in which the catalytic Ser-351 is replaced by Ala, are equally targeted to the Golgi-like structure in control cells, the mutant was much less efficiently targeted to the Golgi-like structure compared with the wild-type protein in cells depleted of endogenous KIAA0725p with siRNA, which suggests that the phospholipase activity of KIAA0725p is important for its Golgi localization. Lysophospholipids formed by KIAA0725p might support the association of KIAA0725p with Golgi membranes. The second factor is the phosphatidylinositol phosphate (PIP)-binding ability of KIAA0725p (36). *In vitro*, KIAA0725p like p125 binds to phosphatidylinositol monophosphates and possibly phosphatidylinositol 4,5-bisphosphate [PI(4,5)P$_2$]. This binding is independent of phospholipase activity. Among PIPs, phosphatidylinositol 4-phosphate [PI(4)P] is known to be abundant in Golgi membranes (38). The notion that the levels of PIPs, including PI(4)P, are important for the association of KIAA0725p with Golgi membranes is supported by the finding that the expression of Sac1, a phosphoinositide phosphatase that prefers PI(3)P and PI(4)P, in the Golgi causes drastic redistribution of KIAA0725p from the Golgi to cytoplasmic punctate structures.

The PIP-binding site has been mapped to the tandem sterile alpha-motif (SAM)-DDHD domain of KIAA0725p. The SAM domain is a putative protein interaction module present in a wide variety of proteins involved in many biological processes (55), and the DDHD domain comprises a ~180 amino acid stretch found in iPLA$_1$ family proteins and some phosphatidylinositol transfer proteins (56). All three mammalian iPLA$_1$ proteins have a DDHD domain, and two of them, KIAA0725p and p125, have a SAM domain (Figure 1B). The lack of the PIP-binding domain (the tandem SAM-DDHD domain) by PA-PLA$_1$ may explain its inability to bind to cellular membranes. In addition to its PIP-binding ability, the

DDHD domain of $iPLA_1$ has two more functions: catalysis and oligomer formation (36). Replacement of Asp-662, Asp-820 and His-839 by Ala in the DDHD domain of PA-PLA_1 abolished the phospholipase activity, whereas Asp-847 replacement had only a marginal effect. The DDHD domain of KIAA0725p contributes to oligomer formation by interacting with the N-terminal half of the protein.

The role of KIAA0725p in membrane trafficking is less clear and controversial. In cultured cells, overexpression of KIAA0725p causes dispersion of the ERGIC and Golgi apparatus, which suggests its involvement in the secretory pathway. Based on the results of a knockdown study, Morikawa et al. (53) suggested that KIAA0725p is involved in brefeldin A-induced retrograde trafficking from the Golgi to the ER. However, as suggested by Sato et al. (54), the results of Morikawa et al. (53) may be artifacts due to an off-target effect. Using several siRNAs, Sato et al. (54) demonstrated that knockdown of KIAA0725p does not inhibit the retrograde trafficking from the Golgi, but causes a partial defect in the trafficking of VSV-G from the Golgi to the plasma membrane. As described above, KIAA0725p is mainly localized on the *cis*-side of the Golgi. How KIAA0725p contributes to the trafficking from the Golgi to the plasma membrane is not clear. One possible explanation is that lysophospholipids produced by KIAA0725p on the *cis*-Golgi side may be important for maturation of the Golgi, and a deficiency of them may affect the function of the *trans*-Golgi network, where transport carriers destined for the cell surface are produced. Recent studies suggested the importance of phospholipid metabolism in the Golgi function (57).

Quite recently, we produced KIAA0725p KO mice. The KO mice grew normally and noticeable defects were not seen at first glance. Differing from p125 KO mice, male infertility was not observed in KIAA0725p KO mice (K. Tani and T. Baba, unpublished observation). It is possible that the KIAA0725p deficiency is compensated for by the presence of other family member(s), likely PA-PLA_1. Further analysis is necessary to elucidate the physiological function of KIAA0725p.

$iPLA_1$ family proteins in other species

In *Saccharomyces cerevisiae*, the *YOR022c* gene encodes a protein similar to PA-PLA_1 (19% identical at the amino acid level), and the null mutation of *YOR022c* reduces the growth rate of the yeast on respiration-dependent carbon sources. The YOR022c protein may be localized in mitochondria (58). A recent genome-wide interaction study showed that *YOR022c* genetically interacts with eight genes, including *MMM1*, a component of the ER–mitochondria encounter structure, and *UGO1*, an outer membrane component implicated in mitochondrial membrane fusion (59). It may be interesting to examine the connection of the YOR022c protein with mitochondria and ER membrane dynamics.

Higher plant *A. thaliana* possesses one $iPLA_1$ protein, shoot gravitropism 2 (SGR2), which is 22% identical to PA-PLA_1 at the amino acid level. In higher plants, the shoot generally shows negative gravitropism; after being placed horizontally, the inflorescence stems bend upwards. The Tasaka group showed that the SGR2 mutant exhibits abnormal gravitropism with respect to the shoot, but the positive root gravitropism is normal (60). The endodermal cells of the shoot are thought to be the gravity-sensing cells, in which the amyloplasts, which sediment in the direction of gravity, act as statoliths (13, 60). In an SGR2 mutant, amyloplast sedimentation is abnormal. The expression of the wild-type SGR2 protein but not the mutant protein, which lacks phospholiase activity, can rescue the phenotype, suggesting the importance of the enzymatic activity of SGR2 for its function.

Shoot endodermal cells are mostly occupied by a large central vacuole, and SGR2 is localized on the vacuole. Interestingly, other SGR mutants, SGR3 and ZIG/SGR4, encode Qa-SNARE syntaxin AtVAM3/SYP22 and Qb-SNARE AtVTI11, respectively, both of which are involved in vesicular transport to the vacuole. Thus, the three SGR gene functions are closely associated with vacuolar biogenesis (61).

Caenorhabditis elegans has one $iPLA_1$ family protein named ipla-1, which is a protein of 840 amino acids and shows 29% identity to human PA-PLA_1. The Arai group showed that ipla-1 deletion alleles are viable but cause vulval defects (14). In ipla-1 mutants, the terminal asymmetric division of seam cells, stem-cell-like epithelial cells that play a role in vulva formation (62, 63), was found to be disturbed. Further investigation revealed the mislocalization of cortical β-catenin (14), a component of the Wnt/β-catenin asymmetry pathway, which determines the polarity of most asymmetric division of stem cells (64). ipla-1 mutant phenotypes are rescued by the expression of catalytically active ipla-1 in seam cells, suggesting the importance of its enzyme activity in these cells. Genetic screening demonstrated that ipla-1 interacts with mon-2, a homologue of an ARF GEF-like protein, and tbc-3, a homologue of a Rab GAP, both of which are regulators of endosome-to-Golgi retrograde transport. Based on these results, Kanamori et al. (14) suggested that ipla-1 controls localization of the cortical asymmetry of β-catenin by regulating membrane trafficking. The Arai group extended that research and showed that ipla-1 is involved in the remodeling of the fatty acid at the *sn*-1 position of PI (65). Genetic and biochemical analyses revealed that the *sn*-1 fatty acid of PI is determined by ipla-1 and three acyltrasferases (*acl-8*, *-9* and *-10*). It has been suggested that the mutation of *ipla-1* causes alteration of the molecular species of PI, which in turn causes abnormal membrane trafficking, leading to mislocalization of β-catenin in seam cells (65).

Remaining questions

The functions of $iPLA_1$ proteins are less understood compared with those of phospholipase A_2 and extracellular PLA_1. However, recent gene manipulation studies have shed light on their physiological roles in several organisms. In mammals, the physiological function of p125 has been

revealed, but those of PA-PLA$_1$ and KIAA0725p remain to be elucidated. If PA-PLA$_1$ functions in sperm formation as predicted (8), it is important to determine whether or not the stage involving PA-PLA$_1$ is the same as that involving p125 in sperm formation. The apparent lack of phenotypes of KIAA0725p KO mice may be due to redundant functions of iPLA$_1$ proteins. As the catalytic activity of PA-PLA$_1$ is much higher than that of KIAA0725p, endogenous PA-PLA$_1$ may fully compensate for KIAA0725p ablation, even though PA-PLA$_1$ transiently, not stably, associates with Golgi membranes. To answer this question, the production of double and perhaps triple KO mice may be necessary. Genetic manipulation revealed that the iPLA$_1$ protein is involved in shoot gravitropism in *Arabidopsis* and vulva formation in *C. elegans*. Although the two phenotypes are very different, membrane trafficking has been suggested to be a possible common cause. The iPLA$_1$ proteins of the two organisms are most similar to PA-PLA$_1$ in mammals, which suggests that they are PA-PLA$_1$ counterparts. In lower eukaryotes and plants, only a PA-PLA$_1$ counterpart protein may be required and enough. Alternatively, one protein in these organisms plays multiple roles equivalent to those of the three proteins in mammals.

The early work by the Glomset group and recent studies by other groups revealed the enzymatic characters of iPLA$_1$ proteins. However, under *in vitro* assay conditions, PA-PLA$_1$ and KIAA0725p cleave a broad spectrum of substrates. Thus, it is difficult to identify physiological substrate(s) through *in vitro* studies. Lipidomic analysis of cells overexpressing or depleted of iPLA$_1$ proteins is required to determine the physiological substrate(s) of iPLA$_1$ proteins. In this context, it should be noted that two independent studies suggest that PI is a physiological *in vivo* substrate of PA-PLA$_1$. In particular, the study of Imae et al. (65) showed that *C. elegans* iPLA$_1$ is involved in the fatty-acid remodeling of PI, which is important in vulva formation. Examination of the possible involvement of PA-PLA$_1$ and/or KIAA0725p in the fatty-acid remodeling of PI is an important issue. It would also be valuable to examine whether or not iPLA$_1$ plays a role in the shaping of membranes. Lysophospholipids are known to induce curvature of membranes, and may play roles in membrane fission, fusion, and tubulation (66).

Nearly 20 years have passed since the discovery of PA-PLA$_1$ by the Glomset group. Many things have been learned about the iPLA$_1$ family proteins. However, understanding of the correlation between enzymatic activity and physiological function remains insufficient. Future analysis will be necessary to clarify these points.

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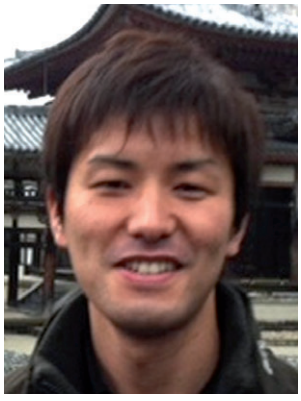

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