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8 Glycosylphosphatidylinositol-anchored proteins in plants

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Abstract

Various eukaryotic cell-surface proteins are attached to the outer plasma membrane leaflet by a glycosylphosphatidylinositol (GPI) anchor. Proteins with the GPI anchor have been implicated in cellular adhesion, signaling, and protection. GPI-anchored proteins (GAPs) differ from transmembrane proteins in their lateral mobility, interactions with lipids, and transport process. GAPs are clustered in lipid microdomains rich in sphingolipids and sterols, and are delivered in a polar manner to specific sites in the plasma membrane. Plant GAPs have not been well

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characterized, but recent studies have demonstrated large sets of plant GAPs, structures of GPI anchors, biosynthesis pathway for the GPI anchor, and contributions of GAPs to plant development.

Introduction

Recent proteomic and genomic analyses have identified multiple families of plant GAPs, including arabinogalactan proteins (AGPs), COBRA family proteins, SKU5-family proteins, lipid-transfer proteins (LTPs), β(1-3) glucanases, extensins, glycerophosphodiesterases, phytocyanins, proteases, and receptor-like proteins (4, 5, 13). We will summarize recent findings of GPI biosynthesis and GAPs in plants, with particular emphasis on genetic analyses of *Arabidopsis* demonstrating the roles of GAPs in plant development and defense response.

Structure of GPI anchors

A detailed structural analysis of the GPI anchor of PcAGP1 (*Pyrus communis* AGP1) showed that the anchor has the minimal core oligosaccharide conserved in animals, yeast, and protozoa, but also contains two novel structural features: a partial β(1-4)-galactosyl substitution on the 6-linked mannose residues, and a phosphoceramide lipid composed of phyto-sphingosine and tetracosanoic acid (Fig. 1, 36). Analysis of AGPs derived from

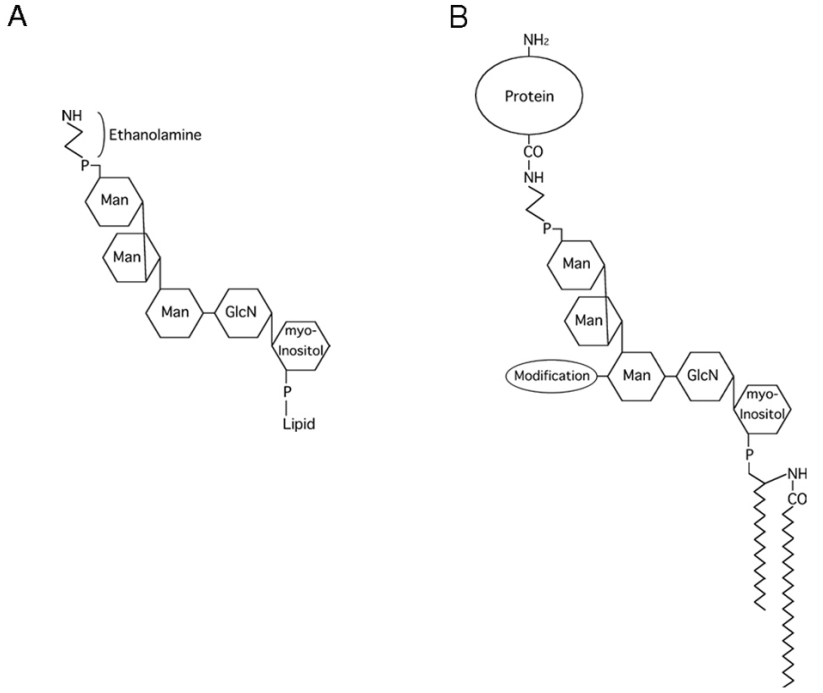

Figure 1. Structure of GPI anchor. A, core structure of GPI anchor commonly found in yeast, mammals, protozoa, and plants. B, structure of plant GPI anchor.

a rose cell suspension indicated similar structural features for plant GPI anchors (57). Interestingly, PcAGP1 secreted into culture medium has a truncated GPI without a lipid moiety, suggesting the release of PcAGP1 *via* the action of unknown phospholipases. Although several plant PI-specific phospholipases have been analyzed (37, 40), there is no direct evidence for the phospholipase-induced release of GPI-anchored proteins from plasma membranes, as occurs in animal cells.

Biosynthesis of GPI anchors

Detailed analyses of the biosynthesis of GPI anchors in mammals, yeast, and protozoa showed that GPI anchors are synthesized in the endoplasmic reticulum in at least ten sequential reaction steps by the addition of *N*-acetylglucosamines, mannoses, fatty acids, and phosphatidylethanolamines to phosphatidylinositol (Fig 2, 22). The first step in GPI-anchor biosynthesis is the transfer of *N*-acetylglucosamines to phosphatidylinositol catalyzed by GPI-*N*-acetylglucosaminyltransferase (GPI-GnT), which is composed of at least seven proteins (PIG-A, PIG-C, PIG-H, PIG-P, PIG-Y, GPI1, and DPM2). The product, *N*-acetylglucosaminylphosphatidylinositol, is deacetylated by PIG-L. *myo*-inositol of glucosaminylphosphatidylinositol is then acylated by PIG-W. The first mannose is added from dolichol-phosphate-mannose (DPM) by PIG-M and PIG-X. The second mannose is added from DPM by PIG-V. The subsequent modification of the first mannose by PIG-N is followed by the transfer of the third mannoses from DPM, catalyzed by PIG-B. Ethanolamine phosphate, the attachment site between GPI anchors and proteins, is added to the

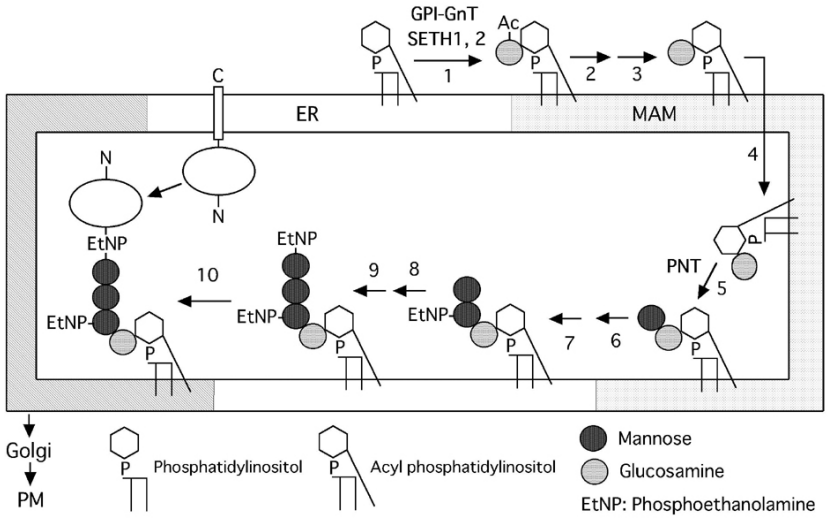

Figure 2. Synthesis of GPI anchors and GAPs. ER, endoplasmic reticulum. GPI-GnT, GPI-*N*-acetylglucosaminyltransferase. MAM, mitochondria associated membrane. PM, plasma membrane. Modified from Kinoshita and Inoue (22).

third mannose by PIG-F and PIG-O. The synthesized GPI anchors are transferred to the carboxy termini of proteins containing the GPI-attachment signal sequence by GPI transamidase, which consists of at least five proteins; GAA1, GPI18, PIG-S, PIG-T, and PIG-U.

Although the biosynthetic pathway of the plant GPI anchor remains to be elucidated, several plant genes have sequence similarities to genes for GPI-anchor biosynthetic enzymes (46). In addition, recent genetic approaches have demonstrated the significance of GPI-anchor biosynthesis in plant development (26, 53). Lalanne et al. (26) isolated the Arabidopsis *seth1* and *seth2* mutants from a screen of mutants with gametophytic defects. The genetic transmission of the *seth1* and *seth2* mutations through the male gamete is blocked, whereas female transmission is normal. *seth1* and *seth2* exhibit defects in pollen germination and pollen tube growth that are correlated with aberrant callose deposition. *SETH1* and *SETH2* encode proteins with significant similarities to human PIG-C and PIG-A, respectively, subunits of GPI-GnT, which catalyzes the first step in GPI-anchor biosynthesis. In addition, transcriptome and proteomic analyses identified 47 genes encoding putative GPI-anchored proteins that are preferentially expressed in pollen. These data indicate that pollen tip growth requires GAPs and/or free GPI. It would be interesting to identify which GAPs are required for pollen growth and to analyze the functions of SETH1 and SETH2 in zygotic development.

The Arabidopsis mutant *peanut* (*pnt*) was isolated, owing to its embryos being swollen radially. Mutations in *PNT* have pleiotropic effects, including defects in embryo and meristem morphology, seedling lethality, reduced pollen viability, reduced cellulose content, increased contents of pectins and neutral sugars, and the ectopic deposition of xyloglucan, pectin, and callose (53). *PNT* encodes a homolog of PIG-M, which is involved in the addition of the first mannose during GPI-anchor synthesis. Interestingly, callus of *pnt* mutants does not exhibit the ectopic accumulation of cell-wall materials found in *pnt* embryos, suggesting lower requirement for GAPs and free GPIs in undifferentiated callus. The absence of GAPs in *pnt* embryos and callus implies their degradation in the absence of functional GPI anchors although the cellular localization of GAPs in the shortage of GPI has not been determined.

These genetic analyses of GPI-anchor biosynthesis demonstrate the critical requirement of GAPs and/or GPI in plant development. Defects in cell wall formation of GPI-biosynthesis mutants recall us yeast GPI-anchor mutants with abnormal cell wall. Further biochemical and genetic analysis of plant GPI-anchor biosynthesis might be essential to understand roles of GPI-anchors in plant development and cell wall formation.

COBRA, a family of GAPs involved in directional growth

The Arabidopsis *cobra* (*cob*) mutant *cob-1* exhibits conditional misoriented expansion of root cells (43). In the region where longitudinal cell expansion normally occurs, *cob-1* root cells undergo an unusual extreme radial expansion instead of longitudinal expansion. Analysis of the null allele *cob-4* revealed that COB regulates anisotropic expansion in aerial organs as well as roots (41). In the root-elongation zone in the wild type, most inner cellulose microfibrils are oriented perpendicularly to the longitudinal elongation axis, allowing them to regulate anisotropic cell expansion. In contrast, cellulose microfibrils in the elongation zone in *cob* are randomly oriented (41). As a secondary effect, the amount of crystalline cellulose in the root growth zone is reduced in *cob* mutants. *COB* encodes a GAP with a potential cellulose-binding domain and belongs to a multigene family (42, 43). Immunolocalization analysis revealed that COB proteins preferentially localize to the longitudinal side of the cell surface and are distributed in a banded pattern transverse to the elongation axis (41, 43). The transverse COB pattern is dependent on cortical microtubules. These results suggest that COB controls anisotropic cellular expansion *via* its association with the orientation of cellulose microfibrils.

The classic rice mutant *brittle culm* (*bc1*) exhibits reduced mechanical strength in the culm along with reduced cell wall thickness and cellulose accumulation (28). Walls of sclerenchyma and vascular cells in culms of the *bc1* mutant accumulate significantly larger amounts of lignin. *BC1* encodes a COBRA-like protein closely related to Arabidopsis COBL4, and is preferentially expressed in developing sclerenchyma cells and vascular bundles (28). These results demonstrate the involvement of COB and COB-like proteins in cell wall formation in different tissues.

SKU5 regulating anisotropic growth

Arabidopsis *sku5* was isolated as a mutant with a defect in directional root growth (49). The roots of *sku5* are skewed strongly to the left, resulting in looped and coiled roots. SKU5 encodes a GAP related to multiple-copper oxidases, but contains no copper center motif, which is essential for enzymatic activity. SKU5 localizes to the plasma membrane and cell wall, and probably controls directional growth *via* an unknown regulatory process of cell wall expansion.

AGPs involved in directional growth and Agrobacterium infection

Arabinogalactan proteins (AGPs) are firstly identified GAPs in plants (36, 47, 56, 57, 65). AGP is a family of plant proteoglycans harboring extensive

O-glycosylation with type II arabinogalactan consisting of a backbone of (1→3)β-D-galactan and side chains of (1→6)β-D-galactan, to which arabinose residues are attached at the O-3 or O-6 positions (46, 50). Among 248 predicted GAPs of *Arabidopsis*, 100 GAPs in various families contain putative arabinogalactan glycosylation modules, of which 29 are classical AGPs (4, 5). A number of AGPs have been isolated from various tissues of diverse plant species, and there are several papers reporting tissue-specific and stage-dependent localization of AGPs.

SALT-OVERLY-SENSITIVE (SOS5), has been found to govern organized plant cell growth (51). Arabidopsis *sos5* was isolated in a screen for mutants hypersensitive to salt. Under salt-stress conditions, roots of the *sos5* mutant exhibit growth inhibition and become swollen radially, reminiscent of *cob* mutant roots. *SOS5* encodes a AGP with two fasciclin-like domains, which are involved in animal cell adhesion. Fasciclin-like arabinogalactan proteins (FLAs) are a subfamily of the AGPs, with at least 21 FLAs present in the *Arabidopsis* genome (19, 47, 48). The *sos5* mutation is a single amino acid substitution in the highly conserved motif of the second fasciclin-like domain, suggesting functional significance of this motif. *sos5* mutant plants have shorter siliques, fewer seeds, longer petioles, and flatter stems, suggesting the requirement of SOS5 for shoot development and reproduction process. The cell walls in *sos5* roots are thinner and unorganized, between the epidermal and cortical cells, demonstrating requirement of SOS5 for cell wall formation. The functional relationships among SOS5, SKU5, and COBRA in regulation of cell wall remain to be elucidated.

A synthetic phenylglycoside, β-glycosyl Yariv reagent (βGlcY), binds specifically to AGPs and inhibits functions of AGPs. The application of βGlcY inhibits the elongation of carrot cultured cells (62), Arabidopsis roots (12, 62), and lily pollen tubes (30), suggesting the involvement of AGPs in directional and coordinated cell expansion. Recently, Lee et al. (27) observed an inhibitory effect of βGlcY on apical cell expansion in the moss *Physcomitrella patens*, and isolated from this organism seven genes encoding arabinogalactan proteins, including two FLAs, suggesting the functional conservation of AGPs in oriented cell expansion in the plant kingdom.

The Arabidopsis *rat1* mutant highlights a role for a GAP in *Agrobacterium tumefaciens* infection (16). *rat1* was isolated as a mutant that shows resistance to *Agrobacterium*. RAT1 is AtAGP17, which belongs to a family of Lys-rich AGPs (16, 55). AGP17/RAT1 is a *bona fide* AGP with a molecular mass of 80 to 150 kDa that is localized on the plasma membrane and Hechtian strands (55). *rat1* mutant roots exhibit decreased binding of *Agrobacterium* and strongly decreased transformation efficiency. The expression of PR genes is downregulated in the wild type after *Agrobacterium* infection, but not in *rat1*

mutant roots. These results suggest that AGP17 is required for reducing the resistance response during the process of *Agrobacterium* infection. Interestingly, another Lys-rich AGP, AtAGP18, is expressed in the female gametophyte, pollen, and embryos, and RNAi-induced AGP18-silenced plants exhibit a specific defect in female gametogenesis (1). Tomato LeAGP-1, the first Lys-rich AGP identified, has been implicated in lateral branching and reproduction (54).

GPI-anchored LTPs involved in vascular formation

GPI-anchored non-specific lipid-transfer proteins (nsLTPs) have been found to be involved in plant vascular development (34, 59). The Arabidopsis dominant mutant *lettuce* (*let*), created using activation tagging, exhibits an ectopic leaf blade in the petiole. This effect is caused by the overexpression of *LEAFY PETIOLE (LEP)*, which encodes an AP2/EREBP family transcription factor (59). *let* shows the additional phenotype of an increased number of vascular cells, caused by the activation of both *LEP* and *VASCULAR TISSUE SIZE* (*VAS*), which encodes a nsLTP with a putative GPI-anchor attachment site. The increase in the xylem cell number in this mutant is due to the overexpression of *LEP*, and the overexpression of *VAS* results in a specific increase in phloem (pro)cambial and pericycle cells (59). The *VAS* promoter is preferentially active in vascular tissue, suggesting the involvement of *VAS* in vascular development.

Although cell-cell interactions are essential for plant vascular formation (14, 31), the mediators of the intercellular communication that regulates vascular development are not well characterized. Local cell-cell interaction was found to induce the differentiation of zinnia mesophyll cells into xylem cells (32). The inductive interaction was mediated by an AGP with a molecular mass of 50,000 to 100,000 (33). The AGP was isolated and named xylogen, based on its biological activity (34). Xylogen is a hybrid molecule with properties of both AGPs and nsLTPs, and has a putative GPI-anchor attachment site at its C terminus (Fig. 3). Xylogen is specifically expressed in procambium and immature xylem cells, and is polarly localized in differentiating tracheary elements, toward immature cells (Fig. 4). Two Arabidopsis genes, *AtXYP1* and *AtXYP2*, have been found to encode the protein backbones of xylogen. The *xyp1 xyp2* double mutants exhibit defects in vascular patterning, including discontinuous veins, misconnected tracheary elements, and loss of loop formation and lateral vines (Fig. 5). These results suggest that xylogen is secreted directionally from differentiating xylem cells, diffuses to adjacent undifferentiated cells, leads the neighboring cells into the pathway of xylem differentiation, and supports the formation of continuous networks of vasculature.

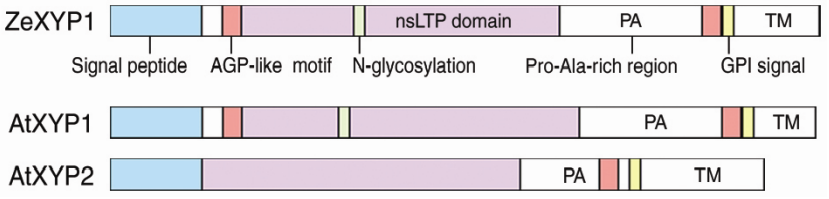

Figure 3. Domain structures of protein backbones of xylogen. ZeXYP1, *Zinnia elegans* xylogen protein 1. AtXYP1, *Arabidopsis thaliana* xylogen protein 1. AtXYP2, *Arabidopsis thaliana* xylogen protein 2. AGP, arabinogalactan protein. nsLTP, non-specific lipid transfer protein. PA, Pro-Ala-rich domain. TM, transmembrane domain. From Motose et al. (34).

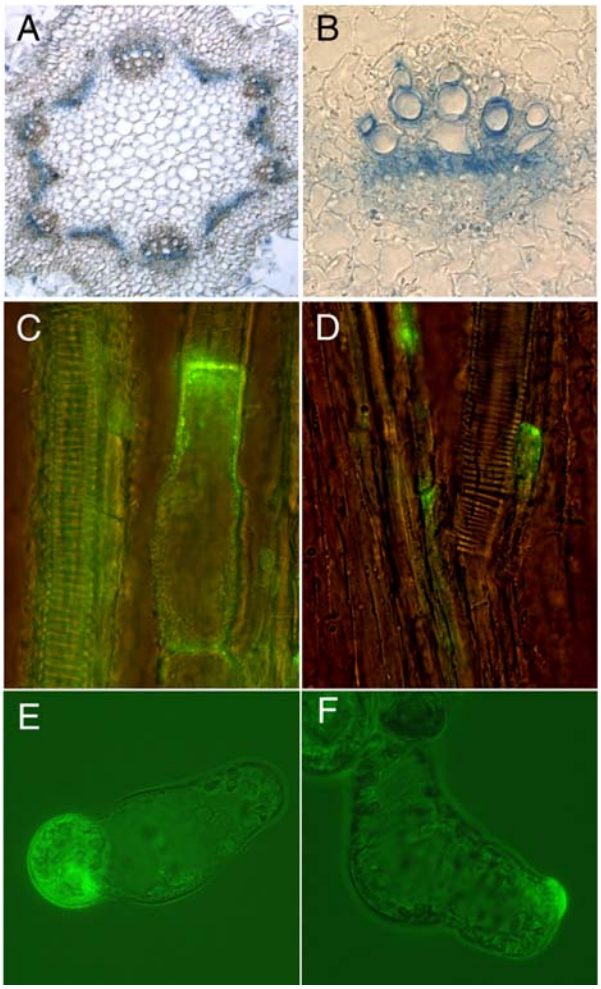

Figure 4. Xylogen accumulated in procambium and immature xylem cells with polarity. A and B, immunohistochemical localization of xylogen (*ZeXYP1*) in procambium and immature xylem cells. C, D, E, and F, polar localization of xylogen detected by indirect fluorescent antibody technique. A and B, cross section of zinnia seedling. C and D, longitudinal section of zinnia stem. E and F, zinnia cell cultured in xylogenic medium for 48 h and 60 h, respectively. From Motose et al. (34).

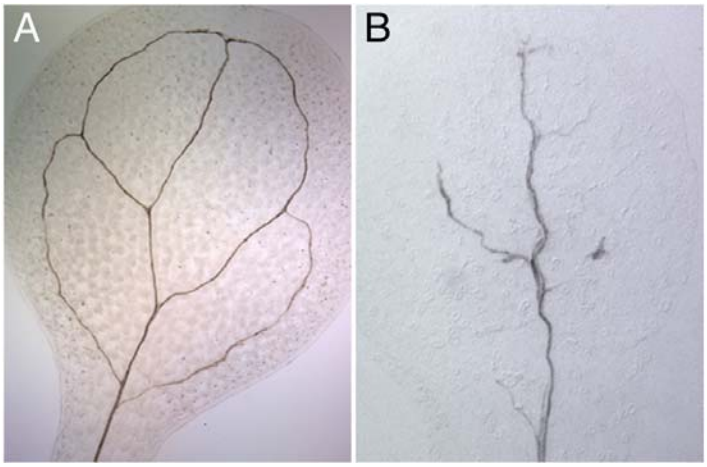

Figure 5. Vascular patterns in the wild type (A) and *xyp1 xyp2* (B). From Motose et al. (34).

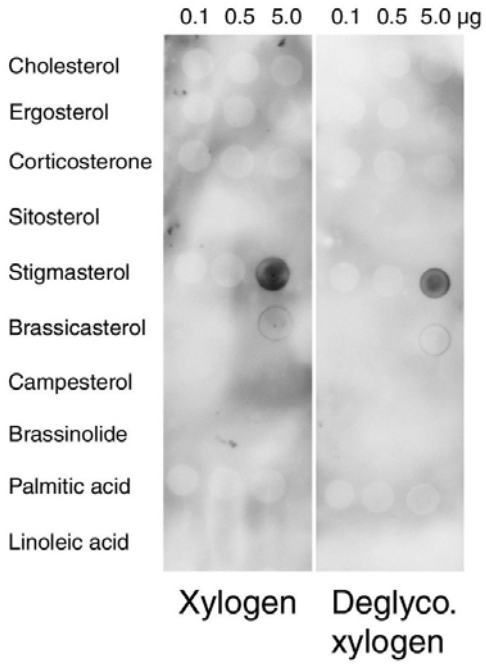

Figure 6. Specific binding of zinnia xylogen to sterols in a protein-sterol overlay assay. From Motose et al. (34).

In addition, the binding of xylogen to some plant sterols implies that xylogen carries sterol-derived signal molecule(s), which induce cellular differentiation (Fig. 6). Several Arabidopsis mutants with discontinuous veins have been isolated and analyzed in detail (2, 7, 8, 23, 24). Among them, mutations in *CVP1*, which encodes sterol methyltransferase 2, a protein involved in the biosynthesis pathway of phytosterols, result in discontinuous veins and defects in cell polarity (7, 8). These results support the possibility

that xylogen functions in concert with a phytosterol or sterol-derived molecule. Other phytosterol mutants also suggest significant roles for phytosterols in embryogenesis (44, 45) and root polarity (63).

VAS, *XYP1*, and *XYP2* belong to a multigene family of GPI-anchored nsLTPs (Table 1, Fig. 7). The Arabidopsis genome contains about 100 genes with nsLTP-like domains, and 25 nsLTPs that harbor putative GPI-anchor attachment sites. Small basic nsLTPs without GPI anchors and glycosylation modules have been isolated from various plant species, and these proteins could transfer lipids between membranes *in vitro*, with broad specificities (20, 21, 64). The expression patterns of these nsLTPs are dependent on cellular differentiation, with some strongly expressed in epidermal cells (52, 58). nsLTPs have been found to be involved in pollen tube adhesion (38), systemic acquired resistance (29), defense responses (15), and cell-wall extension (35). Although most GPI-anchored nsLTPs remain to be characterized, their expression patterns also show developmental and tissue specificity (Fig. 8). Some are expressed in pollen, and others are expressed in seeds (Table 1). GPI-anchored nsLTPs also exhibit characteristic expression patterns during *in-vitro* xylem differentiation of Arabidopsis cultured cells (25). Different from basic small nsLTPs, biosynthesis of GPI-anchored nsLTPs involve multiple steps including the attachment of GPI anchor, *N*-glycosylation, *O*-glycosylation, lipid binding, and cleavage by phospholipases (Fig. 9).

At present, many questions remain in this regard. For example, what are the functions of GPI-anchored nsLTPs, the modes of action of VAS and XYPs, the roles of the GPI anchor, and the functional and biochemical differences between non-GPI-anchored and GPI-anchored nsLTPs? What types of lipids and proteins bind to VAS and XYPs *in vivo*? These questions should be addressed in further analyses.

GAPs involved in defense responses

Mutants in *PMR6* (powdery mildew resistance), which encodes a putative GPI-anchored pectate lyase, exhibit a incompatible interaction with the powdery mildew fungus *Erysiphe cichoracearum* (61). The resistance of *pmr6* is probably due to the loss of a compatible interaction rather than a stimulation of the defense response. Interestingly, mutations in *PMR6* result in reduced plant size, decreased leaf size, and altered cell wall composition, suggesting the involvement of PMR6 in cell expansion and cell-wall loosening.

The Arabidopsis *NDR1* (non-race-specific disease resistance) gene is required for the disease resistance response to bacterial and fungal pathogens (9, 10). NDR1 is essential for the activity of several CC-NB-LRR (coiled-coil nucleotide-binding leucine-rich-repeat) resistance proteins, including RPS2, RPM1, and RPS5, which recognize the cognate effector proteins AvrRpt2,

Table 1. Members of xylogen-like LTP gene family in *Arabidopsis*. a, presence (+) or absence (-) of corresponding cDNA/ESTs in public databases. b, c, and d, presence (+) or absence (-) of putative GPI-anchor attachment site (b), *O*-glycosylation motif (c), and *N*-glycosylation motif (d). e and f, presence of GPI anchor was experimentally confirmed by Bonner et al. (4) and by Motose et al. (unpublished data), respectively. g, expression pattern of XYPs in public databases of microarray analyses (AtGenExpress; http://www.arabidopsis.org/info/expression/ATGenExpress.jsp). h, Expression Atlas of Arabidopsis Development by Weigel and Lohmann. i and j, chemical treatments / stress condtions inducing and reducing gene expression, respectively. ABA, abscissic acid. ACC, 1-aminocyclopropane-1-carboxylic acid. TIBA, 2, 3, 5-triiodobenzoic acid. Pst, infection with *Pseudomonas syringae* pv. tomato.

MIPS code	Family name	Other name	cDNA/ ESTs[a]	GPI[b]	*O*-gly[c]	*N*-gly[d]	Expression[g]		
							Development[h]	Induction[i]	Reduction[j]
At1g05450	AtXYP24		+	+	+	+	Seeds, flowers		
At1g18280	AtXYP16		+	+	+	+	Pollen		
At1g27950	AtXYP25		+	+[e]	+	+	Shoot		
At1g36150	AtXYP13		+	+	+	+	Pollen		
At1g55260	AtXYP12		+	+	-	+	Flowers		ACC
At1g62790	AtXYP26		+	+	+	+	Seeds, roots, all organs		
At1g73890	AtXYP20		-	+	+	+			
At2g13820	AtXYP2		+	+	+	-	Roots, stems		
At2G27130	AtXYP11		+	+	+	+	Seeds, all organs		
At2g44290	AtXYP9	YLS3	+	+	+	+			
At2g44300	AtXYP10		+	+	+	+	Roots, stems, all organs		
At2g48130	AtXYP7		+	+	+	+	Seeds, roots	ABA	
At2g48140	AtXYP6		+	+	+	+	Seeds, roots	ABA	
At3g08770	AtXYP15	LTP6	+	-	-	-	Seeds, flowers		
At3g22600	AtXYP8		+	+	+	+	Seeds, roots, leaves	ABA, TIBA, Pst	
At3g22620	AtXYP18		+	+	+	+	Seeds, roots	ABA, TIBA, Pst	
At3g43720	AtXYP5		+	+	+	+	Shoot, seeds, flowers	Brassinolide	ACC
At3g58550	AtXYP21		+	+	+	+	Seeds, roots		
At4G08670	AtXYP3		+	+	+	+	Pollen		
At4g12360	AtXYP17		+	+	+	+	All organs (low level)		
At4g14805	AtXYP27		-	+	+	+			
At4g14815	AtXYP14		+	+	-	+	Flowers		
At4g22630	AtXYP22		-	+	-	+			
At4g22640	AtXYP23		+	-	+	+	Stamens, roots		
At5g09370	AtXYP4		+	+	+	+	Seeds, carpels		
At5g13900	AtXYP19	VAS	+	+	-	+	Seeds, roots	ABA	
AT5g64080	AtXYP1		+	+[f]	+	+	Seeds, flowers	ABA, Pst	

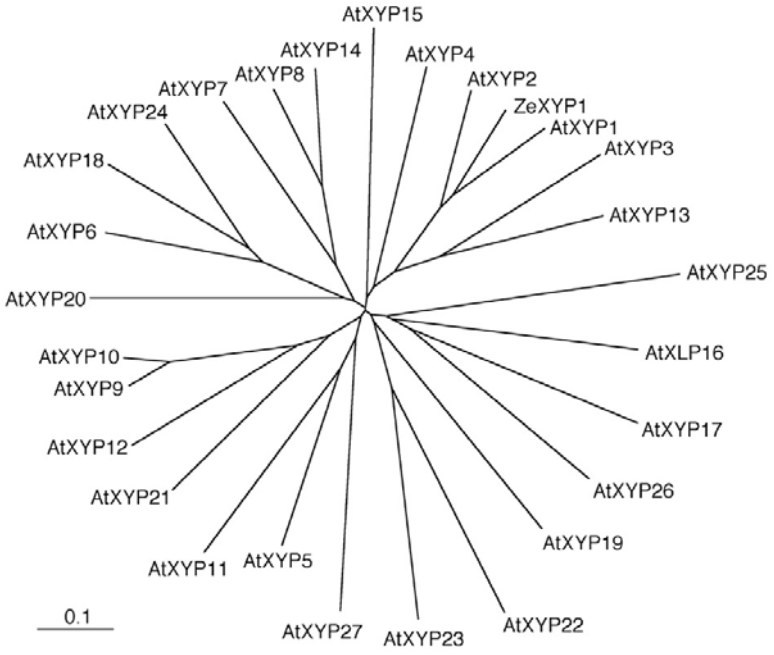

Figure 7. Phylogenetic relationships of xylogen-like LTP gene family. Phylogenetic tree generated from the alignment of nsLTP domains by bootstrap N-J method.

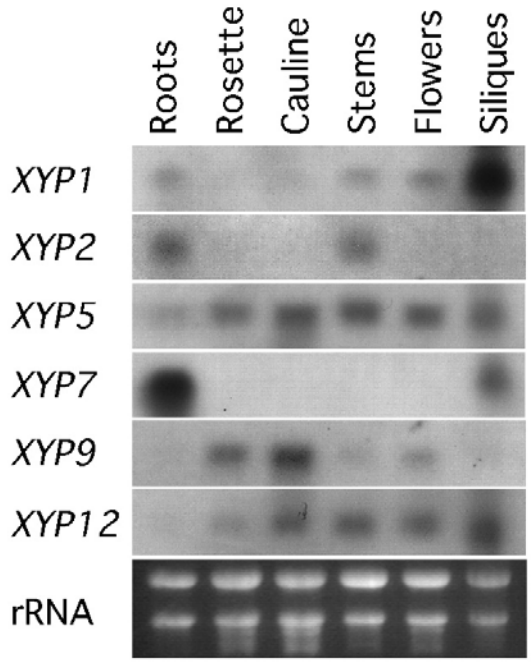

Figure 8. mRNA accumulation of xylogen-like LTP gene family. Total RNA was isolated from organs of one-month-old wild-type Arabidopsis plants (ecotype, Columbia) for RNA gel blot analysis of *AtXYP1*, *AtXYP2*, *AtXYP5*, *AtXYP7*, *AtXYP9*, and *AtXYP12*. Rosette; rosette leaves, Cauline; cauline leaves, Stems; inflorescence stems.

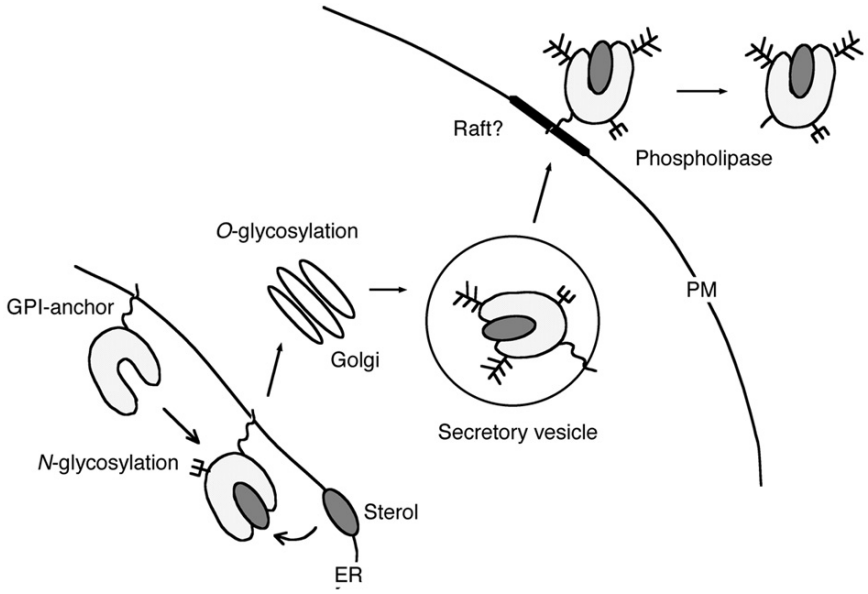

Figure 9. Biosynthetic pathway of xylogen-like GPI-anchored LTPs. ER, endoplasmic reticulum. PM, plasma membrane.

AvrB/AvrRpm1, and AvrPphB, respectively, of *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000 (9, 10). The overexpression of NDR1 induces enhanced resistance to virulent Pst and spontaneous lesions (11). NDR1 is subjected to C-terminal processing and N-glycosylation, and localizes to the plasma membrane (11). Sequence analysis of NDR1 and the presence of an inositol residue on NDR1 suggest that it is modified with a GPI anchor. NDR1 probably acts as an infection sensor and/or a direct receptor of pathogens at the outer surface of the plasma membrane.

Perspectives

Plant GAPs have been implicated in various biological processes, including directional cell expansion, cellular differentiation, and defense responses, but the results are quite fragmentary and many open questions remain. One question concerns the issue of whether the plant GPI anchor biosynthetic pathway differs from that in other systems. The similarities in the structures of the GPI anchors suggest common biosynthesis machinery, but the enzymes catalyzing GPI anchor biosynthesis remain to be characterized in plants. The second question involves the mode of action of GAPs. Studies of Arabidopsis mutants have elucidated the essential functions of GAPs in the regulation of cell walls. Biochemical analysis of GAPs, investigations of the functional relationships among GAPs, and studies of the interactions between GAPs, cell wall components, and proteins would be helpful in answering this question. Another issue is that of the biological significance of GPI anchors.

GAPs localize to lipid microdomains rich in sphingolipids and sterols, which may function as selective sorting platforms for vesicle transport. Lipid microdomains or rafts can be isolated, relying on their behavior as detergent-resistant membranes. Isolated plant detergent-resistant membranes are also rich in sphingolipids, phytosterols, GAPs, flotillin, and several types of plasma-membrane proteins (6, 39). It might be informative to analyze the roles of lipid microdomains and GPI anchors in the transport of GAPs and the formation of cell polarity. The conditional effects of several mutations in GAPs may imply roles for these proteins as sensors of environmental and endogenous conditions. The relatively flexible localization of GAPs in the plasma membrane, cell wall, and the interface between them would aid GAPs in responding to environmental changes, remodeling cell wall matrix, redistributing proteins, and adjusting the direction of growth. Using noninvasive fluorescence-based imaging, Bhat et al. (3) found that the initiation of pathogen entry stimulates localized accumulation of plasma-membrane proteins, which are required for the resistance to penetration by fungal pathogens, in membrane microdomains beneath attempted fungal entry sites. Together with probes of lipids (17, 18) and mutants, sophisticated imaging technologies would be very useful for investigating the redistribution of GAPs during cell polarity formation and morphogenesis. Further analysis of GPI-anchor biosynthesis and GAPs will shed light on the regulation of the plant cell wall and polarity.

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