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Arabinogalactan-proteins and the research challenges for these enigmatic plant cell surface proteoglycans

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Arabinogalactan-proteins (AGPs) are complex glycoconjugates that are commonly found at the cell surface and in secretions of plants. Their location and diversity of structures have made them attractive targets as modulators of plant development but definitive proof of their direct role(s) in biological processes remains elusive. Here we overview the current state of knowledge on AGPs, identify key challenges impeding progress in the field and propose approaches using modern bioinformatic, (bio)chemical, cell biological, molecular and genetic techniques that could be applied to redress these gaps in our knowledge.

INTRODUCTION

Arabinogalactan-proteins (AGPs), ubiquitous cell surface proteoglycans in both terrestrial and aquatic plants (and algae), are proposed to play essential roles in a range of plant growth and development processes, including cell expansion, cell division, reproductive development, somatic embryogenesis, xylem differentiation, abiotic stress responses, and hormone signaling pathways (Selifet and Roberts, 2007; Ellis et al., 2010). These roles emerge from largely indirect evidence and from the “recognition” potential arising from the incredible diversity of their glycan and protein backbone moieties as well as their location; attached to the outer leaflet of the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor and in some instances cross-linked into the wall. Despite intense research to unravel AGP function, their molecular mechanism(s) of action remain elusive. AGPs exhibit complexity at many levels: First, AGP protein backbone genes are part of large gene families, and this makes the study of AGP function through characterization of single AGP mutants a major challenge due to gene redundancy (Ma and Zhan, 2010; Showalter et al., 2010). Second, AGP protein backbones are highly glycosylated, hindering production of antibodies specifically directed to the protein moiety that would allow for identification and evaluation of specific AGPs. Third, the variety of monosaccharides present in AGP carbohydrate moieties, the variety of linkage types arising from the incredible diversity of their glycan and protein backbone moieties, the special arrangement of these linkages and their direct role(s) in biological processes remains elusive. Here we overview the current state of knowledge on AGPs, identify key challenges impeding progress in the field and propose approaches using modern bioinformatic, (bio)chemical, cell biological, molecular and genetic techniques that could be applied to redress these gaps in our knowledge.

STRACTIONS

Arabinogalactan-proteins belong to the hydroxyproline-rich superfamily of glycoproteins (Schultz et al., 2002; Johnson et al., 2003) being composed largely of carbohydrate (90–98% w/w) with some protein typically rich in the amino acids, Hyp/Pro, Ala, Ser/Thr, that is usually covalently modified with a GPI anchor at the C-terminus (see Figure 1). Historically, AGPs were defined if they met three criteria: the presence of arabinogalactan chains, a Hyp-rich protein backbone, and the ability to bind to a class of synthetic phenylalao dyes, the β-glucosyl Yariv reagent (see Fu et al., 1993). The significant advances in our knowledge of their carbohydrate structures, protein backbone sequences, and variability in Yariv binding has considerably complicated how an AGP is defined. For instance, the diversity of protein backbones has led to a classification of the AGPs into different sub-classes based on the presence/absence of particular motifs/domains (Johnson et al., 2003). The carbohydrate moiety is typically in the form of type II arabinogalactans (AGs) although some AGPs also contain short arabino-oligosaccharide chains (Figure 1; Fincher et al., 1983; Tan et al., 2004, 2010; Ellis et al., 2010). Type II AGs have also...
been reported either as free polysaccharides (Ponder and Richards, 1997) or as side chains of rhamnogalacturonan I (RG-I; Caffall and Mohren, 2009). The existence of different forms of type II AGs raises a few questions. Are free type II AGs generated from AGPs by hydrolases in the wall or synthesized de novo? Are AG side chains of RG-I derived from either AGPs by transglycosylases or from covalently linked RG-I–AGP complexes? To understand how this diversity impacts biological function, we face the challenge of isolating "individual" AGPs and sequencing their glycans (and protein backbones).
Another aspect of AGP research is the intriguing possibility that they are one form of covalent cross linker for wall matrix phase polysaccharides. In the early 1970s, Keegstra et al. (1973) hypothesized that Rha residues on AG side chains of AGPs might be attachment sites for RG-I. Since then, AGPs/AGs have been reported to form complexes with pectins (Yamada et al., 1987; Saulnier et al., 1988; Kwon and Morvan, 1991; Pellerin et al., 1990; Yamada, 2000; Duan et al., 2003, 2004) and xylans (Kwon and Morvan, 1995). However, residues involved in the covalent cross-link between AGPs and wall polysaccharides have not been defined. Several major challenges must be addressed to determine if AGP polysaccharide complexes (APCs) exist and to determine the structure and function of any such complexes.

**CHALLENGE 1: ISOLATION AND PURIFICATION OF AGPs**

The incredible heterogeneity of AGP structures has hampered purification of individual AGPs. As a consequence, most studies on AGPs have been on a family of molecules and often in the presence of contaminating polymers. There are a few examples of AGPs purified by a combination of traditional chromatographic methods (for example, anion exchange/lectin affinity/gel permeation using chaotropic reagents) and/or Yariv precipitation that are “pure” AGPs; for example, the AGPs from tobacco floral tissues (Gane et al., 1995) and larch AG exudates (Ponder and Richards, 1997). The application of molecular biology techniques to both isolate heterologously expressed AGP protein backbones or synthetic peptides as green fluorescent protein tagged (GFP)-fusion proteins by the Kieliszewski/Showalter, Matsuoka, and Somerville laboratories (Shpak et al., 1999; Zhao et al., 2002; Tan et al., 2004; Shimizu et al., 2005; Estève et al., 2006) was an ingenious innovation that allowed the purification of AGPs with a single protein backbone and therefore the study of inherent glycan heterogeneity. However, the low DP of these glycans raises some questions of the fidelity of glycosylation in heterologous/high expression systems.

Thus, a combination of purification techniques is necessary to purify relatively homogeneous AGPs (and APC complexes extracted as described below). These techniques take advantage of the heterogeneous structural features of AGPs and wall polysaccharides including size, charge, hydrophobicity (Serpe and Nothnagel, 1996; Lampour et al., 2011), the ability to co-precipitate with Yariv reagents, the availability of anti-AG antibodies (Pattathil et al., 2010), and the use of tagged heterologously expressed protein backbones.

**CHALLENGE 2: EXTRACTION AND PURIFICATION OF PUTATIVE APCs FROM WALLS**

Because pectins and non-cellulosic polysaccharides are embedded within the highly cross-linked wall, the first obstacle to studying putative APCs is to extract intact macromolecules from the wall, especially from secondary walls. Traditional methods to release polysaccharides from the wall include either the use of wall-specific degrading enzymes (York et al., 1986) or the extraction of walls with increasingly harsh solvents (Fry, 1988). Since the enzymatic and strong base treatments could also potentially break covalent linkages between AGPs and wall polysaccharides, the released polymers may only contain partial structural information of potential APCs and may still contain contaminating wall polysaccharides.

To avoid these extraction complications it may be possible to source APCs from potentially rich sources such as suspension culture media, especially of xylogenic calli, polysaccharide-rich seed mucilages, and exudates, such as gums (Defaye and Wong, 1986) and root mucilages (Morothy et al., 1988) since these are released in a “solubilized” form.

**CHALLENGE 3: SEQUENCING OF AGs AND APCs**

Our current knowledge of AG carbohydrate sequences is based on experiments using tools that include monosaccharide composition, linkage analysis, chemical or enzymatic degradation of glycans, mass spectrometry (MS), and NMR analysis. Partial acid hydrolysis (Defaye and Wong, 1986), acetylation, alkaline degradation, and Smith degradation (Charms et al., 1981; Basci et al., 1987) have supported the basic structures summarized in Figure 1 and led to the suggestion that the AG glycans contain a backbone of β-(1,3)-galacto-oligosaccharides interrupted at regular intervals with a periodate-sensitive residue. However, few of the large AG chains have been de novo-sequenced due to the inherent biosynthetic heterogeneity and the current limitations of sequencing technologies.

The availability of linkage-specific enzymes has greatly assisted the sequencing of glycans although their lack of commercial accessibility has hampered progress. Thus a breakthrough in AGP analysis was the identification of an AGP-specific exo-β-(1,3)-galactanase that can bypass the β-(1,6)-galactosyl side chains (Tsumuraya et al., 1990; Kotake et al., 2005; Ichinoe et al., 2006). This enzyme, together with the recently characterized β-glucuronidase (Haque et al., 2005), α-arabinofuranosidase (Hata et al., 1992), and endo-β-(1,6)-galactanase form a enzyme tool kit specific for AG side-chain analysis which enabled Tryfona et al. (2010) to characterize some long β-(1,6)-galacto-oligosaccharide AG side chains with the aid of MS/MS fragmentation (see Oxley et al., 2004). A recent study of Arabidopsis AGP31 (Hizaji et al., 2012), a chimeric AGP, illustrates the power of a multipronged approach to purification and characterization of AGPs.

Therefore, the best solution is to sequence small structural units of AGs, generated using a combination of chemical and enzymatic techniques, and then to re-construct models of the intact AGs. Discovery of new chemicals and enzymes that can selectively cleave AGs would facilitate future progress in the sequencing of the AG glycan chains.

**BIOINFORMATICS**

Genomics and its related technologies have revolutionized the study of biology, facilitated the development of other ‘omics platforms, and created a need for bioinformatics to handle the acquisition, storage, and analysis of the vast amount of data generated from ‘omics and ‘omics-related projects. The AGP field has greatly benefited from genomics and bioinformatics. Given that AGP protein backbone sequences often have low sequence similarity, BLAST-type searches typically identify only a few closely related AGP family members and, therefore, are not a particularly effective means to comprehensively identify members of the AGP family. In contrast, bioinformatics approaches have provided a
broader and more complete picture of AGP gene/protein families. Schultz et al. (2002) conducted the first comprehensive bioinformatic analysis to identify and characterize AGP genes/proteins from the Arabidopsis genome/proteome with respect to their protein backbones. This study was refined by Showalter et al. (2010), who focused on AGPs, including 22 classical AGPs, 5 lysine-rich AGPs, 16 AG peptides, 21 fucosin-rich AGPs (FLAs), 17 plastocyanin AGPs, and 6 other chimeric AGPs. Ma and Zhao (2010) have conducted the only other comprehensive bioinformatics analysis for AGP protein backbones in rice. They identified 69 rice AGP protein backbones from the rice genome/proteome, including 13 classical AGPs, 15 AG peptides, 3 non-classical AGPs, 3 early nodulin-like AGPs (E nods-like AGPs), 8 non-specific lipid transfer protein-like AGPs (nLTP-like AGPs), and 27 FLAs. A few other bioinformatic studies are reported for AGP protein backbones, but these studies were not focused exclusively on AGPs and/or concentrated only on one particular sub-class (e.g., GPI-anchored AGPs or FLAs). For example, Börner et al. (2002, 2003) used bioinformatics to identify GPI-anchored proteins in Arabidopsis from genomic and proteomic data. In addition, Irshad et al. (2008) applied bioinformatic analysis to their cell wall proteomic data in Arabidopsis to identify several AGPs and Fuik et al. (2009) used bioinformatic analyses to identify 34 wheat and 24 rice FLAs. Bioinformatic tools have also been used to provide insight to the glycosyltransferases (GTs) involved in the assembly of AGP glycans (see Biosynthesis of Glycan Moieties) and in this way Börsch and colleagues have proposed that the CaZY GT family comprise putative β-(1,3)-GalTs (Qiu et al., 2005; Ellis et al., 2010; Egelund et al., 2011).

The comprehensive bioinformatic studies on AGPs also took advantage of other related genomic technologies, including microarray data to reveal organ-specific expression patterns, abiotic- and biotic-regulated expression profiles, and genes which are co-expressed. Co-expression analysis has the potential to reveal networks of genes that are related to particular aspects of AGP biology, including their biosynthesis, interacting partners, and physiological functions. These kinds of downstream bioinformatic analyses are just in their infancy and many bioinformatics challenges lie ahead relating to AGPs, as outlined below.

**CHALLENGE 4: IDENTIFYING AND CLASSIFYING AGPs FROM OTHER SEQUENCED PLANT GENOMES**

Over 30 plant and algal genomes/proteomes are now known (see http://en.wikipedia.org/wiki/List_of_sequenced_eukaryotic_genomes#Algae). It would be useful to apply either the current or improved bioinformatics programs to these various datasets. Suggested enhancements to the programs would include making the bioinformatic analysis more automated and integrating the programs for predicting signal peptides, GPI anchor addition sites, gene expression, co-expression analysis, etc. into a single program. In addition, based on existing protein sequence and carbohydrate data on AGPs, a bioinformatics program predicting sites of prolyl hydroxylation and corresponding sites and type of glycosylation (i.e., AGs and arabino-oligosaccharides) could be developed and used. This relies on our knowledge that the types of O-glycosylation on the AGP protein backbone can be predicted from the Hyp-contiguity hypothesis that defines Hyp (arabino)galactosylation as occurring on the clustered, non-contiguous Hyp residues separated by Ala or Ser residues in a protein backbone whereas blocks of contiguous Hyp residues, such as occur in extensins, are arabinosylated with short oligosaccharides (Kieliszewski and Lampert, 1994; Shpak et al., 1999; Goodrum et al., 2000; Zhao et al., 2002). N-glycosylation, is predicted by the universally conserved consensus amino acid sequence Asn-X-Ser/Thr, where X can be any amino acid except Pro. Similarly, the specificity of prolyl hydroxylation by prolyl-4-hydroxylase, although not as well defined in plants as in mammalian systems (Gorés and Raies, 2010), can be used together with the Hyp-contiguity hypothesis to inform design of bioinformatics programs.

**CHALLENGE 5: APPLYING AND IMPROVING BIOINFORMATIC ANALYSES OF MICROARRAY DATA TO ELUCIDATE PATTERNS OF AGP (CO-)EXPRESSION**

Unfortunately, not all of the sequenced plant genomes have extensive publicly available microarray data, unlike Arabidopsis and rice (e.g., see PLEXdb, http://www.plexdb.org/). Thus, in addition to generating new microarray data, it would be convenient to utilize and integrate expression analysis programs like Genestigator and co-expression analyzer tools (see Table 1) to mine data and provide it in a more tailored manner. Analysis of such data can provide remarkable insight into the function (and functional redundancy) of AGP protein backbone genes as well as elucidate networks of AGPs and AGP-related genes involved in various metabolic pathways.

**CHALLENGE 6: IMPROVING AND DEVELOPING NEW BIOINFORMATICS PROGRAMS TO ELUCIDATE MOLECULAR PHYLOGENIES OF AGP PROTEIN BACKBONE GENES**

It would be interesting from an evolutionary standpoint to understand how AGPs are related within and between species, since such analysis may explain how the AGP gene family evolved and provide insight into AGP function. From a functional perspective, it would be useful to be able to identify AGP gene orthologs and paralogs. Software developers would use the gene families identified in Challenge 4 through packages summarized in Table 1 and the extensive web-based resources developed for studying gene ontology to focus on the AGP protein backbone genes.

**CHALLENGE 7: DEVELOP BIOINFORMATICS TOOLS TO IDENTIFY AND CLASSIFY GENES/PROTEINS INVOLVED WITH AGP METABOLISM**

Bioinformatic tools to identify genes involved with the biosynthesis and possible modification and degradation of AGPs would be of great benefit. In particular, bioinformatics analysis has the potential to identify GTs likely to be involved in the biosynthesis of AG chains. Currently, sequence similarities to mammalian GTs represent one approach to identifying these enzymes, for example, as recently described by Egelund et al. (2011) in which the authors adopted a bioinformatic approach to identify and systematically characterize putative GaTs from CaZY GT-family-31 responsible for synthesizing the β-(1,3)-Gal linkage. This study revealed that the Arabidopsis accessions grouped into four plant-specific clades (1, 7, 10, and 11; Table 2). Furthermore, the investigators attempted to predict the possible substrate specificity of these
Table 1 | Bioinformatic programs used to identify and characterize AGPs.

<table>
<thead>
<tr>
<th>Program</th>
<th>Program use</th>
<th>Web address</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAST percentage calculator</td>
<td>Identification of AGP backbones</td>
<td><a href="http://www.adelaide.edu.au/~directory/carolyn.schultz/under_Files">http://www.adelaide.edu.au/~directory/carolyn.schultz/under_Files</a></td>
</tr>
<tr>
<td>BIO OHIO</td>
<td>Identification of AGP backbones and more</td>
<td><a href="http://code.google.com/p/prot-class/">http://code.google.com/p/prot-class/</a></td>
</tr>
<tr>
<td>Plant big-PI predictor</td>
<td>Identification of GPI anchor addition sites</td>
<td><a href="http://mendel.imp.mpg.de/piglo/planter_server.html">http://mendel.imp.mpg.de/piglo/planter_server.html</a></td>
</tr>
<tr>
<td>Genevestigator</td>
<td>Identification of gene expression</td>
<td><a href="https://www.genevestigator.ethz.ch">https://www.genevestigator.ethz.ch</a></td>
</tr>
</tbody>
</table>

Table 2 | The 31 potential GaTs from the Arabidopsis thaliana CAZy GT-family-31 and their proposed function.

<table>
<thead>
<tr>
<th>Sub-clade</th>
<th>Accession</th>
<th>Proposed function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>At1g33250, At5g12460, At4g10240, At4g03300, At1g05770, At4g11560, At2g37770, At1g14320, At1g65230, At4g23490, At1g07950</td>
<td>β-1,3-GalTs; substrate unknown!</td>
</tr>
<tr>
<td>7</td>
<td>At1g27120, At1g24900, At5g26200, At1g06440, At1g06910, At1g21090</td>
<td>β-1,3-GalTs involved in the synthesis of N- and O-glycans, and β-1,3- GlcNAcTs; substrate unknown!</td>
</tr>
<tr>
<td>10</td>
<td>At4g32120, At1g17730, At1g22015, At5g53340, At2g53500, At1g77810, At3g24330, At1g01710, At1g53290, At2g26100, At3g4960, At1g33430, At4g26940, At1g32930</td>
<td>β-GalTs involved in the synthesis of AGPs</td>
</tr>
<tr>
<td>11</td>
<td>At5g57500</td>
<td>β-1,3-GalTs; substrate unknown!</td>
</tr>
</tbody>
</table>

The accessions clustered into four plant-specific sub-groups according to Egelund et al. (2011). Substrate specificity were predicted based on secondary structure and conserved motifs shared with known β-1,3-GalTs (Qu et al., 2008).

GaTs based on secondary structure and conserved motifs shared with known β-1,3-GalTs (Qu et al., 2008). These predictions have formed the basis for detailed biochemical and molecular studies to define the precise substrate specificities of GT-31 family members.

In a similar manner co-expression analysis of either selected AGP or groups of AGP protein backbones provides another, largely unexplored, option to identify candidate GTs responsible for AG biosynthesis (and/or degradation in mura). This idea is based on the premise that once the gene encoding the AGP protein backbone is expressed, other genes needed for AGP or groups of AGP protein backbones provides another, idea is based on the premise that once the gene encoding the AGP protein backbone is expressed, other genes needed for AGP biosynthesis should also be co-expressed. In addition, co-expression analysis in conjunction with computational prediction of sub-cellular location and known protein–protein interaction data of candidate proteins involved in AGP biosynthesis could be used to identify proteins that function together in a complex (Mostafavi et al., 2008; http://www.genemania.org). Such information could be integrated into an “interactome” focusing on AGP biosynthesis.

CHALLENGE 8: DEVELOP BIOINFORMATICS TOOLS TO IDENTIFY REGULATORY SEQUENCES IN AGP PROTEIN BACKBONE GENES

Bioinformatics has the potential to reveal gene regulatory sequences involved in regulated expression of AGP genes with respect to developmental expression (e.g., tissue- and temporal-specific expression) and a variety of stresses. Bioinformatic programs that have the ability to recognize either conserved nucleotide patterns alone or in combination with chromatin immunoprecipitation (ChIP) assays followed by DNA sequencing have the potential to reveal AGP gene regulatory sequences and the corresponding trans-acting factors. Knowledge of such regulatory sequences would reveal commonly networked AGP genes as well as other co-regulated genes. As such, this information may be complementary to co-expression data and would provide another avenue to elucidate AGP function(s).

BIOSYNTHESIS OF GLYCAN MOIETIES

Many mammalian, fungal, and bacterial GTs have been identified, cloned, and biochemically characterized (Cantarini et al., 2009; Ellis et al., 2010). In contrast, only a few plant cell wall polysaccharide/proteoglycan-related GTs have been characterized biochemically (Doblin et al., 2010). From studies of Arabidopsis at the molecular and biochemical level (Strasser et al., 2007; Qu et al., 2008), and from assembly of mammalian proteoglycans, it is expected that AGP glycan chains that decorate AGPs are synthesized by type II membrane-bound GTs located in the Golgi apparatus. This includes members of CAZy GT-family-31 with putative β-(1,3)-GalT activity, that are suggested to be involved in synthesis of the β-(1,3)-Gal backbone in AG glycan chains (Qu et al., 2008; Egelund et al., 2011).

Early studies showed that the Golgi apparatus plays an important role in synthesis of β-(1,6)-Gal of the AG glycan chains of AGPs (Mascara and Fincher, 1982; Shibue et al., 1994), whereas the initial enzyme in the AG biosynthetic pathway, adding the first Gal residue to a Hyp residue on the protein backbone (the Hyp-O-galactosyltransferase or HGTT), is predominantly located in the ER (Okk et al., 2010). Outside of the development of in vitro assays to monitor GaT activity (Qu et al., 2008; Liang et al., 2010;
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An alternative approach to the one described in Challenge 7, centred on the analysis of Gum Arabic, a tree exudate from the Acacia species, whose main fraction is an AG (Defaye and Wong, 1986; Randall et al., 1989; Al-Assaf et al., 2005). AG chains comprise as much as 90–98% of the gum exudate (Osman et al., 1993), thus making Gum Arabic-producing cells from the Acacia tree an obvious choice as starting material to identify enzymes involved in AG biosynthesis.

**CHALLENGE 9: AN ALTERNATIVE APPROACH FOR THE IDENTIFICATION OF THE GLYCOSYLATION MACHINERY INVOLVED IN AG CHAIN SYNTHESIS**

An alternative approach to the one described in Challenge 7, centers on the analysis of Gum Arabic, a tree exudate from the Acacia species, whose main fraction is an AG (Defaye and Wong, 1986; Randall et al., 1989; Al-Assaf et al., 2005). AG chains comprise as much as 90–98% of the gum exudate (Osman et al., 1993), thus making Gum Arabic-producing cells from the Acacia tree an obvious choice as starting material to identify enzymes involved in AG biosynthesis.

**FUNCTION**

Arabinogalactan-protein glycan-specific antibodies and β-Gal Vari悦 reagent have recently been published (Li et al., 2010a; Ellis et al., 2010). The current use of these two indirect tools continues to provide information on AGP activity in new biological systems, e.g., European larch, Larix decidua (Rafinska and Bednarska, 2011), and little studied developmental processes, in this case, ovule development in gymnosperms, confirming the relevance and conservation of function of these molecules within the plant kingdom. Unfortunately, the broad specificity of these techniques makes it impossible to assign function to a single AGP. This limitation has been partially overcome by genetic and molecular studies, including the characterization of AGP single or double mutants, RNAi and over-expressing lines, although these approaches also have complications.

**SCREENING SYSTEM**

Expression of non-cellulosic/cellulosic plant GTs in functional assay systems remains a key challenge. The past lack of success of this approach has been ascribed to the mismatch between biochemical assays and native activity, failure of the expressed protein to accumulate to sufficient levels, incorrect folding or improper post-translational modifications (Petersen et al., 2009). The most obvious choice would be to develop an “in planta” system, however, the endogenous GT activities can make it difficult to distinguish the specific activity of the expressed protein (Petersen et al., 2009). Prokaryotes, of which some have limited capacity for post-translational processing, pose other problems. We therefore suggest developing multiple heterologous expression systems to maximize the likelihood that at least one will allow for successful expression where the biochemical activity is retained. Additionally, testing new expression systems that may prove “universal” (e.g., Aspergillus), which has served as one of the preferred expression systems in the biotechnology industry, as well as cell-free expression systems may prove useful for heterologous expression of plant GTs.
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The characterization of the promoters of AGP genes specifically expressed in pollen, have been demonstrated to be involved in the control of timing of pollen germination, as pollen FLA4/SOS5 in maintaining proper cell expansion under salt-stressed conditions. The apparent diversity of AGP function may be due to the ability of FLAs to mediate protein-protein interactions with cell wall or plasma membrane-associated ligands via their fasciclin-like domains, shown in other eukaryotic systems to facilitate cell adhesion.

In addition to FLA3, AGP6, and AGP11, two classical AGPs specifically expressed in pollen, have been demonstrated to be involved in the control of timing of pollen germination, as pollen of the agp11 agp12 double mutant germinates precociously inside the anthers (Coimbra et al., 2010). How the presence of AGP6 and AGP11 avoids precocious pollen germination is unknown, but it may occur by regulating water uptake.

The Lys-rich AGP sub-family has been the focus of several studies in tomato and Arabidopsis. Functional characterization of AtAGP18, one of the three Lys-rich AGPs, by over-expression of the genomic sequence in Arabidopsis indicates that AGP18 plays a role in vegetative growth and sexual reproduction (Acosta-Garcia and Vieille-Calzada, 2004; Zhang et al., 2011a). The busby phenotype resembles that of tomato lines over-expressing LeAGP1 and is similar to tobacco plants over-producing cytokinins (Zhang et al., 2011b) leading to the suggestion that it may participate in a cytokinin signal transduction pathway as a co-receptor of cytokinins. A similar model has been proposed for FLA4/SOS5 in its interactions with two members of the leucine-rich repeat receptor-like kinase family, FEI1 and FEI2, shown by double mutant analyses to have non-additive genetic interactions with FLA4/SOS5 (Xu et al., 2008). SOS5 has been hypothesized to act as the ligand of a signal molecule that then either binds directly to FEI1/FEI2 or assists in presenting the signal molecule to FEI1/FEI2, initiating a signaling cascade that regulates the synthesis of cellulose and ultimately cell growth.

Several questions arise from this ligand model of AGP function. Given the effects on cellulose in the fla11 fla12 double null mutant (MacMillan et al., 2010) and the abnormal cellulose deposition fla3 fla3 RNAS lines (Li et al., 2010), may FLA11, FLA12, and FLA3, as well as other GPI-anchored or non-anchored AGPs, also be a part of this same network of components involved in wall sensing? Does this model explain the observation of AGPs as cell fate markers in tissues undergoing cell differentiation? Considering that the appearance of AGPs during specific developmental stages has been described using antibodies that recognize AGP-carbohydrate epitopes, is the heterogeneity of AGP glycosylation also involved in providing the necessary specificity to interact with different signal molecules and generate specific responses? What is the relevance of the presence and number of fasciclin domains of FLAs? Further investigation of the possible function of AGPs in wall sensing is of fundamental importance to uncover some of the components and mechanisms involved in the regulation of wall biosynthesis and ultimately plant cell growth. To address some of these challenges, we propose the use of the following experimental approaches, techniques, and resources:
CONCLUSION

In this brief overview we have attempted to summarize what we believe to be the major challenges facing the research community in attempting to unravel the structure, function, and biosynthesis of AGPs and to provide some indicators on how we might progress. In addition, we believe there is much to be learnt from advances in their glycomodules, genetic and functional. Multifunctional protein-glycan fields have made and encouraged our colleagues to embrace these findings as a guide to advancing AGP research.

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