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Arabinogalactan-proteins: structure, expression and function

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Abstract. Arabinogalactan-proteins (AGPs) are a family of extensively glycosylated hydroxyproline-rich glycoproteins that are thought to have important roles in various aspects of plant growth and development. After a brief introduction to AGPs highlighting the problems associated with defining and classifying this diverse family of glycoproteins, AGP structure is described in terms of the protein component (including data from molecular cloning), carbohydrate component, processing of AGPs (including recent data on glycosylphosphatidylinositol membrane anchors) and overall molecular shape. Next, the expression of AGPs is examined at several different levels, from the whole plant to the cellular levels, using a variety of experimental techniques and tools. Finally, AGP function is considered. Although the existing functional evidence is not incontrovertible, it does clearly point to roles for AGPs in vegetative, reproductive, and cellular growth and development as well as programmed cell death and social control. In addition and most likely inextricably linked to their functions, AGPs are presumably involved in molecular interactions and cellular signaling at the cell surface. Some likely scenarios are discussed in this context. AGPs also have functions of real or potential commercial value, most notably as emulsifiers in the food industry and as potential immunological regulators for human health. Several important questions remain to be answered with respect to AGPs. Clearly, elucidating the unequivocal functions of particular AGPs and relating these functions to their respective structures and modes of action remain as major challenges in the years ahead.

Key words. Arabinogalactan-proteins; cell surface; cell wall; hydroxyproline-rich glycoprotein; plant growth and development; plasma membrane.

Introduction

AGPs are a family of highly glycosylated hydroxyproline-rich glycoproteins (HRGPs) analogous to animal proteoglycans. These glycoproteins are expressed throughout the plant kingdom, mainly at cell surfaces, where they are thought to have important roles in plant growth and development. Certain AGPs, particularly those found in plant gums, are also of commercial interest for the valuable chemical properties that they confer in various industrial applications.

An AGP consists of a hydroxyproline-rich core protein which is decorated by arabinose and galactose-rich polysaccharide units; moreover, AGPs are operationally defined by their ability to react with a synthetic chemical reagent, a phenylazoglycoside dye called Yariv reagent (fig. 1) [1, 2]. Such generalities, however, are too narrow to account for all AGPs given that some AGPs are hydroxyproline poor, lightly glycosylated and largely unreactive with Yariv reagent. It is worth remembering that it is human nature to group and classify things to facilitate their comprehension and discourse, whereas Mother Nature simply constructs biological entities, including AGPs, using material at hand with blatant, pedagogical disregard.

Structure

Protein moiety

Knowledge of the protein moieties of AGPs has mostly come from purifying AGPs, deglycosylating them and analyzing their respective core proteins by amino acid analysis and, to a more limited extent, by sequence analysis (fig. 2) [3–10]. More recently, molecular cloning of several confirmed and putative AGP core proteins has

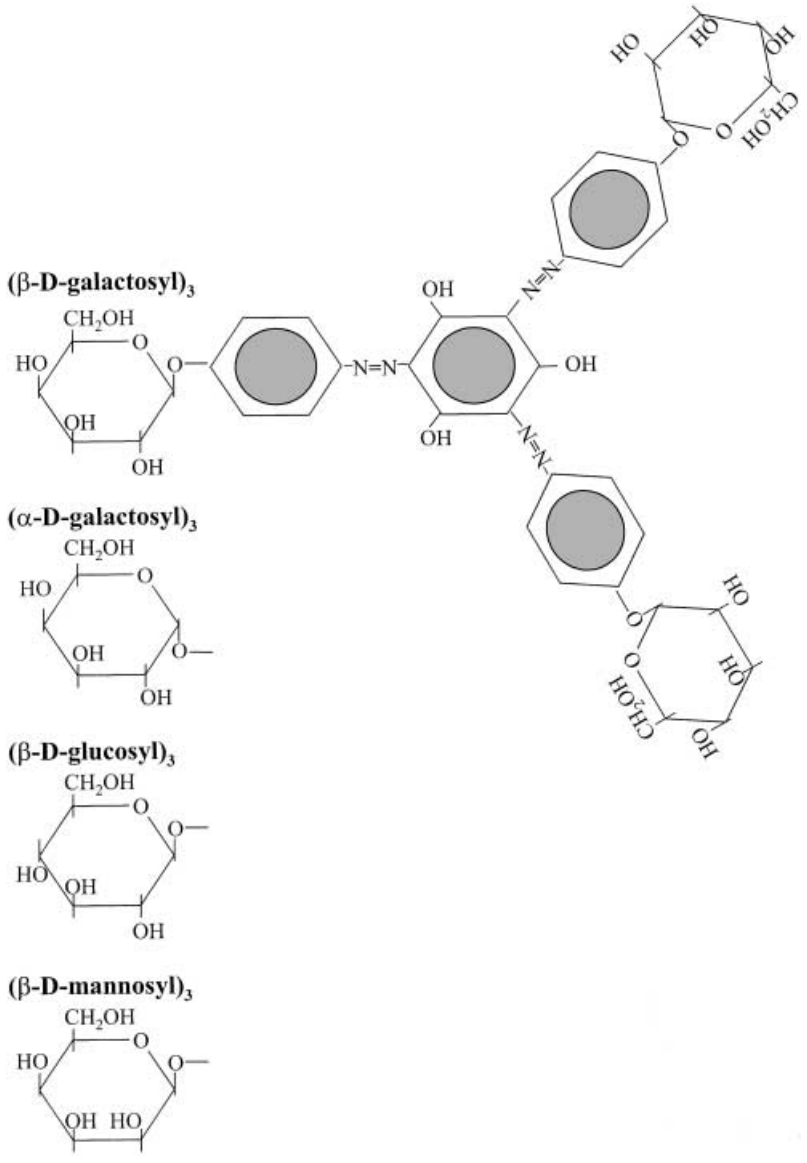

Figure 1. The chemical structure of Yariv reagent. Yariv reagent can be used to stain AGPs a reddish-brown color in plant sections and to bind and precipitate AGPs for quantitation or purification. The three terminal sugars on this reagent are critical for AGP binding. Certain sugars in these positions allow for AGP binding, whereas other sugars do not and serve as important control reagents. For example, (β-D-galactosyl)$_3$ and (β-D-glucosyl)$_3$ Yariv reagents bind AGPs, whereas (α-D-galactosyl)$_3$ and (β-D-mannosyl)$_3$ Yariv reagents do not bind AGPs. Note that (β-D-galactosyl)$_3$ Yariv reagent is depicted here with its three (β-D-galactosyl) arms; the names and terminal sugar structures of other commonly used Yariv reagents also are indicated.

greatly increased our understanding of their structure and diversity, although posttranslational modifications of the core protein (e.g. hydroxylation and glycosylation) can only be inferred from cloning data [7–21].

AGPs are currently divided into two classes depending upon their core protein: 'classical' and 'nonclassical' AGPs (fig. 3) [8, 15]. Undoubtedly, these assignments will be revised as we gather further information and attempt to classify Mother Nature's 'experiments' in this field. Classical AGPs are defined by the core protein and contain hydroxyproline (Hyp), Ala, Ser, Thr and Gly as the major amino acid constituents, whereas nonclassical AGPs have their carbohydrate moieties attached to core proteins which are different from the classical ones in any number of ways. For, example Hyp-poor AGPs, Cys-rich AGPs and Asn-rich AGPs all belong to this group [8, 15, 22–24].

It is interesting to note the extensive sequence divergence in these various AGP core proteins within a species, although at least one pair of orthologous AGP genes apparently do exist in tomato (i.e. *LeAGP-1*) and tobacco (*Na* AGP4), and show a high degree of sequence similarity

AGP	Sequence
Carrot	Asp-Glu-Ala-Hyp-Ala-Hyp-Ala-Hyp-Ser-Hyp-Met
	Hyp-Ala-Hyp-Ala-Hyp-Ala-Hyp
Ryegrass	Ala-Glu-Ala-Hyp-Ala-Hyp-Ala-Hyp-Ala-Ser
Rose	Asp-Ala-Hyp-Ala-Hyp-Ser-Hyp-Val
Maize	Asn-Ala-Hyp-Hyp-Hyp-Ala-Ala-His-Tyr
	Ala-Hyp-Hyp-Ala-Pro-Ala-Pro
Pear (AGP *Pc*1)	Ala-Lys-Ser-Hyp-Thr-Ala-Thr-Hyp-Hyp-Thr-Ala-Thr-Hyp-Hyp-Ser-Ala-Val
	Val-Thr-Ala-Hyp-Thr-Hyp-Ser-Ala-Ser-Hyp-Hyp-Ser-Ser-Thr-Hyp-Ala
Pear (AGP *Pc*2)	Ala-Glu-Ala-Glu-Ala-Hyp-Thr-Hyp-Ala-Leu-Gln-Val-Val-Ala-Glu-Ala-Hyp-Glu-Leu-Val-Hyp-Thr-Hyp-Val-Hyp-Thr-Hyp-Ser-Tyr
Tobacco (AGP *Na*1)	Leu-Ala-Ser-Hyp-Hyp-Ala-Hyp-Hyp-Thr-Ala-Asp-Thr-Hyp-Ala-Phe-Ala-Hyp-Ser-Gly-Gly-Val-Ala-Leu-Pro-Hyp-Ser
Tomato (LeAGP-1)	Hyp-Ala-Ala-Ala-Hyp-Thr-Lys-Pro-Lys
	Ala-Hyp-Ala-Ser-Ser-Hyp-Hyp-Val-Gln-Ser-Hyp-Hyp-Ala-Hyp-Ala-Hyp-Glu-Val-Ala-Thr-Hyp-Hyp-Ala-Val

Figure 2. Amino acid sequences determined for various AGP core proteins directly. Since these data are directly from peptides, as opposed to proteins predicted from cloned sequences, hydroxyproline (Hyp) residues are shown. In these sequences, relatively few Pro are not hydroxylated, and the occurrence of Hyp-Ala and Ala-Hyp dipeptide repeats is common. References for these data are as follows: carrot [3], ryegrass [4], rose [5], maize [6], pear (Pc1) [7], pear (Pc2) [8], tobacco [9] and tomato [10].

[24A]. Nonetheless, standard computer databank searches typically fail to identify other AGP core protein sequences; instead, similarities between AGP core proteins are best seen in hydropathy plots, at least in the case of the classical AGPs. Moreover, hydropathy plots predict a hydrophobic C-terminal tail in all of the classical AGPs. This prediction served as an important clue which led to the discovery that classical AGPs are glycosylphosphatidylinositol (GPI)-anchored proteins, a topic that is addressed more fully below [25]. It remains to be seen whether other classical AGPs are similarly modified and whether any nonclassical AGPs are modified in this way. To date, however, none of the nonclassical AGP clones predict a C-terminal hydrophobic tail, so it is unlikely that they have a GPI anchor.

One notable exception to the generally unfruitful homology searches is seen in examining the nonclassical carrot AGP clone [21]. This clone was demonstrated to have a high degree of similarity to a previously reported proline-rich protein (PRP) in bean that is downregulated by elicitor treatment [17]. Further work on this bean PRP has shown it to be extensively glycosylated, which is atypical for PRPs, which are generally regarded to be nonglycosylated or lightly glycosylated. Thus, it is likely that this bean PRP is actually a nonclassical AGP. Moreover, the carrot AGP shows a substantial degree of similarity to a set of related tobacco clones, *Na*PRP4, TTS-1 and TTS-2 [16, 18]. The conservation of the number and relative positions of Cys residues in these encoded proteins is also noteworthy. The TTS and *Na*PRP4 clones and their encoded proteins are clearly related, with ~96% similarity at both the nucleotide and amino acid, but are regarded as AGPs and a PRP by the two groups who identified them. This identification was based in part on the ability of Yariv reagent to recognize the TTS proteins in one lab and the failure of Yariv reagent to recognize the *Na*PRP4 protein (which is also known as the galactose-rich-style glycoprotein or GaRSGP) to any substantial extent in the other lab [26, 27]. Whereas the classification is still a point of contention and highlights the difficulties in classifying a spectrum of related molecules, it would appear that classification of TTS-1, TTS-2 and *Na*PRP4 as nonclassical AGPs is supported by their similarity to the carrot AGP.

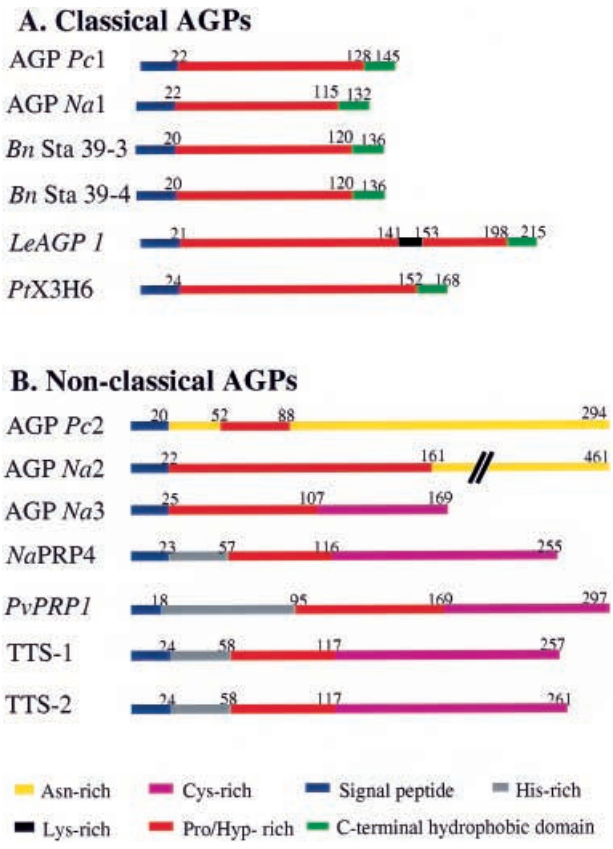

Figure 3. Domain structures encoded by various bona fide and suspected classical and nonclassical AGP clones. References for these data are as follows: AGP *Pc*1 [7], AGP *Na*1 [9], *Bn* Sta 39-3 [11], *Bn* Sta 39-4 [11], *LeAGP-1* [12,13], *Pt*X3H6 [14], AGP *Pc*2 [8], AGP *Na*2 [8], AGP *Na*3 [15], *Na*PRP4 [16], *PvPRP1* [17], TTS-1 [18], and TTS-2 [18].

Carbohydrate moiety

As their name implies, AGPs are rich in arabinose and galactose, and in some cases glucuronic acid, along with other less-abundant sugars. The arabinose and galactose residues are arranged in polysaccharide units that are attached to multiple sites on the core protein. These polysaccharide units vary in size from 30–150 sugar residues, but inevitably exhibit a so-called type II arabinogalactan glycan structure consisting of a (1–3)-β-D-galactan backbone having (1–6)-β-D-galactan side chains, which in turn are modified by arabinose and other less-abundant sugars, including L-rhamnose, D-mannose, D-xylose, D-glucose, L-fucose, D-glucosamine, D-glucouronic acid and D-galacturonic acid [28, 29]. Short arabinose oligosaccharide chains additionally decorate at least some AGPs, just as they do in the extensins [6, 30].

Essentially no information exists on the sequence of the polysaccharide units. Also, the question of whether certain polysaccharide units contain *N*-acetylglucosamine units remains an open and important question, given that certain AGPs are suspected substrates for chitinase (see function section).

Carbohydrate-core protein linkages

The polysaccharide chains appear to be attached to Hyp residues and possibly to Ser and Thr residues, whereas the short arabinose oligosaccharides, if present, are attached to Hyp. Evidence for galactosyl-*O*-Hyp, galactosyl-*O*-Ser, and arabinosyl-*O*-Hyp and unidentified glycosyl residues linked to Thr are reported for several AGPs [29]. Based on the Hyp-contiguity hypothesis, which briefly states that contiguous Hyp residues are glycosylated with oligoarabinosides, whereas single noncontiguous Hyp residues are glycosylated with polysaccharide units, glycosylation patterns can be predicted for AGP core protein sequences [31]. In other words, for AGPs, isolated Hyp residues appearing in the core protein are predicted to be the points of attachment of polysaccharide chains, whereas clusters of Hyp residues are predicted to be the potential sites of attachment for oligoarabinoside chains approximately four to six residues in length. Such predictions need to be tested further, but in the limited number of cases tested thus far for various plant HRGPs, the predictions are well supported [32].

Some AGPs may also contain N-linked glycans as evidenced by the ability of peptide: *N*-glycosidase F to cleave TTS and *Na*PRP4 (i.e. GaRSGP) glycoproteins as well by direct sequencing of the N-linked chain of GaRSGP [26, 27, A. Bacic, personal communication]. Also, a few AGPs, such as LeAGP-1 and PtX14A9, contain recognition sites for N-glycosylation, but further investigation is required to determine whether these sites are utilized [12–14].

Processing of AGPs and GPI anchors

AGPs are extensively modified by posttranslational modifications, most notably hydroxylation of prolyl residues and glycosylation; however, relatively little is known about subsequent processing of AGPs. The finding that GPI anchors exist on at least some classical AGPs represents a recent, important contribution to the field [25, 33–35A]. These anchors have the general structure depicted in fig. 4 and are added to a specific amino acid in the C-terminal portion of classical AGPs concomitant with the elimination of the C-terminal hydrophobic tail from these AGPs. This modification occurs in the endoplasmic reticulum (ER) and anchors the AGP to the lumenal face of the ER membrane and then the Golgi membrane before secretion to the outer face of the plasma membrane. Such a plasma membrane AGP can be further processed, presumably by the action of an endogenous phospholipase C or D, so as to release this AGP from the plasma membrane for cell wall or extracellular destinations. It is interesting to note that in the limited number of AGP genes characterized to date (e.g., LeAGP-1 and AGP *Na*4), a separate exon encompasses the entire consensus region for GPI anchor addition, indicating this

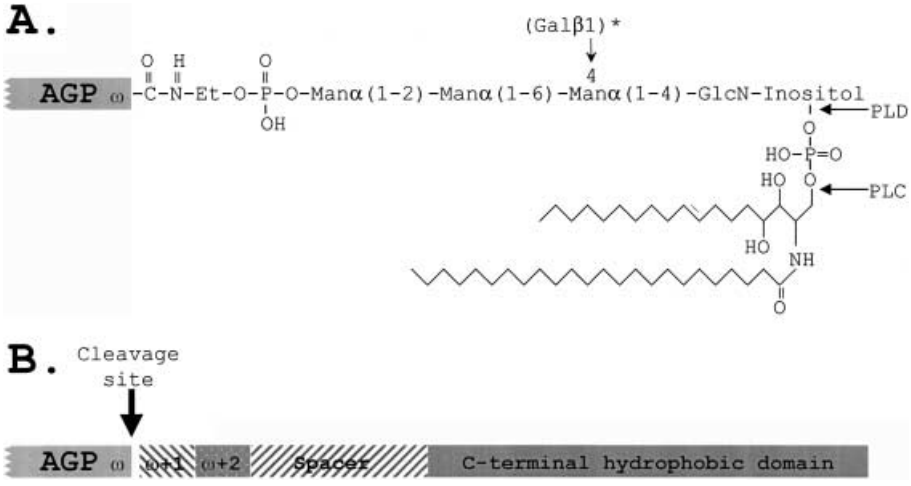

Figure 4. GPI anchor structure and the consensus sequence for its addition. (*A*) Structure of the GPI anchor found in the pear AGP, AGP *Pc*1. The GPI anchor has a partial *β*-galactosyl substitution (*) of its core oligosaccharide and includes a phosphoceramide lipid composed primarily of phytosphingosine and tetracosanoic acid [35]. Potential sites of cleavage by phospholipase C (PLC) and phospholipase D (PLD) are also indicated; cleavage at one or both of these sites would release the AGP from the plasma membrane into the extracellular matrix. (*B*) Consensus sequence present in the C-terminal portion of classical AGPs for the addition of a GPI anchor in the AGP core protein. The amino acid residue designated ω is the site of GPI anchor addition, while the remaining C-terminal residues are removed during anchor addition. Generally, the ω residue is Ser, Asn, Ala, Gly, Asp, or Cys, whereas the $\omega+2$ residue is Ala, Gly, Thr, or Ser. The $\omega+1$ residue is less critical. A 4–8 amino acid spacer region follows which often contains a basic residue (e.g. Arg or Lys) just before the terminal 14–18-amino acid hydrophobic tail.

may be an important functional domain [13, P. Gilson, personal communication].

It also appears that at least some nonclassical AGPs (i.e. AGP *Pc*2 and AGP *Na*2) will be processed so as to remove amino acid sequences which are encoded by complementary DNA (cDNA) sequences, but which apparently are not present in the corresponding AGPs [8]. Direct evidence for such proteolytic processing remains to be obtained and represents an intriguing area of study.

AGPs are apparently rapidly synthesized, secreted to the cell surface and then turned over. This conclusion is based on two independent pulse chase studies using radioactive glucose and arabinose, respectively [36, 37]. In addition, protoplasts synthesize and secrete AGPs into the extracellular medium during the process of cell wall regeneration. Some AGPs are present in multivesicular bodies, and this has led to the suggestion that these AGPs are destined for degradation in the vacuole [38, 39]. Several plant glycosidases (e.g., *α*-L-arabinofuranosidases, *β*-galactosidases) are known which could theoretically break down AGPs; however, direct testing of these enzymes on AGP substrates and determining conclusive cellular localizations for them remain to be performed [29]. Such processing events may represent important ways in which AGP functions are regulated.

Molecular shape and aggregation of AGPs

Based on transmission electron microscopic imaging of AGPs, some AGPs are globular, whereas others are rod-like molecules. Two models of molecular organization correspond to these observed shapes: the 'wattle blossom' model and the 'twisted hairy rope' model (fig. 5) [30, 40]. In the wattle blossom model, the polysaccharide chains are folded into globular units which decorate the core protein so as to generate an overall spheroidal shape, whereas in the twisted hairy rope model, the polysaccharide chains as well as oligoarabinosides are postulated to wrap around the rodlike core protein. One or both of these models may prove to be correct, as different AGPs may have different molecular shapes. Visualization of three different AGPs using transmission electron microscopy provides support for both models. Specifically, the Hyp-poor carrot AGP and the TTS proteins appeared spheroidal, whereas an AGP from gum arabic was rodlike [22, 26, 30]. Moreover, it is also likely that these models will be modified. For example, oligoarabinoside chains may exist along with polysaccharide chains, as predicted by the Hyp-contiguity hypothesis, within the general content of the wattle blossom model. In any event, more research will be required before well-substantiated models emerge.

AGPs are known to aggregate in vitro and may do so in vivo as well. Such aggregates were observed in imaging the Hyp-poor carrot AGP and the TTS proteins by electron microscopy [22, 26]. These self-associations are consistent with the adhesive nature of AGPs and may relate to the observed cross-linking of AGPs [41].

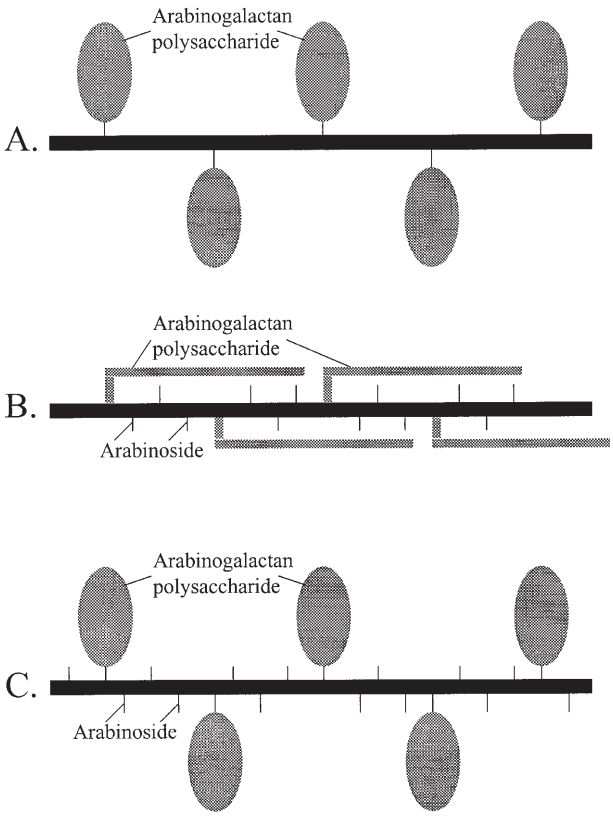

Figure 5. Three proposed models for AGPs showing their general molecular organization and shapes: (*A*) the 'wattle blossom' model, (*B*) the 'twisted hairy rope' model and (*C*) a modified 'wattle blossom' model with oligoarabinoside chains.

Expression

Tools for studying AGP expression

Several tools and techniques are used to examine and localize expression of AGPs in plants at several levels from whole plants to cells. Such studies typically employ Yariv reagent, biochemical isolation, antibodies and molecular cloning/hybridization, either individually or in combinations. A summary of various antibody probes for AGPs or putative AGPs is shown in table 1 for convenience and complements the list of molecular probes (i.e. clones) already presented (fig. 3) [4, 10, 20, 27, 42–58].

AGP expression in the plant kingdom

AGPs are broadly distributed and probably universal in the plant kingdom. Although this early survey work was largely based on positive reactivity of plant extracts or tissue sections with Yariv reagent, other tools were subsequently used to confirm that numerous angiosperms, gymnosperms and lower plants (e.g. bryophytes, algae) contain AGPs [29, 40, 59–61].

AGP expression in plant organs and tissues

At the organ level, AGPs are found in leaves, stems, roots, floral parts and seeds [29, 40]. Similarly, AGPs are found in many tissues, and are especially abundant and well documented in xylem, stylar transmitting tissue and cell suspension cultures. AGPs, as a collective group or family, are widely distributed in organs and tissues; however, individual AGP family members (as defined by their particular core protein) demonstrate varying degrees of organ-specific and tissue-specific developmental expression (tables 2 and 3) [7–10, 12–18, 39, 45, 46, 62–76]. Biochemical characterization of particular AGP family members, Yariv-stained crossed electrophoresis patterns of extracts and hybridization of AGP clones to Northern blots of RNA isolated from various organs of a single plant illustrate this point. Moreover, AGPs demonstrate temporal patterns of developmental expression. For example, in tomato, *LeAGP-1* messenger RNA (mRNA) is found in young but not old stems and fruit [12, 13]. Interestingly, the LeAGP-1 glycoprotein is detected at both temporal stages, providing some support to the notion that AGPs are stable molecules once produced and may accumulate over time [75].

At the tissue (and cellular) level, immunolocalization studies have revealed developmental patterns of AGP expression. Several monoclonal antibodies exist with reactivity directed against carbohydrate epitopes found on AGPs and were used to localize sets of AGPs to various tissues in selected plant organs (tables 1 and 2). Although this has proven to be an extremely useful approach, it should be noted that these antibodies may react with carbohydrate epitopes which are displayed on different AGP core proteins (family members), on certain glycoforms of a particular AGP core protein (i.e. AGP subfamily members) or even in other molecules (e.g. pectin). On the other hand, as these epitopes are more precisely defined, these antibodies may provide a means to probe organ- and tissue-specific glycosylation/deglycosylation events involving AGPs. Such events may be important in regulating AGP interactions and cellular signaling as subsequently discussed in the function section. In contrast, few AGP antibodies are directed against the core protein, which is typically extensively glycosylated. Two notable exceptions are the PAP antibody, which has reactivity directed against a putatively unglycosylated, Lys-rich region of a tomato AGP known as LeAGP-1, and an antibody which has reactivity directed against a bacterially expressed tobacco TTS core protein (table 1) [10, 58]. These antibodies have proven useful in localizing the respective expression of an individual AGP core protein (i.e. family member) and its associated glycoforms (subfamily members).

Whereas AGPs clearly demonstrate developmentally regulated expression, other factors regulate AGP expression, too. Specifically, wounding regulates steady-state AGP mRNA levels at least in some instances. For exam-

Table 1. Antibody probes for AGPs[a].

Antibody/antigen[b]	Epitope	Reference
Monoclonal		
J539	unsubstituted (1 → 6)-β-D-galactan	42
PCBC3	*t*-α-L-Ara*f*[c]	43
PN16.4B4	carbohydrate portion of AGP	44
MAC207	β-D-Glc*p*A-(1 → 3)-α-D-Gal*p*A-(1 → 2)-L-Rha	45
JIM4	β-D-Glc*p*A-(1 → 3)-α-D-Gal*p*A-(1 → 2)-L-Rha	45
JIM8	carbohydrate portion of AGP	46
JIM13	β-D-Glc*p*A-(1 → 3)-α-D-Gal*p*A-(1 → 2)-L-Rha	45
JIM14	carbohydrate portion of AGP	47
JIM15	β-D-Glc*p*A	45
JIM16	unknown	47
PCBC4	substituted (?)(1 → 6)-β-D-galactan	48
CCRC-M7	arabinosylated (1 → 6)-β-D-galactan	49
ZUM15	unknown	50
ZUM18	unknown	50
LM2	β-D-Glc*p*A	51
Polyclonal		
Gladiolus AGPs	arabinosylated (1 → 6)-β-D-galactan	52
Radish AGP	substituted (?)(1 → 6)-β-D-galactan	53
Phenyl-α-L-Ara*f*	t-α-L-Ara*f*	54
Phenyl-α-L-Ara*f*	t-α-L-Ara*f*	55
Ryegrass AGP	deglycosylated protein	4
Gum arabic	β-D-Glc*p*A-(1 → 6)-D-Gal	56
Gum arabic	α-L-Ara*f*-(1 → 4)-D-GlcA	56
$(\beta\text{-}(1 \rightarrow 6)\text{-D-Gal})_4$	unsubstituted (1 → 6)-β-D-galactan	57
N. tabacum TTS-2	protein produced from engineered bacteria	58
N. alata 120 kDa	deglycosylated protein	48
Cotton H6	synthetic peptide	20
*N. alata Na*PRP4	deglycosylated protein	27
PAP (LeAGP-1)	synthetic peptide of Lys-rich subdomain	10

[a] Modified from [29]. [b] Antibody name for monoclonals or antigen injected for polyclonals. [c] For sugar residues: *f*, furanosyl; *p*, pyranosyl; *t*, terminal.

ple, *LeAGP-1* and the carrot Hyp-poor AGP mRNA rapidly disappear in response to wounding [12, 13, 21]. Similarly, *PvPRP1* mRNA rapidly disappears in response to elicitor treatment [17]. Notably, all three of these clones have a repeated nucleotide sequence motif in their respective 3′ untranslated sequences, which is associated with rapid turnover of mRNAs. It also should be noted that gum arabic AGP is secreted only upon wounding, and this secretion occurs only at the wound site [28]. Other factors, including treatment with various plant hormones and pathogen infection, also may serve to regulate AGP expression, but remain to be investigated.

To date, few published reports on in situ localization of AGP mRNAs exist [18]. Given that the majority of the antibodies against AGPs apparently recognize more than one AGP family member, such experiments should provide useful gene-specific data. Alternatively, reporter gene fusions to AGP promoters would be another useful avenue to elucidate gene-specific expression patterns as well as the sequences controlling such expression; however, only a limited number of genomic clones for AGPs are available, and this area has received little attention to date.

AGP expression in plant cells

For the most part, immunolocalizations at the transmission electron microscope level have identified specific cell types that express AGPs (table 2). Such studies, along with isolation and characterization of AGPs following subcellular fractionation, have elucidated their subcellular locations.

AGPs are generally found in plasma membranes, cell walls or as secretions to intercellular spaces, culture media or the environment [29]. AGPs are also found in intracellular, multivesicular bodies; this localization likely reflects turnover of AGPs [38], whereas the other locations suggest particular functions, as discussed in the next section.

Can a particular AGP family member exist at more than one cell surface location? The answer appears to be yes, and it is based on several lines of evidence. The finding that GPI anchors exist on AGPs and are responsible for their localization to the plasma membrane is a key point here [25, 33–35]. In animals, GPI-anchored proteins can be enzymatically cleaved in vitro and in vivo to release plasma membrane-bound proteins to the extracellular matrix. Likewise in plants, GPI-anchored proteins can be

Table 2. Developmentally regulated expression of AGP epitopes as revealed by a variety antibodies.

Species	Organ/tissue	Antibody	Labeling[a]	Cell type(s)	Localization	Reference
Daucus carota		MAC207	IF	protoplasts	plasma membrane	62
Beta vulgaris		MAC207	IF	protoplasts	plasma membrane	62
Daucus carota	root	MAC 207	IF	cortex	cell surface	62
Pisum sativum	root	MAC 207	IF	cortex	cell surface	62
Allium cepa	root	MAC 207	IG	cortex	plasma membrane	62
Cucurbita pepo	hypocotyl	MAC 207	IG	mesophyll cell	plasma membrane	62
Beta vulgaris	anther	MAC 207	IG	pollen vegetative cell	plasma membrane	62
Daucus carota		MAC 207	IG	cultured cells	plasma membrane	62
Hyacinthoides non-scriptus	leaf	MAC 207	IG	mesophyll cell	plasma membrane	62
Daucus carota	root	JIM4	IF	pericycle cells	cell surface	63
Pisum sativum	embryogenic apex (meristems, primordia)	MAC207	IF	cells of future stamen filaments, petal primordia, outer pollen sac boundary	plasma membrane	64
	stamen	MAC207	IF	pollen vegetative cell	plasma membrane	64
	carpel	MAC207	IF	cells of integuments and heart- and torpedo-stage embryos	plasma membrane	64
Daucus carota	hypocotyl	JIM4	IF	epidermal cells and vascular cylinder cells	cell surface	65
	preembryogenic masses (PEMs)	JIM4	IF	surface cells	cell surface	65
	PEM embryos:					
	– globular stage	JIM4	IF	surface layers center on the shoot end	cell surface	65
	– early heart stage	JIM4	IF	two groups of internal cells (reflect cotyledonary ridges)	cell surface	65
	– late heart stage	JIM4	IF	epidermal cells at the shoot apical end	cell surface	65
	– early torpedo stage	JIM4	IF	provascular tissue cells	cell surface	65
	– late torpedo stage	JIM4	IF	provascular tissue cells, isolated epidermal cells and future shoot apex cells	cell surface	65
Daucus carota		JIM8	IF	protoplasts	plasma membrane	46
Brassica napus	anther	JIM8	IF	endothecium, middle layer, tapetum, microspore tetrads, vegetative cells and sperm cells	plasma membrane	46
	ovule	JIM8	IF	nucellus, synergid cells and egg cell	plasma membrane	46
	embryo	JIM8	IF	zygote, embryo proper and suspensor cells	plasma membrane	46
	anthers	JIM8	IG	vegetative cell and sperm cell in pollen	outer face of the plasma membrane	46
Daucus carota		MAC207 and JIM4	IF	plasmolysed cultured cells	outer face of the plasma membrane	47
	root	JIM4	IF	future pericycle cell	cell surface	47
	root	JIM13	IF	epidermal cells, developing xylem elements, root cap cells	cell surface	47
	root	JIM14	IF	all cells	cell surface	47
	root	JIM15	IF	all cells except epidermal and future xylem cells	cell surface	47
	root	JIM16	IF	all cells	cell surface	47
		MAC207	IG	plasmolysed cultured cells	outer face of the plasma membrane	47
		JIM4	IG	cultured cells	cell wall and plasma membrane	47

Table 2 (continued)

Nicotiana tabacum	flowers	JIM8 and MAC207	IG	pollen grains	intine, cytoplasm including vesicles, Golgi apparatus	66
	flowers	JIM8 and MAC207	IG	generative cell	cell wall and cytoplasm including vesicles	66
	flowers	JIM8 and MAC207	IG	pollen tube	periodically along the outer wall of the tube except the tip	66
Arabidopsis thaliana	root	JIM13	IF	initial of central metaxylem vessels, other pre-metaxylem elements, parenchyma cells, endodermal and pericycle cells)	cell surface	67
	root	JIM13	IG	differentiating metaxylem vessel elements	outer face of plasma membrane and cell wall	67
Zea mays	coleoptiles	MAC207	IG	all cells	plasma membrane	39
	coleoptiles	JIM13	IG	maturing sclerenchyma cells	plasma membrane invaginations, multivesicular bodies	39
				differentiating tracheid cells	secondary wall thickenings	
	coleoptiles	JIM14	IG	maturing sclerenchyma cells	innermost wall layer	39
Daucus carota		JIM8	IF	cultured cells	cell surface	68
Arabidopsis thaliana	root	CCRC-M7	IF	columellar root cap cells epidermal cells, cortical, endodermal and pericycle cells	cell surface	69
	root	CCRC-M7	IG	endodermal, pericycle and phloem cells	cell wall	69
Lilium longiflorum	flowers	JIM13	IF	pollen tube	tube tip	70
		JIM13	IF	stylar transmitting tract epidermal cells (TTEs)	cell surface	70
	flowers	LM2	IF	all cells in the style except the TTEs	cell surface	70
	flowers	JIM13	IG	pollen tube	cell wall, plasma membrane and cytoplasmic vesicles	70
	flowers	JIM13	IG	generative cell	plasma membrane	70
Lilium longiflorum	flowers	JIM13	IG	(β-D-glucosyl)$_3$ Yariv reagent-treated pollen tubes	secretory vesicles, plasmalemma, electron-translucent areas within the expanded periplasm	71
Amaranthus hypochondriacus	young flower	MAC207	IF	all cells but nucellar cells	cell surface	72
	young flower	JIM8	IF	all cells with nucellus selectively labeled on the micropylar cells	cell surface	72
	ovule with mature embryo sac	JIM8	IF	synergid cells with filiform apparatus, integument and micropylar nucellus cells	cell surface	72
	ovule with mature embryo sac	MAC207	IF	micropylar nucellus cells, filiform apparatus and integument cells	cell surface	72
	ovule with young embryo	JIM8	IF	embryo proper, suspensor cells and filiform apparatus	cell surface	72
	ovule with young embryo	MAC207	IF	filiform apparatus	cell surface	72
	ovule with young globular embryo	JIM8	IF	micropylar nucellus cells, embryo proper, suspensor and integument cells	cell surface	72

Table 2 (continued)

Species	Organ/tissue	Antibody	Labeling[a]	Cell type(s)	Localization	Reference
Daucus carota	root	JIM4	IF	pericycle cells, and protoxylem elements	cell surface	73
	root	JIM13	IF	developing xylem cells	cell surface	73
Raphanus sativus	root	JIM4	IF	pericycle cells in front of both the phloem and xylem	cell surface	73
	root	JIM13	IF	developing xylem cells	cell surface	73
Pisum sativum	root	JIM13	IF	xylem and pericycle cells in front of the phloem	cell surface	73
	root	JIM4	IF	none	–	73
Allium cepa	root	JIM13	IF	cells in phloem region,	cell surface	73
	root	JIM4	IF	none	–	73
Zea mays	root	JIM8	IF	developing and mature sieve element	sieve plates, sieve element reticulum	74
	root	JIM13	IF	sieve elements and adjacent pericycle and companion cells	sieve plates, cortical ER[b] and developing sieve element plasmodesmata	74
	root	JIM8	IG	sieve elements	ER, plasma membrane	74
	root	JIM13	IG	sieve elements, pericycle and companion cells	ER, plasma membrane	74
	root	LM2	IG	all cells except sieve elements	ER, Golgi apparatus and tonoplast	74
Lycopersicon esculentum		PAP	IF	cultured cells	cell surface	10
		PAP	IG	cultured cells	cell wall	10
	petiole	PAP	IF	developing metaxylem, outer phloem	cell surface	10, 75
	petiole	PAP	IG	developing metaxylem	secondary cell wall	10, 75
	first internode	PAP	IF	developing metaxylem, outer and inner phloem	cell surface	10, 75
	first internode	PAP	IG	developing metaxylem	secondary cell wall	10, 75
	style	PAP	IF	transmitting tissue	not determined	10
	style	PAP	IG	transmitting tissue	intercellular space, cell wall	10

[a] IF, immunofluorescence; IG, immunogold.
[b] ER, endoplasmic reticulum.

Table 3. Organ- and tissue-specific patterns of AGP expression as revealed by RNA blotting using AGP clones as probes.

Plant	Organ or tissue	mRNA size	AGP probe	Reference
Tomato	young stem, flower, roots, green fruit, cultured cells	980 nt	*LeAGP-1*	12, 13, 76
Tomato	leaves*, old stem*, red fruit*	980 nt	*LeAGP-1*	12, 13
Pear	cultured cells	900 nt	AGP *Pc1*	7
N. alata	root, style, ovary, petal, stem, leaf	700–750 nt	AGP *Na1*	9
N. alata	anther*	700–750 nt	AGP *Na1*	9
Pear	cultured cells	1000 nt	AGP *Pc2*	8
Pear	styles, petals, pedicels	not detected	AGP *Pc2*	8
N. alata	cultured cells	1700 nt	AGP *Na2*	8
N. alata	leaves*, stems*, roots*	1700 nt	AGP *Na2*	8
N. alata	pollen*, styles*	1000 nt	AGP *Na2*	8
N. alata	petals	not detected	AGP *Na2*	8
N. alata	mature pistils	780 nt	AGP *Na3*	15
N. alata	immature pistils, ovary, petal, anther, stem, leaf, roots, cultured cells	not detected	AGP *Na3*	15
N. alata	mature styles	1000 nt	*NaPRP4*	16
N. alata	immature styles*, cultured cells*	1000 nt	*NaPRP4*	16
N. alata	stems, leaves, roots, pollen, petals	not detected	*NaPRP4*	16
N. tabacum	pistils, flowers, styles, stigmas	1000 nt	TTS1 and 2	18
N. tabacum	stems, leaves, roots, petals, sepals, ovaries	not detected	TTS1 and 2	18
P. vulgaris	cultured cells, hypocotyls	1100 nt	*PvPRP1*	17
Pine	xylem	1200 nt	*Pt*X3H6	14
Pine	needles*	1200 nt	*Pt*X3H6	14
Pine	megagametophyte, embryo	not detected	*Pt*X3H6	14

* Little expression observed.

cleaved in vitro and in vivo to release proteins, including AGPs, from the plasma membrane [33, 34]. Thus, the model that emerges is that AGPs containing a GPI anchor are associated with the plasma membrane and are cleaved so as to release the AGP to and beyond the cell wall. Immunolocalizations as well as biochemical isolations of identical, or nearly identical AGPs (family members), in multiple surface locations (e.g. cell wall and culture media; plasma membrane, cell wall and culture media) provide evidence for multiple surface locations for a given AGP and are consistent with the above processing model for GPI-anchored AGPs [5, 10, 77, 78]. Nonetheless, the possibility of exclusively targeting certain AGPs to specific cell surface locations cannot be excluded, and the contribution of particular sequence determinants in the carbohydrate or protein moieties to such a process remains to be investigated.

Function

AGPs are thought to function in various aspects of plant growth and development based on several lines of evidence. Most of this evidence is correlative and infers a particular function, whereas some is circumstantial based on the unique characteristic(s) of AGPs. Moreover, whereas experiments designed to probe AGP functions often reflect observable effects at the cellular level, the underlying molecular mechanisms of AGP action remain unclear. Below is a summary of this information relating to AGP function at the plant, cellular and molecular levels appended with speculation.

Vegetative growth and development

Organ-, tissue- and cell-type-specific expression patterns elucidated for AGPs indicate that AGPs are markers of cellular identity and fate [46, 62, 64]. The question, however, remains whether such AGPs are responsible for cellular differentiation or just useful markers of the process. Support for the former, active role comes from studies on a leafy liverwort in which vegetative growth of leaf primordia is suppressed by treatment with various biosynthesis inhibitors of AGPs, and other HRGPs [61]. Currently, work in my laboratory involving expression of an antisense *LeAGP-1* gene in tomato also argues for a functional role for this particular AGP in cellular differentiation and development [H. Lua, M. Gao and A. M. Showalter, unpublished].

Certain AGPs are associated with xylem development (see table 2). More specifically, such AGPs are associated with, and hypothesized to function in, secondary cell wall thickening and programmed cell death (PCD) [39, 75]. Here, AGPs which are located at the cell surface of differentiating xylem elements may serve to position or interact with wall components, including themselves, and

thus contribute to wall thickening. Moreover, since these differentiating cells ultimately undergo PCD in order to allow for water transport, it is hypothesized that AGPs are involved in this PCD process. Support for this hypothesis is based not only on AGP deposition in such developing xylem elements but also on experiments demonstrating that Yariv reagent induces PCD in cell cultures, thereby implicating AGP involvement [79]. Clearly, more research needs to be done to test this hypothesis, but the evidence to date supports this idea.

AGPs also possess the ability to control plant embryogenesis. Evidence for such control comes from adding purified AGPs to embryogenic cell cultures and finding that certain AGPs will stimulate, whereas other AGPs will inhibit somatic embryogenesis [50, 80–82]. Interestingly, the addition of chitinase to such experimental preparations can greatly enhance somatic embryogenesis as measured by counting the number of preembryogenic masses (PEMs) [83]. Thus, the possibility that certain AGPs contain *N*-acetyl-glucosamine (GlcNAc) residues that may be processed to produce a cellular signal warrants further examination. Consistent with this somatic embryogenesis work, immunolocalization studies correlate expression of specific AGP epitopes with embryogenesis [65, 84].

AGPs are extremely hydroscopic molecules. Their water-holding ability may be related to their proposed roles as structural molecules involved in the physical organization of the plant extracellular matrix or as protective molecules. A protective role, or role in wound healing, is also likely for the AGPs present in gum arabic.

Reproductive growth and development

The regulated expression and abundance of particular AGPs in the stigma, stylar transmitting tissue and pollen has led to the suggestion that AGPs are important for plant reproduction (see table 2). This suggestion is more vigorously supported from several, additional experiments. For example, a purified tobacco AGP, which is specifically expressed in the stylar transmitting tissue (i.e. TTS protein) was shown to enhance pollen tube growth in vitro and to attract pollen tubes in a semi-in vivo system [26]. It should be noted, however, that similar experiments in another tobacco species using the TTS homolog (i.e. the NaPRP-4-encoded glycoprotein called GaRSGP) demonstrated essentially none of these effects; the reasons for such discrepancies may relate to GaRSGP being a less glycosylated component of the TTS glycoprotein spectrum [85, 85A]. Moreover, transgenic tobacco plants successfully engineered for downregulated TTS expression demonstrated reduced pollen tube growth in the style and reduced seed production compared with wild-type plants [26]. In another set of experiments, Yariv reagent was applied to lily pollen tubes and shown to inhibit their growth and to produce a bulbous morphology at the pollen tube tip [70, 71]. Furthermore, the normal influx of calcium at the tip was inhibited by the addition of Yariv reagent.

Cellular growth and development

The ability of Yariv reagent to inhibit cell division in cell suspension cultures and to inhibit cell expansion in cell cultures and roots provides support for the action of AGPs at the cellular level [86–88]. In the case of Yariv-inhibited root growth, the epidermal cells in the region of root elongation demonstrated a bulbous morphology, similar to the Yariv-inhibited pollen tube tip [87, 88]. Similarly, the elongation of suspension-cultured carrot cells was inhibited by the addition of Yariv reagent [87].

Experiments in my lab are consistent with these above data. Specifically, we have used Yariv reagent to inhibit cell growth in tomato suspension-cultured cells as well as to inhibit tomato seedling root growth. Root growth inhibition encompassed reductions in overall root length, epidermal root cell elongation, root cell numbers and the number of root hairs. We have also found that Yariv reagent effectively blocks water uptake by the roots of tomato seedlings [88A].

Programmed cell death and social control

In plants, PCD is a normal developmental process involved in anther, megagametophyte and vascular tissue development as well as in senescence, pollination and sex determination [89–98]. Plants also employ PCD as a precisely controlled response to different biotic and abiotic stimuli [96–104].

In my laboratory, we have shown that $(\beta$-D-galactosyl$)_3$ Yariv reagent inhibits the growth of *Arabidopsis* suspension-cultured cells by inducing these cells to undergo PCD in a time- and dose-dependent manner [79]. These results implicate that AGPs are involved in PCD in plants, and indicate that AGPs may be an important component of the signal transduction pathway for this process. Furthermore, these results are in agreement with previous work by Langan and Nothnagel, who noted that $(\beta$-D-galactosyl$)_3$ Yariv reagent was able to kill *Arabidopsis* cell cultures, although they did not determine whether this represented PCD or necrosis [105].

Related to this PCD issue is the idea of social control of cell survival. AGPs may indeed represent an important component for cellular growth and survival. This conclusion is suggested based on the following evidence. Carrot cells cultured at low density are known to activate a PCD pathway, which can be prevented by addition of a cell-free, cell-conditioned growth medium [103]. Similarly, animal cells grown in medium absent of growth factors initiated PCD, which can be suppressed by signal molecules released by other cells [106, 107]. We have also ob-

served that our *Arabidopsis* suspension cells when cultured at low density have a much higher percentage of cells undergoing PCD than when cultured at high density. Thus, 'social control' of cell death, as observed in animal cells and carrot cells and *Arabidopsis* cells, may operate in all of these systems. Indeed, these observations, coupled with (β-D-galactosyl)$_3$ Yariv reagent's ability to induce cell death in *Arabidopsis* cell cultures, indicate that AGPs may represent an important component for plant cell growth and survival.

Molecular interactions and signaling

AGPs possess a rich array of biochemical information that presumably is involved with interactions with other cell surface molecules and with cellular signaling [108]. These two potentially interrelated areas represent fertile ground for future AGP research, although relatively little is known at present. For the record, however, it should be noted that several investigators have found that AGPs often copurify with pectin [77, 86, 109–112]. One interpretation of this phenomenon is that at least some AGPs can interact with pectin, most likely through ionic interactions. For example, negatively charged galacturonic acid residues in pectin could interact (i) indirectly with negatively charged AGPs (i.e. AGPs having glucoronic acid in the polysaccharide side chains) through Ca^{++} bridges or (ii) directly with clusters of basic amino acid residues (i.e. positively charged regions) found in some AGPs, such as the carrot Hyp-poor AGP and tomato LeAGP-1. Furthermore, Baldwin and co-workers have provided some support to this idea in their study of the Hyp-poor carrot AGP, which appears to show some degree of Ca^{++}-mediated binding of pectin in blotting experiments performed in vitro [22]. However, the observed binding appears weak, and this entire area of molecular interactions with AGPs requires additional, rigorous study.

It is also possible that AGPs can associate with one another. Such associations may involve oxidative crosslinking, ionic interactions similar to those suggested above for pectin, or by plant analogs of Yariv reagent [41]. Flavonol glycosides are one possible class of plant analogs, because they are capable of inhibiting the interaction between Yariv reagent and AGPs [113].

Clearly, the carbohydrate moieties of AGPs contain a reservoir of structural information that could serve as potential chemical signals consistent with oligosaccharide signaling in plants as championed by Peter Albersheim [114]. In addition, limited regions of AGP core proteins may also be accessible, as appears to be the case for at least some AGPs such as LeAGP-1, and may also provide chemical signaling information [10]. Many scenarios or models can be envisioned whereby AGPs serve as cell-signaling and/or cell adhesion molecules involved in various aspects of growth and development already mentioned above. These hypothetical models, which are in need of testing and/or further refinement, are summarized in figure 6 and discussed briefly in subsequent paragraphs. It should be emphasized that construction of these speculative models is based on limited information and is often analogous to animal models involving either proteoglycans or other GPI-anchored cell membrane proteins.

First, given that AGPs carry many, information-rich carbohydrate chains and that oligosaccharides are involved in signal transduction in plants, AGPs may be enzymatically processed to release oligosaccharide signals which then could bind to an appropriate cell membrane receptor tied into a signal transduction system (fig. 6A). Such a model could include extracellular, cell wall and plasma membrane AGPs as potential substrates. Interestingly, a tetrasaccharide with a structure similar to the terminal sequence of an AGP accumulates in developing rice anthers, but the origin and role of this oligosaccharide remains unknown [115]. As already mentioned, chitinase apparently acts on AGPs so as to enhance somatic embryogenesis [83]. Naturally, it will be important to demonstrate the presence of GlcNAc residues and a chitinase binding site [i.e. $(GlcNAc)_3$] in such AGPs. Nonetheless, it is tempting to speculate that such an oligosaccharide released from AGPs by chitinase action interacts with a plasma membrane receptor to stimulate somatic embryogenesis. Alternatively, the altered AGPs, rather than the oligosaccharide, may act to stimulate somatic embryogenesis following this unmasking process.

It is also conceivable that the protein moiety could serve as a signaling molecule. However, with few exceptions, such as that illustrated by LeAGP-1's Lys-rich domain that apparently is not glycosylated, the core proteins of the classical AGPs are covered by carbohydrate, and would require at least some degree of deglycosylation before the core protein would be available for further processing or signaling. Knowledge of deglycosylation reactions occurring in vivo for AGPs is largely unknown. The nonclassical AGPs are another matter, since they possess and possibly proteolytically release non-AGP protein domains that could act in signaling or other functions.

Second, AGPs may directly or indirectly interact with a plasma membrane receptor (fig. 6B). In the case of direct interaction, the AGP would bind to a receptor in any number of ways, as many potential sites of interaction exist, and tie into a signal transduction system. This idea is perhaps most analogous to the animal model in which contactin, a GPI-anchored membrane protein in neurons, interacts with contactin associated protein (Caspr). Caspr is a transmembrane protein that is also found in neuronal membranes and its cytoplasmic domain supposedly triggers a signal transduction cascade [116]. In the case of an

indirect interaction, AGPs are envisioned to interact with and collect ligand molecules for effective presentation to an appropriate ligand receptor. This idea is analogous to that supported in animals for the manner in which fibroblast growth factor (FGF), the ligand, is collected by a heparin sulfate proteoglycan (HSPG) for presentation to and subsequent dimerization of the FGF receptor [117]. The idea here is that the assembled complex of FGF ligands collected and appropriately displayed by HSPG allows for more effective (i.e. high-affinity) binding to the FGF receptor in contrast to less efficient (i.e. low-affinity) binding of individual FGF molecules to the receptor. Here, AGPs are analogous to HSPG; however, the plant analogs for FGF and the FGF receptor await identification. Moreover, whereas plasma membrane AGPs fit this model best, cell wall or extracellular AGPs could also interact with such receptors.

Third, AGPs may serve in cell-cell interactions and/or corresponding signal transduction events. In this model, plasma membrane AGPs may interact with one another, perhaps by one of the means already mentioned above (i.e. ionic interactions, Ca^{++} bridges) or by the presence of AGP receptors on neighboring cells (fig. 6C). Although the manner in which a signal would be sent by an AGP-AGP interaction here is unclear (i.e. one might have to invoke general perturbations of the membrane so as to open Ca^{++} channels or release some other type of signal), a receptor model has the benefit that it could be directly tied into a signal transduction system. This later scenario is precisely the case in animals where contactin, that GPI-anchored protein in neurons, binds to a receptor protein tyrosine phosphatase β that is expressed on neighboring glial cells to effect cell adhesion and neurite outgrowth [118]. AGPs secreted from neighboring cells may act in such an intercellular signaling capacity, and would not require direct cell-cell contact.

Fourth, in the case of GPI-anchored plasma membrane AGPs, the AGP moiety is apparently released from the membrane to the extracellular milieu by the action of a phospholipase, such as phospholipase C or D (PLC or PLD) (see fig. 4A). These enzymes, which are commercially available and purified from animal sources, serve to release AGPs from plasma membranes of cells in suspension culture. To date, PLC is reported to exist in peanut [119]. Other plant sources of PLC as well as the identification of PLD in plants await further investigation. Bearing in mind these caveats, the GPI anchors for such processed AGPs could serve to generate phosphatidyl-inositol and inositol phosphoglycan and ceramides that could be used as intracellular messengers (fig. 6D) [120].

Fifth, AGPs may serve as cell adhesion molecules necessary for normal growth and development. As already noted, Yariv reagent is capable of inducing PCD in *Arabidopsis* cell suspension cultures and implicates the involvement of AGPs in this process [79]. The signal transduction process for this event will be interesting to dissect and relate to some of the models already presented. It is likely that Yariv reagent could induce abnormal clustering of AGPs at the plasma membrane surface and/or disrupt essential AGP connections required for normal growth and development (e.g. potential AGP-mediated connections between cells or between plasma membrane AGPs and the extracellular matrix). In animals, it is already clear that disrupting cell membrane-extracellular matrix connections leads to PCD [121–125]. Thus, in plants, AGPs may serve as cell adhesion molecules that serve to connect the cell membrane with the cell wall (fig. 6E).

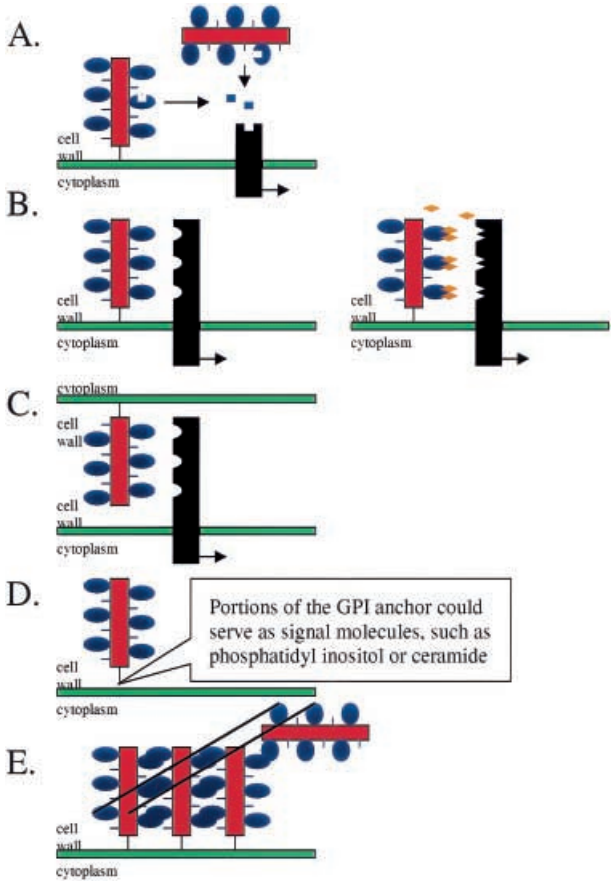

Figure 6. Models for AGP involvement in cellular signaling and cell adhesion. (*A*) signaling by the release of a carbohydrate epitope (small squares) from an AGP which binds to a plasma membrane receptor capable of initiating an intercellular signaling cascade, (*B*) signaling by an AGP directly or indirectly after binding ligand molecules (diamonds) for presentation to an appropriate plasma membrane receptor, (*C*) intercellular signaling by an AGP from one cell interacting with a plasma membrane receptor on an adjacent cell, (*D*) signaling by the GPI anchor (or a portion thereof) following release of a GPI-anchored AGP from the plasma membrane, (*E*) AGPs as cell adhesion molecules capable of aggregation on the plasma membrane and forming a plasma membrane-cell wall connection essential for normal growth and development; these connections may involve cell wall AGPs or other cell wall molecules (black lines) such as pectin.

The manner by which an external signal or disruption of such a connection would be transduced inside the cell is unclear, and not as straightforward as the case for animal integrins. Moreover, if AGPs do serve as cell adhesion molecules, the presence of a GPI anchor and its subsequent processing could facilitate cell wall remodeling which occurs as an integral part of plant development.
In any event, these models are intriguing, highly speculative, and hopefully thought provoking. Clearly, much work will need to be done before we have a clear picture in terms of how AGPs work in various aspects of growth and development.

Commercial applications of AGPs

In addition to the functions of AGPs in plants, AGPs have commercial importance. AGPs are significant components of a number of plant gums or exudates and confer special properties on these plant products. For example, gum arabic that is harvested from wounded *Acacia senegal* trees represents one of the most commercially important gums. The ability of gum arabic to suspend flavorings and colorings, and to do so with low viscosity, makes it an extremely valuable additive in the food industry [126]. Moreover, gum arabic is used in the candy industry to slow the hardening process in the manufacture of hard candy and as an adhesive in the stamp industry. A major question which remains to be resolved is the extent to which the AGPs in gum arabic are responsible for these remarkable properties.
AGPs and type II arabinogalactans may also have applications in medicine. Both of these molecules reportedly stimulate animal immune systems, in some cases by activating the complement system and in other cases by enhancing the cytotoxic activity of natural killer cells [127]. Here again, more extensive investigations are warranted.

Concluding remarks and important questions

It should also be recognized that AGPs possess unique sets of structural characteristics and properties that undoubtedly are linked to their functions. With the successful cloning of several AGP core proteins, the production of useful AGP antibodies, a knowledge of various AGP expression patterns at the organ, tissue and cellular levels, the identification of GPI-anchored plasma membrane AGPs and some extremely tantalizing functional implications for AGPs, the future looks bright for AGP research. Armed with well-characterized individual AGPs and some knowledge of their structure and expression, researchers are now in a position to address several important and challenging questions in the field. Here are but a few of these questions:

- What are the primary structures of polysaccharide chains on AGPs, and how are these chains assembled and attached to their corresponding core protein?
- How do the polysaccharide chains vary within an AGP family member and between family members?
- Why are AGP core protein sequences so diverse? Do they have a role beyond just providing a scaffold for the carbohydrate?
- How are AGPs arranged in three-dimensional space?
- How does Yariv reagent interact with AGPs and exert its physiological effects? Do flavanol glycosides or other molecules serve as endogenous plant analogs of Yariv reagent?
- Which AGPs have GPI anchors? How and why are such AGPs processed?
- Can AGP mutants be identified from mutagenized plants or created by using transgenic plant technology to help elucidate AGP functions?
- What molecules interact with AGPs in plants and what is the molecular nature and location of such interactions?
- Can the function of a particular AGP be clearly and convincingly demonstrated? For example, do certain AGPs function in cell adhesion or cell signaling? If so, how?

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