Biochemistry (Moscow), Vol. 70, No. 10, 2005, pp. 1073-1085. Translated from Biokhimiya, Vol. 70, No. 10, 2005, pp. 1301-1317.

REVIEW

Arabinogalactan Proteins: Involvement in Plant Growth and Morphogenesis

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Received November 23, 2004
Revision received March 2, 2005

Abstract—Arabinogalactan proteins (AGPs) are highly glycosylated hydroxyproline-containing variously located proteoglycans dynamically regulated in the course of plant ontogenesis. Special functions of AGPs are still unclear, but their involvement in vegetative growth and reproduction of plants is well established. This review considers data on the structure, biosynthesis, and metabolism of AGPs. Special attention is given to involvement of AGPs in growth and morphogenesis, and possible mechanisms of their regulatory action are considered. AGPs are also compared with animal proteoglycans.

Key words: arabinogalactan proteins, *O*-glycosylation, cell wall, plasmalemma, cytoskeleton, plant growth, cell differentiation, somatic and zygotic embryogenesis, heparan sulfates

Arabinogalactan proteins (AGPs) constitute an abundant class of highly glycosylated proteins characterized by complicated structure and various location, which are regulated during plant ontogenesis. AGPs belong to the family of hydroxyproline-rich proteins, which also includes extensins, proline-rich proteins, and lectins [1]. AGPs are found in different taxons of both higher and lower plants [2, 3], and the retention of these proteins during evolution suggests importance of their functions. AGPs can be located in the cell wall, on the outer side of plasmalemma, in vacuoles, in intercellular spaces, and in different secretions and mucilages. AGPs are actively released into the nutritional medium by suspension cultures [4]. Many researchers are interested in this class of proteoglycans, and this has resulted in obvious progress in studies on the structure, location, expression, and possible functions of AGPs [4-10]. At present the involvement of AGPs in the regulation of somatic and zygotic embryogenesis is best established [11-15], although AGPs are also shown to play an important role in vegetative growth and reproductive development of plants. But the supposed mechanisms of this regulation are still speculative. Because studies on mechanisms involved in the regulation of growth and morphogenesis are fundamental trends in modern biology, we here attempt to generalize the data on AGP involvement in the regulation of these processes and discuss putative mechanisms of their regulatory action.

CLASSIFICATION, CHEMICAL COMPOSITION, AND STRUCTURE OF AGPs

AGPs are traditionally set off from the family of hydroxyproline-rich proteins by the linkages of their arabinosyl and galactosyl residues and ability for complexing with Yariv reagents, or synthetic trivalent phenylglycoside dyes, such as Yariv reagent $(\beta\text{-D-glucosyl})_3$ (GlYR) (1,3,5-tris[4-β-D-glucopyranosyl oxyphenylazo]2,4,6-trihydroxybenzene) or Yariv reagent $(\beta\text{-D-galactosyl})_3$ (GalYR) [16, 17]. Structural features of Yariv reagents precipitating AGPs are presented in a review by Nothnagel [7]. The mechanism of this reaction is unknown. This interaction is probably due to the ability of AGPs to complex with certain β-bound glycans of cell walls. Cellulose is the main β-1,4-bound glycan of cell walls [18]. However, structures similar to Yariv reagents can also be present in other polysaccharides and phenols of cell walls [7]. In particular, flavone glycosides are such analogs because they display considerable affinity for cell walls [19] and inhibit the interaction between Yariv reagents and AGPs [20]. In practice, the specific precipitation of AGPs with GlYR is used to isolate and purify them. In general, the optimum precipitation occurs at the ratio of 1 : 1 between AGP and Yariv reagent [21]. It should be noted that molecules similar in composition to

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AGP, such as arabinogalactans, which have a similar carbohydrate moiety but lack a protein core, cannot be precipitated with GlYR. AGPs are chemically stable molecules resistant to high temperature and cold alkali [6].

Electron microscopy has shown that AGPs can have either linear structure of a twisty hairy rope or globular shape of wattle blossom (models are presented in reviews [1, 10]). A scheme of the structure of an AGP molecule is shown in Fig. 1. Most AGPs studied so far contain a core polypeptide, which contributes 1-10% of the molecular weight [2]. The carbohydrate component is prevalent in AGP molecules, and they are therefore classified as proteoglycans [7]. However, some AGPs have high protein content from 30 to 65% [22-24], thus division of hydroxyproline-containing proteins into proteoglycans and glycoproteins is a matter of convention. The molecular weights of AGPs are usually 60-300 kD [7], but there are also AGPs with molecular weight higher than 1 MD [25]. Depending on amino acid composition of the core protein, AGPs are subdivided into classical and nonclassical AGPs [7]. The protein moiety of classical AGPs mainly contains Hyp/Pro, Ala, Ser, Thr, and Gly. Amino acid composition of nonclassical AGPs varies considerably: thus, Hyp-poor AGPs [26-28], a His-rich AGP [29], an Ala-poor AGP [30], and Asp-rich AGPs have been described [31]. So far genes encoding the protein moiety of AGPs are identified only for some plant species, such as pine [32, 33] and *Arabidopsis* [34]. Classic AGPs of *Arabidopsis* have been subdivided as follows: proper classical AGPs with polypeptide of 85-151 amino acids; arabinogalactan peptides (AG-peptides) with peptide of 10-13 amino acids; basic AGPs containing a short lysine-rich region, and also fasciclin-like AGPs (FLA) [9, 35]. FLAs are a new class of classical AGPs, which, in addition to AGP-specific regions rich with Pro/Hyp, Ala, Ser, and Thr, contain one or two β-Ig-H3/fasciclin-like domains [34-36]. Forty-seven genes of classical AGPs were identified in work [34].

Note that analysis of DNA sequences of all known classical AGP clones has predicted them to have a hydrophobic transmembrane domain on the C-end [10]. In the mature AGP molecule, this hydrophobic domain is substituted by a glycosyl phosphatidylinositol anchor (GPI) [37-41]. The structure of this anchor is comprehensively studied only for AGP from a pear suspension culture [40]. The GPI anchor consists of a core oligosaccharide produced by mannose residues (mannose can also be replaced by β-galactose) and acetylglucosamine bound via ethanolamine phosphate to the C-end of the protein. On the reducing end, glucosamine is bound through inositol with a phosphoceramide consisting of phytosphingosine and tetracosanic acid (Fig. 1, [4, 8, 9]). Studies on DNA sequences of nonclassical AGP

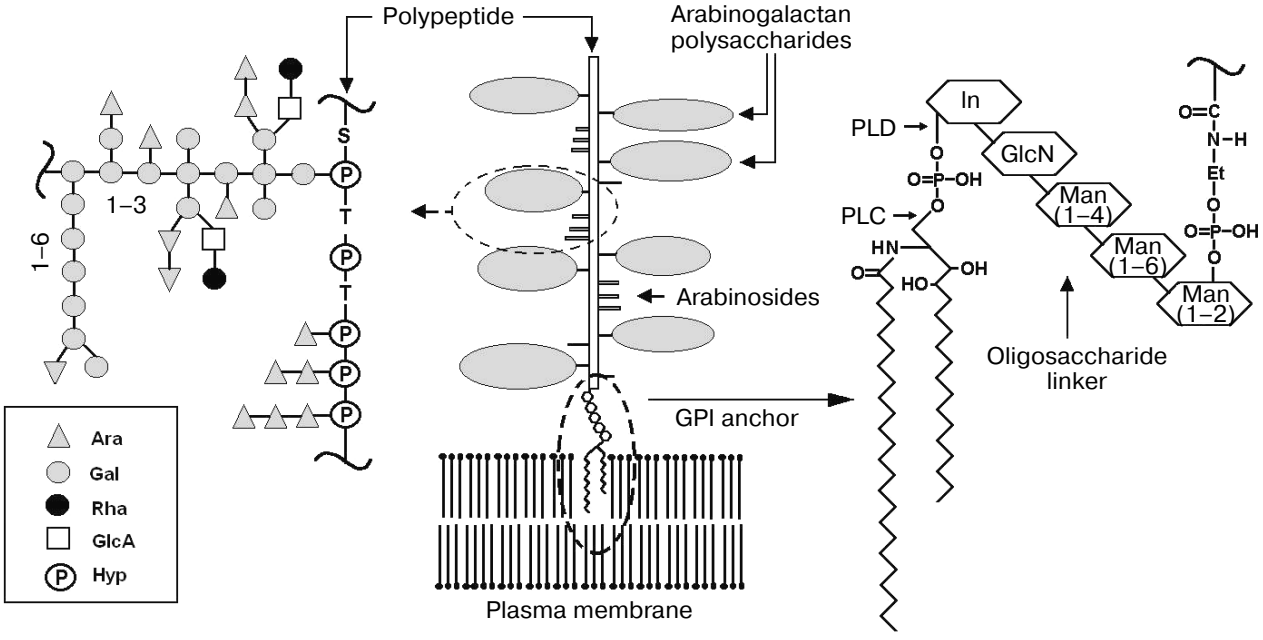

Fig. 1. A hypothetical structural model of an AGP molecule based on schematic models presented in reviews [4, 8, 9]. To the AGP core polypeptide, polysaccharide units (type II arabinogalactans) and arabinooligosaccharides are bound through oxygen of hydroxyproline. According to the hypothesis of Kieliszewski [29, 46, 47], isolated hydroxyproline residues are sites for binding polysaccharide units, whereas sequentially arranged hydroxyproline residues are arabinosylated. The C-terminal end of the AGP polypeptide is modified by binding of a GPI anchor consisting of ethanolamine, an oligosaccharide linker, and a lipid submerged into the plasma membrane. The sites specific for cutting by phospholipase C (PLC) or phospholipase D (PLD) are indicated.

clones have in no case predicted the presence of the hydrophobic C-end in the protein moiety; therefore, nonclassical AGPs are unlikely to have a GPI anchor [10].

AGPs are *O*-glycosylated proteins: polysaccharide and oligosaccharide units in them are bound to the protein core through the oxygen of hydroxyproline (Fig. 1). In hydroxyproline-free nonclassical AGPs, e.g., DcAGP1 of carrot, serine or threonine residues seem to be glycosylated [28]. Carbohydrate side chains of AGPs can contain more than 95-120 monosaccharide residues [42], and side chains of AGPs from coffee beans are supposed to consist of 1000 monosaccharide residues [43].

Carbohydrate chains of AGPs are type II arabinogalactans [18]. Usually, to hydroxyproline of the protein core β-1,3-D-galactan chains are connected, and to their galactose residues in the sixth position short β-1,6-galactan chains can be attached, which are modified with L-arabinose or less often with L-rhamnose, D-mannose, D-xylose, D-glucose, L-fucose, D-glucosamine, and D-glucuronic and D-galacturonic acids [10] (Fig. 1). As distinguished from the type II arabinogalactans found in plants and microorganisms, type I arabinogalactans are characterized by β-1,4-D-galactopyranosyl bonds [2]. Short oligosaccharide chains consisting of arabinose residues can also decorate some AGPs, although this modification is more specific for extensins [29, 30]. Polysaccharide chains of AGPs are connected to hydroxyproline and, possibly, serine and threonine residues of the protein core, whereas oligosaccharide fragments consisting of arabinose are connected only to hydroxyproline [44]. Using synthetic genes encoding different arrangements of hydroxyproline residues in the protein moiety of AGP, it has been shown that sequential hydroxyproline residues (in such amino acid sequences as $(Ser\text{-}Hyp\text{-}Hyp\text{-}Hyp\text{-}Hyp)_n$ or $(Ser\text{-}Hyp\text{-}Hyp)_n$) are decorated only with arabinosyl oligosaccharides (arabinosides). However, separate hydroxyproline residues in the chain, e.g., in the sequence $(Ser\text{-}Hyp\text{-}Ser\text{-}Hyp)_n$, are sites for binding polysaccharide units [45]. Thus, isolated hydroxyproline residues in AGPs are galactosylated and the adjacent sequentially arranged hydroxyproline residues are arabinosylated. The establishment of this regularity, which determines the type of *O*-glycosylation in different hydroxyproline-containing proteins, formed the basis of the Hyp contiguity hypothesis [29, 46, 47]. Additional data have been recently obtained in favor of this hypothesis, with DNA-constructions of the "natural" gene *LeAGP-1* encoding one of the prevalent AGPs in tomato suspension [48].

Although the presence of *O*-glycans is specific for all AGPs, there are also AGPs that possess both *O*- and *N*-connected glycomodification. These AGPs include a transmitting tissue specific protein (TTS) isolated from pistils of *Nicotiana tabacum* [49] and also fasciclin-like AGPs (FLA) [50].

POST-TRANSLATIONAL MODIFICATION AND SUBSEQUENT PROCESSING OF AGPs

Data on post-translational modification of AGPs are extremely important because just this modification seems to be crucial for functioning of AGPs. The post-translational modification of AGPs includes hydroxylation of proline residues and glycosylation. An important modification of classical AGPs is separation of the C-hydrophobic end and its replacement by the GPI anchor. In some cases, nonclassical AGPs undergo proteolytic processing [31].

The processing of classical AGPs is presented in Fig. 2 [10]. The cDNA of classical AGPs encodes proteins with a characteristic three-domain structure: the N-terminal secretion signal, central domain enriched with Pro/Hyp, Ala, Ser, and Thr, and the C-terminal hydrophobic domain. The N-terminal secretion signal is cut off on termination of the protein synthesis, and the C-terminal transmembrane domain undergoes processing on attachment of the GPI anchor. The GPI modification

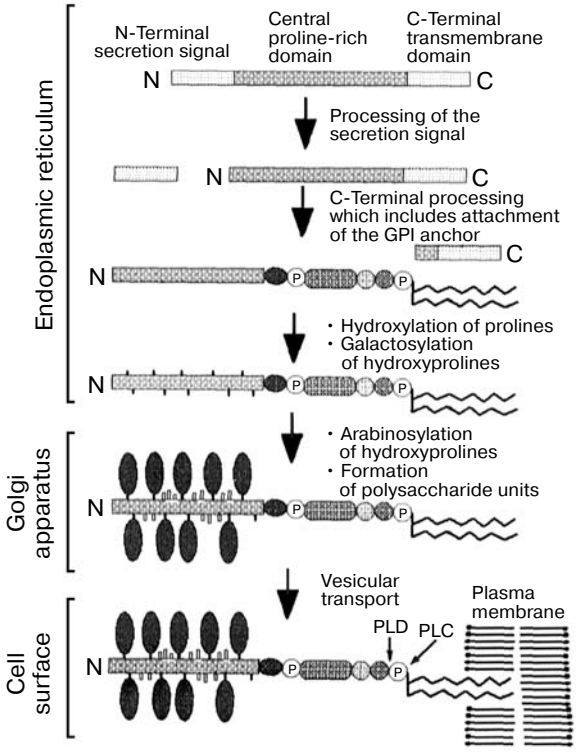

Fig. 2. Sequential processing of AGPs, which includes removal of the N-terminal secretion signal, attachment of the GPI anchor, hydroxylation of prolines, and *O*-glycosylation. The attachment of the GPI anchor promotes incorporation of AGPs into the membrane of vesicles, which later are transported towards the surface and incorporated into the plasma membrane. Separation of the GPI anchor with involvement of phospholipase C or phospholipase D promotes the separation of the AGP molecule from the plasma membrane and its secretion into the cell wall.

in plants (the separation of the C-terminal domain and attachment of the GPI anchor) seems to occur in the endoplasmic reticulum (ER), similarly to that observed in animals, yeast, and protozoa [9]. The attachment of the GPI anchor fastens the proteins on the membrane side turned to the ER cavity and then on the Golgi apparatus membrane. The movement of secretory vesicles to the plasmalemma and the following fusion with it results in location of the plasmalemma-connected proteins on its external side turned into the periplasmic space. AGPs anchored on the plasmalemma can be subjected to the further processing, possibly with involvement of phospholipase C or D that leads to secretion of AGPs into the cell wall and then into the extracellular space.

Proline residues are hydroxylated and β-galactosylated in the ER, whereas polysaccharide units are arabinosylated and modified in the Golgi apparatus [37] (Fig. 2). In classical AGPs, 80-90% of proline residues are hydroxylated [37]. The degree of proline hydroxylation depends on the neighboring amino acids. For example, in extensins and other hydroxyproline-containing proteins proline residues are always hydroxylated in the sequences Ala-Pro, Pro-Ala, Pro-Pro, Pro-Val, and Ser-Pro, whereas the sequences Lys-Pro, Tyr-Pro, and Phe-Pro are not hydroxylated [46, 51]. Synthetic gene constructions were of help in detection of hydroxylation of only 70-80% of proline residues in the sequences (Thr-Pro-Thr-Pro)$_n$ and (Val-Pro-Val-Pro)$_n$ [44]. And only 50-60% of them are hydroxylated via attachment of arabinans. However, in the sequence (Ala-Pro-Ala-Pro)$_n$ all proline residues are hydroxylated and glycosylated with arabinans. These data suggest that the sequence of flanking amino acids influences not only the hydroxylation degree of proline residues but also the glycosylation type of hydroxyproline.

High heterogeneity of polysaccharide chains of AGPs is rather intriguing, and its significance is insufficiently understood. Because most AGPs have complicated structure, numerous glycosyltransferases are suggested to be involved in glycosylation. And then the fully assembled polysaccharide moiety of AGPs seems to undergo processing with involvement of glycosidases [6]. It is clear that in addition to exoglycosidases degrading the side chains, other enzymes are involved in the processing of AGPs, and they all provide a tissue-specific regulatory mechanism; however, these enzymes are not yet tested directly with AGP substrates and their intracellular location has not been determined.

METABOLISM OF AGPs

Using labeled hydroxyproline, the half-life of AGP in suspension culture of *Phaseolus vulgaris* was shown to be 10-15 min [52]. The half-life of AGP was longer in segments of *P. vulgaris* hypocotyls than in the cells of the suspension culture, as much as 120 min. The short-time presence of AGP molecules in the tissues seems to be due to their active degradation. Internalization of AGPs into multivesicular bodies and vacuole has been described [53-56]. Generation of multivesicular bodies in animal cells is believed to be associated with degradation [57]. Using various monoclonal antibodies to AGPs (LM2, JIM4, JIM13, MAC207), we found AGP location in vacuoles of the subsurface cells of pro-embryonic cell complexes of the embryogenic callus of Tatar buckwheat [58], and this seems to be associated with specific secretory processes in this culture. We consider vacuoles of the subsurface cells to be not lytic but storage. It seems that on excess production of the secreted protein, only part of it is released from the cell, and the remaining part can be internalized into the vacuole. The secretion onto the surface increases with growth of the storing vacuole and reaches the maximum on destruction of the AGP-containing compartment, i.e., the storing vacuole. However, secretion of AGPs into the vacuole is doubtful, because AGPs, similarly to most of soluble cytoplasmic proteoglycans, are mainly *O*-glycosylated. Nevertheless, despite the *O*-glycan prevalence in AGPs, potential sites for *N*-glycosylation specific for vacuolar proteoglycans can be present in some AGPs.

INTERACTION OF AGPs WITH COMPONENTS OF CELL WALLS, PLASMALEMMA, AND CYTOSKELETON

Intracellular adhesion in plant tissues is known to occur via middle lamellae, but the mechanism of this adhesion is still unknown [59]. Middle lamellae mainly consist of pectins, but there are data on AGP location in them [60]. The molecular nature of adhesion contacts between the plasmalemma and cell wall is also insufficiently studied. The cell walls of plants are mainly built of polysaccharides, whereas extracellular matrix of animal cells mainly contain proteins, the plant cells are often subjected to hyperosmotic stress which requires the plasmalemma to be rapidly separated from the cell wall, the inability of plants for movement and, as a result, the development of specific adaptive mechanisms to various stresses—these seem to be the main reasons for different molecular composition and organization of adhesion sites in plants and animals. Linkers connecting the plasmalemma and cell wall are now thought to include the following molecules: wall-associated kinase (WAK), AGPs, pectins, cellulose synthase [61], and also formins, plant-specific class VIII myosin, phospholipase D, and callose synthase [62].

AGPs are mainly located on the outer side of the plasma membrane [10]. A high density of the plasmalemma-bound AGPs seems to be favorable for production of periplasmic glycocalix, a kind of network called plas-

malemma reticulum by Gens et al. [63]. But information about the mode of interaction of AGP with the plasmalemma is extremely scarce. Animal proteoglycans can interact with the plasmalemma by three pathways: a complete submergence of the protein core into the lipid matrix; via the GPI anchor submerged in the plasmalemma by its lower part; and noncovalent binding with other membrane components [64]. So far only AGP interaction with the plasmalemma via the GPI anchor has been shown.

AGPs are preferentially located on the outer side of the plasmalemma; therefore, it was suggested that AGPs could interact with cell wall components and, thus, provide for the plasmalemma attachment to the cell wall. AGPs are suggested to interact with cell wall polysaccharides, mainly with pectins [18, 23], because the narrow zone of the cell wall adjacent to the plasmalemma is often enriched with non-esterified homogalacturonan [65], rhamnogalacturonan-II [66, 67], β-1,4- and β-1,6-bound galactan [68, 69]. The pectin fraction can contain AGPs [43, 70, 71], and extraction conditions optimal for isolation of AGPs from cell walls of rose suspension were also favorable for extraction of pectin polymers [72, 73]. The interaction of pectins and AGPs was more directly shown by Baldwin et al. [23] who revealed by dot-blotting the calcium-dependent binding of AGPs of carrot with the pectin fraction from the cell walls of carrot suspension. However, the structure of AGPs promotes the binding not only with cell wall polysaccharides but also with proteins. Asparagine-rich domains can promote the transverse binding of proteins in the cell wall [9]. Such domains are present in nonclassical AGPs such as AGPNa2 and AGPPc2 [31]. Intermolecular bridges also seem to be produced between tyrosine residues [74, 75] or tyrosine and lysine residues [76], which are present in some AGPs [10].

The intermolecular attachment of AGPs can also be associated with accumulation of hydrogen peroxide generated from superoxide, in particular, during oxidative stress. Addition of H_2O_2 and horseradish peroxidase to the partially purified fraction of plasmalemma-bound AGPs with molecular weight of 82 and 98 kD resulted in appearance of an additional band of a 170-kD protein in the electrophoregram [77]. The 170-kD protein was dominating in the AGPs isolated from wounded leaves of sugar beet. But the mechanism of the H_2O_2-dependent transverse attachment of AGPs is still unknown. Adhesion properties of AGPs can also be provided by the multigenic family FLA, similarly to animals, insects, algae, and bacteria, in which β-Ig-H3/fasciclin-like domains are involved in cell adhesion [78].

The involvement of AGPs in adhesion between the cell wall and plasmalemma is also evidenced by data obtained with gene engineering constructions of the *LeAGP-1* gene encoding the extracellular AGP of tomato suspension used for transformation of *Nicotiana tabacum* suspension [48]. During the cell plasmolysis, AGP was found to be located in Hectian strands connecting the cell wall and plasmalemma. These sites are likely to be a result of adhesion of AGP regions of the plasmalemma and cell wall (or pectins), e.g., on formation of Ca-bridges between glucuronic acid residues, AGPs, and cell wall pectins. These regions are supposed to be specific adhesion microdomains involved in the cell wall assemblage and metabolism [79] or anchor sites, which facilitate mitosis and cytokinesis [80].

A possible involvement of AGPs in adhesion of some endomembranes was shown recently [81]. In sieve-like elements of maize roots, AGPs were found in dense contacts of sieve-like element reticulum (SER) and plasma membrane, plastids, and mitochondria. AGPs were also found in the plasmodesmata connecting the sieve-like elements and satellite cells. Based on these findings, it was suggested that AGPs should be responsible for adhesion of the plasma membrane of the plasmodesma channel and cell wall. Unfortunately, so far no data have been obtained on other objects and tissues to support this hypothesis.

Can AGPs interact, although indirectly, with the cytoskeleton elements? In this connection, work [82] should be mentioned. Different exposures (cold, colchicine, cytochalasin B) inhibiting cytoskeleton polymerization after 7-8 h of culture destroyed the specific fibrillar network on the surface of chicory proembryo, with a concurrent increase in the amount of AGPs in the culture medium. The released AGPs were supposed to be constituents of the fibrillar network of the chicory proembryo and interact with the cytoskeleton molecules via membrane ligands or cytoskeleton-associated proteins. Thus, the authors consider the fibrillar network molecules to be a component of the continuum consisting of the extracellular matrix, plasmalemma, and cytoskeleton. Depolymerization of the cytoskeleton molecules (both microtubules and actin filaments) disturbs this connection and destroys the surface fibrillar network. We proposed a similar concept earlier [83] based on studies of secretion of proteins and polysaccharides under the influence of colchicine in buckwheat calluses with varied morphogenicity. Destruction of the fibrillar network covering the proembryo of *Drosera rotundifolia* L. treated with trifluralin and colchicine was also recorded in [84]. Nevertheless, another hypothesis was proposed to explain the effect of the poisons used on destruction of the fibrillar network [84]: the destroyed microtubular cytoskeleton was unavailable for involvement in the polar transport of the fibrillar network component onto the cell surface. We think that the observed destruction of the fibrillar network can be caused, on one hand, by disorders in secretory processes associated with destruction of the cytoskeleton and, on the other hand, by activation of enzymes hydrolyzing AGPs and other components of the fibrillar network on the cell surface. The existence of the AGP-

containing fibrillar network seems to be determined by the balance between secretion of the polymers onto the surface and the rate of their degradation, and this balance can be changed by some exposures.

INVOLVEMENT OF AGPs IN GROWTH REGULATION

The growth of a plant is associated with division and expansion of the cells. Some specialized cells, such as pollen tubes and root hairs, grow only by expansion. Some AGP-like molecules have been directly shown to play an important role in the growth of pollen tubes. Thus, an increase in the TTS protein content in the culture medium from 0 to 2 μg/ml accelerated the growth of *Nicotiana tabacum* pollen tubes from 50 to 150 μm/h [24]. TTS proteins adhere to pollen tubes during their growth inside the pistil tissues or *in vitro* in medium containing TTS proteins [85]. The interaction of TTS proteins with the pollen tubes results in their deglycosylation [85]. Note that the deglycosylating activity was closely associated with the pollen tube wall or plasma membrane, and deglycosylated TTS proteins appear in the pistil tissues after the pollination [86]. However, even in unpollinated pistils there was a kind of gradient of TTS protein glycosylation: the glycosylation degree was higher near the ovule and lower near the stigma [85]. The authors suggest that the glycosylation gradient can point the growth of pollen tubes towards the seed bud. In transgenic tobacco plants with a considerably decreased content of TTS proteins than in the control, the growth of pollen tubes was weaker and the fertility of the female gametophyte was lower [49].

AGPs seem to be involved in the control of orientation of the cell expansion. AGP epitopes were immunocytochemically detected on the surface of root hairs of maize seedlings during their development [87]. AGP epitopes were especially abundant on bulges, or sites of root hair initiation, and also on tips of the growing root hairs. Secretion of AGPs into the cell walls seemed to be involved in modification and expansion of cell walls of these huge cells. Possibly, functions of AGPs in the growing root hairs are also determined by their high water-holding ability that is directly associated with the physiological function of root hairs. Studies on the *reb-1* mutation in *Arabidopsis* also confirmed the involvement of AGPs in the growth of root hairs [88]. This mutation was manifested by disorders in the initial stage of development of many trichoblasts (at the stage of bulge formation) and, as a result, lack of growth of most root hairs. Anomalous trichoblasts contained no specific AGP epitopes.

It should be noted that involvement of AGPs in the regulation of plant growth has been mainly shown in experiments with Yariv reagents: the treatment with these reagents inhibited the growth of cell cultures [71], pollen tubes [89], seedlings [90], and development of somatic embryos [91]. The growth inhibition caused by Yariv reagents could be associated with inhibition of both the cell expansion [90] and cell proliferation [91]. There are some reasons for thinking the AGP involvement in the cell expansion. First, releasing of AGPs from the cell between cellulose fibrils can lead to separation of microfibrils and thus decrease the rigidity of the cell walls [92]. Another opinion was stated in [93]. In cell walls of di- and monocotyledonous plants, ferulic acid residues can be bound to polysaccharides by ether bonds [74, 94]. Via oxidation, these bound ferulic acids can produce diferulic bridges, which attach polysaccharides together and thus limit the expansion of cell walls and impart them rigidity. Ferulic acid conjugates with polysaccharides have a structure similar to that of Yariv reagent phenyl-β-glycosides except for the ether bond between the ferulic acid residues and polysaccharides. The secreted AGPs are likely to bind with ferulic acid residues and thus prevent the production of diferulic bridges and weaken the cell wall rigidity. In both cases, the binding of AGPs with Yariv reagents will prevent the cell wall expansion.

Addition of GlYR to rose suspension culture inhibited the culture growth, and the effect depended on concentration, with complete growth inhibition at 50 μM GlYR [71]. Because the cell size was the same in the control and experiment, the inhibition of the culture growth was suggested to be associated with suppression of cell division. The binding of 95% of GlYR with the cell wall was recorded, whereas the remaining GlYR could be connected with the plasmalemma, because molecules of Yariv reagents are known [95] to produce large aggregations unable to penetrate through the plasmalemma. Measurement of the lateral diffusion of the plasmalemma components showed that GlYR did not change the lipid diffusion but increased the variability of diffusion coefficients and decreased the mobile fraction of membrane proteins and glycoconjugates [71] that suggested changes in the transverse binding of membrane components. Such bonds are known to be involved in information transmission across the plasma membrane; therefore, it is very likely that perturbation of the plasmalemma AGPs caused by GlYR can disturb the regulation of many vitally important cell processes including the regulation of the cell cycle.

Destruction of specific interactions between the plasmalemma and extracellular matrix activates in animal cells the program of internal suicide, i.e., programmed cell death (PCD) [96]. The plasmalemma separation from the cell wall is a sign of PCD in cultured plant cells [97]. AGPs seem to play a certain role in triggering PCD. Studies on the AGP distribution in maize seedlings revealed location of the JIM13 epitope on the plasma membrane and multivesicular bodies of the future cells of sclerenchyma and also in thickenings of the secondary cell walls of the cells which had to form the xylem tracheids [56]. Based on these findings, certain AGPs were

supposed to be markers of the cells entering the pathway to PCD. In some plant cell cultures sensitive to Yariv reagent the treatment with high concentrations of this reagent resulted in a rapid death of the cells within 2-3 days [71, 98, 99]. The death of the suspension culture of *Arabidopsis* under the influence of GalYR was not caused by necrosis but displayed signs specific for PCD [99]. It was suggested that Yariv reagent disturbed the interaction between the plasmalemma and cell wall and thus activated the signaling cascade, which caused escape of the cell from the cell cycle and switched on PCD.

INVOLVEMENT OF AGPs IN DIFFERENTIATION OF PLANT CELLS

Changes in AGP epitopes during differentiation and morphogenesis are described in many works, but the functional meaning of these changes is still unclear. According to a hypothesis supported by many researchers, AGPs are involved in plant morphogenesis as markers of the cell identity or even as regulatory molecules. This hypothesis was initially based on the finding that expression and/or modification of some AGPs occur in different tissues or cells and at different time in correlation with definite stages in the plant development. Because carbohydrate side chains of AGPs can be subjected to a partial tissue-specific degradation and their modification can be regulated, AGPs conform to the role of cell identity markers. It was shown with monoclonal antibodies (MAB) MAC207 [100] and JIM8 [101] specific to AGPs that the expression of certain AGP epitopes is strictly controlled in correlation with the flower differentiation: the MAC207 epitope was found only in vegetative but not in reproductive cells. The spatial regulation of expression of AGP epitopes was also observed in the pistil tissues of lily [102]: MAB LM2 bound to the surface of all cells of the style, whereas MAB JIM13 bound only to epidermal cells of the conducting tract and their secretion.

MABs were useful in demonstrating the regulated expression of AGPs also during the development of root. The JIM4 epitope was located in the cells of the carrot root tip, whereas the MAC207 epitope was found in all cells of the root [103]. Later MAB JIM13 was shown to bind with the surface of epidermal cells of the root apex and future cells of the xylem [104]. The type of the root apex labeling with MAB JIM15 was complementary to that observed with MAB JIM13: with MAB JIM15 the cells were labeled which remained unlabeled with MAB JIM13. Correlation between the appearance of the JIM13 epitope and the subsequent development of xylem vessels was also observed in the roots of *Arabidopsis thaliana* [105] and in cultured cells of *Zinnia elegans* [106]. Notwithstanding changes in the presence of different AGP epitopes in certain tissues during plant development, there was no correlation between their appearance and development of definite tissue (cells) in different plant species. Thus, the JIM13 epitope in the carrot root was found in the epidermis and future cells of the xylem [104], whereas in the maize coleoptiles it was located only in the future cells of sclerenchyma and tracheids of vascular bundles [56]. On the other hand, the JIM14 epitope was found in all cells of the carrot root and only in the sclerenchyma cells of the maize coleoptiles. The expression of individual AGPs belonging to different subclasses of classical AGPs (classical, Lys-rich, AG-peptides, FLA) varies differently on beginning of the root tissue dedifferentiation (callus formation) and redifferentiation (bud formation from the callus or root) [34]. Note that the bud formation from the root tissue and the callus obtained from the root was accompanied by different expression of one-two AGPs of different subclasses. Based on these examples, we conclude that AGPs are not tissue-specific markers but more likely determine distinctions between the cells from different regions, possibly by encoding position-related information.

Data on interaction between AGPs and plant hormones [36, 107] would considerably contribute to elucidation of the putative involvement of AGPs in differentiation. The expression of *AGP2* of *Arabidopsis* is, at least partially, controlled by ethylene [107], and the synthesis of mRNA on three genes of *Arabidopsis* encoding FLA rapidly decreases in response to abscisic acid (ABA) [36].

Unfortunately, the fundamental question whether the appearance of certain AGP epitopes on the surface is a result of differentiations or, in contrast, the generation of new epitopes induces differentiation is not settled definitely. However, an extracellular protein factor xylogen, which diffuses between adjacent cells and is necessary for differentiation of the mesophyll cells of *Zinnia* into tracheids, was recently shown to be a nonclassical AGP [108, 109]. The appearance of AGP epitopes preceded development of the mesophyll cells of *Zinnia* into tracheid elements [106], the effect of xylogen was strictly time-limited (36-60 h of culture), and was observed 12-36 h before the appearance of thickenings in the secondary cell wall [108]. Therefore, we conclude that the differentiation followed the appearance of certain AGP epitopes and changes in the cell surface.

ROLE OF AGPs IN SOMATIC AND ZYGOTIC EMBRYOGENESIS

There are now rather convincing arguments in favor of the involvement of AGP in both somatic and zygotic embryogenesis. In plant cell cultures, somatic embryos develop from specialized structures, proembryogenic masses (PEMs) or, in other terms, proembryonic cell complexes (PECCs) [110]. The somatic embryogenesis is *in vitro* induced by decrease or removal of auxin 2,4-D

from the culture medium. In the carrot cell culture in the presence of 2,4-D, only individual surface cells of PEM expressed the JIM4 epitope [111]. A decrease in the 2,4-D content in the culture medium resulted in appearance of this AGP epitope on the surface of nearly all PEM cells. On passage of the somatic carrot embryo to the heart-shape stage, the JIM4 epitope appeared on the boundary between the future root and stalk and in the provascular tissue cells on the torpedo-like stage. With the anti-JIM8 MAB it was shown that the epitope JIM8 appeared in the cell walls of carrot cells within a very short period of culture, concurrently with the induction of the embryogenic program [55]. Initiation of the culture was not accompanied by expression of the JIM8 epitope, and this epitope rapidly disappeared during the PEM formation. Based on these findings, appearance of the JIM8 epitope in the walls of definite cells was suggested to "mark" their turning to somatic embryogenesis. But later the development of the cells labeled with JIM8 was traced [12], and only some of these cells did develop into somatic embryos. The hypothesis [55] was revised later [13]. It was concluded that only small, spherical, vacuolated cells with the JIM8 antigen in the cell wall were embryogenic. These cells denoted as the type B were later polarized and divided asymmetrically. One of the daughter cells (the F type) was small, spherical, and vacuolated, with the JIM8 antigen on the surface. The other cell (the C type) was small, spherical, but with a dense cytoplasm, and with no JIM8 antigen on the surface. Only the C type cells could develop into the somatic embryo, but soluble signaling molecules entering from the B type cells or other cells of the JIM8(+) population were required.

Somatic embryogenesis in suspension culture of carrot changed on addition of exogenous AGPs [11]. The AGPs isolated from an old nonmorphogenic culture inhibited the morphogenic potential of young cultures capable of somatic embryogenesis. In contrast, the total fraction of AGPs from carrot seeds increased the number of embryogenic cells or even recovered the embryogenic potential of an old culture that had lost regenerability. But when MAB were prepared to certain fractions of the purified mixture of AGPs [112], the AGPs from carrot seeds bound by MAB ZUM15 were found to decrease the embryogenic potential of the carrot cell culture, whereas the AGPs bound by MAB ZUM18 increased it. Thus, the stimulating or inhibiting effect of an AGP fraction on the cells was determined by the ratio of concrete AGP epitopes constituting the total fraction of AGPs. The effect of the ZUM18 fraction of AGPs varied depending on the concentration used, and the effect of the added factor was manifested even at ~2 nM. Moreover, the activity of this fraction did not depend on the species: the ZUM18 fraction of AGPs from tomato seeds increased the embryogenic potential of carrot suspensions and the ZUM18 fraction of AGPs from carrot seeds stimulated somatic embryogenesis in the cell strains of cyclamen [112, 113]. However, results of similar investigations of Toonen et al. [12] only partially coincided with data of Kreuger and van Holst [11]: the total fraction of AGPs from carrot seeds stimulated indeed the somatic embryogenesis, but the fraction ZUM18 of AGPs failed to increase it.

The effects of exogenous AGPs were studied on development of the A and B groups of Norway spruce somatic embryos [114]. The group A embryos had a large embryogenic region and a small suspensor, whereas the group B embryos had a small embryogenic area and no suspensor. On addition of ABA only the group A embryos "ripened" and developed further to plants. But addition of an aqueous extract from spruce seeds to somatic embryos of the B group converted them into embryos morphologically like those of the group A. Note that the total fraction of AGPs prepared from the seed extract only partially promoted this conversion, whereas the conditioned medium from culture A stimulated the further development of the group B embryos. Thus, both AGPs and other, yet unidentified, compounds were required for development of Norway spruce somatic embryos.

Scanning and transmission electron microscopy revealed [82, 115-118] that the proembryos formed *in vitro* from explant tissues or PECC of embryogenic cultures were covered with a fibrillar network which was termed extracellular matrix surface network (EMSN) [119]. The fibrillar network of proembryogenic units of maize contained AGPs detectable with the JIM4 monoclonal antibodies [119]. AGPs were also identified with the MAB LM2, JIM13, and JIM16 in the fibrillar material on the surface of chicory proembryo [120]. Using immunocytochemical and biochemical approaches, we have established that AGPs are constituents of the surface fibrillar network of PECC of morphogenic calluses of Tatar buckwheat [58]. The structure of the fibrillar network changed depending on the size and stage of PECC development, and the maximum secretion onto the surface preceded a new cycle of PECC production in the callus [118]. No fibrillar network was found on the surface of nonmorphogenic calluses. The fibrillar network seems to have the following functions. First, AGPs as molecules with high water-retaining capacity [7] can regulate the water balance of the PECC surface and prevent the cell drying up in the absence of protoderm. Second, the keeping of a small size is known to be a prerequisite for maintaining the meristematic state of the cell. Because AGPs can be transversally attached via oxidation [77], the fibrillar network on the PECC surface is likely to prevent the expansion of PECC cells and hold the integrated state of this structure. Third, components of the fibrillar network are likely to be a pool of molecules regulating somatic embryogenesis, because the fibrillar network is a dynamic structure that is dissembled previously to the protoderm generation in a globular embryoid [117] or the new cycle of PECC production [118]. Finally, the fibrillar network molecules can support the growth of new PECCs.

In contrast to the nonembryogenic carrot culture, cells of the embryogenic culture produced AGPs, and *N*-acetylglucosamine was about 0.2% of the amino sugars in the AGP carbohydrate moiety and seemed to be also present in the side polysaccharides of AGPs [14]. Treatment of AGPs with chitinase increased their biological activity as compared to untreated AGPs. Addition into the culture medium of the class IV endochitinase allowed the deficient embryos of the mutant strain *ts11* of the *Daucus carota* suspension culture to develop into full plants [121, 122]. The class IV chitinases hydrolyze β-1,4-bonds between the neighboring *N*-acetylglucosamine residues. However, AGPs contain carbohydrate chains consisting of no less than five *N*-acetylglucosamine residues, because only chitooligosaccharides with more than four *N*-acetylglucosamine residues are physiologically active [123-125]. AGPs from the unripe endosperm of carrot seeds, similarly to AGPs isolated from the culture medium of the embryogenic carrot suspension, at the concentration 15 μg/ml nearly 40-fold increased the production of somatic embryos in the nonmutant strain of carrot [14]. Addition of EP3 class IV endochitinase also increased the frequency of somatic embryogenesis, but only eightfold. Paradoxically, the combined addition of AGPs and EP3 chitinase decreased the number of somatic embryos more strongly than the addition of endochitinase alone! However, AGPs pretreated with endochitinase and reprecipitated with the Yariv reagent before addition into the culture medium increased 50% the number of somatic embryos compared to the first variant (i.e., nearly 70-fold compared to the control). The authors' explanation of this stimulating effect was as follows: an inhibitor which seemed to be produced on the treatment of AGPs with endochitinase was not precipitated by the Yariv reagent together with the AGPs and, consequently, failed to affect the somatic embryogenesis. Thus, it was reliably proved that the somatic embryogenesis in suspension cultures of carrot was controlled by AGPs containing *N*-acetylglucosamine and glucosamine. The AGP processing by endochitinase occurs not only *in vitro* but also *in vivo* during zygotic embryogenesis [14]. Note that both the presence of a site for cutting with endochitinase and the ability of stimulating somatic embryogenesis were specific only for AGPs isolated from the seeds with endosperm at the cellular stage of the development. Some authors [126] think that somatic and zygotic embryogeneses are stimulated by not whole AGP molecules but by endochitinase-produced chitooligosaccharides, which have been shown to be involved in morphogenesis of both animals [127] and plants [125, 128]. However, based on data on modification of the protein and carbohydrate moieties of AGPs isolated from the endosperm of carrot seeds, van Hengel et al. [14] concluded that the regulation of somatic embryogenesis needed the whole AGP molecule rather than its small chitinase-modified chitosan fragment. Partial hydrolysis of the protein moiety of AGP did not affect its regulatory functions. In contrast, incubation of AGPs with such hydrolases as endogalactosidase and endo- and exoarabine furanosidase completely inactivated the embryogenic activity of AGPs. Nevertheless, these findings did not fully exclude the "chitooligosaccharide" effect, because chitooligosaccharides might be coprecipitated with the isolated AGPs.

SUPPOSED MECHANISMS OF THE AGP SIGNALING EFFECT

The role of the GPI anchor in functioning of AGPs remains unclear. In animal cells more than 150 proteins are now known which bind to the outer side of the plasmalemma via a highly conservative core with structure like that of the GPI anchor of the classical AGPs. These proteins function as receptors, enzymes, antigens, and/or matrix proteins [129]. So far only a few proteins are identified among plant proteins with the GPI anchor. However, based on the computer-aided analysis of the *Arabidopsis* genome, it was suggested that the number of such proteins should be more than 200, and AGPs constitute about 16% of these [130]. Some animal proteins with the GPI anchor are involved in cascades of signal transduction [131-133]. Because the signal is transduced via interaction with other membrane-bound proteins, the plant AGPs would interact with molecules having both the intra- and extracellular domain. The number of such integral proteins of the plasmalemma identified in plants is growing every year, e.g., the cell wall-associated kinase (WAK) and somatic embryogenesis receptor kinase (SERK) [134]. Nevertheless, at present the interaction of AGP with one of these proteins, WAK, is shown only indirectly [63].

Another mechanism responsible for the signaling function of AGPs with the GPI anchor is associated with AGP separation from the plasmalemma with involvement of phospholipase C or D. In this case, the remaining part of the GPI anchor can be recycled with production of secondary messengers, such as phosphatidylinositol, phosphoglycan, and ceramide [135]. To have the regulatory functions, the AGP molecule separated from the plasmalemma has at least to be able to diffuse across the barrier of cell walls. Because the addition of nanomolar amounts of AGPs to suspension cultures changed the direction of their development [11], it was suggested that AGPs could pass across the cell walls and change the direction of cell development. However, it is not quite clear how such large molecules can penetrate the cell walls. The molecular weights of globular proteins that can move from the apoplast across the primary cell walls to the plasmalemma vary from 17 [136] to 60 kD [137]. For penetration of a 17-kD protein across the cell wall, the pores must be ~5 nm in diameter. However, cell walls can

have channels with diameter up to 50 nm, as it has been shown by the freeze–fracture technique for the cell walls of papillas of the *Gladiolus gandavensis* stigma [138]. Moreover, the ability to pass through the pores considerably depends on the spatial structure of the molecule, and penetration of AGPs across the cell wall is associated with changes in their conformation [30]. Consequently, the migration across the cell walls even of such large molecules as AGPs can actually occur. It seems also that less than whole AGP molecules can possess regulatory properties, i.e., their fragments. Free tetrasaccharides with the structure of β-L-Ara-(1→3)-α-L-Ara-(1→3)-β-D-Gal-(1→6)-β-D-Gal, which is similar to the structure of the terminal oligosaccharides of AGPs of *Acacia senegal* and *Acacia xanthoploea*, are accumulated in the developing anthers of rice [139, 140]. Thus, AGPs can act indirectly as substrates for production of chitooligosaccharides and oligosaccharins. In other words, a small oligosaccharin can perform a signaling function and the remaining part of AGP will be recycled, and this is consistent with data on the rapid metabolism of AGPs. It has already been mentioned that nonclassical AGPs, especially poorly glycosylated ones, can undergo proteolytic processing associated with separation of protein domains. Theoretically, these protein molecules can also perform signaling functions. Consequently, under certain environmental conditions and the presence of such enzymes as endochitinases, endoglycosidases, phospholipases, etc., AGPs can produce a variety of molecules that control cell differentiation and proliferation. Whole AGP molecules (possibly, endochitinase-modified) as well as oligosaccharides produced from them are believed to provide different signals [141]. Unfortunately, molecular mechanisms of the interaction of AGP with the supposed receptors are not established.

COMPARISON OF PLANT AGPs AND ANIMAL PROTEOGLYCANS

Based on the principle of evolutionary economy, molecular regulatory mechanisms of the main processes were kept, if only generally, in different groups of multicellular organisms. Therefore, data on the structure, location, and functions of animal proteoglycans can be of value for studies on possible functions of AGPs and also of molecular mechanisms involved in their realization. Heparan sulfates (HS), which are predominant proteoglycans of the plasma membranes in animals, seem to be the most similar to AGPs. The core polypeptide of HS varies in size from 20 to 40 kD [142]. The HS core polypeptide is glycosylated by multiple straight ~30 kD glucosaminoglycan chains, and the total molecular weight of HS is, on average, 300 kD [81]. Glucosaminoglycan chains consist of repeated disaccharides glucosamine and uronic acid, and both residues can be modified by sulfation or otherwise, with production of numerous structural variants of HS [142, 143]. Glucosaminoglycan chains are connected with the core polypeptide through a specific tetrasaccharide, which has xylose and serine residues bound via *O*-glycosyl bond. Some HS (e.g., glypicans) have GPI anchors [145].

Although HS and AGPs are alike in structure only generally, their similar location and expression regulated during ontogenesis suggest similar functions. Similarly to AGPs, HSs are mainly located on the membranes and can be released into the extracellular space. Expression of both HSs and AGPs is regulated during ontogenesis [146]. HSs are involved in the regulation of development and cell differentiation. Thus, mutation in *Drosophila*'s gene *dally* encoding a protein of the glypican family [147] results in multiple disorders in the development of the nervous system. The *dally* gene is also necessary for the morphogenesis of wings, antennas, and genitals. The *dally* gene is a haplolethal locus [148, 149], and a 50% decrease in its expression as compared to the wild genotype is lethal. The human Simpson–Golabi–Behmel syndrome characterized by pre- and postnatal overgrowth of tissues and organs and an increased risk of certain tumors is caused by deletion or mutation of the gene encoding glypican-3 [150]. A mutation was recently found in mice associated with the absence of the gene encoding glypican-3 [151]. The phenotypic manifestation of this mutation has much in common with the Simpson–Golabi–Behmel syndrome. Both mutations seem to result in disorders in cell control caused by HS-induced modulations of activities of growth factors [150]. The regulation of growth factors by HSs was also shown in [132]. Interactions between AGPs and plant hormones were also shown [107, 152, 153].

Modifications in the regions consisting of glucosamine residues seem to be crucial for the activities of HSs [149]. Modifications and enzymatic degradation of the carbohydrate moiety also affect the activity of AGPs [154] and disturb their ability of producing oligomers [49]. HSs can trigger apoptosis [155]. In this review we have already considered a possible involvement of AGPs in development of programmed cell death [56, 99]. HSs are involved in cell adhesion [156], wound healing [145], microbial invasion [157], viral infection [158], and carcinogenesis [132]. The involvement of AGPs in adhesion has been described above. Some AGPs are known to be involved in wound healing; thus, wound can regulate the level of AGP mRNA [159, 160], and gum arabic is secreted only in response to a wound and in its region [2]. Expression of AGPs also increases in response to biotic and abiotic stress. Infection of *Arabidopsis* plants with *X. campestris* [107], as well as cultivation of *Arabidopsis* in the presence of $AlCl_3$ [40], increases the expression of *AGP2*.

Although animal proteoglycans have been studied for a long time, works concerning mutations in the genes of proteoglycans and their *in vivo* functions have appeared

only recently. According to an apt expression of Lander and Selleck [161], "cell biologists have had a love–hate relationship with proteoglycans almost since their discovery" because of their uncommon heterogeneity, difficulties for biochemical analysis, multiple putative functions which are not easy to be proved, along with abundance of their molecules on the plasmalemma and in the extracellular matrix. The same seems to be true for AGPs, the functions of which are known still worse. Molecular mechanisms of even known functions of AGPs are waiting to be found. Although there are many lines in studies on AGPs because of their extreme structural and functional heterogeneity, the main trends seem to be as follows.

1. Elucidation of functions of individual AGPs. The main approach seems to be obtaining of AGP gene mutants and also of transformants with a decreased expression of definite AGP genes by means of recombinant constructions. The latter approach seems to be the most promising, and it was used in the recent finding [162] that the *AGP18* gene encoding a classical AGP is required for initiation of female gametogenesis in *Arabidopsis*.

2. Identification of genes and enzymes involved in the assemblage and modification of carbohydrate chains of AGPs, and also elucidation of the role of post-translational modification of the carbohydrate moiety of AGPs in their functioning.

3. Study on signaling functions of AGPs and search for receptor molecules interacting with AGPs. The treatment with Yariv reagent and wounding were shown [163] to cause a similar expression of genes in cultured *Arabidopsis* cells. It remains to be determined whether the effect of Yariv reagents is associated with activation of the mechanism involving AGPs as signal molecules, or it is realized via a general mechanical perturbation of the cell surface.

This work was supported by the Russian Foundation for Basic Research (project Nos. 02-04-48904 and 05-04-49433).

REFERENCES

1. Du, H., Clarke, A. E., and Bacic, A. (1996) *Trends in Cell Biology*, **6**, 411-414.
2. Clarke, A. E., Anderson, R. L., and Stone, B. A. (1979) *Phytochemistry*, **18**, 521-540.
3. Fincher, G. B., Stone, B. A., and Clarke, A. E. (1983) *Annu. Rev. Plant Physiol.*, **34**, 47-70.
4. Serpe, M. D., and Nothnagel, E. A. (1999) *Adv. Bot. Res.*, **30**, 207-289.
5. Knox, J. P. (1996) in *Membranes: Specialized Functions in Plants* (Smallwood, M., Knox, J. P., and Bowels, D. J., eds.) Bios, Guildford, pp. 93-102.
6. Kreuger, M., and van Holst, G.-J. (1996) *Plant Mol. Biol.*, **30**, 1077-1086.
7. Nothnagel, E. A. (1997) *Int. Rev. Cytol.*, **174**, 195-291.
8. Majewska-Sawka, A., and Nothnagel, E. A. (2000) *Plant Physiol.*, **122**, 3-9.
9. Gaspar, Y., Johnson, K. L., McKenna, J. A., Bacic, A., and Schultz, C. J. (2001) *Plant Mol. Biol.*, **47**, 161-176.
10. Showalter, A. M. (2001) *Cell Mol. Life Sci.*, **59**, 1399-1417.
11. Kreuger, M., and van Holst, G.-J. (1993) *Planta*, **189**, 243-248.
12. Toonen, M. A. J., Schmidt, E. D. L., van Kammen, A., and de Vries, S. C. (1997) *Planta*, **203**, 188-195.
13. McCabe, P. F., Valentine, T. A., Forsberg, L. S., and Pennel, R. I. (1997) *Plant Cell*, **9**, 2225-2241.
14. Van Hengel, A. J., Tadesse, Z., Immerzeel, P., Schols, H., van Kammen A., and de Vries, S. C. (2001) *Plant Physiol.*, **125**, 1880-1890.
15. Van Hengel, A. J., van Kammen, A., and de Vries, S. C. (2002) *Physiol. Plant.*, **114**, 637-644.
16. Yariv, J., Rappoport, M. M., and Graf, L. (1962) *Biochem. J.*, **85**, 383-388.
17. Yariv, L., Lis, H., and Katchalski, E. (1967) *Biochem. J.*, **105**, 1-2.
18. Carpita, N. C., and Gibeaut, D. M. (1993) *Plant J.*, **3**, 1-30.
19. Schwalb, P., and Feucht, W. J. (1998) *Appl. Bot.*, **72**, 157-161.
20. Jermyn, M. A. (1978) *Aust. J. Plant Physiol.*, **5**, 697-705.
21. Jermyn, M. A., and Yeow, Y. M. (1975) *Aust. J. Plant Physiol.*, **2**, 501-531.
22. Norman, P. M., Kjellbom, P., Bradley, D. J., Hahn, M. G., and Lamb, C. J. (1990) *Planta*, **181**, 365-373.
23. Baldwin, T. C., McCann, M. C., and Roberts, K. (1993) *Plant Physiol.*, **103**, 115-123.
24. Cheung, A. Y., Wang, H., and Wu, H.-M. (1995) *Cell*, **82**, 383-393.
25. Classen, B., Witthohn, K., and Blaschek, W. (2000) *Carbohydr. Res.*, **327**, 497-504.
26. Hillestead, A., Wold, J. K., and Engen, T. (1977) *Phytochemistry*, **16**, 1953-1956.
27. Mollard, A., and Joseleau, J.-P. (1994) *Plant Physiol. Biochem.*, **32**, 703-709.
28. Baldwin, T. C., Domingo, C., Schmidler, T., Seetharaman, G., Stacey, N. J., and Roberts, K. (2001) *Plant Mol. Biol.*, **45**, 421-423.
29. Kieliszewski, M. J., Kamyab, A., Leykam, J. F., and Lamport, D. T. A. (1992) *Plant Physiol.*, **99**, 536-547.
30. Qi, W., Fong, C., and Lamport, D. T. A. (1991) *Plant Physiol.*, **96**, 848-855.
31. Mau, S.-L., Chen, C.-G., Pu, Z.-Y., Moritz, R. L., Simpson, R. J., Bacic, A., and Clarke, A. E. (1995) *Plant J.*, **8**, 269-281.
32. Youl, J. J., Bacic, A., and Oxley, D. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 7921-7926.
33. Svetek, J., Yadav, M. P., and Nothnagel, E. A. (1999) *J. Biol. Chem.*, **274**, 14724-14733.
34. Sherrier, D. J., Prime, T. A., and Durpee, P. (1999) *Electrophoresis*, **20**, 2027-2035.
35. Oxley, D., and Bacic, A. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 14246-14251.
36. Takos, A. M., Dry, I. B., and Soole, K. I. (2000) *Plant J.*, **21**, 43-52.
37. Loopstra, C. A., Puryear, J. D., and No, E.-G. (2000) *Planta*, **210**, 686-689.
38. Zhang, Y., Sederoff, R. R., and Allona, I. (2000) *Tree Physiol.*, **20**, 457-466.
39. Schultz, C. J., Rumsewicz, M. P., Johnson, K. L., Jones, B. J., Gaspar, Y. M., and Bacic, A. (2002) *Plant Physiol.*, **129**, 1448-1463.

40. Schultz, C. J., Johnson, K. L., Currie, G., and Bacic, A. (2000) *Plant Cell*, **12**, 1751-1767.
41. Johnson, K. L., Jones, B. J., Bacic, A., and Schultz, C. J. (2003) *Plant Physiol.*, **133**, 1911-1925.
42. Gane, A. M., Craik, D., Munro, S. L. A., Howlett, G. J., Clarke, A. E., and Bacic, A. (1995) *Carbohydr. Res.*, **277**, 67-85.
43. Redgwell, R. J., Curti, D., Fischer, M., Nicolas, P., and Fay, L. B. (2002) *Carbohydr. Res.*, **337**, 239-253.
44. Tan, L., Leykam, F., and Kieliszewski, M. J. (2003) *Plant Physiol.*, **132**, 1362-1369.
45. Kieliszewski, M. J., and Shpak, E. (2001) *Cell. Mol. Life Sci.*, **58**, 1386-1398.
46. Kieliszewski, M. J., and Lamport, D. T. A. (1994) *Plant J.*, **5**, 157-172.
47. Kieliszewski, M. J., O'Neill, M., Leykam, J., and Orlando, R. (1995) *J. Biol. Chem.*, **270**, 2541-2549.
48. Zhao, Z. D., Tan, L., Showalter, A. M., Lamport, D. T. A., and Kieliszewski, M. J. (2002) *Plant J.*, **31**, 431-444.
49. Wu, H.-M., de Graaf, B., Mariani, C., and Cheung, A. Y. (2001) *Cell. Mol. Life Sci.*, **58**, 1418-1429.
50. Johnson, K. L., Schultz, C. J., and Bacic, A. (2001) *Proc. 9th Int. Cell Wall Meeting*, 77.
51. Sommer-Knudsen, J., Clarke, A. E., and Bacic, A. (1996) *Plant J.*, **9**, 71-83.
52. Van Holst, G.-J., Klis, F. M., de Wildt, P. J. M., Hazenberg, C. A. M., Ruijs, J., and Stegwee, D. (1981) *Plant Physiol.*, **68**, 910-913.
53. Sedgley, M., and Clarke, A. E. (1986) *Nord. J. Bot.*, **6**, 591-598.
54. Herman, E. M., and Lamb, C. J. (1992) *Plant Physiol.*, **98**, 264-272.
55. Pennell, R. I., Janiche, L., Scofield, G. N., Booij, H., de Vries, S. C., and Roberts, K. (1992) *J. Cell Biol.*, **119**, 1371-1380.
56. Schindler, T., Bergfeld, R., and Scopfer, P. (1995) *Plant J.*, **7**, 25-36.
57. Stahl, P., and Schwartz, A. L. (1986) *J. Clin. Invest.*, **77**, 657-662.
58. Rumyantseva, N. I., Samaj, J., Akulov, A. N., Lebedeva, V. V., Ensikat, H.-J., Baluska, F., and Volkmann, D. (2003) *Abst. VIII Int. Conf. "The Biology of Plant Cells in Vitro and Biotechnology"*, Saratov, September 9-13, pp. 274-275.
59. Knox, J. P. (1992) *Plant J.*, **2**, 137-141.
60. Northcote, D. H., Davey, R., and Lay, J. (1989) *Planta*, **178**, 353-366.
61. Kohorn, B. D. (2000) *Plant Physiol.*, **124**, 31-38.
62. Baluska, F., Samaj, J., Wojtaszek, P., Volkmann, D., and Menzel, D. (2003) *Plant Physiol.*, **133**, 482-491.
63. Gens, J. S., Fujiki, M., and Pickard, B. G. (2000) *Protoplasma*, **212**, 115-134.
64. Yanagishita, M., and Hascall, V. C. (1992) *J. Biol. Chem.*, **267**, 9451-9454.
65. Knox, J. P., Linstead, P. J., King, J., Cooper, C., and Roberts, K. (1990) *Planta*, **181**, 512-521.
66. Williams, M. N. V., Freshour, G., Darvill, A. G., Albersheim, P., and Hahn, M. G. (1996) *Plant Cell*, **8**, 673-685.
67. Matoh, T., Takahashi, M., Takabe, K., and Kobayashi, M. (1998) *Plant Cell Physiol.*, **39**, 483-491.
68. Jauneau, A., Roy, S., Reis, D., and Vian, B. (1998) *Int. J. Plant Sci.*, **159**, 1-13.
69. Sorensen, S. O., Pauly, M., Bush, M., Skjot, M., McCann, M. C., Borkhardt, B., and Ulvskov, P. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 7639-7644.
70. O'Neill, M. A., and Selvendran, R. R. (1985) *Biochem. J.*, **227**, 475-481.
71. Serpe, M. D., and Nothnagel, E. A. (1994) *Planta*, **193**, 542-550.
72. Carpita, N. C. (1989) *Phytochemistry*, **28**, 121-125.
73. Serpe, M. D., and Nothnagel, E. A. (1995) *Plant Physiol.*, **109**, 1007-1016.
74. Epstein, L., and Lamport, D. T. A. (1984) *Phytochemistry*, **23**, 1241-1246.
75. Fry, S. C. (1986) *Annu. Rev. Plant Physiol.*, **37**, 165-186.
76. Schnabelrauch, L. S., Kieliszewski, M., Upman, B. L., Alizedeh, H., and Lamport, D. T. A. (1996) *Plant J.*, **9**, 477-489.
77. Kjellbom, P., Snogerup, L., Stohr, C., Reuzeau, C., McCabe, P. F., and Pennell, R. I. (1997) *Plant J.*, **12**, 1189-1196.
78. Kawamoto, T., Noshiro, M., Shen, M., Nakamasu, K., Hashimoto, K., Kawashima-Ohya, Y., Gotoh, O., and Kato, Y. (1998) *Biochim. Biophys. Acta*, **1395**, 288-292.
79. Garcia-Gomez, B. L., Campos, F., Hernandez, M., and Covarrubias, A. A. (2000) *Plant J.*, **22**, 277-288.
80. Cleary, A. L. (2001) *Protoplasma*, **215**, 21-34.
81. Samaj, J., Baluska, F., and Volkmann, D. (1998) *Protoplasma*, **204**, 1-12.
82. Chapman, A., Helleboid, S., Blervacq, A.-S., Vasseur, J., and Hilbert, J.-L. (2000) *Plant Sci.*, **150**, 103-114.
83. Rumyantseva, N., Valieva, A., and Mukhitov, A. (1997) *Cell Biol. Int.*, **21**, 891-892.
84. Bobak, M., Hlavaeka, A., Oveeka, M., and Samaj, J. (1999) *J. Plant Physiol.*, **155**, 387-392.
85. Wu, H.-M., Wang, H., and Cheung, A. Y. (1995) *Cell*, **82**, 395-403.
86. Wang, H., Wu, H.-M., and Cheung, A. Y. (1993) *Plant Cell*, **5**, 1639-1650.
87. Samaj, J., Braun, M., Baluska, F., Ensikat, H.-J., Tsumuraya, Y., and Volkmann, D. (1999) *Plant Cell Physiol.*, **40**, 874-883.
88. Andeme-Onzighi, C., Sivaguru, M., Judy-March, J., Baskin, T. I., and Driouich, A. (2002) *Planta*, **215**, 949-958.
89. Roy, S., Jauh, Y. J., Hepler, P. K., and Lord, E. M. (1998) *Planta*, **204**, 450-458.
90. Willats, W. G. T., and Knox, J. P. (1996) *Plant J.*, **9**, 919-925.
91. Thompson, H. J. M., and Knox, J. P. (1998) *Planta*, **205**, 32-38.
92. Schopfer, P. (1990) *Planta*, **183**, 139-142.
93. Zhu, J.-K., Bressan, R. A., and Hasegawa, P. M. (1993) *Planta*, **190**, 221-226.
94. Fry, S. C. (1983) *Planta*, **157**, 111-123.
95. Nothnagel, E. A., and Lyon, J. L. (1986) *Plant Physiol.*, **80**, 91-98.
96. Raff, M. C. (1992) *Nature*, **356**, 397-400.
97. Pennell, R. I., and Lamb, C. (1997) *Plant Cell*, **9**, 1157-1168.
98. Langan, K. J., and Nothnagel, E. A. (1997) *Protoplasma*, **196**, 87-98.
99. Gao, M., and Showalter, A. M. (1999) *Plant J.*, **19**, 321-331.
100. Pennell, R. I., and Roberts, K. (1990) *Nature*, **344**, 547-549.
101. Pennell, R. I., Janniche, L., Kjellbom, P., Scofield, G. N., Peart, J. M., and Roberts, K. (1991) *Plant Cell*, **3**, 1371-1380.
102. Jauh, G. Y., and Lord, E. M. (1996) *Planta*, **199**, 251-261.
103. Knox, J. P., Day, S., and Roberts, K. (1989) *Development*, **106**, 47-56.
104. Knox, J. P., Linstead, P. J., King, J., Cooper, C., and Roberts, K. (1991) *Plant J.*, **1**, 317-326.

105. Dolan, L., Linstead, P., and Roberts, K. (1995) *Protoplasma*, **189**, 149-155.
106. Stacey, N. J., Roberts, K., Carpita, N. C., Wells, B., and McCann, M. C. (1995) *Plant J.*, **8**, 891-906.
107. Hall, A. E., Chen, Q. G., Findell, J. L., Schaller, G. E., and Bleeker, A. B. (1999) *Plant Physiol.*, **121**, 291-299.
108. Motose, H., Fukuda, H., and Sugiyama, M. (2001) *Planta*, **213**, 121-131.
109. Motose, H., Sugiyama, M., and Fukuda, H. (2001) *Plant Cell Physiol.*, **42**, 129-137.
110. Williams, E. G., and Maheswaran, G. (1986) *Ann. Bot.*, **57**, 443-462.
111. Stacey, N. J., Roberts, K., and Knox, J. P. (1990) *Planta*, **180**, 285-292.
112. Kreuger, M., and van Holst, G.-J. (1995) *Planta*, **197**, 135-141.
113. Kreuger, M., Postma, E., Brouwer, Y., and van Holst, G.-J. (1995) *Physiol. Plant.*, **94**, 605-612.
114. Egertsdotter, U., and von Arnold, S. (1995) *Physiol. Plant.*, **93**, 334-345.
115. Sondahl, M. R., Salisburi, J. L., and Sharp, W. R. (1979) *Z. Pflanzenphysiol.*, **94**, 185-188.
116. Dubois, T., Dubois, J., Guedira, M., Diop, A., and Vasseur, J. (1992) *Ann. Bot.*, **70**, 119-124.
117. Samaj, J., Bobak, M., Blehova, A., Kristin, J., and Auxtova-Samajova, O. (1995) *Protoplasma*, **186**, 45-49.
118. Rumyantseva, N. I., Samaj, J., Enzikat, H.-J., Salnikkov, V. V., Kostukova, Yu. A., Baluska, F., and Volkmann, D. (2003) *Dokl. Ros. Akad. Nauk*, **391**, 123-127.
119. Samaj, J., Baluska, F., Bobak, M., and Volkmann, D. (1999) *Plant Cell Rep.*, **18**, 369-374.
120. Chapman, A., Blervacq, A.-S., Vasseur, J., and Hilbert, J.-L. (2000) *Planta*, **21**, 305-314.
121. De Jong, A. J., Cordewener, J., Lo Schiavo, F., Terzi, M., Vandekerckhove, J., van Kammen, A., and de Vries, S. C. (1992) *Plant Cell*, **4**, 425-433.
122. Kragh, K. M., Hendriks, T., de Jong, A. J., Lo Schiavo, F., Bucherna, N., Hojrup, P., Mikkelsen, J. D., and de Vries, S. C. (1996) *Plant Mol. Biol.*, **31**, 631-645.
123. Baureithel, K., Felix, G., and Boller, T. (1994) *J. Biol. Chem.*, **269**, 17931-17938.
124. Minami, E., Kouchi, H., Cohn, J. R., Ogawa, T., and Stacey, N. J. (1996) *Plant J.*, **10**, 23-32.
125. Dyachok, J. V., Wiweger, M., Kenne, L., and von Arnold, S. (2002) *Plant Physiol.*, **128**, 523-533.
126. Von Arnold, S., Sabala, I., Bozhkov, P., Dyachok, Yu., and Filonova, L. (2002) *Plant Cell Tissue Organ Culture*, **69**, 233-249.
127. Bakkers, J., Semino, C. E., Stroband, H., Kijne, J. W., Robbins, P. W., and Spaink, H. P. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 7982-7986.
128. De Jong, A. J., Heidstra, R., Spanik, H. P., Hartog, M. V., Meijer, E. A., Hendriks, T., Lo Shiavo, F., Terzi, M., Bisseling, M. T., van Kammen, A., and de Vries, S. C. (1993) *Plant Cell*, **5**, 615-620.
129. Thompson, G. A., Jr., and Okuyama, H. (2000) *Progr. Lipid Res.*, **39**, 19-39.
130. Borner, G. H. H., Sherrier, D. J., Stevens, T. J., Arkin, I. T., and Dupree, P. (2002) *Plant Physiol.*, **129**, 486-499.
131. Peles, E., Nativ, M., Lustig, M., Grumet, M., Schilling, J., Martinez, R., Plowman, G. D., and Schlessinger, J. (1997) *EMBO J.*, **16**, 978-988.
132. Kleeff, J., Ishiwata, T., Kumbasar, A., Friess, H., Buchler, M. W., Lander, A. D., and Korc, M. (1998) *J. Clin. Invest.*, **102**, 1662-1673.
133. Resta, R., Yamashita, Y., and Thompson, L. F. (1998) *Immunol. Rev.*, **161**, 95-109.
134. Lease, K., Ingham, E., and Walker, J. C. (1998) *Curr. Opin. Plant Biol.*, **1**, 388-392.
135. Munnik, T., Irvine, R. F., and Musgrave, A. (1998) *Biochim. Biophys. Acta*, **1389**, 222-272.
136. Carpita, N., Dubularse, D., Montezions, D., and Delmer, D. P. (1979) *Science*, **205**, 1144-1147.
137. Tepfer, M., and Taylor, I. E. P. (1981) *Science*, **213**, 761-763.
138. Clarke, A. E., Abbot, A., Mandel, T. E., and Pettit, J. M. (1980) *J. Ultrastruct. Res.*, **73**, 269-281.
139. De Pinto, G. L. (1991) *Carbohydr. Res.*, **220**, 229-242.
140. Kawaguchi, K., Shibuya, N., and Ishii, T. (1996) *Plant J.*, **9**, 777-785.
141. Schultz, C. J., Gilson, P., Oxley, D., Youl, J. J., and Bacic, A. (1998) *Trends Plant Sci.*, **3**, 426-431.
142. David, G. (1993) *FASEB J.*, **7**, 1023-1030.
143. Gallagher, J. T. (1989) *Curr. Opin. Cell. Biol.*, **1**, 1201-1218.
145. Bernfield, M., Gotte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Lincecum, J., and Zako, M. (1999) *Annu. Rev. Biochem.*, **68**, 729-777.
146. Salmvirta, M., and Yalkanen, M. (1995) *Experientia*, **51**, 863-872.
147. Nakato, H., Futch, T. A., and Selleck, S. B. (1995) *Development*, **121**, 3687-3702.
148. Lin, X., and Perrimon, N. (1999) *Nature*, **400**, 281-284.
149. Tsuda, M., Kamimura, K., Nakato, H., Archer, M., Staatz, W., Fox, B., Humphrey, M., Olson, S., Futch, T., and Kaluza, L. (1999) *Nature*, **400**, 276-280.
150. Pilia, C., Hughes-Benzie, R. M., MacKenzie, A., Baybayan, P., Chen, E. Y., Huber, R., Neri, G., Cao, A., Foarabosco, A., and Schlessinger, D. (1996) *Nat. Genet.*, **12**, 241-247.
151. Cano-Gauci, D. F., Song, H. H., Yang, H., McKerlie, C., Choo, B., Shi, W., Pullano, R., Piscione, T. D., Grisaru, S., and Soon, S. (1999) *J. Cell Biol.*, **146**, 255-264.
152. Suzuki, Y., Kitagawa, M., Knox, J. P., and Yamaguchi, I. (2002) *Plant J.*, **29**, 733-741.
153. Park, M. H., Suzuki, Y., Chono, M., Knox, J. P., and Yamaguchi, I. (2003) *Plant Physiol.*, **131**, 1450-1459.
154. Yu, K. W., Kiyohara, H., Matsumoto, T., Yang, H. C., and Yamada, H. (1998) *Planta Med.*, **64**, 714-719.
155. Gonzalez, A. D., Kaya, M., Shi, W., Song, H., Testa, J. R., Penn, I. Z., and Filmus, J. (1999) *J. Cell Biol.*, **141**, 1407-1414.
156. LeBaron, R. G., Esko, J. D., Woods, A., Johansson, S., and Hook, M. (1988) *J. Cell Biol.*, **106**, 945-952.
157. Van Putten, J. P., and Paul, S. M. (1995) *EMBO J.*, **14**, 2144-2154.
158. Shieh, M. T., WuDunn, D., Montgomery, R. I., Esko, J. D., and Spear, P. G. (1992) *J. Cell Biol.*, **116**, 1273-1281.
159. Pogson, B. J., and Davies, C. (1995) *Plant Mol. Biol.*, **28**, 347-352.
160. Li, S.-X., and Showalter, A. M. (1996) *Plant Mol. Biol.*, **32**, 641-652.
161. Lander, A. D., and Selleck, S. B. (2000) *J. Cell Biol.*, **148**, 227-232.
162. Acosta-Garcia, G., and Vielle-Calzada, J.-P. (2004) *Plant Cell*, **16**, 2614-2628.
163. Guan, Y., and Nothnagel, A. (2004) *Plant Physiol.*, **135**, 1346-1366.