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Arabinogalactan-Proteins: Getting to the Core

All organisms produce glycoconjugates, oligosaccharide-linked proteins and lipids. An enormous range of possible biological roles have been proposed, and in some cases demonstrated, for these molecules, especially those of animals (for review, see Varki, 1993). Some glycoconjugates, particularly proteoglycans, appear to play structural or organizational roles, whereas others, in particular glycoproteins, act as specific receptors. Still others mediate cell-cell or cell-matrix interactions, some of which guide normal development or differentiation.

The differences in their structures and developmental strategies make it likely that glycoconjugates will turn out to play somewhat different roles in plants and animals. Nevertheless, evidence is accumulating pointing to the possibility that a particular class of plant glycoproteins and proteoglycans, the arabinogalactan-proteins (AGPs), play important roles in plant development. Assessing their exact roles has been complicated by the fact that no AGP core protein had ever been cloned and sequenced. Two reports from Adrienne Clarke's group now remedy this deficit: Du and coworkers report on pages 1643–1653 of this issue the isolation of a cDNA for an AGP secreted by Nicotiana alata styles, and Chen et al. (1994) have reported the isolation of a cDNA for an AGP secreted by cultured pear cells. The cloning of these genes will make it possible to explore a number of questions about AGP function and regulation.

AGPs can be detected in a number of different plant organs by staining with the synthetic phenylglycoside known as Yariv reagent, which binds to and precipitates AGPs (for reviews, see Clarke et al., 1979; Fincher et al., 1983). Some AGPs appear to be membrane associated, whereas others are secreted in large amounts, both in the plant and by cultured cells. AGPs are highly heterogeneous, judging by both their size range and their differential reactivity with anti-AGP monoclonal antibodies; each organ and cell type is associated with a characteristic subset of AGPs. Most of the AGP molecule—typically at least 95% by weight—is carbohydrate, principally galactopyranose and arabinofuranose residues. The sugar groups are O-linked to hydroxy amino acids in the core protein, which is usually rich in Ser, Ala, Gly, and Hyp residues. The secreted AGPs contain polysaccharides and are thought to be proteoglycans, whereas the membrane-associated AGPs are linked to oligosaccharides and appear therefore to be glycoproteins. One of the many mysteries about AGPs is how these two classes are related. Are the secreted forms cleavage products of the membrane bound forms, or do the two classes have completely different core proteins?

The pronounced stickiness and water-holding capacities of AGPs suggest an array of possible functions for these molecules. One possible general function is simply in controlling water balance. Another is in internalizing periplasmic material for vacuole-mediated degradation; this is suggested by the finding (Herman and Lamb, 1992) that a monoclonal antibody that reacts with a glycan epitope found on a family of membrane-associated AGPs labels not only the plasma membrane but also multivesicular plasma membrane–derived bodies that may be part of an endocytotic pathway. The inherent adhesiveness of AGPs could enable them to stick to, and thereby mediate the disposal of, extraneous molecules, and even pathogens, in the extracellular matrix. Their stickiness also raises the possibility that AGPs are involved in adhesion between the pollen and stigma or between adjacent cell walls. Yet another possibility is that AGPs play a nutritive role, with those in the style, for example, providing carbohydrate precursors for the growing pollen tube wall.

Mounting evidence also raises the possibility that AGPs are involved in growth regulation. For example, treatment of suspension-cultured rose cells with a phenylglycoside that binds AGPs inhibits cell division in a reversible fashion, whereas a phenylglycoside that does not bind AGPs has no effect on cell proliferation (Serpe and Nothnagel, 1994). The involvement of AGPs in cell proliferation may reflect their participation in wall expansion (Zhu et al., 1993), or it may reflect a more direct effect on cell division per se. Secreted AGPs have also been found to influence somatic embryogenesis (Kreurer and van Holst, 1993). The pattern of AGPs secreted by a carrot cell line changes as its embryogenic potential changes, and addition of AGPs secreted by an embryogenic carrot cell line to a nonembryogenic line can actually induce embryogenic potential in that line. Conversely, addition of AGPs from a nonembryogenic cell line can prevent a carrot explant culture from becoming embryogenic. In leafy liverworts, different AGPs are produced depending on whether or not place-dependent suppression of leaf production is released (e.g., by culturing in the presence of ammonium ions) (Basile and Basile, 1993), although it is not known whether these proteoglycans are actually able to influence leaf cell proliferation.

Whatever their roles in guiding development, it is clear that AGP expression is exquisitely sensitive to developmental cues, not only in leafy liverworts but also in higher plants. For example, antibody staining experiments indicate that one arabino- containing epitope, which is also present on a secreted AGP, is associated with the plasma membrane of all cells of pea plants except those of developing sexual organs and the young embryo (Pennell and Roberts, 1990). Other monoclonal antibodies that also react with both secreted and plasma membrane AGPs recognize epitopes expressed in the developing root in spatially restricted patterns.
that reflect cell position rather than cell lineage and that change during root development (Knox et al., 1989, 1991). Another AGP epitope shows a temporally and spatially modulated expression pattern in flowers and embryos of oilseed rape (Pennell et al., 1991).

These results raise an important question: What accounts for the highly regulated modulations in epitope expression? That is, do changes in glycosylation remove or mask epitopes? Do interactions with other proteins mask epitopes? Or do entirely new AGPs (i.e., with different protein cores) replace the epitope-bearing ones? In theory, one way to investigate this question would be to probe with antibodies to the protein part of the AGP—but this approach is hindered by the fact that most anti-AGP antibodies recognize carbohydrate rather than protein epitopes. Indeed, any protein epitopes would probably be masked by carbohydrate anyway. On the other hand, it should be possible to produce antibodies to deglycosylated AGPs and use them to probe tissue sections that have been treated to remove O-linked sugars.

An alternative approach to investigate the developmental regulation of AGPs is to examine the expression pattern of the core protein gene, but cloning AGPs has been hampered by the difficulties in isolating single AGP species. That is, because multiple AGPs tend to copurify, it is not always clear whether two peptide sequences derive from a single protein. And even when peptide sequence is available, designing and using the appropriate probes can be problematic because the amino acids that predominate in AGP are encoded by degenerate codons that are rich in GC. Despite these obstacles, Du and coworkers were able to purify an AGP from extracts of N. alata styles sufficiently that a long peptide sequence could be deduced. By making a probe to the least redundant and most AT-rich portion of the peptide, they were then able to isolate a cDNA.

The deduced protein product of the cDNA is a small protein with an N-terminal signal peptide and an abundance of Ala, Ser, and Pro residues. The C-terminal region is very hydrophobic, and the authors suggest that it could form a membrane-spanning helix. The presence of a membrane-spanning domain in a secreted protein is intriguing; one possible explanation is that the secreted form purified by the authors is proteolytically cleaved off of a membrane-bound form. Alternatively, the C-terminal region may not in fact be a membrane anchor. Interestingly, the predicted overall structure of the N. alata AGP core protein is similar to that deduced from the cDNA for an AGP secreted by suspension-cultured pear cells (Chen et al., 1994), although the primary sequences of the two proteins are different.

Transcripts for the N. alata protein are detected in a number of organs, both reproductive and vegetative, which raises the possibility that at least some of the different N. alata AGPs are created by differential modification of a common core protein. If it turns out that glycosylation is regulated, then it will be important to identify the glycosyltransferases and determine how they, in turn, are regulated. Interestingly, cell-specific glycosylation is one feature of the regulation of the activity of a set of vertebrate proteins, the mucins, that bear striking similarities to AGPs. The mucins are very large and heavily O-glycosylated proteins that, by binding to the cell surface receptors known as selectins, mediate the adhesive interactions between leukocytes and endothelial cells that allow leukocytes to migrate out of the circulatory system and into sites of injury or inflammation (Shimizu and Shaw, 1993). It is particular carbohydrate groups of the mucins that are involved in selectin recognition, although the protein core is also important in specificity. Another interesting, although possibly speculative, feature of the regulation of the AGPs is that allows them to project out far from transmembrane tails.

Because of steric constraints, the glycosylated regions of the mucins and other O-glycosylated animal proteins have been proposed to adopt an extended structure that allows them to project out far from the plasma membrane (Jentoft, 1990). This is predicted to facilitate their interactions with specific receptors on other cells. A tantalizing possibility is that AGPs similarly extend well beyond the glycosalyx and can therefore interact, if not with receptors on adjacent cells, then with components of the wall.

So far, the function of AGPs remains speculative. Like mucins, they may well play some general roles— but they may also play some specific roles as well, judging from their highly regulated expression patterns. With the cloning of AGPs, it should begin to be possible to ask not only what accounts for the variations in epitope expression but also what is the significance of this variation. Disrupting expression with antisense or sense gene expression should help reveal whether the epitope variations reflect the existence of cell-cell inductive events or whether they are merely markers for the early commitment to differentiate in a particular way.

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REFERENCES


