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Arabinogalactan-proteins (AGPs) and Plant Cell Development

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Summary

Arabinogalactan-proteins (AGPs) are a class of proteoglycans found at the surface of plant cells and in plant gums and secretions. Their functions in these locations are far from clear although various studies involving genetic, inhibitor and immunochemical analyses are indicating roles in cell expansion and the mediation of local cell interactions that underpin developmental events. AGPs are structurally diverse within a plant: *Arabidopsis thaliana* has at least 40 genes encoding AGP core proteins. Proline-rich domains that carry AGP glycans can be associated with a range of other protein motifs including fasciclin domains. AGP glycan components are decorated arabino-3,6-galactans and are highly heterogeneous. Monoclonal antibodies to AGP glycan epitopes indicate extensive regulation of glycan structure in relation to developmental events in meristems and that the precise developmental occurrence of an AGP epitope can vary between species. The relationship between AGP glycans and core protein diversity has not been fully elucidated and understanding the precise biochemical/cell biological functions of individual AGPs remains a challenge. A chemical disruptor of AGPs, a synthetic phenyl glycoside, known as β-glucosyl Yariv reagent (βGlcY) is a useful tool for the functional analysis of AGPs. The application βGlcY to living cells can result in the disruption of cell proliferation, disruption of cell expansion or modified cell development. In the *Arabidopsis* seedling root the application of βGlcY blocks the final stage of accelerated cell elongation and also disrupts a staged occurrence of pectin-associated 1,4-galactan. Analysis of the moss *Physcomitrella patens* indicates the presence of several classes of AGP core protein sequences and the presence of AGP glycan epitopes at all plasma membranes. *Physcomitrella* grows by tip-extending apical cells and this cell expansion is sensitive to βGlcY application. Genetic and experimental tractability makes this a suitable system for the structural and functional analysis of AGPs as well as providing an evolutionary perspective.

1. Introduction

The retention of cellulose-reinforced composites, cell walls, at the surface of all plant cells determines the distinctive modes of cell development in plants. A lack of relative cell movement and the generation of form through controlled proliferation and expansion of adhered cells in meristems underlies development and the continuous production of organs throughout the life of a plant. Cells walls are structurally complex and remain a challenging area of plant molecular and cell biological sciences which aim to generate a full mechanistic understanding of growth and development [1,2]. In addition to the major cell wall

polysaccharide groups of cellulose, hemicelluloses (cross-linking glycans) and pectic polysaccharides, cell surfaces contain a range of proteins and glycoproteins. The arabinogalactan-proteins (AGPs) are a class of highly glycosylated proteins that are viewed as being at one end of a continuum of plant cell surface components that range from proline-rich proteins through extensin glycoproteins to AGP proteoglycans [3~5]. AGPs appear to be relatively abundant features of all plant cell surfaces and are associated with plasma membranes, cell walls and secretions.

2. Dissection of AGP structures

Through the availability of encoding gene sequences and biochemical analyses the protein cores of AGPs are now relatively well understood in terms of primary structure [4,6]. In the genome of the model organism *Arabidopsis thaliana* there are over 40 sequences encoding AGP core proteins. A significant feature of these AGP sequences, which can be grouped in relation to sequences and modular structure, is that they are often hybrid [6]. That is, a proline-rich domain, characteristic of AGPs, can be associated with a non-AGP protein domain such as a fasciclin domain or a non-specific lipid-transfer protein [6,7].

The proline-rich regions of AGP core proteins are decorated with AGP glycans. AGP glycans are short chains of arabinose residues or heteropolymers based on arabino-3,6-galactans substituted with a range of other sugars including uronic acids [3~5]. Some understanding of how amino acid sequences in the protein can determine the sites and nature of *O*-glycosylation are emerging [8,9]. AGP glycans are less tractable for analysis than the protein cores. Some insights into their structure and their regulation within plant materials have arisen from the use of anti-glycan monoclonal antibodies. The major observation is that some AGP glycan epitopes are developmentally regulated within meristems and patterns of occurrence reflects early events involved in cell distinctions and cell specialization [10]. Several anti-AGP glycan antibodies with novel cell recognition patterns were selected during immunological dissection of complex cellular antigens and the AGP epitopes have generally been difficult to define in structural terms although the presence of the epitopes in acacia and other gums has been a useful resource to support these retrospective characterization studies [10~12]. Major outstanding questions about AGP glycan structures/epitopes concern their relationship to individual core proteins. What is the precise glycan structure carried by an individual core protein? Do all copies of that protein in that cell or organ carry identical glycans? Is a developmentally restricted AGP glycan epitope carried by one core protein or a range of core proteins?

AGPs are generally linked to plasma membranes and for most of the classes of AGP core proteins this is by means of a glycosylphosphatidylinositol (GPI) anchor with the large glycan domains residing on the outer face of the plasma membrane [13,14]. These GPI anchors may confer residence in lipid raft components of plasma membranes [15]. The location of AGP glycans at the outer face of the plasma membrane means that they are at the interface with cell walls and that phospholipase cleavage of GPI anchors therefore has the capacity to release AGPs into the cell wall and for secretion. AGPs are known to be abundant in plant secretions such as wound exudates and culture media but the significance of their loss from a cell surface is not known.

3. AGPs and the evolution of land plants

AGP glycan epitopes, recognised by monoclonal antibodies, can occur at the surface of groups of cells that are differentiating from neighbouring cells. For example, the JIM4 AGP epitope occurs on the plasma membrane of cells of the developing protoderm in somatic embryos of *Daucus carota* [16]. In *Euphorbia pulcherrima* this cell layer is marked, not by the JIM4 epitope, but by the AGP glycan epitope bound by monoclonal antibody LM2 [17]. These observations exemplify sets of observations that have been made with these and other anti-AGP glycan monoclonal antibodies in a range of species in that the occurrence of a

specific epitope in one species cannot be used to predict the occurrence of the same epitope in the equivalent organ of another species. A feature of these patterns is that they are often complementary in terms of cell recognition patterns to other cell wall glycoprotein epitopes [10]. Another example involves the JIM13 AGP glycan epitope and examples are shown in Figure 1. This epitope is specifically associated with differing aspects of developing xylem in a range of dicotyledon roots (*Arabidopsis thaliana, Daucus carota, Pisum sativum, Rhaphanus sativus*) but it is associated specifically with phloem cells in the roots of the monocotyledons *Zea mays* and *Allium cepa* [18] indicating a taxonomic significance to the cellular occurrence of epitopes. Indeed, an extended analysis of plasma membrane AGP glycan epitopes in a range of species representative of wide range of families has found that epitope occurrence varies taxonomically suggesting an intimate association of epitope presence and absence with evolutionary events [19]. For example, although the presence of the MAC207 AGP glycan epitope is generally present in species in the superorder Asteridae it is absent from a related group of families that includes the Solanaceae and the Rubiaceae [19].

AGPs occur in bryophytes [20,21], the earliest extant land plants, indicating that this group of complex macromolecules have been present throughout land plant evolution. The moss *Physcomitrella patens* has emerged as a model organism for the genetic analysis of this group of plants with developing genetic and genomic resources [22]. Bioinformatic and biochemical analyses of *Physcomitrella* have indicated the presence of at least three classes of AGP core proteins known for higher plants [23]. An interesting aspect of *Physcomitrella* AGPs is that although a preparation of AGPs contains the JIM13 epitope the most abundant glycan epitope detected to-date is 1,5-arabinan which is predominantly a feature of the pectic rhamnogalacturonan-I (RG-I) polymer group in higher plants [23,24].

Taken together, these observations suggest that the molecular action of AGPs may be involved in generating aspects of plant form that are associated with evolutionary events. An intriguing set of experiments has involved the application of a synthetic inhibitor of AGP action, β-glucosyl Yariv reagent (β GlcY), to Streptocarpus prolixus belonging to the Gesneriaceae angiosperm family. Germination in the presence of 30 μM β GlcY resulted in morphological changes that were characteristic of presumed more primitive species as well as species in

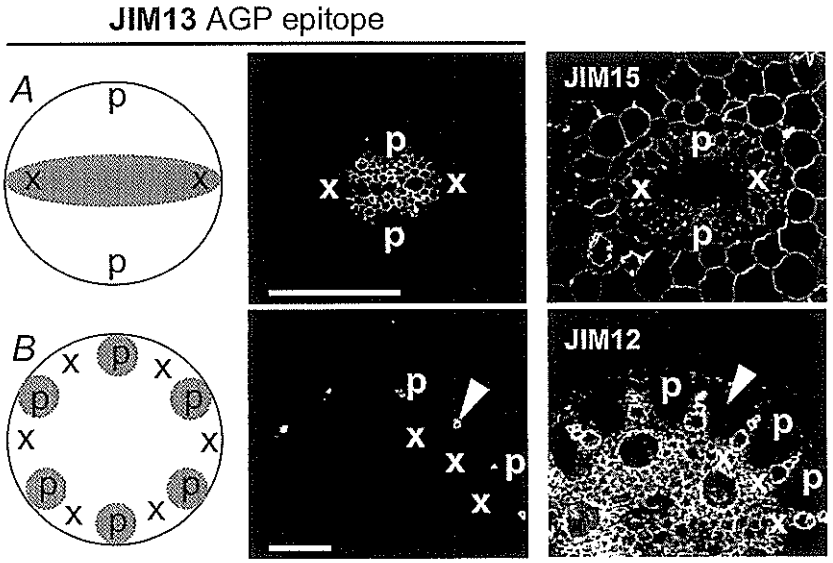

Fig. 1. Examples of a taxonomic distinction in the occurrence of an AGP glycan epitope in relation to cell differentiation
A) Schematic representation of the occurrence of the JIM13 epitope (shaded zone) in relation to the vascular tissues of the carrot (*Daucus carota*) root with micrographs of TS carrot root showing immunofluorescence labelling with JIM13 and an equivalent section labelled with anti-AGP antibody JIM15. B) Schematic representation of the occurrence of the JIM13 epitope (shaded zone) in relation to the vascular tissues of the maize (*Zea mays*) root with micrographs of TS maize root showing immunofluorescence labelling with JIM13 and an equivalent section labelled with anti-extensin antibody JIM12. Arrowhead indicates phloem sieve element. x = xylem, p = phloem, scale bars = 100 μm.

other genera of the Gesneriaceae family 25).

4. The search for cellular functions of an individual AGPs

What it is important to know, in addition to the structure of a single AGP within a plant organ, is its role(s) in the organ at a cellular level. The application of isolated AGPs or AGP-binding β GlcY to plant systems and the genetic disruption or over-expression of AGP core proteins has implicated AGPs in roles connected with cell proliferation, cell expansion cell differentiation and cell death processes amongst others [3,5,26~29] - all of which may influence plant development. In no case has the role of a single AGP been fully defined in a process in terms of it cell biological function i.e. in mechanistic terms how it effects or influences these processes. A good advance has been made in recent work that indicates that a factor required for vascular tissue development, termed xylogen, is a hybrid molecule consisting of a proline-rich domain with AGP glycan (conferring binding by β GlcY and the monoclonal antibody JIM13) and a non-specific lipid transfer protein domain [30]. This factor is essential for correct patterning of the vascular tissue in *Arabidopsis* cotyledons [30]. This work indicates that xylogen is a component of signals passing between cells that coordinate cell differentiation events. Xylogen is located in and secreted from cells in a polar manner [30]. A complexity arising from the functional dissection hybrid molecules is to determine the specific properties provided by the component domains. For example, what properties do AGP glycan carrying domains impart to such hybrid molecules? Do they direct location to a specific domain of the plasma membrane or promote specific interactions with cell wall components?

5. AGPs and cell expansion

For some time, evidence has been accumulating indicating a role for AGPs in the processes that underpin plant cell expansion. Over-expression or disruption of AGP core proteins and application of exogenous AGPs or β GlcY can alter plant growth in a range of systems [3,5,31~34]. We have been studying cell elongation in the *Arabidopsis* seedling root. In this system the application of 30 μM β GlcY specifically stops the final phase of cell elongation resulting in shorter roots and bulged epidermal and cortical cells [35]. Analysis of cell wall components in this system indicates that a 1,4-glactan polymer, associated with pectic RG-I, is associated with the acceleration of cell elongation into this final phase but that it is absent from cell walls in the final stages of cell elongation [36]. It is of interest that β GlcY application, alone among factors that can cause reduced cell elongation, results in the cell wall 1,4-galactan marker persisting into the mature root indicating that AGPs may be involved in the remodelling of the cell wall pectic polymers that appears to be required for cell elongation [36] as shown in Figure 2.

In the multicellular systems, such as the *Arabidopsis* seedling root, cell expansion is coordinated across the

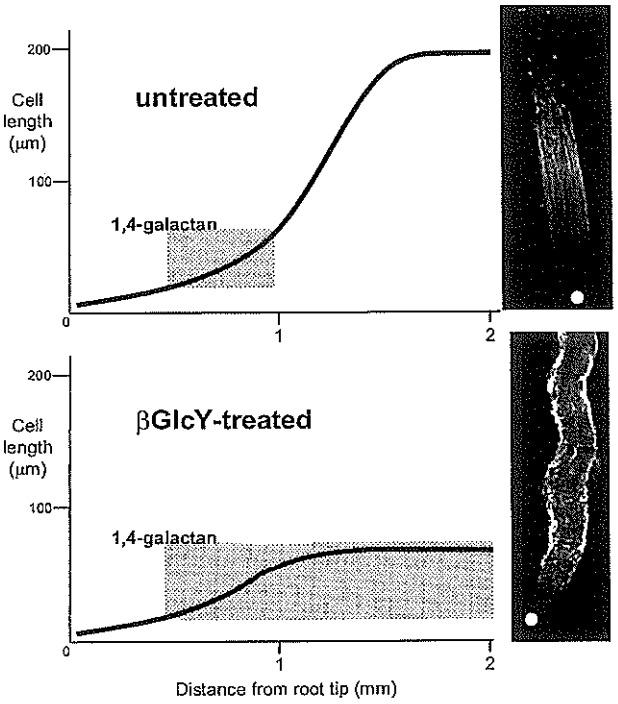

Fig. 2. Schematic representations of cell elongation dynamics at the *Arabidopsis* seedling root apex in control (untreated) and seedlings treated with AGP disruptor βGlcY in relation to the occurrence of a 1,4-galactan epitope in epidermal and cortical cells walls
Micrographs showing the immunofluorescence labelling of the surface on intact roots with the anti-1,4-galactan antibody are shown alongside. White dots indicate the most distal regions of the root tips.

adhered cells of the root organ. The gametophyte of the bryophyte *Physcomitrella* is a series of protonemal filaments in which cells are adhered only end to end and that extend by tip growth of apical cells. This presents an excellent accessible single-celled system for the analysis of cell walls and AGPs in relation to cell expansion and other aspects of cell development [23]. Protonemal apical cell extension in *Physcomitrella* is blocked by the application of 1 μM β GlcY and evidence suggests that this may be associated with the disruption in the tip-located release of AGPs from the plasma membrane into the cell wall [23]. It is of interest that the growth of *Physcomitrella* appears to be associated with the abundant secretion of AGPs into the medium. Can the released AGPs act as cell wall loosening factors in some way?

An important point concerning these observations that use β GlcY as a tool to dissect AGP function, is that this inhibitor is not specific to individual AGPs and that it is likely to disrupt a wide range of AGPs in each case. However, the biological actions of β GlcY are a useful guide to dissect aspects of AGP function within organs or cells. Technologies are required that can selectively disrupt specific AGPs, defined in terms of both protein and glycan. *Physcomitrella* has the experimental advantage that it can undergo a high frequency of homologous recombination of DNA sequences during transformation and this allows the careful one by one functional dissection of AGP core proteins and other associated proteins in this system.

6. Prospects

The last few years have seen an increase in knowledge and understanding of the structural aspects of AGPs and this has informed and supported approaches to the determination of their functions in plant cell development. It has long been a matter of speculation, building on observation in a range of systems, that AGPs have fundamental roles involved in cell specialisation and signalling within meristems and organs. Recent work indicates that AGPs are set of macromolecules with an ancient origin in the context of land plants and they are intimately involved in developmental events. Technologies for refined structural and functional dissection of AGP glycan structures are required and the identification of specific glycosyltransferases that underpin the synthesis of these will be an important factor. It is also important to maintain an overview of the taxonomic dimension to AGP protein and AGP glycan occurrence to gain insight into the roles that they may have played in evolutionary events.

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