

Plant Cell Biology

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Arabinogalactan-proteins (AGPs), the major constituent of gum arabic, have been used for centuries as emulsifiers and stabilizing agents. They are also abundant cell surface proteoglycans consisting of >90% carbohydrate linked to a minor (<10%) protein backbone. In many cases a glycosylphosphatidylinositol (GPI) anchor attaches then to the outer leaflet of the plasma membrane (see Figure). Despite their abundance and industrial utility, the *in vivo* function(s) of AGPs remains unknown. The broad objective of our program is to define the molecular mechanisms of AGP function using *Arabidopsis thaliana* as a model system.

Bioinformatics

We developed custom software in order to identify AGPs from the *Arabidopsis* genome based on the biased amino acid composition of AGP backbones (AGPs are rich in Pro, Ala, Ser, and Thr). More than 50 different genes encoding AGP protein backbones have been identified and these are classified into several sub-groups.

These include:

1. Classical AGPs,
2. AGPs containing a lysine rich region - the *Arabidopsis rat1* mutant, resistant to *Agrobacterium tumefaciens* transformation, is due to a mutation in the gene for the Lys-rich AGP, AtAGP17,
3. Fasciclin-like AGPs (FLAs), that contain AGP domains and fasciclin-like domains. In other eukaryotes (e.g. fruitfly - *Drosophila melanogaster*, humans - *Homo sapiens*, algae – *Volvox carteri*) fasciclin domain-containing proteins are involved in cell adhesion,
4. AG-peptides where the protein backbone is only 10-17 amino acids in length. We are using a combination of functional genomics and biochemical approaches to identify AGP function(s).

Molecular Biology

Most single gene *Arabidopsis* AGP mutant and transgenic plants do not show phenotypes under standard glasshouse conditions. We are challenging them under various conditions (e.g. hormones, pathogens) based on data from microarray and other experiments.

We are also crossing lines to make multiple mutants where data suggests that they are from the same functional class and/or expressed under similar conditions.

We are also using multiple RNAi for reducing the expression of several genes in a single plant. We have generated a construction vector where several independent RNAi modules can be inserted using Gateway™. We have also generated several AGP native promoter::GFP/GUS lines to visualise expression of the genes.

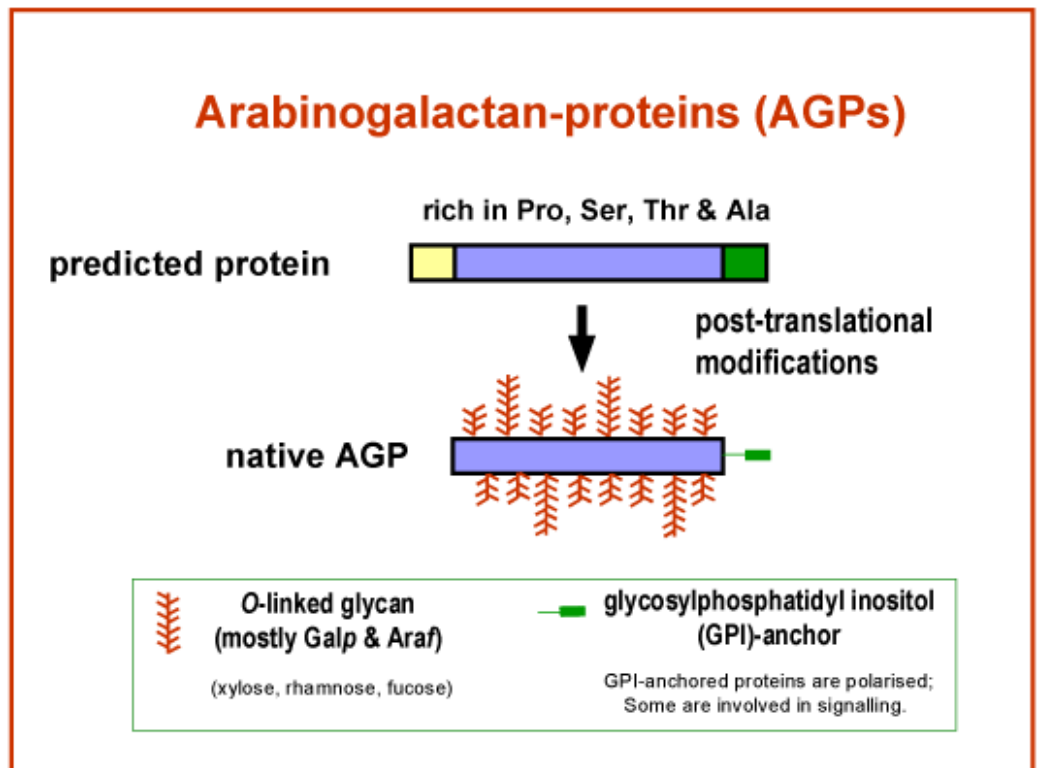
Biosynthesis / Biochemistry

We are also focussing on the biosynthesis of AGPs. AGPs are extensively modified post-translationally. We have confirmed that AG-peptides are *bona fide* AGPs in that they precipitate with single Yariv reagent. using MALDI-TOF MS/MS sequencing we have also determined the precise cleavage site for GPI-anchor additions for 8/12 AG-peptides. Protein sequencing of AG-peptides has also revealed that, in addition to the expected Ala-Pro, Ser-Pro motifs, the Gly-Pro motif can be hydroxylated *in vivo*. The knowledge of the post-translational modification of the protein backbone assist in developing and refining prediction tools for the primary structure of AGPs.

The other significant modification of AGPs is the addition of AG polysaccharides. A family of Type II glycosyltransferases have been identified by bioinformatic analysis of the *Arabidopsis*

genome and using the CAZY database. We are using a combination of molecular and biochemical approaches to test whether the genes are responsible for the synthesis of the β (1-3) galactan backbone of AGPs.

The AGP research program benefits from an active collaboration with a group at INRA, Versailles, France (Professor Herman Hofte and Dr Gregory Mouille) to investigate changes to cell wall structures using Fourier Transform Infrared (FTIR) microspectroscopy. An understanding of the *in planta* function of AGPs has the potential to benefit both industrial and agricultural sectors of the economy.



Recent Publications

Gaspar YM, Nam J, Schultz CJ, Lee L-Y, Gilson PR, Gelvin SB, Bacic A (2004) Reduced expression of an *Arabidopsis* lysine-rich arabinogalactan-protein, *AtAGP17*, results in a decreased efficiency of *Agrobacterium* transformation. *Plant Physiology* First published on July 30, 2004; 10.1104/pp.104.045542.

Schultz C, Ferguson, Bacic A (2004) Post-translational modifications of arabinogalactan-peptides of *Arabidopsis thaliana*: ER and GPI-anchor signal cleavage sites and hydroxylation of proline. *Journal of Biological Chemistry* (in press).

Eisenhaber BJ, Wildpaner M, Schultz CJ, Borner GHH, Dupree P, Eisenhaber F (2003)

Glycosylphosphatidylinositol lipid anchoring of plant proteins. Sensitive prediction from sequence- and genome-wide studies for *Arabidopsis* and rice. *Plant Physiology* 133:1691-1701.

Johnson K, Jones B, Schultz CJ, Bacic A (2003) Non-enzymic cell wall (glyco)proteins. In: The Plant Cell Wall, J Rose (ed), Blackwell Publishing, UK, ch 4, pp. 111-154.

Johnson KL, Jones BJ, Bacic A, Schultz CJ (2003) The fasciclin-like arabinogalactan proteins of *Arabidopsis*. A multigene family of putative cell adhesion molecules. *Plant Physiology* 133:1911-25.

Schultz CJ, Rumsewicz MP, Johnson KL, Jones BJ, Gaspar YM, Bacic A (2002) Using genomic resources to guide research directions. The arabinogalactan protein gene family as a test case. *Plant Physiology* 129:1448-63.

Gaspar Y, Johnson KL, McKenna JA, Bacic A, Schultz CJ (2001) The complex structures of arabinogalactan-proteins and the journey towards understanding function. *Plant Molecular Biology* 47:161-76.

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