Proposition d'un sujet de stage au M2 ADAM (2017-2018)

Titre	**Rôle des protéines à domaine PAC dans les parois cellulaires végétales**
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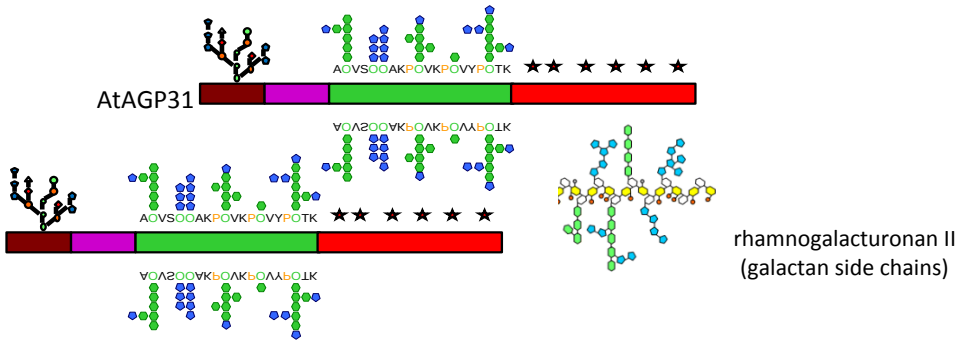
Résumé	Plant cell walls are the major component of plant biomass and play important roles during plant development and in response to environmental constraints. Plant cell walls are natural composite structures, mostly made of polysaccharides, proteins and lignin. Polysaccharides represent up to 95% of the primary cell wall mass, whereas cell wall proteins (CWPs) account for 5-10%. Cell wall proteomics has revealed the high diversity of protein content [Jamet *et al.* 2008, Proteomics 8: 893] and CWPs play many roles in plant cell wall plasticity [Frankova and Fry 2013, J Exp Bot 12: 3519]. However, not much is known about the cell wall supramolecular architecture in which CWPs are certainly major players via protein/protein or /polysaccharide interactions. The *Arabidopsis thaliana* AtAGP31 (ArabinoGalactan Protein 31) has been identified as a major protein in actively growing etiolated hypocotyls. From N- to C-terminus, it comprises several domains such as a predicted signal peptide, a short AGP domain, a His-stretch, a proline (Pro)/hydroxyproline (Hyp)-rich domain, and a PAC (Pro-rich protein, AGP, Cysteine containing) domain. It should be noted that proteins having a PAC-domain belong to multigene families which are found in all land plants including mosses and liverworts, *e.g.* *Physcomitrella patens* and *Marchantia polymorpha*. The PAC domain has been shown to interact *in vitro* with cell wall polysaccharides among which galactans and with *O*-glycans of the AtAGP31 Pro-Hyp-rich domain, thus leading to the formation of AtAGP31 oligomers [Hijazi *et al.* 2014, Ann Bot 114: 1087]. CWPs containing PAC domains are assumed to be part of non covalent protein/protein networks in cell walls allowing cell wall elongation [Hijazi *et al.* 2014, Front Plant Sci 5: 395]. This project will have three parts: (i) completion of the phylogenic study of PAC domain proteins using newly released genomic sequencing data to understand the origin of associated domains; (ii) characterization of the binding properties of available recombinant PAC domains to cell wall polysaccharides, based on preliminary results showing different specificities; (iii) obtention of knock out mutants of *Marchantia polymorpha* defective in either or both of the two PAC domain proteins using the CRISPR/CAS9 technology. The latter part of the project will pave the way for a PhD project. Indeed, the PAC domain protein family comprises only two members in *M. polymorpha*, compared to 14 members in *A. thaliana*. It will be easier to characterize a phenotype in *M. polymorpha* because of limited possible functional redundancy. In addition, complementation studies with *A. thaliana* proteins will be feasible. These mutants will provide a valuable tool to understand the function of PAC domain proteins in plant cell walls.
Illustration	 A model for protein/protein non-covalent networks in plant cell walls: the AtAGP31 protein as a candidate. Its His-rich domain is represented as a pink rectangel, its AGP domain as a brown one, its Pro/Hyp-rich domain as a green one and its PAC domain as a red one. Stars stand for conserved Cys residues. The *O*-glycosylations on the AGP and Pro/Hyp-rich domains are shown.