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The *Arabidopsis thaliana FASCICLIN LIKE ARABINOGALACTAN PROTEIN 4* gene acts synergistically with abscisic acid signalling to control root growth

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• **Background and Aims** The putative *FASCICLIN-LIKE ARABINOGALACTAN PROTEIN 4* (*At-FLA4*) locus of *Arabidopsis thaliana* has previously been shown to be required for the normal growth of wild-type roots in response to moderately elevated salinity. However, the genetic and physiological pathway that connects *At-FLA4* and normal root growth remains to be elucidated.
• **Methods** The radial swelling phenotype of *At-fla4* was modulated with growth regulators and their inhibitors. The relationship of *At-FLA4* to abscisic acid (ABA) signalling was analysed by probing marker gene expression and the observation of the *At-fla4* phenotype in combination with ABA signalling mutants.
• **Key Results** Application of ABA suppresses the non-redundant role of *At-FLA4* in the salt response. *At-FLA4* positively regulates the response to low ABA concentration in roots and is required for the normal expression of ABA- and abiotic stress-induced genes. The *At-fla4* phenotype is enhanced in the *At-abi4* background, while two genetic suppressors of ABA-induced gene expression are required for salt oversensitivity of *At-fla4*. Salt oversensitivity in *At-fla4* is suppressed by the CYP707A inhibitor abscinazole E2B, and salt oversensitivity in *At-fla4* roots is phenocopied by chemical inhibition of ABA biosynthesis.
• **Conclusions** The predicted lipid-anchored glycoprotein At-FLA4 positively regulates cell wall biosynthesis and root growth by modulating ABA signalling.

Key words: Fasciclin, arabinogalactan protein, root growth, cell wall, abscisic acid, plant cell wall signalling, *Arabidopsis thaliana*, *At-FLA4*.

INTRODUCTION

Among the biopolymers of the plant cell wall, the vast group of hydroxyproline-rich glycoproteins (Showalter *et al.*, 2010) comprises lightly glycosylated proline-rich proteins, moderately glycosylated extensins and highly glycosylated arabinogalactan-proteins (AGPs). The latter, structurally highly diverse family of glycoproteins has been implicated to have a wide range of biological roles; however, there is still an overall lack of understanding of the biophysical and biochemical mode of action of any individual AGP (Seifert and Roberts, 2007; Ellis *et al.*, 2010; Tan *et al.*, 2012).

A sub-group of AGPs, specified by the presence of one or two copies of a fasciclin domain (Fas1), have been termed fasciclin-like AGPs or FLAs (Johnson *et al.*, 2003). The Fas1 domain was named after glycoproteins found in the axonal fascicles of grasshoppers (Bastiani *et al.*, 1987); however, Fas1-containing proteins occur across all phyla (Moody and Williamson, 2013). Fas1 proteins mediate the adhesion between cells and their matrix, e.g. in bacterial biofilms (Moody and Williamson, 2013), or in animal cells, where Fas1 proteins such as periostin and transforming growth factor-β-induced matrix protein (βIG-h3) interact with extracellular matrix receptors of the integrin type (Gillan *et al.*, 2002; Kim *et al.*, 2002). Like many other Fas1-containing proteins, most FLAs localize to the outer leaflet of the plasma membrane via a lipid moiety – specifically a glycosylphosphatidylinositol (GPI) anchor – and associate with putative membrane nanodomains also known as lipid rafts (Borner *et al.*, 2003). The biological roles of FLAs in plant growth and development follow the theme of an involvement in cell wall deposition. The *Arabidopsis thaliana FLA11* and *FLA12* genes are preferentially expressed in secondary cell wall- (SCW) forming cells (Ito *et al.*, 2005; Persson *et al.*, 2005), similar to their orthologues from various other plant species (Dahiya *et al.*, 2006; MacMillan *et al.*, 2010; Liu *et al.*, 2013). Supporting a role in SCWs, the *At-fla11/At-fla12* double mutant displays a reduction in cellulose content accompanied by reduced tensile strength and tensile modulus of elasticity. This suggests an effect of FLAs both on cellulose deposition and on cell wall matrix integrity (MacMillan *et al.*, 2010). The authors of this paper propose a direct biomechanical role for At-FLA11 and At-FLA12 proteins in the cell wall matrix and also consider a role in signalling that might influence cellulose deposition. Indeed, there is accumulating evidence for FLAs to modulate signalling upstream of cell wall polymer biosynthesis. Overexpression of the cotton orthologue of *At-FLA12,* named *Gh-FLA1* (Liu *et al.*, 2013), in transgenic cotton, results in an increased rate of fibre initiation and elongation and causes an upregulation of a suite of genes related to cell wall biosynthesis including other *FLA* genes, whereas antisense suppression has the opposite effect (Huang *et al.*, 2013). This gain-of-function phenotype shows that FLAs can control the transcriptional programme for cell wall formation, which is best explained by a function in signalling.