Title: Plant Fucosyltransferases and the Emerging Biological Importance of Fucosylated Plant Structures

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Abstract

Plants frequently incorporate the monosaccharide L-fucose (Fuc; 6-deoxy-L-galactose) into glycans and glycopolymers located in diverse cellular locations. The incorporation of Fuc onto these varied glycans is carried out by fucosyltransferases (FUTs), that make up a protein superfamily with equally varied and diverse functions. The structures wherein Fuc is found have numerous proposed and validated functions, ranging from plant growth and development, cell expansion, adhesion and signaling, to energy metabolism, among others. FUTs from several different plant species have been identified and described; however, very few of them have been extensively characterized biochemically and biologically. In this review, we summarize plant FUTs that have been biochemically characterized and biologically investigated for associated phenotypes, offering greater insight and understanding into the physiological importance of Fuc in plants and in plant cell wall structures, glycans, and proteins.

Keywords: Fucosyltransferase, xyloglucan, arabinogalactan proteins, rhamnogalacturonan, *N*-glycan, *O*-fucosylation

Introduction

Fucose (Fuc; 6-deoxy-L-galactose) is a deoxyhexose sugar that is found in the glycans of diverse macromolecules in numerous species of plants, bacteria, fungi, mammals, and invertebrates. Fuc and other sugars are incorporated into macromolecules via the action of glycosyltransferases (GTs), which are enzymes that catalyze the transfer of a sugar from an activated sugar donor containing a phosphate leaving group. The incorporation of Fuc into these varied structures is carried out by specific enzymes, fucosyltransferases (FUTs), which are Leloir glycosyltransferases that catalyze the transfer of Fuc from guanidine 5'-diphosphate-β-L-Fucose (GDP-Fuc) to a suitable acceptor substrate, often an *N*-glycan, polysaccharide, or protein. FUTs belong to an enzyme superfamily that is sub-categorized based on the linkage in which Fuc is added onto the acceptor substrate, as follows: α-(1,2) FUT, α-(1,3) FUT, α-(1,4) FUT, α-(1,6) FUT, protein *O*–fucosyltransferase family 1 (POFUT1) and protein *O*–fucosyltransferase family 2 (POFUT2) (Martinez-Duncker, et al., 2003). Furthermore, these enzymes are classified into GT families, including; GT10, GT11, GT23, GT37, GT41, GT65, and GT68 in the Carbohydrate-Active enZYmes Database (CAZy, www.cazy.org); however, only GT10, GT37, and GT41 FUTs have been found in plants thus far (Both et al., 2011; Cantarel et al., 2009; Coutinho et al., 2003; Lombard et al., 2014).

In plants, Fuc has been found in the hemicellulosic polysaccharide, xyloglucan (XyG) in an α-(1,2)-linkage (Pauly and Keegstra, 2016); in the

pectic polysaccharides, rhamnogalacturonan I (RG-I) in an α-(1,2)-linkage, and rhamnogalacturonan II (RG-II) in both α-(1,2) and α-(1,4) linkages (Atmodjo et al., 2013); and on the extracellular, arabinogalactan proteins (AGPs) in an α-(1,2)-linkage (Tan et al., 2012), all of which are cell wall glycans. In addition, Fuc can also be present attached to proteins, either on *N*-glycans in an α-1,3 linkage to the proximal *N*-acetyl glucosamine (GlcNAc) of the core, in an α-1,4 linkage to the terminal GlcNAc residue of complex-type *N*-glycans (Staudacher et al., 1999); or directly attached to serine/threonine residues of proteins, in an *O*-linkage (Hallgren *et al.*, 1975; Figure 1).

A common feature of FUTs is that they all use the activated sugar nucleotide, GDP-Fuc as a donor. GDP-Fuc is synthesized from GDP-Mannose (GDP-Man), in a pathway consisting of three steps: 4,6-dehydration, 3,5-epimerization, and 4-reduction (Reiter and Vanzin, 2001). In the model plant species, *Arabidopsis thaliana* (*A. thaliana*) these three reactions are carried out by two separate types of enzymes. The first are GDP-D-Man-4,6-dehydratases encoded by isoforms *GMD1* and *GMD2. GMD2* was first identified in a mutagenesis screen as *AtMUR1*, taken from the Latin word, murus, or wall *(Reiter et al., 1993, 1997)*. The second enzyme is a GDP-4-keto-6-deoxy-D-Man (GDP-KDM) 3,5-epimerase-4-reductase, encoded by *GER1* (Bonin et al. 1997; Bonin and Reiter 2000). Interestingly, the *GMD2* (*AtMUR1*) gene contributes to the *de novo* biosynthesis of GDP-Fuc in most tissues, while *GMD1* contributes to its synthesis in a limited number of cell

types (Bonin et al., 2003). GDP-Fuc can also be synthesized from a salvage pathway that involves the direct phosphorylation of free Fuc followed by the attachment of guanosine monophosphate (GMP) (Feingold and Avigad, 1980).

In this review, we will offer an overview of plant FUTs that have been biochemically and biologically investigated and characterized. Although the number of plant FUTs that have been extensively characterized are few, the FUTs responsible for the addition of Fuc to many known fucosylated plant cell wall polysaccharides and other glycan structures have been identified, with the exception of the FUTs specific for RG-I and RG-II. Though relatively few in number, the plant FUTs included in this review offer valuable insight into the wide diversity of activities and specificities of these plant enzymes.

Xyloglucan-specific FUTs

XyGs are a family of hemicellulosic polysaccharides that have a β-(1,4)-linked-Glucose (Glc) backbone with sidechains that are initiated at the *O*-6 position with α-D-xylose (Xyl) (Pauly and Keegstra, 2016). XyGs are thought to contribute to cell wall strengthening in dicots and non-graminaceous monocots by binding to the hydrophobic surfaces of cellulose fibrils (Cosgrove, 2014; Darvill et al., 1985). To date, 19 different XyG sidechains have been identified from various plant species, and are described using an accepted single-letter nomenclature (Fry *et al.*, 2006; Tuomivaara *et al.*, 2015; Figure 1A). For example, an unsubstituted Glc is denoted by the letter

G, a backbone residue appended with α-D-Xyl is termed **X**, and when this xylosyl residue is further substituted by β-D-Gal it is called **L**. The **F** sidechain, characteristic of fucogalactoxyloglucan, consists of a backbone Glc residue that is substituted with α-L-Fuc-(1,2)-β-D-Gal-(1,2)-α-D-Xyl (Fry et al., 1993; Tuomivaara et al., 2015).

Three XyG-specific FUTs have been identified and characterized in plants, all of which are classified in the plant-specific GT37 family. The first XyG-specific FUT to be identified was isolated and purified from microsomal fractions of etiolated pea, *Pisum sativum*, stems (Farkas and Maclachlan, 1988). The enzyme, called *Ps*FUT1, was demonstrated to catalyze the *in vitro* transfer of radiolabeled Fuc from GDP-Fuc onto a Gal residue of exogenously available XyG acceptors. *Ps*FUT1 was shown to prefer tamarind XyG, where almost all Gal residues are not fucosylated, over XyG isolated from wildtype (WT) pea cell walls, where most Gal residues are already fucosylated. In the process of characterizing *Ps*FUT1 and its corresponding gene in pea, *AtFUT1* in *A. thaliana* was identified, based on sequence similarity to the pea gene. The corresponding gene in *A. thaliana* is also listed as *MUR2*, and was initially identified by screening chemically mutagenized *A. thaliana* plants for changes in neutral monosaccharide content of their walls (Reiter et al., 1997). The mutation responsible for the *mur2* chemotype was eventually shown to be in the gene *AtFUT1* (Faïk et al., 1997; Perrin et al., 1999). Heterologous expression of *AtFUT1* in a mammalian COS cell line yielded 41 times higher fucosyltransferase activity for tamarind XyG relative to a control

COS cell line expressing an empty vector, confirming that *At*FUT1, like *Ps*FUT1, is a XyG-specific FUT (Perrin et al., 1999). Interestingly, *in planta* *At*FUT1 has also been shown to fucosylate galacturonic acid (GalA) in certain types of XyGs, demonstrating that *At*FUT1 is capable of recognizing at least two XyG acceptor residues, Gal and GalA (Peña *et al.*, 2012).

In *A. thaliana*, XyG is produced by a Golgi-localized multi-protein complex that consists, at a minimum, of three xylosyltransferases (XXTs), XXT1, XXT2, and XXT5 as well as one β-(1,4)-glucan synthase, Cellulose Synthase-Like C4 (CSLC4) in the trans-Golgi network (TGN) (Chou et al., 2012). *At*FUT1 can simultaneously form homo-complexes through disulfide bonds or heterocomplexes via two interaction surfaces on the protein. Two separate heterocomplexes formed by *At*FUT1 have been documented, one with the galactosyltransferases (MUR3 and XLT2), another with XXT2 and XXT5 (Chou et al., 2015; Lund et al., 2015). Together these results suggest that *At*FUT1 along with the galactosyltransferases MUR3 and XLT2 also form part of the multi-protein complex involved in XyG biosynthesis (Chou et al., 2012, 2015; Lund et al., 2015).

In addition to the biochemical research done to determine the activity and specificity of *At*FUT1, structural studies have led to its successful crystallization (Rocha et al., 2016; Urbanowicz et al., 2017) and detailed analysis of its mechanism of activity (Urbanowicz *et al.*, 2017). Subsequent analyses of the enzyme structure determined that it adopts the glycosyltransferase B (GT-B) fold and is metallo-independent, like all other

FUT proteins that have been structurally characterized to date. A third XyG-specific FUT was identified in rice, *Oryza sativa,* by phylogenetic and coexpression analyses, and was subsequently named *Os*MUR2. Although the *Os*MUR2 protein has yet to be biochemically or biologically characterized in rice, the XyG Fuc deficiency in the *mur2 A. thaliana* mutant was successfully rescued when this mutant line was transformed with *OsMUR2*, indicating that *At*FUT1 and *Os*MUR2 are functionally equivalent *in planta (Liu et al., 2015; Vanzin et al., 2002)*.

In addition to being implicated in cell wall strengthening, fucosylated XyG has long been postulated to be involved in several plant growth responses (Pauly and Keegstra, 2016). To characterize the function of *Ps*FUT1 *in planta*, pea hairy root lines expressing full-length *PsFUT1* antisense mRNA were constructed (Wen et al., 2008). Hairy root lines expressing the *PsFUT1* antisense mRNA had 40-50% of the WT levels of *Ps*FUT1 mRNA. Emerging root tips appeared WT in morphology; however, elongating cells developed bulges that progressed into undifferentiated calluses within 2-4 weeks (Wen et al., 2008). Additionally, antisense hairy root tips surface labeled with the CCRC-M1 monoclonal antibody, that specifically recognizes α-L-fucosylated XyG (Puhlmann et al., 1994), displayed labeling patterns that differed from those observed in WT hairy root cells. This was due to cells being collapsed and wrinkled, which inhibited recognition and binding by CCRC-M1, as was discovered upon visualization with scanning electron microscopy (SEM) (Wen et al., 2008).

Similar disruptions to morphology have been reported for the trichomes of *mur*2 *A. thaliana* mutants, which have less than 2% of WT levels of fucosylated XyG (Vanzin et al., 2002). Accordingly, *mur2* mutants lack fucosylated XyG in all major plant organs, indicating that *At*FUT1 is solely responsible for the fucosylation of XyG. Despite the severe reduction of fucosylated XyG throughout the entire plant, *mur2* mutant plants grow indistinguishably from WT plants when grown under normal conditions, as well as under cold, heat, and salt stress, with the only detectable phenotype being the previously mentioned disruptions to trichomes (Vanzin et al., 2002).

AGP-Specific FUTs

AGPs are an abundant and diverse family of cell wall glycoproteins, with numerous and varied functions in plants, including cellular growth and stress responses. AGPs contain abundant amounts of hydroxyproline (Hyp), Ala, Ser, and Thr residues, and are extensively glycosylated on non-contiguous Hyp residues. Polysaccharide chains on the glycan portions of AGPs consist of β-(1,3) linked galactose (Gal) backbones decorated with β-(1,6) linked Gal side-chains that are further modified with α-linked arabinose (Ara) residues, as well as α-(1,2) linked Fuc, α-linked rhamnose (Rha), α-linked glucuronic acid (GlcA), and other sugars to a lesser extent (Showalter and Basu, 2016).

One AGP-specific FUT, α-L-FTase, from radish (*Raphanus sativus* L.), and two AGP-specific FUTs from *A. thaliana*, *At*FUT4 and *At*FUT6, have been identified and studied (Liang et al., 2013; Misawa et al., 1996; Tryfona et al.,

2012, 2014; Wu et al., 2010). α-L-FTase was identified in microsomal preparations from roots of 6-day old radish seedlings. Enzyme activity for α-L-FTase was measured fluorimetrically, and it was found that the enzyme successfully fucosylated a pyridylaminated (PA) trisaccharide consisting of L-Ara*f*-α-(1,3)-D-Gal*p*-β-(1,6)-D-Gal*p* (AraGalGal-PA). Subsequent chemical and enzymatic analyses of the fucosylated reaction product, (FucAraGalGal-PA), confirmed that fucosylation occurred on the *O*-2 of L-Ara*f* attached to β-(1,6)-linked D-Gal (Misawa et al., 1996). *At*FUT4 and *At*FUT6 are members of the plant-specific GT37 family and were initially postulated to be putative FUTs based on their sequence similarity to *At*FUT1 (Sarria et al., 2001). Early studies conducted on *At*FUT4 and *At*FUT6 were done using tobacco Bright Yellow-2 (BY-2) suspension-cultured cells that make non-fucosylated AGPs. Transient overexpression of *At*FUT4 and *At*FUT6 in BY-2 cells resulted in the production of AGPs with a Fuc moiety appended to *O*-2 of L-Ara*f* (Wu et al., 2010). However, *At*FUT4 and *At*FUT6 were unable to add Fuc to other glycopolymers such as RG-I and XyG *in vitro*, demonstrating the specificity of these two FUTs for AGPs. While *At*FUT4 and *At*FUT6 appear to have similar activities *in vitro*, their expression patterns *in planta* differ. *AtFUT6* is only expressed in the root, while *AtFUT4* is expressed in both the leaf and root (Sarria et al., 2001). Due to differences in their expression patterns, studies have demonstrated that *At*FUT4 is solely responsible for the fucosylation of leaf AGPs, while *At*FUT4 and *At*FUT6 are both required for the fucosylation of root AGPs (Liang et al., 2013; Tryfona et al., 2012, 2014).

Characterization of *fut4*, *fut6*, and *fut4/fut6* single and double mutants in *A. thaliana* revealed that the loss of these genes does not seriously impact plant growth. More specifically, when grown under normal physiological conditions *fut4*, *fut6*, and *fut4/fut6* grew comparably to WT plants when evaluated for phenotypes such as rosette size, height, branch number, dry weight, and flowering time (Tryfona et al., 2014). Interestingly, the *fut4/fut6* double mutant displayed an observable phenotype that was detected when mutant plants were subjected to stressful growth conditions, particularly salt stress. Under salt-stress conditions, ranging from 100-150 mM NaCl, *fut4/fut6* double mutants had significantly shorter roots relative to WT control plants also grown under salt stress (Liang et al., 2013; Tryfona et al., 2014). This observation supports the hypothesis that fucosylated AGPs are involved in some aspect of cell expansion in elongating root cells. Furthermore, these results suggest that the presence or absence of Fuc on AGP glycan structures may be a key determinant for proper cell growth under osmotic, or potentially other extracellular/environmental stresses.

This finding is in support of previous studies on *mur1* mutants of *A. thaliana*, which are impaired in Fuc biosynthesis. Accordingly, the AGPs isolated from *mur1* mutants are not substituted with Fuc in leaves and roots. Furthermore, these mutants also exhibited decreased root growth resulting from concurrent regions of normal and abnormal cell elongation. Despite phenotypic similarities, *mur1* mutants lack Fuc in all analyzed fucosylated glycopolymers, including AGPs, XyG, *N*-glycans, RG-I, and RG-II. Thus, the

root growth phenotype of *mur1* plants cannot be solely ascribed to the lack of fucosylated AGPs, but rather an overall reduction of Fuc in plant structures (Bonin et al., 1997). Regardless, the decreased root growth of *fut4/fut6* and *mur1* mutants appear to be related to the under-fucosylation of AGPs and, possibly other structures, suggesting the importance of Fuc attached on oligosaccharides and/or glycoproteins for proper cell expansion and elongation in plants.

More recent findings on the *At*FUT4 and *At*FUT6 proteins suggest that they are functionally equivalent *in vitro*, as both are able to fucosylate various arabinogalactan (AG)-related oligosaccharide structures (unpublished results of the authors). Furthermore, the differences in expression patterns of the *AtFUT4* and *AtFUT6* genes at the cellular level, suggest that *At*FUT4 is responsible for the majority of AGP fucosylation throughout the plant body, while both *AtFUT4* and *AtFUT6* work concurrently in the root, albeit in different locations. *AtFUT4* expression localizes only to the basal regions of the tap root and emerging lateral roots, while *AtFUT6* is expressed only in the tips of the tap root and emerging lateral roots (unpublished results of the authors).

Pectic Polysaccharides

In addition to XyG and AGPs, RG-I and RG-II are two other major classes of cell wall polysaccharides that contain Fuc. The pectic polysaccharides RG-I and RG-II are among the most structurally complex cell wall polysaccharides in plants. RG-I has a backbone of repeating [α-(1,4)-D-GalA-α-(1,2)-L-Rha]$_n$

units, with sidechain modifications of variously linked arabinose and galactose residues that also contain Fuc and GlcA to a lesser extent (Ridley et al., 2001). RG-II consists of an α-(1,4)-linked galacturonic acid (GalA) backbone, modified with sidechains A-F that consist of 12 different monosaccharides, including Fuc and 2-*O*-methyl-L-Fuc (MeFuc) present in sidechains A and B, respectively (Ndeh et al., 2017). RG-I and RG-II are implicated in various plant functions, ranging from cellular growth and expansion to wall porosity (Darvill et al., 1985; Mohnen, 2008; Ridley et al., 2001; Willats et al., 2001).

While Fuc has long been known to be present on RG-I and RG-II, the FUTs specific for adding Fuc to these polysaccharides remain unidentified. The Fuc found on RG-I is α-(1,2)-linked, and as such, the FUT responsible for this fucosylation is potentially one of the 7 uncharacterized members of GT37, which are predicted to be α-(1,2) FUTs in *A. thaliana (Sarria et al., 2001)*. RG-II also has two well characterized L-Fuc residues and a terminal L-Gal, which only differs from L-Fuc by having a hydroxymethyl group at C-6. There is a terminal non-reducing 2-*O*-Me-α-L-Fuc residue that is α-(1,2)-linked to D-Gal in sidechain B that is often acetylated. The Gal-Fuc disaccharide structure in sidechain B of RG-II is identical to that found in XyG; therefore, we hypothesize that the FUT responsible for catalyzing the transfer of Fuc to this Gal is related to the XyG-specific *At*FUT1 and also is a member of GT37. The second Fuc in RG-II is a 3,4-linked α-L-Fuc residue in the core oligosaccharide structure of sidechain A. This Fuc is more likely added by a FUT from an

entirely different GT family, possibly a member of the GT10 family that include α-(1,3)- and α-(1,4)-specific FUTs (Martinez-Duncker et al., 2003). However, three FUTs from this family, one in *A. thaliana (Wilson et al., 2001)*, one in mung bean (*Vigna radiata*) (Leiter et al., 1999), and one from tomato (*Solanum lycopersicum*) (Wilson, 2001), have been characterized, and all three are involved in *N*-glycosylation. Interestingly, there is also a terminal L-Gal present in sidechain A that is α-(1,2)-linked to D-GalA. Prior work on the *mur1* mutant of *A. thaliana*, which encodes GMD2, results in plants that lack L-Fuc and substitute L-Fuc with L-Gal (O'Neill et al., 2001; Reuhs et al., 2004), indicating that the FUTs catalyzing the synthesis of these glycans can also utilize GDP-L-Gal as a donor. Taken together, we hypothesize that the enzyme responsible for catalyzing the addition of the non-reducing terminal L-Gal on side chain A is also a member of GT37. The identification and detailed characterization of these additional FUTs would provide a more complete view on the fucosylation of cell wall polysaccharides, providing additional comparative insight into the specific activities of the GT37 FUTs, as well as the possible GT10 FUT involved in the synthesis of RG-II sidechain A.

_N_-glycan Specific FUTs

N-glycosylation is a highly conserved modification in plants and animals and is one of the most important post-translational modifications of proteins. *N*-glycosylation involves the attachment of oligosaccharides to asparagine residues with an Asn-X-Ser/Thr consensus sequence, termed a sequon, with

X being any amino acid other than proline (Staudacher et al., 1999). Unlike, mammalian *N*-glycans, plants often incorporate an α-(1,3)-linked Fuc onto the proximal *N*-acetylglucosamine (Glc*NAc*) of the core oligosaccharide attached to the protein (Strasser et al., 2004). This fucosyl residue is the key element that makes plant *N*-glycans antigenic to mammals, and has hindered the use of plants for the production of recombinant glycoproteins for medical applications (Bardor et al., 2003; Harmoko et al., 2016). The α-(1,3) and α-(1,4) FUTs required for *N*-linked glycan biosynthesis are more closely related to each other than to the α-(1,2) FUTs of GT37, such as those responsible for the fucosylation of XyGs and AGPs, and are therefore separately classified in GT10 in the CAZy database (Martinez-Dunker *et al.*, 2003). The first FUT with *N*-glycan core α-(1,3)-fucosyltransferase activity was identified and purified from mung bean (*Vigna radiate)* seedlings (Leiter et al., 1999; Staudacher et al., 1995). The enzyme was demonstrated to transfer Fuc from GDP-Fuc onto the Asn-linked Glc*NAc* core residue of *N*-glycans, as well as onto *N*-glycopeptides and oligosaccharides with the Glc*NAc*$_2$Man$_3$Glc*NAc*$_2$ glycan structure. The enzyme was unable to transfer onto *N*-glycans without terminal Glc*NAc* residues or onto *N*-acetyllactosamine, lacto-*N*-biose and *N*-acetylchito-oligosaccharides (Leiter et al., 1999; Staudacher et al., 1995). Following the characterization of the α-(1,3) FUT from mung bean, three genes related to the mung bean gene sequence were identified in *A. thaliana*; *AtFucTA* (*AtFUT11*), *AtFucTB* (*AtFUT12*), and *AtFucTC* (*AtFUT13*) (Wilson et al., 2001). Of the three, only

AtFucTA (*AtFUT11*) was successfully expressed in *Pichia pastoris*, and was demonstrated to catalyze the same reaction as the FUT from mung bean (Wilson *et al.*, 2001). Finally, an α-(1,4) FUT from tomato, expressed in *Pichia pastoris*, was demonstrated to have Lewis-a activity on the *N*-glycans of tomato, catalyzing the transfer of Fuc from GDP-Fuc to lacto-*N*-tetraose as well as β-(1,3) and β-(1,4)-galactosylated *N*-glycans (Wilson, 2001). Although *N*-glycan specific FUTs have been identified and biochemically studied in other plant species (Table 1), no follow up studies have been conducted for phenotypes associated with their mutations in those plant species, and as such, they will not be discussed in the scope of this review.

Few studies have been carried out to understand what, if any, impact the loss of α-(1,3) and α-(1,4) FUTs would have in plants. The *A. thaliana fucTA*, *fucTB*, and *fucTC* mutants have yet to be characterized. However, *A. thaliana* mutants that are otherwise impaired in the plant *N*-glycosylation pathway are generally embryo lethal or developmentally impaired and are, therefore, unable to be bred (Boisson et al., 2001; Lukowitz et al., 2000; von Schaewen, et al., 1993). Studies to elucidate the physiological significance of α-(1,3) and α-(1,4) *N*-glycan fucosylation have been successfully carried out in other model plant species, like rice and tobacco (Harmoko et al., 2016; Joly et al., 2002; Sim et al., 2018). Two independent studies conducted on T-DNA insertion lines for an α-(1,3)-fucosyltransferase gene in rice, *Os*08g36840, found that mutants are impaired in a number of features, including shoot growth, root elongation, flowering time, and plant height. Furthermore, these

plants are also impaired in their ability to respond to stresses such as high salinity, and the rice pathogen *Magnaporthe oryzae* (Harmoko et al., 2016; Sim et al., 2018). Mutants were also found to have lower levels of auxin-related transcription factors relative to their progenitor lines, and were accordingly determined to be impaired in polar auxin transport, the primary mechanism for the transport of auxin in the vascular meristem (Harmoko et al., 2016; Helen & Goldsmith, 1977). Studies on an α-(1,4) FUT protein in tobacco flowers showed that a constant, but relatively low level of expression (~20 pmol Fuc h^{-1} mg^{-1} protein) could be detected in different parts of the tobacco flower, and a 3-fold increase in activity was detected in both the stamen during anthesis and in pollinated pistils, with the highest levels of activity (~120 pmol Fuc h^{-1} mg^{-1} protein) being measured in mature pollen grains. The basal FUT activity detected in tobacco flowers suggest that α-1,4 fucosylation of *N*-glycans is a basic requirement during tobacco flower maturation, while the peaks in activity during pollen maturation could be ascribed to microgametogenesis and pollen tube elongation; no analyses on mutations in tobacco FUT proteins or genes have been conducted (Čapková et al., 1997; Joly et al., 2002).

POFUTs

As with *N*-glycan fucosylation, protein *O*-fucosylation is conserved between plants and other organisms, and entails the transfer of Fuc from GDP-Fuc directly onto a serine/threonine residue of proteins, an activity that was first identified in human urine (Hallgren et al., 1975). Protein *O*-fucosylation in

mammals and invertebrates is found on folded Epidermal Growth Factor-like (EGF) repeats and Thrombospondin Type 1 repeats (TSRs) and occurs in the endoplasmic reticulum (ER), where it is catalyzed with strict specificity by POFUT1 and POFUT2, respectively (Luo et al., 2006; Wang et al., 2001). About 100 potential human proteins have EGF repeats that POFUT1 could target, with the Notch receptor family being the most prevalent protein family to contain this motif (Okajima and Irvine, 2002; Shi and Stanley, 2003). The Notch signaling pathway is widely conserved evolutionarily and has been implicated in neurogenesis and embryonic development (Imayoshi and Kageyama, 2011). About 49 proteins in humans contain the TSR sequence targeted by POFUT2, most of which are secreted factors destined for the extracellular matrix, or are cell surface proteins that are involved with modulating cell signaling (Schneider et al., 2017).

The putative POFUTs in plants are unrelated to the POFUT1 and POFUT2 families found in other organisms and were classified by the presence of a domain of unknown function (DUF) 246 (PF03138/IPR024709) and are distantly related to CaZY family GT65, sometimes termed plant GT65R proteins (Hansen et al., 2012). They appear to be prevalent in plant genomes, with *A. thaliana* having 39 predicted POFUT-like genes (Hansen et al., 2012; Smith et al., 2018a) and are involved in growth and reproduction (Smith et al., 2018b). Despite their predicted prevalence, this family of GTs is by far the most understudied, with studies on members of this family having only been published within the last decade. Those that have been identified,

though, have not been biochemically characterized until recently, as described below.

Plants carrying mutations in proteins with a DUF246 domain have been investigated due to the variety of interesting phenotypes exhibited by plants when these genes are lost or disrupted, including the effects on diverse cell wall polymers. A Golgi-localized DUF246 containing protein, FRIABLE1 (FRB1), was found to affect cell adhesion and organ fusion in *A. thaliana*, and was the first member of this family to be identified in plants (Neumetzler et al., 2012). Loss of the *FRB1* gene product resulted in pleiotropic effects on cell wall architecture, particularly cell adhesion. This was due to alterations in both extensins and pectins that resulted in changes to the structure of the cell wall and middle lamella and consequently affected cell adhesion (Neumetzler et al., 2012). Interestingly another member of this family, ESMERALDA1 (ESMD1) did not exhibit any associated phenotype when the *esmd1* single mutant plant were generated. However, *frb1-2/esmd1-1* double mutants showed a rescue of the cell adhesion defect associated with *frb1 (Verger et al., 2016)*. In another suppressor screen, *quasimodo* mutants, defective in the putative pectin methyltranferase gene *QUASIMODO2* (*TSD2*, *OSU1*) similarly show a cell-detachment phenotype (Verger et al., 2016) that was rescued in the *qua2-1/esmd1-1* double mutant. Furthermore, a *qua2-1/frb1-2/esmd1-1* triple mutant also showed rescue of the cell-detachment phenotype, indicating that knocking out *ESMD1* rescues the cell adhesion defects caused by single mutations in *QUA2* and *FRB1(Verger et*

al., 2016). Recently, four members of the DUF246 family were biochemically characterized for the first time and shown to be UDP-β-L-Rha dependent 4-α-rhamnosyltransferases (RRTs) involved in the synthesis of the repeating disaccharide unit [2)-α-L-Rha-(1,4)-α-D-GalA-(1] of the RG-I backbone (Takenaka et al., 2018). This family is now classified as a new plant-specific GT family, GT106. The functional characterization of these enzymes calls into question the original bioinformatics predictions that this family is involved in protein fucosylation; however, more members will need to be biochemically characterized to elucidate the role of this protein family in plants (Takenaka et al., 2018).

The putative POFUT, SPINDLY (SPY) is classified as a GT41 enzyme and was recently shown to *O*-fucosylate DELLA proteins. DELLA proteins are negative transcriptional regulators of gibberellin (GA) signaling (Zentella et al., 2017). In *A. thaliana*, *O*-fucosylation activates DELLA proteins, so that they are then able to interact with other transcription factors involved in, for example, brassinosteroid and light signaling pathways (Zentella et al., 2017). Finally, the most recently studied putative plant POFUT, is *A. thaliana O*-FUCOSYLTRANSFERASE1 (*At*OFUT1). Mutant analyses showed that this protein is involved in pollen-pistil interactions, where a pollen tube physically penetrates specialized tissues during fertilization and germination (Smith et al., 2018a). Phylogenetic analysis indicated that *At*OFUT1 is more similar to metazoan POFUT1s, which are GDP-Fuc dependent FUTs that fucosylate specific Ser or Thr residues in CXXXX(S/T)C consensus sequences within EGF

repeat or TSR domains (Smith et al., 2018). In contrast to other putative or known plant POFUTs, *At*OFUT1 is categorized as a non-classified glycosyltransferase (GTnc) in the CAZy database. *Atoft*1 mutants were significantly impaired in the ability of their pollen tubes to penetrate the stigma-style interface, resulting in an almost 2,000-fold decrease in pollen transmission efficiency, and consequently displayed 5 to 10-fold decreased seed set (Smith et al., 2018). However, more data will be needed to confirm the biochemical function of *At*OFUT1.

Plant FUT phylogeny

Although the activities that plant FUTs catalyze are broad and diverse due to the innate complexities of plant cell wall polysaccharides, proteins and associated glycans, the plant FUTs are also distinguishable in how they relate phylogenetically to each other and to FUTs from the other kingdoms of life. Unlike vertebrate FUTs that form clades based on predicted specificity and function (Martinez-Duncker et al., 2003), the few and limited trees that have been published on plant FUTs exhibit an unusual relationship, with clades largely forming by species rather than predicted function (Sarria et al., 2001; Liu, Paulitz and Pauly, 2015).

A much larger phylogenetic analysis, generated for this review, of 206 plant FUTs sequences from 33 species corroborates this unique phylogenetic relationship among plant FUTs, with terminal clades generally comprising single-, or closely related species (Figure 2). This unique phylogenetic relationship, overall, suggests that sequence homology alone cannot be used

to deduce functional homology of FUTs from one plant species to another. This is exemplified by the case of the rice XyG FUT, *Os*MUR2, that is phylogenetically distinct from its functional homolog in *A. thaliana*, *At*FUT1 (Liu et al., 2015) (Figure 2).

It is interesting to note that while the FUTs from monocot grasses cluster within one sector of the phylogenetic tree distinct from other plant FUTs (Figure 2), the FUTs from any single monocot grass species are dispersed among the various terminal clades within the monocot grass domain of the tree. This pattern suggests that there might be functional orthologies among FUTs from different grass species, but this awaits experimental verification. It is also interesting that monocot grasses have large FUT families (>10) in spite of the fact that two commonly fucosylated cell wall glycans, XyGs and rhamnogalacturonans, are significantly less abundant in monocot grass walls than in walls from dicots and monocots outside of the Poales.

The unusual phylogenetic tree structure for plant FUTs also suggests that these proteins have very species-specific functions, perhaps even down to the cellular level. The three GT37 FUTs biochemically characterized thus far in *A. thaliana*, *At*FUT1, *At*FUT4, and *At*FUT6, exemplify this, as *At*FUT1 is XyG-specific, while *At*FUT4 and *At*FUT6 are both AGP-specific, but sub-localize to two distinct regions of the developing root (Sarria *et al.*, 2001; unpublished results of the authors). As we have alluded to throughout this review, a greater number of plant FUTs need to be functionally characterized to see if this hypothesis is valid. Unfortunately, the unusual phylogenetic relationship

exhibited by known and putative plant FUTs will complicate the functional characterization of additional FUTs in diverse plant species.

Conclusions

The carbohydrate-active enzymes involved in the biosynthesis of the plant cell wall are varied and unique in their activities and functions, and are typically encoded by large gene families, with the various known and putative FUTs being no exception to this pattern. While the activities of plant FUTs and the fucosylation of diverse glycans and proteins have been studied readily across many organisms, the biological importance of fucosylation *in planta* is just starting to be understood. With suggested and proven functions ranging from cellular communication and growth to cellular adhesion, the presence or absence of Fuc on various plant structures appears to have serious implications for proper plant development and response to diverse stimuli and stress. The FUTs specific for XyG fucosylation are by far the most thoroughly studied and well-understood. However, recent progress on the activities of the AGP-specific FUTs has offered additional insights into the activities and specificities of the plant-specific GT37 family. *N*-glycan fucosylation and the recent identification of the downstream targets of POFUTs, offer insight into the involvement of Fuc modifications in structures beyond the cell wall, as well as into the differences between conserved pathways in plants and vertebrates.

Characterization of FUTs in plant species other than *A. thaliana* has proven difficult, but not impossible. The continued research into the identification

and characterization of functional homologs from additional plant species, as well as the identification of the FUTs specific for RG-I and RG-II fucosylation promise to extend our understanding of the physiological role and importance of Fuc in plant cell wall polysaccharides. In addition, the characterization of more FUTs from other plant species would aid in understanding the unique evolutionary diversification pattern exhibited by this important family of biosynthetic enzymes in plants.

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The authors declare no competing interests.

Data and materials availability:

The data that support the findings of this study are present in the paper and any data are available from M.G.H. and B.R.U. upon reasonable request.

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Tables

FUT Type	Plant Species	Citation
α-(1,3) FUT	*Zea mays*	Bondili et al., 2006
α-(1,4) FUT	*Silene alba*	Léonard *et al.*, 2005
α-(1,4) FUT	*Vaccinium myrtillus L.*	Palma et al., 2001
α-(1,4) FUT	*Mangifera indica L.*	Okada et al., 2017
Undetermined	*Ricinus communis*	Roberts, Mellor and Lord, 1980

Table 1. Plant FUTs from additional plant species. These FUTs have been biochemically characterized to varying extents, but no mutational studies have been conducted for associated phenotypes.

Figure Legends

Figure 1. Fucosylated cell wall poly- and oligosaccharides. (A) Xyloglucan, (B) Arabinogalactan proteins, (C) *N*-Glycans, (D) Rhamnogalacturonan II, and (E) Rhamnogalacturonan I. Glc, glucose; Ara*f*, arabinofuranose; Ara*p*, arabinopyranose; GlcA, glucuronic acid; Gal, galactose; GalA, galacturonic acid; Kdo, 3-deoxy-D-*manno*-2-octulosonic acid; GlcNAc, *N*-acetylglucosamine; Dha, 3-deoxy-D-*lyxo*-2-heptulosonic acid; Xyl, xylose; Man, mannose; Rha, rhamnose; Fuc, fucose; Hyp, hydryxoproline; Ser, serine; Thr, threonine.

Figure 2. Phylogenetic tree of 206 plant FUTs from 33 species. A multiple sequence alignment of the amino acid sequences of these genes was truncated from position 1-340 and from positions 1,156-1,178 to omit large, poorly resolved gaps in the alignment. The truncated alignment was then used to make a phylogenetic tree by Neighbor-Joining with 200 bootstraps and rooted with a *Physcomitrella* clade consisting of the genes Physcomitrella|Pp3c6_13740V3.1 and Physcomitrella|Pp3c6_13730V3.1; both the alignment and tree were made in Geneious. Highlighted in red are the ten *A. thaliana* genes, nine of which form a terminal clade. The phylogenetically distinct, yet functional homolog to *At*FUT1 in rice, *Os*MUR2, is highlighted in purple. Finally, three more species are highlighted: banana in green, in which 12 out of 15 genes form a terminal clade; clubmoss in blue, in which five out of seven genes form a terminal clade; and *Populus* in orange, in which seven out of eight genes form terminal clades. These three additional clades are highlighted as further examples of the unusual, species-specific phylogenetic grouping of the plant FUTs.

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