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Title
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Permalink
https://escholarship.org/uc/item/1zv6q9sz

Journal
Critical Reviews in Plant Sciences, 38(4)

ISSN
0735-2689

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Publication Date
2019-07-04

DOI
10.1080/07352689.2019.1673968

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1 Title: Plant Fucosyltransferases and the Emerging Biological Importance of
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19 Keywords: Plant Cell Walls, Glycosyltransferase, Arabinogalactan Proteins,
20 Xyloglucan, Fucosyltransferase
21

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

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.

45 **Keywords**: Fucosyltransferase, xyloglucan, arabinogalactan proteins,
46 rhamnogalacturonan, *N*-glycan, *O*-fucosylation
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51 **Introduction**

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

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

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

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

97 types (Bonin et al., 2003). GDP-Fuc can also be synthesized from a salvage
98 pathway that involves the direct phosphorylation of free Fuc followed by the
99 attachment of guanosine monophosphate (GMP) (Feingold and Avigad,
100 1980).

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

109

110 **Xyloglucan-specific FUTs**

111 XyGs are a family of hemicellulosic polysaccharides that have a β-(1,4)-
112 linked-Glucose (Glc) backbone with sidechains that are initiated at the *O*-6
113 position with α-D-xylose (Xyl) (Pauly and Keegstra, 2016). XyGs are thought
114 to contribute to cell wall strengthening in dicots and non-graminaceous
115 monocots by binding to the hydrophobic surfaces of cellulose fibrils
116 (Cosgrove, 2014; Darvill et al., 1985). To date, 19 different XyG sidechains
117 have been identified from various plant species, and are described using an
118 accepted single-letter nomenclature (Fry *et al.*, 2006; Tuomivaara *et al.*,
119 2015; Figure 1A). For example, an unsubstituted Glc is denoted by the letter

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

125 Three XyG-specific FUTs have been identified and characterized in plants,
126 all of which are classified in the plant-specific GT37 family. The first XyG-
127 specific FUT to be identified was isolated and purified from microsomal
128 fractions of etiolated pea, *Pisum sativum*, stems (Farkas and Maclachlan,
129 1988). The enzyme, called *Ps*FUT1, was demonstrated to catalyze the *in vitro*
130 transfer of radiolabeled Fuc from GDP-Fuc onto a Gal residue of exogenously
131 available XyG acceptors. *Ps*FUT1 was shown to prefer tamarind XyG, where
132 almost all Gal residues are not fucosylated, over XyG isolated from wildtype
133 (WT) pea cell walls, where most Gal residues are already fucosylated. In the
134 process of characterizing *Ps*FUT1 and its corresponding gene in pea, *AtFUT1*
135 in *A. thaliana* was identified, based on sequence similarity to the pea gene.
136 The corresponding gene in *A. thaliana* is also listed as *MUR2*, and was
137 initially identified by screening chemically mutagenized *A. thaliana* plants for
138 changes in neutral monosaccharide content of their walls (Reiter et al.,
139 1997). The mutation responsible for the *mur2* chemotype was eventually
140 shown to be in the gene *AtFUT1* (Faïk et al., 1997; Perrin et al., 1999).
141 Heterologous expression of *AtFUT1* in a mammalian COS cell line yielded 41
142 times higher fucosyltransferase activity for tamarind XyG relative to a control

143 COS cell line expressing an empty vector, confirming that *At*FUT1, like
144 *Ps*FUT1, is a XyG-specific FUT (Perrin et al., 1999). Interestingly, *in planta*
145 *At*FUT1 has also been shown to fucosylate galacturonic acid (GalA) in certain
146 types of XyGs, demonstrating that *At*FUT1 is capable of recognizing at least
147 two XyG acceptor residues, Gal and GalA (Peña *et al.*, 2012).
148 In *A. thaliana*, XyG is produced by a Golgi-localized multi-protein complex
149 that consists, at a minimum, of three xylosyltransferases (XXTs), XXT1,
150 XXT2, and XXT5 as well as one β-(1,4)-glucan synthase, Cellulose Synthase-
151 Like C4 (CSLC4) in the trans-Golgi network (TGN) (Chou et al., 2012). *At*FUT1
152 can simultaneously form homo-complexes through disulfide bonds or
153 heterocomplexes via two interaction surfaces on the protein. Two separate
154 heterocomplexes formed by *At*FUT1 have been documented, one with the
155 galactosyltransferases (MUR3 and XLT2), another with XXT2 and XXT5 (Chou
156 et al., 2015; Lund et al., 2015). Together these results suggest that *At*FUT1
157 along with the galactosyltransferases MUR3 and XLT2 also form part of the
158 multi-protein complex involved in XyG biosynthesis (Chou et al., 2012, 2015;
159 Lund et al., 2015).
160 In addition to the biochemical research done to determine the activity and
161 specificity of *At*FUT1, structural studies have led to its successful
162 crystallization (Rocha et al., 2016; Urbanowicz et al., 2017) and detailed
163 analysis of its mechanism of activity (Urbanowicz *et al.*, 2017). Subsequent
164 analyses of the enzyme structure determined that it adopts the
165 glycosyltransferase B (GT-B) fold and is metallo-independent, like all other

166 FUT proteins that have been structurally characterized to date. A third XyG-
167 specific FUT was identified in rice, *Oryza sativa,* by phylogenetic and
168 coexpression analyses, and was subsequently named *Os*MUR2. Although the
169 *Os*MUR2 protein has yet to be biochemically or biologically characterized in
170 rice, the XyG Fuc deficiency in the *mur2 A. thaliana* mutant was successfully
171 rescued when this mutant line was transformed with *OsMUR2*, indicating that
172 *At*FUT1 and *Os*MUR2 are functionally equivalent *in planta (Liu et al., 2015;*
173 *Vanzin et al., 2002)*.
174 In addition to being implicated in cell wall strengthening, fucosylated XyG
175 has long been postulated to be involved in several plant growth responses
176 (Pauly and Keegstra, 2016). To characterize the function of *Ps*FUT1 *in planta*,
177 pea hairy root lines expressing full-length *PsFUT1* antisense mRNA were
178 constructed (Wen et al., 2008). Hairy root lines expressing the *PsFUT1*
179 antisense mRNA had 40-50% of the WT levels of *Ps*FUT1 mRNA. Emerging
180 root tips appeared WT in morphology; however, elongating cells developed
181 bulges that progressed into undifferentiated calluses within 2-4 weeks (Wen
182 et al., 2008). Additionally, antisense hairy root tips surface labeled with the
183 CCRC-M1 monoclonal antibody, that specifically recognizes α-L-fucosylated
184 XyG (Puhlmann et al., 1994), displayed labeling patterns that differed from
185 those observed in WT hairy root cells. This was due to cells being collapsed
186 and wrinkled, which inhibited recognition and binding by CCRC-M1, as was
187 discovered upon visualization with scanning electron microscopy (SEM) (Wen
188 et al., 2008).

189 Similar disruptions to morphology have been reported for the trichomes of
190 *mur*2 *A. thaliana* mutants, which have less than 2% of WT levels of
191 fucosylated XyG (Vanzin et al., 2002). Accordingly, *mur2* mutants lack
192 fucosylated XyG in all major plant organs, indicating that *At*FUT1 is solely
193 responsible for the fucosylation of XyG. Despite the severe reduction of
194 fucosylated XyG throughout the entire plant, *mur2* mutant plants grow
195 indistinguishably from WT plants when grown under normal conditions, as
196 well as under cold, heat, and salt stress, with the only detectable phenotype
197 being the previously mentioned disruptions to trichomes (Vanzin et al.,
198 2002).

199 **AGP-Specific FUTs**

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

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

212 2012, 2014; Wu et al., 2010). α-L-FTase was identified in microsomal
213 preparations from roots of 6-day old radish seedlings. Enzyme activity for α-
214 L-FTase was measured fluorimetrically, and it was found that the enzyme
215 successfully fucosylated a pyridylaminated (PA) trisaccharide consisting of L-
216 Ara*f*-α-(1,3)-D-Gal*p*-β-(1,6)-D-Gal*p* (AraGalGal-PA). Subsequent chemical and
217 enzymatic analyses of the fucosylated reaction product, (FucAraGalGal-PA),
218 confirmed that fucosylation occurred on the *O*-2 of L-Ara*f* attached to β-(1,6)-
219 linked D-Gal (Misawa et al., 1996). *At*FUT4 and *At*FUT6 are members of the
220 plant-specific GT37 family and were initially postulated to be putative FUTs
221 based on their sequence similarity to *At*FUT1 (Sarria et al., 2001). Early
222 studies conducted on *At*FUT4 and *At*FUT6 were done using tobacco Bright
223 Yellow-2 (BY-2) suspension-cultured cells that make non-fucosylated AGPs.
224 Transient overexpression of *At*FUT4 and *At*FUT6 in BY-2 cells resulted in the
225 production of AGPs with a Fuc moiety appended to *O*-2 of L-Ara*f* (Wu et al.,
226 2010). However, *At*FUT4 and *At*FUT6 were unable to add Fuc to other
227 glycopolymers such as RG-I and XyG *in vitro*, demonstrating the specificity of
228 these two FUTs for AGPs. While *At*FUT4 and *At*FUT6 appear to have similar
229 activities *in vitro*, their expression patterns *in planta* differ. *AtFUT6* is only
230 expressed in the root, while *AtFUT4* is expressed in both the leaf and root
231 (Sarria et al., 2001). Due to differences in their expression patterns, studies
232 have demonstrated that *At*FUT4 is solely responsible for the fucosylation of
233 leaf AGPs, while *At*FUT4 and *At*FUT6 are both required for the fucosylation of
234 root AGPs (Liang et al., 2013; Tryfona et al., 2012, 2014).

235 Characterization of *fut4*, *fut6*, and *fut4/fut6* single and double mutants in
236 *A. thaliana* revealed that the loss of these genes does not seriously impact
237 plant growth. More specifically, when grown under normal physiological
238 conditions *fut4*, *fut6*, and *fut4/fut6* grew comparably to WT plants when
239 evaluated for phenotypes such as rosette size, height, branch number, dry
240 weight, and flowering time (Tryfona et al., 2014). Interestingly, the *fut4/fut6*
241 double mutant displayed an observable phenotype that was detected when
242 mutant plants were subjected to stressful growth conditions, particularly salt
243 stress. Under salt-stress conditions, ranging from 100-150 mM NaCl,
244 *fut4/fut6* double mutants had significantly shorter roots relative to WT
245 control plants also grown under salt stress (Liang et al., 2013; Tryfona et al.,
246 2014). This observation supports the hypothesis that fucosylated AGPs are
247 involved in some aspect of cell expansion in elongating root cells.
248 Furthermore, these results suggest that the presence or absence of Fuc on
249 AGP glycan structures may be a key determinant for proper cell growth
250 under osmotic, or potentially other extracellular/environmental stresses.

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

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

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

276 **Pectic Polysaccharides**

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

281 units, with sidechain modifications of variously linked arabinose and
282 galactose residues that also contain Fuc and GlcA to a lesser extent (Ridley
283 et al., 2001). RG-II consists of an α-(1,4)-linked galacturonic acid (GalA)
284 backbone, modified with sidechains A-F that consist of 12 different
285 monosaccharides, including Fuc and 2-*O*-methyl-L-Fuc (MeFuc) present in
286 sidechains A and B, respectively (Ndeh et al., 2017). RG-I and RG-II are
287 implicated in various plant functions, ranging from cellular growth and
288 expansion to wall porosity (Darvill et al., 1985; Mohnen, 2008; Ridley et al.,
289 2001; Willats et al., 2001).

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

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

322 ***N*-glycan Specific FUTs**

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

327 X being any amino acid other than proline (Staudacher et al., 1999). Unlike,
328 mammalian *N*-glycans, plants often incorporate an α-(1,3)-linked Fuc onto
329 the proximal *N*-acetylglucosamine (Glc*NAc*) of the core oligosaccharide
330 attached to the protein (Strasser et al., 2004). This fucosyl residue is the key
331 element that makes plant *N*-glycans antigenic to mammals, and has
332 hindered the use of plants for the production of recombinant glycoproteins
333 for medical applications (Bardor et al., 2003; Harmoko et al., 2016). The α-
334 (1,3) and α-(1,4) FUTs required for *N*-linked glycan biosynthesis are more
335 closely related to each other than to the α-(1,2) FUTs of GT37, such as those
336 responsible for the fucosylation of XyGs and AGPs, and are therefore
337 separately classified in GT10 in the CAZy database (Martinez-Dunker *et al.*,
338 2003). The first FUT with *N*-glycan core α-(1,3)-fucosyltransferase activity
339 was identified and purified from mung bean (*Vigna radiate)* seedlings (Leiter
340 et al., 1999; Staudacher et al., 1995). The enzyme was demonstrated to
341 transfer Fuc from GDP-Fuc onto the Asn-linked Glc*NAc* core residue of *N*-
342 glycans, as well as onto *N*-glycopeptides and oligosaccharides with the
343 GlcNAc_2Man$_3$GlcNAc_2 glycan structure. The enzyme was unable to transfer
344 onto *N*-glycans without terminal Glc*NAc* residues or onto *N*-
345 acetyllactosamine, lacto-*N*-biose and *N*-acetylchito-oligosaccharides (Leiter
346 et al., 1999; Staudacher et al., 1995). Following the characterization of the α-
347 (1,3) FUT from mung bean, three genes related to the mung bean gene
348 sequence were identified in *A. thaliana*; *AtFucTA* (*AtFUT11*), *AtFucTB*
349 (*AtFUT12*), and *AtFucTC* (*AtFUT13*) (Wilson et al., 2001). Of the three, only

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

360 Few studies have been carried out to understand what, if any, impact the
361 loss of α-(1,3) and α-(1,4) FUTs would have in plants. The *A. thaliana fucTA*,
362 *fucTB*, and *fucTC* mutants have yet to be characterized. However, *A. thaliana*
363 mutants that are otherwise impaired in the plant *N*-glycosylation pathway
364 are generally embryo lethal or developmentally impaired and are, therefore,
365 unable to be bred (Boisson et al., 2001; Lukowitz et al., 2000; von Schaewen,
366 et al., 1993). Studies to elucidate the physiological significance of α-(1,3)
367 and α-(1,4) *N*-glycan fucosylation have been successfully carried out in other
368 model plant species, like rice and tobacco (Harmoko et al., 2016; Joly et al.,
369 2002; Sim et al., 2018). Two independent studies conducted on T-DNA
370 insertion lines for an α-(1,3)-fucosyltransferase gene in rice, *Os*08g36840,
371 found that mutants are impaired in a number of features, including shoot
372 growth, root elongation, flowering time, and plant height. Furthermore, these

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

391 **POFUTs**

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

396 mammals and invertebrates is found on folded Epidermal Growth Factor-like
397 (EGF) repeats and Thrombospondin Type 1 repeats (TSRs) and occurs in the
398 endoplasmic reticulum (ER), where it is catalyzed with strict specificity by
399 POFUT1 and POFUT2, respectively (Luo et al., 2006; Wang et al., 2001).
400 About 100 potential human proteins have EGF repeats that POFUT1 could
401 target, with the Notch receptor family being the most prevalent protein
402 family to contain this motif (Okajima and Irvine, 2002; Shi and Stanley,
403 2003). The Notch signaling pathway is widely conserved evolutionarily and
404 has been implicated in neurogenesis and embryonic development (Imayoshi
405 and Kageyama, 2011). About 49 proteins in humans contain the TSR
406 sequence targeted by POFUT2, most of which are secreted factors destined
407 for the extracellular matrix, or are cell surface proteins that are involved with
408 modulating cell signaling (Schneider et al., 2017).

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

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

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

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

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

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

473 **Plant FUT phylogeny**

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

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

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

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

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

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

513 **Conclusions**

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

532 Characterization of FUTs in plant species other than *A. thaliana* has proven
533 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.

Acknowledgements

Work on fucosyltransferases in the authors' laboratories has been supported by the National Science Foundation Plant Genome Program (IOS-0923992) and the Center for Bioenergy Innovation (Oak Ridge National Laboratory), a US Department of Energy (DOE) Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science. The authors also acknowledge the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the US Department of Energy through grant DE-SC0008472 for funding studies of cell-type specific pectins in plant cell walls. The authors are grateful to Alan Darvill for a critical reading of the manuscript and to Jim Leebens-Mack for providing the dataset used for generation of the phylogenetic tree.

Author Contributions:

M.J.S, B.R.U., and M.G.H. wrote the manuscript.

Competing interests:

557 The authors declare no competing interests.

558 **Data and materials availability:**

559 The data that support the findings of this study are present in the paper and

560 any data are available from M.G.H. and B.R.U. upon reasonable request.

561

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860

862 **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

863

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

867

868 **Figure Legends**

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

877

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

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