deletion results in a reading frame shift and the introduction of a stop codon at amino acid 181, changing the last two amino acids from Glu-Asn to Asp-Leu. This mutated locus encodes a truncated Ig-α chain harboring 21 of the 61–amino acid cytoplasmic tail.
10. Bone marrow, spleen, lymph node, and peritoneal exudate cells were isolated from *mb*-$1^{\Delta c/\Delta c}$ or control (C57BL/6 × CB.20)F_2 littermates. Cells were stained directly with fluorescein isothiocyanate (FITC)– or phycoerythrin-coupled reagents or indirectly with biotinylated antibodies followed by streptavidin-CyChrome (Pharmingen, San Diego) and analyzed with a FACScan (Becton Dickinson). Monoclonal antibodies used were anti-CD43 (S7), anti-IgM (R33-24), anti-B220/CD45R (RA3-6B2), anti-IgM^a (RS3.1), anti-IgM^b (MB86), anti-CD3 (145-2C11), anti-CD5 (53-6.7), anti-IgD (1.3-5), and anti-CD22 (Lyb 8.2). For intracellular staining, cells were fixed in paraformaldehyde and stained in 0.5% saponin (Sigma) as described [M. Assenmacher, J. Schmitz, A. Radbruch, *Eur. J. Immunol.* **24**, 1097 (1994)] with either FITC-conjugated anti-IgM heavy chain (M41) or anti-κ (R33-18).
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21. Spleens were cut in 6-μm sections and stained with biotinylated anti-IgM or anti-CD4 (GK1.4) after acetone fixation. Antibody was detected with streptavidin–alkaline phosphatase and substrate followed by hematoxylin counterstaining according to standard procedures.
22. C57BL/6 *mb*-$1^{\Delta c/\Delta c}$ or control littermates (9 to 12 weeks of age) were immunized intraperitoneally with either 100 μg of alum-precipitated NP_{15}-CG or 5 to 10 μg of NP_{27}-Ficoll and serum collected 7, 10, or 14 days later (*12*). NP-specific λ1 and IgG3 (TI) or IgG1 (TD) Ig titers were assayed by enzyme-linked immunosorbent assay (ELISA) as described [J. Roes and K. Rajewsky, *J. Exp. Med.* **177**, 45 (1993)]. The limit of detection for NP-specific IgG3, IgG1, or λ1 ELISA is 0.064 μg/ml. Five mutant and control mice were used for each immunization.
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24. Splenic B cells were enriched from control or *mb*-$1^{\Delta c/\Delta c}$ mice by $B220^+$ magnetic cell sorting and cultured in vitro for 18 hours at 1×10^6 cells/ml with anti-IgM. Polyclonal goat anti-mouse IgM $F(ab')_2$ (Jackson ImmunoResearch Labs) or monoclonal antibody b7.6 were used for anti-IgM stimulation. CD86 expression was revealed by flow cytometric analysis with anti-B7.2 (GL-2). In a titration with goat anti-mouse IgM $F(ab')_2$ or monoclonal antibody b7.6, control and mutant splenocytes exhibited a similar dose-response relation in terms of CD86 upregulation.
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26. The antibody response from control animals was distributed over a wide range of titers and gave geometric means that were 700- and 1000-fold over the assay limit of detection for IgG3 and λ, respectively (Fig. 4B). A similar variation in the TI antibody response from *mb*-$1^{\Delta c/\Delta c}$ mice should have resulted in titers that were within the range of detection, at least for some of the animals, if this response were reduced to a similar extent as the TD antibody response. In independent immunizations, however, a measurable response was never elicited in the mutant animals.
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35. We gratefully acknowledge K. Hathcock for providing monoclonal antibody GL-2; C. Uthoff-Hachenberg and E. Popa for technical assistance; R. Pelanda, R. Rickert, and A. Tarakhovsky for critical reading of the manuscript; and U. Ringeisen for assistance with the art work. Supported by the Deutsche Forschungsgemeinschaft through SFB 243, The Land Nordrhein-Westfalen, and the Human Frontier Science Program. R.M.T. was supported by fellowships from the Human Frontier Science Program Organization and Kölner Verein zur Förderung der Immunologie.

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Substitution of L-Fucose by L-Galactose in Cell Walls of *Arabidopsis mur1*

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An *Arabidopsis thaliana* mutant (*mur1*) has less than 2 percent of the normal amounts of L-fucose in the primary cell walls of aerial portions of the plant. The survival of *mur1* plants challenged the hypothesis that fucose is a required component of biologically active oligosaccharides derived from cell wall xyloglucan. However, the replacement of L-fucose (that is, 6-deoxy-L-galactose) by L-galactose does not detectably alter the biological activity of the oligosaccharides derived from xyloglucan. Thus, essential structural and conformational features of xyloglucan and xyloglucan-derived oligosaccharides are retained when L-galactose replaces L-fucose.

To date only one mutant of *Arabidopsis thaliana* has been shown to affect the glycosyl compositions of cell wall polysaccharides (*1*). Plants carrying the *mur1* mutation are more brittle than wild-type plants, are slightly dwarfed, and have an apparently normal life cycle. The scarcity of cell wall mutants suggests that such mutations are lethal if they eliminate sugar residues essential for the integrity of the cell wall or the function of cell wall–derived oligosaccharins (*2*). The *mur1* mutation offers an opportunity to study the effects of the greatly reduced amounts of L-fucose (L-Fuc) on the structure and function of the

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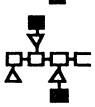
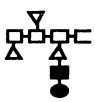
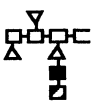
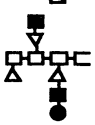
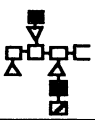
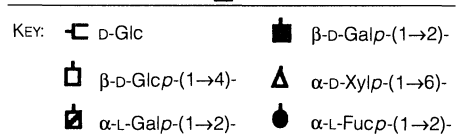
Table 1. XG subunit composition of *A. thaliana* wild-type and *mur1* plants (*26*)

Oligosaccharide	Structure	Composition (mol%)	
		Wild-type	*mur1*
XXXG		37.6	28.2
XLXG		5.9	2.1
XXLG		2.2	28.2
XLLG		2.2	24
XXFG		20.2	0
XXJG		0	6.5
XLFG		31.9	0
XLJG		0	11



cell wall and its derived oligosaccharins.

The primary cell walls of higher plants typically contain cellulose, three pectic polysaccharides, at least two hemicelluloses, and often small amounts of structural proteins (*3*). Fucose is a component of the structurally unrelated pectic polysaccharides rhamnogalacturonan-I and -II and the hemicellulose xyloglucan (XG) (*3*). Xyloglucan is believed to noncovalently cross-link cellulose microfibrils and to act as a load-bearing structure in primary cell walls (*4*–*6*). About 75% of the 4-linked β-D-glucosyl residues in the backbone of the XGs of most dicotyledonous plants are substituted at O-6 with an α-D-xylosyl residue (*6*). From 20 to 30% of these α-D-xylosyl residues are substituted (Table 1) at O-2 with β-D-galactopyranosyl (Gal*p*) or α-L-Fuc*p*-(1→2)-β-D-Gal*p* moieties (where Fuc*p* is fucopyranosyl) (*7*).

We isolated and characterized XG and its subunit oligosaccharides (*8*) from *mur1* plants. The nona- and decasaccharide subunits of XG from *mur1* plants (Table 1) do not contain terminal α-L-Fuc*p* residues characteristic of the corresponding subunits of XG in wild-type plants (*9*). However, the nona- and decasaccharide subunits of *mur1* plants contain an α-L-Gal*p*-β-D-Gal*p*-α-D-Xyl*p* (where Xyl*p* is xylopyranosyl) side chain in which a terminal α-L-galactosyl residue, a sugar not otherwise found in XGs, replaces the normal terminal α-L-fucosyl residue (Fig. 1 and Table 1). α-L-Galactose (Gal) is stereochemically similar to α-L-Fuc and has been found in some higher plants (*10*). However, the absolute configuration (D or L) of galactose is rarely determined. We used a combination of fast atom bombardment–mass spectrometry (FAB-MS) (*11*), ^{1}H-nuclear magnetic resonance (NMR) spectroscopy (*12*), and gas chromatography (GC) analysis (*13*) of the trimethylsilylated (+ and −)-2-butyl glycosides of the galactosyl residues (Fig. 1 and Tables 1 and 2) to determine the structures, including the anomeric and absolute configurations of the terminal galactosyl residues of the nona- and decasaccharide subunits of *mur1*. The terminal α-L-Fuc*p* residues of the XG nona- and decasaccharides of the wild-type *A. thaliana* plants are replaced in the *mur1* mutants by terminal α-L-Gal*p* residues.

The aerial portions of *mur1* plants grown in the presence of L-Fuc contain normal amounts of L-Fuc (*1*). Thus, the enzyme in *mur1* that catalyzes the transfer of fucosyl residues from guanosine diphosphate (GDP)-L-Fuc to XG is not altered. The *mur1* mutation probably incapacitates one of the enzymes responsible for the conversion of GDP-D-mannose to GDP-L-Fuc (*14*).

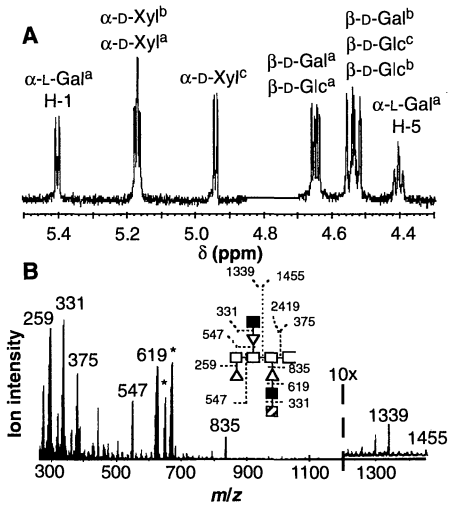


Fig. 1. (**A**) Partial 500-MHz ^{1}H-NMR spectrum of XLJGol from *A. thaliana mur1* showing all the α-anomeric resonances and the H-5 resonance of α-L-Gal*p*. See Tables 1 and 2 and (*27*) for nomenclature and chemical shift data. The structure of XLJGol is identical to that of XLLGol (*7*) except that the side chain attached to β-D-Glc^a is extended by the addition of an α-L-Gal*p* residue. The ^{1}H-NMR spectrum of XLJGol differs from that of XLLGol (*7*) in that it contains additional resonances due to the α-L-Gal*p* residue. Furthermore, the chemical shifts of the β-D-Gal^a residue bearing this α-L-Gal*p* residue are affected (Table 2). The H-5 protons of the α-L-Gal*p* residue in XLJGol [δ 4.409 ppm (parts per million)] and H-5 of the α-L-Fuc*p* residue in XLFGol (δ 4.523) (*7*) both resonate at an unusually high frequency, probably as a result of conformationally induced deshielding. This suggests that the replacement of α-L-Fuc*p* with α-L-Gal*p* does not significantly alter the conformation of the oligosaccharide. (**B**) The positive-ion FAB mass spectrum of per-*O*-acetylated XLJGol (*11*). The series of ions at mass-to-charge ratio (*m/z*) 331, 619, and 835 confirms the presence of the triglycosyl side chain α-L-Gal*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p*. The ions at *m/z* = 547 and 1339 confirm that the triglycysol side chain is not attached to either Glc^b or Glc^c. The ion at *m/z* = 1455 confirms that the triglycosyl side chain is attached to Glc^a. Asterisks identify ions due to a noncarbohydrate contaminant. The portion of the spectrum beyond 1300 is magnified ×10 in intensity.

Table 2. ^{1}H-NMR assignments for the glycosyl residues of XLLGol and XLJGol (*27*). The columns labeled Δδ refer to the change in chemical shift in parts per million (ppm) that occurs upon conversion of XLLGol to XLJGol by addition of the α-L-Gal residue.

Glycosyl residue	H-1		Δδ (ppm)	H-2		Δδ (ppm)
	XLLGol	XLJGol		XLLGol	XLJGol	
α-L-Gal^a	–	5.398	–	–	3.839	–
β-D-Gal^a	4.559	4.653	0.094	3.618	3.771	0.153
β-D-Gal^b	4.559	4.551	−0.008	3.618	3.614	−0.004
α-D-Xyl^a	5.161	5.163	0.002	3.679	3.677	−0.002
α-D-Xyl^b	5.175	5.171	−0.004	3.672	3.668	−0.004
α-D-Xyl^c	4.943	4.941	−0.002	3.545	3.541	−0.004
β-D-Glc^a	4.635	4.645	0.010	3.440	3.432	−0.008
β-D-Glc^b	4.545	4.526	−0.019	3.419	3.423	0.004
β-D-Glc^c	4.535	4.529	−0.006	3.341	3.338	−0.003

L-Galactose differs from L-Fuc only by the presence of an oxygen atom attached to C-6. If the fucosyltransferase can use GDP-L-Gal as a substrate, the presence of terminal α-L-Gal*p* residues on *mur1* XG can be accounted for. The possibility that the transferase can use L-Gal*p* as a substitute for L-Fuc*p* is made more likely by the recent demonstration that a GDP-L-Gal, substituted at C-6 with an eight-atom spacer linked to either biotin or a blood group A–active trisaccharide, is an efficient substrate for a mammalian fucosyltransferase (*15*).

Enzymatic and chemical methods release similar amounts of XG and XG oligosaccharides from *mur1* and wild-type *A. thaliana* cell walls (Table 1). However, the amount of terminal α-L-Gal*p* in *mur1* XG is less than about half of the amount of α-L-Fuc*p* in wild-type XG (Table 1). Thus, if the fucosyltransferase is responsible for incorporating α-L-Gal*p* into the XG of the mutant, then either GDP-L-Gal is a poorer substrate for the transferase than is GDP-L-Fuc or there is less GDP-L-Gal available in mutant plants than there is GDP-L-Fuc in wild-type plants.

The fucosylated XG nonasaccharide (XXFG; see Table 1 for the corresponding structure of each oligosaccharide referred to with a four-letter abbreviation) inhibits auxin-stimulated pea stem elongation (*16*) and is therefore an oligosaccharin. The terminal α-L-Fuc*p* residue of XXFG is required for this growth inhibition (*17*, *18*). We tested the ability of the L-galactosyl–containing nonasaccharide (XXJG) to inhibit auxin-stimulated pea stem elongation. XXJG and XXFG inhibit, at the same concentrations and to the same extent, auxin-stimulated pea stem elongation (Fig. 2). Thus, XXJG produced by *mur1* is an oligosaccharin; additionally, L-Gal acts both as a functional and structural homolog of L-Fuc in *Arabidopsis* XG.

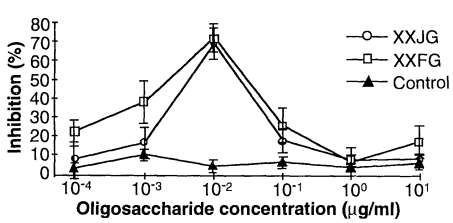


Fig. 2. Effect of XXJG and XXFG on 2,4-dichlorophenoxyacetic acid (2,4-D)–stimulated elongation of pea stem segments (*28*). (○) XXJG, (□) XXFG, and (△) control mixture of nonfucosylated XG oligosaccharides isolated from tamarind seed. Values are percentage of inhibition as described in (*18*). Each value is the average of four treatments. Error bars indicate the standard deviation. The value of each treatment was calculated from measurements of 10 segments. Concentration of 2,4-D was 5 μM.

The slightly smaller stature and fragility of the stems of *mur1* plants (*1*) may result from one or more of the following: (i) α-L-Gal*p* does not fully mimic the function of α-L-Fuc*p*, (ii) the lower content of XXJG and XLJG in the XG of *mur1* plants compared with the content of XXFG and XLFG in the XG of the wild-type plant, or (iii) incomplete substitution of α-L-Fuc*p* in other cell wall polysaccharides. The results presented here provide support for the importance of defined structures for the biological activity of oligosaccharins and the integrity and functioning of the plant cell wall.

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8. Cell walls of *mur1* plants were prepared and XG was isolated by the procedures used for wild-type XG (*9*). XG subunit oligosaccharides from *mur1* were generated (*9*) and then separated on an analytical CarboPac-I PA1 anion-exchange column installed in a Dionex BioLC system. The column was eluted at a rate of 1 ml/min with 50 mM NaOAc in 100 mM NaOH (0 to 1.1 min) followed by a linear gradient of 50 to 70 mM NaOAc in 100 mM NaOH (1.1 to 40 min). The oligosaccharides in the eluant were monitored by pulsed amperometric detection. Masses of the subunit oligosaccharides were determined by matrix-assisted laser desorption (MALDI)–time of flight (TOF) MS, performed on a Hewlett Packard LDI 1700XP spectrometer (*19*). 2,5-Dihydroxybenzoic acid was used as the matrix. All determined masses were within 0.1% of the calculated chemical mass. Glycosyl-residue and glycosyl-linkage compositions of the oligosaccharides were determined as described (*20*). Partially methylated alditol acetate derivatives of *t*-Xyl*p*, 2-Xyl*p*, *t*-Gal*p*, 2-Gal*p*, 6-Glc*p*, 4,6-Glc*p*, and 4-glucitol (4-Glcol) were recovered from XLJGol (*12*) in a molar ratio of 1:2:2:0.8:1.3:2:0.7. Relative amounts of XG subunit oligosaccharides were determined by reversed-phase high-performance liquid chromatography (HPLC) of their *N*-(*p*-nitrobenzyloxy)-aminoalditol derivatives (*21*), the structures of which were confirmed by MALDI-TOF MS.
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11. XLJG, prepared as described (*8*), was reduced with $NaBH_4$ and acetylated (*22*). The FAB mass spectrum was recorded with a JEOL JMS-SX/SX102A tandem mass spectrometer operating at low resolution (1:1000) with an accelerating voltage of 10 kV.
12. XLJG was purified as described (*8*) and converted to XLJGol by reduction with $NaBH_4$. Exchangeable protons were replaced with deuterons and the 500-MHz ^{1}H-NMR spectrum recorded at 300 K as described (*22*).
13. The absolute configurations (D and L) of the galactosyl residues in the *mur1* XG oligosaccharides were determined by capillary GC analysis, on a fused silica DB-1 column (*20*), of their trimethylsilylated (+ or −) -2-butyl α- and β-glycoside derivatives (*23*). D- and L-Gal were present in a 2:1 ratio. A mixture of authentic D-Xyl, D-glucose (D-Glc), D-Gal, and L-Gal in a molar ratio of 3:4:2:1 produced a GC profile identical to that obtained from authentic XLJG.
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26. XG oligosaccharide subunits were obtained from wild-type or *mur1* plants of *A. thaliana*, or both (*8*). The letter codes identifying the subunits follow the accepted XG nomenclature (*24*). The letter J is used to represent the β-D-Glc*p*- residue bearing an α-L-Gal*p*-β-D-Gal*p*-α-D-Xyl*p* side chain, first detected in jojoba seed XG (*25*).
27. Chemical shifts are measured relative to internal acetone (δ 2.225 ppm). Assignments for XLJGol are based on analysis of the COSY (correlated spectroscopy) spectrum. Assignments for XLLGol are taken from (*7*). The superscripts a, b, and c refer to the position of the residue relative to the glucitol moiety [Glc^c→Glc^b→Glc^a→Glcol] (*7*). Specific side chain residues are indicated by using the superscript letter of the β-D-Glc*p* residue to which the side chain is attached. β-D-Gal^a is the glycosyl residue in XLJGol bearing the α-L-Gal residue at O-2. The α-L-Gal*p* resonances not listed in Table 2 were assigned as follows: H-3 at δ 3.890, H-4 at δ 3.998, H-5 at δ 4.409, and H-6/H-6′ at δ 3.767 ppm.
28. Jojoba seeds are a rich source of XGs (*25*), particularly of XXJG. Therefore, the XXJG used in the bioassay was isolated from jojoba seeds. Jojoba seeds were milled and the resulting powder defatted with hexanes (40 g of powder suspended in 500 ml of hexanes for 15 hours). The defatted powder was washed with deionized H_2O by centrifugation. XG was extracted from the washed powder with 4 M KOH, and oligosaccharide subunits were generated and purified from the neutral fraction (*9*). The pea stem elongation assay was performed essentially as described (*18*), except stems were taken from seedlings after onset of development of the 3rd node (in preparation).

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