

In Vivo Colocalization of Xyloglucan Endotransglycosylase Activity and Its Donor Substrate in the Elongation Zone of Arabidopsis Roots

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We have developed a method for the colocalization of xyloglucan endotransglycosylase (XET) activity and the donor substrates to which it has access *in situ* and *in vivo*. Sulforhodamine conjugates of xyloglucan oligosaccharides (XGO-SRs), infiltrated into the tissue, act as acceptor substrate for the enzyme; endogenous xyloglucan acts as donor substrate. Incorporation of the XGO-SRs into polymeric products in the cell wall yields an orange fluorescence indicative of the simultaneous colocalization, in the same compartment, of active XET and donor xyloglucan chains. The method is specific for XET, as shown by competition experiments with nonfluorescent acceptor oligosaccharides, by negligible reaction with cello-oligosaccharide-SR conjugates that are not XET acceptor substrates, by heat lability, and by pH optimum. Thin-layer chromatographic analysis of remaining unincorporated XGO-SRs showed that these substrates are not extensively hydrolyzed during the assays. A characteristic distribution pattern was found in Arabidopsis and tobacco roots: in both species, fluorescence was most prominent in the cell elongation zone of the root. Proposed roles of XET that include cell wall loosening and integration of newly synthesized xyloglucans could thus be supported.

INTRODUCTION

The primary cell walls of flowering plants consist fundamentally of a framework of cellulose microfibrils embedded in a matrix of hemicellulose, pectins, and structural proteins (Carpita and Gibeau, 1993; Brett and Waldron, 1996). Xyloglucan, the major hemicellulosic polysaccharide in the primary cell wall matrix of dicots, consists of a backbone of β -(1 \rightarrow 4)-linked D-glucose residues, the majority of which are α -D-xylosylated at O-6. Some xylose residues are further substituted by galactosyl and fucosyl-galactosyl groups. Other, minor carbohydrate side chains and O-acetyl groups are also present (Fry, 1989a; Hayashi, 1989). Because xyloglucans can form tight hydrogen bonds with cellulose microfibrils (Valent and Albersheim, 1974; Hayashi et al., 1987, 1994a, 1994b; Hayashi, 1989), they may thereby tether adjacent microfibrils (Fry, 1989b). A proportion of the xyloglucan molecules are covalently attached to acidic pectins (Thompson and Fry, 2000). Xyloglucans also serve as storage polysaccharides in some seeds (Edwards et al., 1985).

For plant cells to expand, cellulose microfibrils in parallel alignment need to move apart or past one another, and this movement may create the possibility for newly synthesized xyloglucan molecules to become hydrogen-bonded (Fry, 1989b). Because xyloglucan tethers are thought to be the principal tension-bearing molecules in the cell wall, breaking of the tethers has been proposed as a mechanism for achieving reversible cell wall loosening in elongating tissue without compromising strength (Fry, 1989b; Hayashi, 1989; Hoson et al., 1991). Although the cell wall contains numerous enzymes that can modify polysaccharides (Fry, 1995), xyloglucan endotransglycosylases (XETs) seem well suited to play a predominant role in expansion. XET cleaves a xyloglucan chain (the donor substrate) endolytically and forms a covalent polysaccharide-enzyme complex (Sulová et al., 1998; Steele and Fry, 1999); a new bond then forms between the new (potentially reducing) end and the free nonreducing end of another xyloglucan chain or of a suitable xyloglucan-derived oligosaccharide (XGO; the acceptor substrate) (Baydoun and Fry, 1989; Smith and Fry, 1991; Fry et al., 1992; Nishitani and Tominaga, 1992; Lorences and Fry, 1993).

Fry et al. (1992) hypothesized that XET-catalyzed transglycosylation reversibly loosens the cell wall, as is required

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for turgor-driven cell expansion, and some findings favor this hypothesis. XET activity is often correlated with growth rate (Fry et al., 1992; Hetherington and Fry, 1993; Pritchard et al., 1993; Potter and Fry, 1994; Xu et al., 1995; Palmer and Davies, 1996; Antosiewicz et al., 1997; Catalá et al., 1997). Xyloglucan turnover is correlated with auxin-induced elongation (Labavitch and Ray, 1974; Nishitani and Masuda, 1982), and in dicots, both auxin-induced elongation and xyloglucan breakdown are inhibited by lectins and by antibodies that bind xyloglucans and thereby presumably shield them from enzymic attack (Hoson and Masuda, 1991; Hoson et al., 1991).

Potentially contradictory evidence, however, was obtained by McQueen-Mason et al. (1993), who found that extracts containing active XETs from cucumber hypocotyls were unable to cause wall extension in hypocotyls in which the endogenous proteins had been denatured and that expansins (proteins that did induce extension in this system; McQueen-Mason et al., 1992) did not exhibit any measurable XET activity. Nevertheless, their work did not establish whether the exogenous XETs permeated the cell walls and catalyzed any transglycosylation reactions there. Although extractable XET activity exhibits a marked coincidence with the initiation of extension in maize roots and leaves, substantial activity could also be detected in mature tissue that was still turgid but had ceased extension (Pritchard et al., 1993; Palmer and Davies, 1996). Thus, wall-tightening processes may be capable of overriding the wall-loosening effects of XET.

Besides the proposed role of XETs in cell wall loosening, these enzymes may also favor integration of newly synthesized xyloglucans into the cell wall (Xu et al., 1996; Nishitani, 1997). Such integration is another necessary element for continued cell expansion. A role for XET in xyloglucan integration has been supported by the demonstration that newly secreted xyloglucan chains undergo interpolymeric transglycosylation at the time of their binding to the cell wall (Thompson et al., 1998).

Roles other than the two mentioned above can also be suggested. An enzyme with XET and xyloglucan endohydrolase activity is involved in the postgerminative mobilization of xyloglucan storage reserves in nasturtium (*Tropaeolum majus*) cotyledons (Farkaš et al., 1992; Fanutti et al., 1993). XET activity increases during fruit ripening of kiwi (Redgwell and Fry, 1993), tomato (Maclachlan and Brady, 1994), and persimmon (Cutillas-Iturralde et al., 1994), suggesting a role in softening the cell wall and possibly in preparing it for further modification by other wall-associated (e.g., pectolytic) enzymes. Because Arabidopsis XETs accumulate in epidermal tissues under mechanical stress, XETs may contribute to the strengthening of cell walls to minimize any mechanically induced cell and tissue damage. They may also partially lyse walls to allow formation of xylem and phloem conducting elements and intercellular spaces (Saab and Sachs, 1995; Antosiewicz et al., 1997).

Diverse factors regulate the expression of XETs and XET-

related (XTR) proteins, including auxin (Xu et al., 1995, 1996; Catalá et al., 1997), abscisic acid (Wu et al., 1994), gibberellin (Potter and Fry, 1993; Smith et al., 1996), ethylene (Redgwell and Fry, 1993), and brassinosteroids (Zurek and Clouse, 1994). Upregulation is also triggered by various environmental stimuli, including touch, darkness, temperature shock (Xu et al., 1995, 1996), wind (Antosiewicz et al., 1997), and flooding (Saab and Sachs, 1995).

To date, the best-documented but still hypothetical role of XET is its involvement in cell wall loosening during cell growth. Further evidence for or against this proposed role would be gained by in situ colocalization of XET activity and appropriate donor substrates. Several assays are currently available for extracted XET activity in vitro (Fry et al., 1992; Nishitani, 1992; Lorences and Fry, 1993; Sulová et al., 1995; Fry, 1997), but in all these assays, an exogenous donor substrate is supplied, precluding any conclusions about substrate-enzyme colocalization. The method described by Fry (1997) is based on incorporating sulforhodamine-labeled oligosaccharides of xyloglucan (XGO-SRs), which act as acceptor substrates, into high- M_r products to yield a fluorescent polymer (xyloglucan-SR) that remains hydrogen-bonded to filter paper after the remaining unreacted XGO-SRs have been washed off. The method has been used for semiquantitative in vitro assays with "dot-blot," having been shown to work for tissue prints of celery petioles (Fry, 1997), for example, and zymograms (Iannetta and Fry, 1999). The method has now been adapted for in situ work, and here we report on the in vivo colocalization of XET activity and accessible donor substrate xyloglucans in various plant organs.

RESULTS

In an initial experiment, transverse sections of celery petioles were assayed for simultaneous colocalization of XET activity and xyloglucan by using exogenous XGO-SRs. Bright orange fluorescence, resulting from the formation of wall-bound xyloglucan-SR, was typical of the collenchyma strands, the vascular bundles, and the epidermis, whereas the cortical cell walls displayed a fainter staining, as shown in Figure 1A. The image of the larger vascular bundle (Figure 1B) confirms the fluorescence in both xylem and phloem and the fainter staining in cell walls of cortical cells. In the upper and lower left-hand corners of Figure 1B, parts of two brightly stained collenchyma strands can be seen.

Figure 1C depicts a 2-day-old Arabidopsis root, which displays a very distinct pattern of fluorescence. The root tip is almost devoid of fluorescence, the zone just behind the tip shows very bright fluorescence, and the more basal part of the root contains almost no detectable fluorescent staining except in the vascular tissue. The bright-field image of the same root helps to localize the zones shown in Figure 1C. A close-up of the bright zone near the tip, illustrated in

Figure 1E, shows that the fluorescent cells increase in size going from the tip toward the base of the root (from left to right on the image), indicating that the area that is especially brightly stained is the cell elongation zone of the root.

Fluorescence and bright-field images of 5- and 13-day-old *Arabidopsis* roots are depicted in Figures 1F and 1J, respectively. The size of the brightly fluorescent zone—the cell elongation zone of the root—increased with the age of the root. Figures 1H and 1L illustrate the fluorescence pattern arising from XET–donor–substrate colocalization in lateral roots. In Figure 1H, a very young lateral root (at the left) exhibits no fluorescence, and an older one (at the right) shows some vague fluorescence at its base, where cells probably start to elongate. The epidermal cells of the main root show more intense fluorescence where the lateral roots emerge. In Figure 1L, the fluorescence pattern in an older lateral root resembles that in a young main root, with a distinct area of strong fluorescence in the elongation zone. Farther back along this lateral root, young root hairs are also seen to be associated with intense fluorescence. In general, the root hair cell walls were uniformly stained.

To check whether this specific labeling pattern was more general, roots of *Arabidopsis*, grown in soil under greenhouse conditions, and tobacco roots were assayed. The *Arabidopsis* root in Figure 2A shows a labeling pattern consistent with that seen in roots grown *in vitro*. In tobacco (Figure 2G), the fluorescence pattern and intensity are comparable to those of the *Arabidopsis* roots (Figures 1C, 1F, and 1J), although the elongation zone is longer.

To verify that XET activity was being assayed, we included several controls in our measurements. Roots showed no autofluorescence in response to green light excitation after incubation in Mes buffer that lacked XGO–SR. Figure 2C illustrates the results for an *Arabidopsis* root assayed after it had been boiled for 2 min in water. The structure of the root can be seen in the bright-field image, whereas the fluorescence image shows that boiling resulted in the total loss of ability to yield any fluorescence detectable with the same manipulations and camera settings used for experimental roots that had not been boiled.

When *Arabidopsis* roots were incubated with cellobiose–SR instead of XGO–SR, little or no fluorescent product was discernible. (A specimen in which a small amount of fluorescence was detected is shown in Figure 2E.) Incubation with cellobiose–SR gave similar results. Compared with the normal fluorescence pattern (Figures 1C, 1F, 1J, and 1L), the elongation zone of the roots was not appreciably stained. Instead, a vague signal was generated in a zone nearer to the root tip.

When *Arabidopsis* and tobacco roots were assayed with XGO–SR solution supplemented with 0.08 or 1 mM unlabeled XGOs, fluorescence drastically decreased for both XGO concentrations (results not shown).

Arabidopsis and tobacco roots were incubated with XGO–SR in 25 mM Mes buffer at various pH values. In roots incubated at pH 4.0 (results not shown), fluorescence intensity

was much less than in roots treated at the usual pH of 5.5 (Figures 1C, 1F, and 1J for *Arabidopsis*; Figure 2G for tobacco). Comparison of fluorescence intensities in roots incubated at pH 5.5 and 7.0 showed no appreciable difference (results not shown).

Figure 3 shows that the thin-layer chromatographic (TLC) profile of XGO–SRs reextracted from tobacco roots after a 1-hr incubation (Figure 3, lane 2) was similar to that of the starting material (Figure 3, lane 3), indicating that little hydrolysis of the fluorescently labeled substrate had occurred during the assay. Lanes 4 to 8 establish the relationship between chromatographic mobility and molecular mass for a range of oligosaccharide–SR conjugates. A trace of glucose–SR was present in most preparations, probably indicating a slight nonenzymic hydrolysis of the terminal glycosidic linkage. XGO–SRs reextracted from *Arabidopsis* roots (Figure 3, lane 1) showed evidence of partial conversion of the nonasaccharide (XLLG–SR) to octasaccharide(s) (probably XXLG–SR or XLXG–SR [or both]) and smaller products, indicating some glycosidase activity; however, throughout the 1-hr incubation, the majority of the XGO–SRs remained large enough to be acceptor substrates for XET.

DISCUSSION

Most studies of XET expression in relation to cell expansion have used (1) measurement of extractable enzyme activity assayed *in vitro* (Fry et al., 1992; Hetherington and Fry, 1993; Pritchard et al., 1993; Potter and Fry, 1994; Xu et al., 1995; Palmer and Davies, 1996); (2) immunocytochemistry with antibodies against the XET protein (Antosiewicz et al., 1997); or (3) assessment of XET mRNA concentrations (Zurek and Clouse, 1994; Xu et al., 1995, 1996; Catalá et al., 1997; Nishitani, 1997). None of these approaches is ideal. Method 1 has limited spatial resolution and may fail to extract all the active XET from the tissue; in particular, covalent polysaccharide–enzyme complexes (Sulová et al., 1998; Steele and Fry, 1999) may not be eluted from the cell wall. Method 1 may also extract any intraprotoplasmic XETs, which would not be capable of acting on wall xyloglucans *in vivo* because of compartmentalization. Method 2 detects XETs and related proteins regardless of whether they possess enzymic activity. Method 3 takes no account of the possible translational or post-translational control of XET expression.

A fourth approach, reported here, is the *in situ* colocalization of XET activity and accessible donor substrates. The fluorescent acceptor substrates used (XGO–SRs) are relatively large (~1.6 to 2.0 kD), hydrophilic molecules that are not taken up by living protoplasts; *in vivo*, therefore, the method detects only extraprotoplasmic XETs, that is, those enzyme molecules that potentially have access to substrates in the cell wall. Furthermore, because our assay relies on endogenous extraprotoplasmic xyloglucan as the donor substrate, it cannot give false positives attributable to

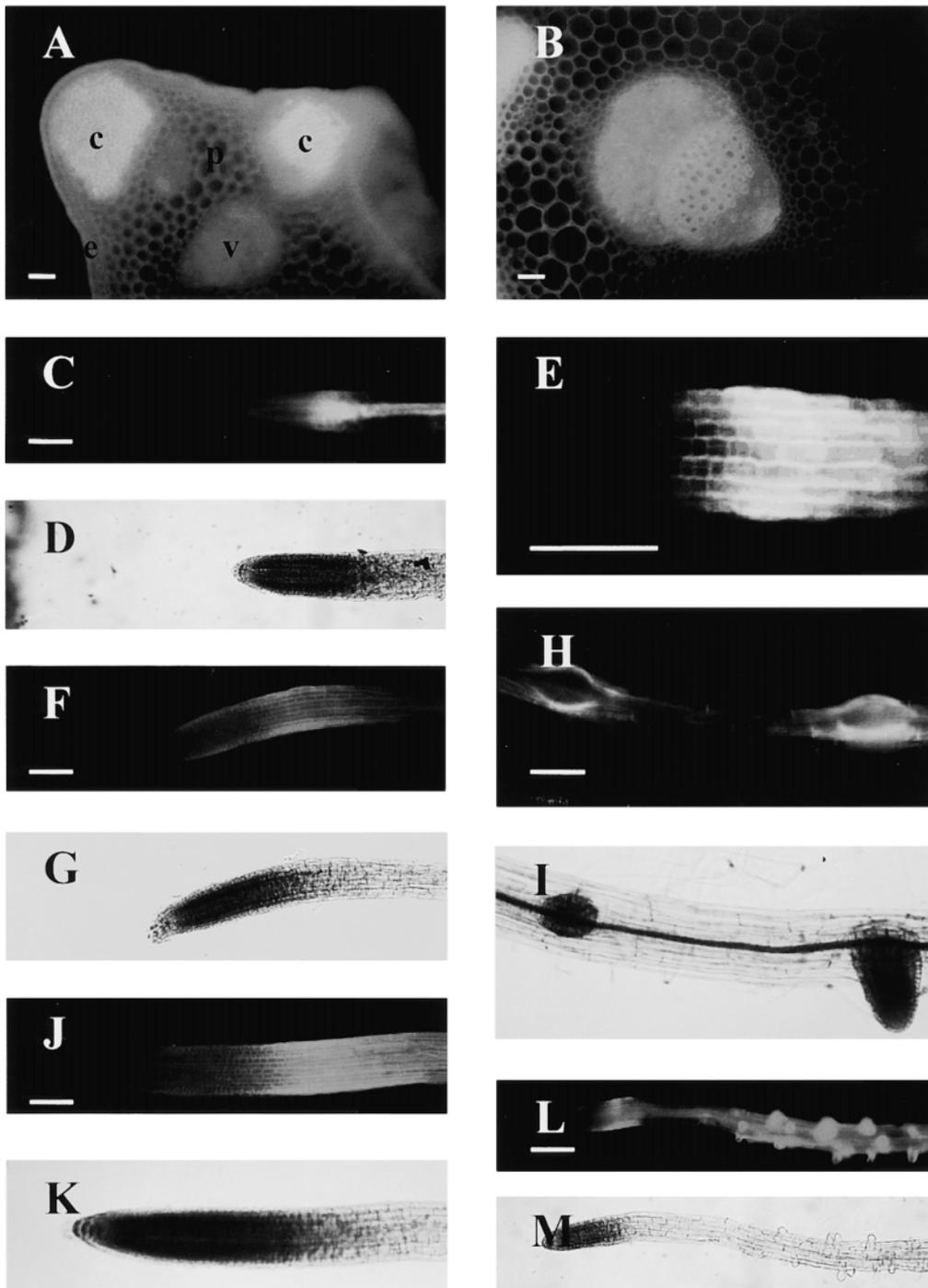


Figure 1. XET Activity–Donor–Substrate Colocalization in Celery Petioles and Arabidopsis Roots.

(A) Celery petiole section showing high XET–donor colocalization, especially in collenchyma (c, collenchyma; e, epidermis; p, parenchyma; v, vascular bundle).

(B) A larger vascular bundle showing bright fluorescent staining.

(C), (D), (F), (G), (J), and (K) XET–donor colocalization in Arabidopsis roots.

(C) Root from a 2-day-old Arabidopsis plant showing a distinct pattern of XET–donor colocalization coinciding with the elongation zone.

(D) Bright-field image of the specimen shown in (C).

the presence in the cell wall of active XETs that are incorrectly located to come into contact with a suitable donor substrate. In addition, our method clearly detects only active XETs, not inactive proenzymes or other structurally related proteins that lack transglycosylase activity. Finally, unlike method 3, it cannot give false positives caused by the failure to take into account the possible regulation of XET gene expression at the translational or post-translational level.

The product of the assay, xyloglucan–SR, could be either an integral cell wall component or a compound that is soluble in the apoplast. Our first washing solvent (ethanol/formic acid/water, 15:1:4 [v/v/v]) in which XGO–SRs are soluble but polysaccharides are insoluble would retain both types of product in situ; the second solvent (5% formic acid) would remove any soluble apoplastic xyloglucan–SR and leave only those products that were firmly wall bound. In the present work, we restricted our observations to the latter.

In fresh sections of celery petioles, the strong XET–donor–substrate colocalization seen in the collenchyma, vascular bundles, and epidermis in comparison with that in cortical cells confirms and adds spatial resolution to tissue print patterns obtained by Fry (1997). The collenchyma is composed of thick-walled cells that provide mechanical support to the petiole but are nevertheless capable of growth in length. It seems reasonable to suggest that elongation of the thick collenchyma cell walls requires particularly high quantities of wall-loosening activities such as that of XET.

A very characteristic distribution pattern of XET–donor–substrate colocalization was found in *Arabidopsis* and tobacco roots. In both species, strong fluorescence is limited to the cell elongation zone of the root. Other regions exhibit very low fluorescence, supporting the correlation between XET action and cell extension. This correlation is further highlighted since the strong fluorescence signal, mainly restricted to the zone of cell elongation, increased steadily during the ageing of the roots as does the growth zone of the roots (Beemster and Baskin, 1998). Lateral roots also mirrored this behavior.

Several controls provided evidence that the formation of the fluorescent product was the result of XET activity rather than a physical artifact or the action of different wall enzymes (Fry, 1995). The loss of activity after boiling is an indication that the fluorescent signals obtained in experimental roots were the result of enzymic activity and not adsorption

of the substrate. The failure of other fluorescent substrates such as cellotetraose–SR (chemically identical to the backbone of XGO–SR but lacking the nonreducing terminal xylose residue that is essential for recognition as an acceptor substrate by XET [Lorences and Fry, 1993]) and cellobiose–SR to give strong fluorescence in the zone of cell elongation indicates that the activity is specific for XET. Competition experiments with unlabeled XGOs confirmed the specificity of the enzymic activity. XETs have a K_m for XGOs of ~ 20 to $80 \mu\text{M}$ (Fry et al., 1992; Purugganan et al., 1997); therefore, XGOs added at or above this concentration would be expected to compete with the XGO–SRs and diminish the XET-catalyzed production of xyloglucan–SR, as we observed.

Analysis by TLC of the nonincorporated XGO–SRs that remained showed that β -galactosidase activity in the wall was only slight. If present, high β -galactosidase activity, followed by the action of α -xylosidase and β -glucosidase (Koyama et al., 1983), would have digested the XGO–SRs, eventually forming XET-inactive products such as the trisaccharide Xyl-Glc-Glc-SR (XG-SR) (Lorences and Fry, 1993). The small extent of such digestion shows that the XGO–SRs did not undergo major competing side-reactions that would have compromised our assays of XET–donor–substrate colocalization.

Known XETs, isolated from or typically expressed in expanding cells, generally have a pH optimum of ~ 5.5 to 6.5 (Fry et al., 1992; Purugganan et al., 1997; Campbell and Braam, 1999; N.M. Steele and S.C. Fry, manuscript submitted). Lowering the pH of the reaction medium to 4.0 decreased the yield of xyloglucan–SR, as would be expected for a reaction catalyzed by XETs. At pH 7.0, however, the yield was not noticeably less than at pH 5.5—an observation that could point to the activity of multiple isoforms of XETs in vivo differing in pH optimum. Several XET isoforms have been described (de Silva et al., 1993, 1994; Okazawa et al., 1993; Zurek and Clouse, 1994; Arrowsmith and de Silva, 1995; Saab and Sachs, 1995; Xu et al., 1995, 1996; Steele and Fry, 1999); in the XTR-encoding gene family of *Arabidopsis*, for example, the encoded proteins are 37 to 84% identical (Xu et al., 1996). Alternatively, the imposed high apoplastic pH could activate plasma membrane proton pumps, restoring the slightly acidic pH of the wall compartment during the assay. Finally, the pH optima of the enzymes in situ in the cell wall could also differ from those measured in vitro.

Figure 1. (continued).

(F) and **(G)** As in **(C)** and **(D)** except that the root is from a 5-day-old plant.

(J) and **(K)** As in **(C)** and **(D)** except that the root is from a 13-day-old plant.

(E) Close-up of the brightly fluorescent cell elongation zone near the root tip.

(H) and **(I)** Young, emerging lateral roots. Fluorescence **(H)** and bright-field image **(I)**.

(L) and **(M)** An older lateral root, showing bright fluorescent staining of its elongation zone, as in the main roots. Fluorescence **(L)** and bright-field image **(M)**.

Bars = 100 μm .

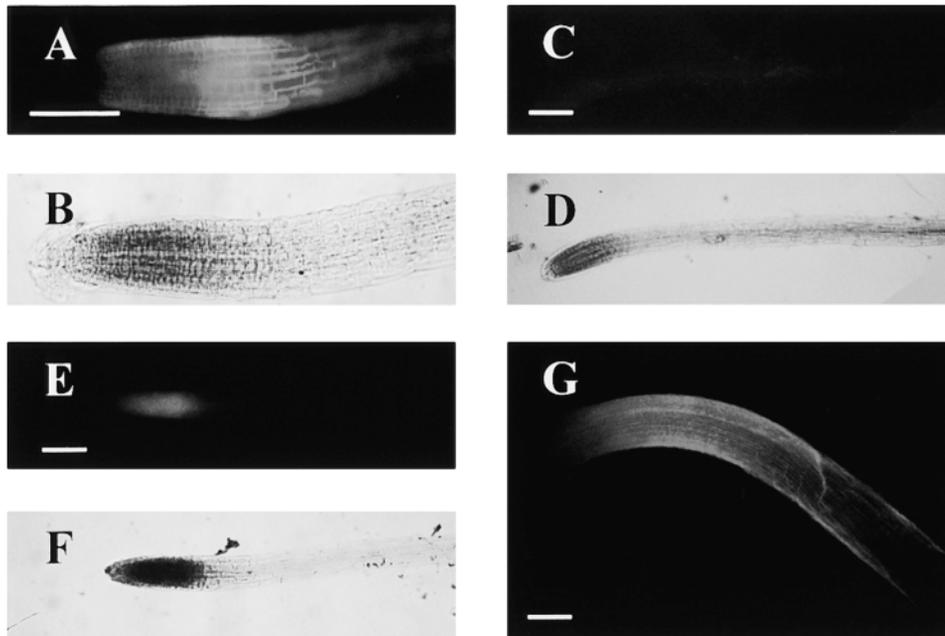


Figure 2. XET Activity–Donor–Substrate Colocalization in Arabidopsis and Tobacco Roots.

(A) and (B) Root of a 5-day-old Arabidopsis plant grown in soil and assayed for XET–donor colocalization (A); the corresponding bright-field image is shown in (B).

(C) and (D) Controls showing lack of XET activity in a boiled root. Fluorescence (C) and bright-field image (D).

(E) and (F) Control showing assay with cellotetraose–SR as the potential acceptor substrate. Fluorescence (E) and bright-field image (F).

(G) Tobacco root assayed for XET–donor colocalization (fluorescence).

Bars in (A), (C), and (E) = 100 μm ; bar in (G) = 300 μm .

In summary, we have visualized XET–donor–substrate colocalization with excellent spatial resolution in growing cells and also in some mature tissues. The results obtained with the collenchyma, and especially with the roots, are compatible with a role for XET in elongation. This role could be wall-loosening by reversible cleavage of tethers, or it could involve the integration of new xyloglucan chains during wall assembly. These two processes probably occur concurrently in elongating cells.

With the methodology recently developed in various laboratories, it is now possible to localize the XET mRNA, the protein itself, and its action in vivo. The near future will undoubtedly bring new and important information about the regulation of cell wall assembly and loosening.

METHODS

Plants and Cultures

Plants of *Arabidopsis thaliana* (wild type) and *Nicotiana tabacum* cv Petite Havana SR1 were grown from seed under sterile conditions on a Murashige and Skoog medium without hormones (4.7 g/L; Duchefa,

Haarlem, The Netherlands), supplemented with 10 g/L sucrose and solidified with 4 g/L Gelrite (Duchefa), pH 5.7. Arabidopsis was also grown in soil. Healthy Arabidopsis roots were obtained at various times, as explained in the text.

Celery (*Apium graveolens*) was obtained commercially. Celery petiole transverse sections (200 μm thick) were cut with a hand microtome (Jung, Heidelberg, Germany). The sections were then treated the same as the roots.

Cytochemical Assays

Unlabeled xyloglucan oligosaccharides (XGOs) were a mixture containing principally XLLG (nonasaccharide) > XXLG (octasaccharide) > XXXG (heptasaccharide) (see Fry et al. [1993] for nomenclature), kindly donated by K. Yamatoya (Dainippon Pharmaceutical Co., Osaka, Japan). The XGO mixture was converted to XGO–SRs (XLLG–SR > XXLG–SR > XXXG–SR, all of which act as fluorescent acceptor substrates for XETs) by the method of Fry (1997). The XGO–SRs at 6.5 μM (sulforhodamine basis) were dissolved in 25 mM Mes buffer, pH 5.5 (different conditions are mentioned in Results). Unlabeled XGOs (~0.08 or 1 mM) were dissolved in the same buffer. Roots and celery sections were incubated in the XGO–SRs alone or supplemented with the unlabeled XGOs in the dark for 1 hr followed by a 10-min wash in ethanol/formic acid/water (15:1:4 [v/v/v]) to remove any remaining unreacted XGO–SRs; a further incubation overnight in

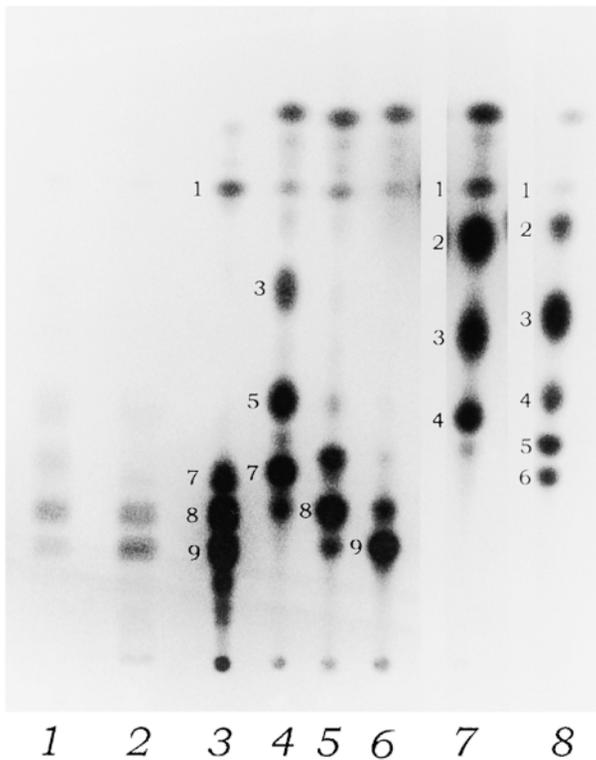


Figure 3. TLC of XGO-SRs before and after incubation with Arabidopsis and Tobacco Roots.

After incubation in the presence of the roots for 1 hr, remaining XGO-SRs were reextracted and analyzed by TLC. This image is a negative (dark spots indicate fluorescence). Lane 1 contains XGO-SRs reextracted from Arabidopsis root; lane 2, XGO-SRs reextracted from tobacco root; lane 3, XGO-SR mixture initially supplied to the roots; lanes 4 to 6, XGO-SRs (including XLLG-SR [DP9], XXLG-SR [DP8], XXXG-SR [DP7], XXG-SR [DP5], XG-SR [DP3], and glucose-SR [DP1]); lane 7, a mixture of cello-oligosaccharide-SRs spanning DP4 (cello-tetraose-SR) to DP1 (glucose-SR); lane 8, a mixture of malto-oligosaccharide-SRs spanning DP6 (malto-hexaose-SR) to DP1 (glucose-SR). Numbers to the left of major spots indicate the degree of polymerization of the oligosaccharide moiety. In each case, the former reducing group of the carbohydrate is a 1-amino-1-deoxy-D-glucitol moiety, to which sulforhodamine is attached by a sulfonamide bond.

5% formic acid removed apoplastic, non-wall-bound xyloglucan-SR. Samples were then inspected under a fluorescence microscope (Leitz Wetzlar, Orthoplan, Germany) using green (540-nm) excitation light. Images were taken with a Nikon 35-mm camera on Fujichrome Sensia II 400 ASA film.

Chromatographic Analysis

For analysis of remaining low- M_r XGO-SRs after incubation in the presence of plant tissues for 1 hr, the plant material was rinsed, freeze-dried, and extracted with 50% ethanol; the extracts were then

resolved by thin-layer chromatography (TLC) on silica gel in butan-1-ol/acetic acid/water (2:1:1[v/v/v]). The plate was illuminated with green light as above, and the fluorescent spots from the sulforhodamine conjugates were photographed through an orange filter.

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In Vivo Colocalization of Xyloglucan Endotransglycosylase Activity and Its Donor Substrate in the Elongation Zone of Arabidopsis Roots

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