

Invited Article

Endo-Xyloglucan Transferase, a New Class of Transferase Involved in Cell Wall Construction

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Cell shape in plants is constrained by cell walls, which are thick yet dynamic structures composed of crystalline cellulose microfibrils and matrix polymers. Xyloglucans are the principal component of the matrix polymers and bind tightly to the surface of cellulose microfibrils and thereby cross-link them to form an interwoven xyloglucan-cellulose network structure. Thus, cleavage and reconnection of the cross-links between xyloglucan molecules are required for the rearrangement of the cell wall architecture, the process essential for both cell wall expansion and the wall deposition occurring during cell growth and differentiation. Endoxyloglucan transferase (EXT) is a newly identified class of transferase that catalyzes molecular grafting between xyloglucan molecules. This enzyme catalyzes both endo-type splitting of a xyloglucan molecule and reconnection of a newly generated reducing terminus of the xyloglucan to the non-reducing terminus of another xyloglucan molecule, thereby mediating molecular grafting between xyloglucan cross-links in plant cell walls. Molecular cloning and sequencing of EXT-cDNAs derived from five different plant species including *A. thaliana* and *V. angularis* has revealed that the amino acid sequence of the mature protein is extensively conserved in the five different plant species, indicating that EXT protein is ubiquitous among higher plants. This structural study has also disclosed the presence of a group of xyloglucan related proteins (XRP) with transferase activity in higher plants. Current data strongly suggest that these proteins are involved in a wide spectrum of physiological activities including cell wall expansion and deposition in growing cell walls.

Key words: Cell wall deposition — Endo-xyloglucan transferase (EXT) — Gene expression — Molecular grafting — Xyloglucan — Xyloglucan related protein (XRP)

Morphological change of a plant organ, such as stem elongation, and root development, is primarily a result of a coordinated enlargement of individual cells generated at respective meristems. The cell enlargement process or cell growth is a distinctive characteristic of morphogenesis in plants. Since a plant cell is enclosed by a thick, yet dynamic wall architecture that constrains the cell shape, structural changes in the cell wall play critical roles in regulation of cell growth and differentiation, and hence morphogenesis in plants. The cell wall changes including extension and deposition are considered to be regulated via a rearrangement or reconstruction process of the cell wall framework primarily composed of cellulose microfibrils and matrix polymers, particularly xyloglucans. Endo-xyloglucan transferase is a transferase, recently purified and identified, that can catalyze the molecular grafting reaction between xyloglucan molecules and thereby mediate rearrangement of the cell wall architecture in plant tissues (Nishitani and Tominaga 1991). Characterization of EXT has disclosed its unique function in cell wall construction (Nishitani and Tominaga 1992). This article will deal with the enzymology and molecular biology of EXT and its related proteins.

1) Structural features of plant cell wall

Structural studies on the plant cell wall originally started more than three centuries ago when Robert Hooke observed cell wall frameworks in the cork layer of an oak tree and coined the term “cells” in his “Micrographia” published in 1665. Shortly after his publication, Marcello Malpighi and Nehemiah Grew described in more detail the structure of plant tissues with rigid cell wall frameworks in “Anatomiae Plantarum” and “The Anatomie of Plantae”, respectively. Investigations into the chemistry of plant cell walls during the nineteenth century revealed that the cell wall consisted of several components including cellulose, pectin and hemicellulose (Schulze 1891 and references in Preston 1974). X ray-diffraction analysis together with electron microscopy has disclosed that cellulose exists as microfibril, a crystalline structure composed of several dozen (1-4)- β -D-glucan molecules (Preston 1974). The glucan is a ribbon-like linear molecule composed of several thousands of glucose residues. The glucan chains are tied up in a bundle by means of both intramolecular and intermolecular hydrogen-bond-

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Abbreviations: EXT, endo-xyloglucan transferase; GPC, gel permeation chromatography; XRP, xyloglucan related protein; XET, xyloglucan endotransglycosylase.

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ings among hydroxyl groups in the glucosyl residues to form a tough crystalline fiber. Recent electron microscopic observations have clarified that the microfibrils are about 10 nm wide and are laid down in the primary cell wall wrapping around a whole cell (McCann and Roberts 1990).

Linkage analyses of matrix polymers, which were made possible by methylation techniques coupled with gas-chromatography/mass-spectrometry (Waeghe *et al.* 1983) have shown that xyloglucans (Bauer *et al.* 1973, Kato and Matsuda 1976), glucuronoxylans (Darvill *et al.* 1980,) and rhamnogalacturonans (Talmadge *et al.* 1973) are the principal wall components common to most flowering plants. Utilization of linkage-specific glycanases or "appendage dependent glycanases", such as endopolygalacturonase (English *et al.* 1972), xyloglucan specific endoglucanase (Bauer *et al.* 1973) and glucuronoxylanase (Nishitani and Nevins 1991), has revealed that matrix polysaccharides are basically composed of repeating structural units.

Xyloglucans are characterized as β -(1-4)-glucans with α -xylosyl side chains attached at the 6-O position of the glucosyl residues. Their basic repeating structural unit consists of three consecutive glucosyl residues with xylosyl side chains followed by a single unsubstituted glucosyl residue. Occasionally, the xylosyl side chains are further substituted with β -(1-2)-galactosyl or α -(1-2)-fucosyl- β -(1-2)-galactosyl side chains. Xyloglucans form a sheath structure around each microfibril. Some xyloglucan molecules are intercalated into the noncrystalline space on each microfibril and bind tightly to the cellulose microfibril by hydrogen bonding (Keegstra *et al.* 1973, Hayashi 1989). Recent electron-microscopic observations have indicated that cellulose microfibrils are actually separated from each other by 20 to 40 nm (McCann and Roberts 1990, McCann *et al.* 1991). Xyloglucans are string-like molecules with an average molecular length of 200 nm, and are long enough to extend from one microfibril to another. Cellulose microfibrils are interconnected by these molecules to form a cellulose-xyloglucan network structure (Talbot and Ray 1992a, Carpita and Gibeaut 1993). Accordingly, xyloglucan cross-links can function as load-bearing molecules in the wall of growing cells that withstand mechanical stresses caused by cell turgor pressure.

2) Dynamic aspects of xyloglucans in plant cell walls

The plant cell wall is better considered as a supermolecular network structure with dynamic properties. During cell growth and differentiation the molecular architecture is always changing so as to allow expansion and deformation of the cell wall, which are essential processes for cell growth and differentiation (Masuda 1990). In an attempt to analyze the dynamic properties of the cell wall, Yamamoto *et al.* (1970) and Cleland and Haughton (1971) measured stress-relaxation process of the cell wall and simulated it with a viscoelastic model consisting of Maxwell elements, i.e. spring elements and dashpot

elements. In their model the viscoelastic properties of the cell wall were represented by four physically defined parameters (Yamamoto and Masuda 1971). These analyses have revealed that cell expansion growth primarily depends on alteration of the viscoelastic properties of the cell wall, the process designated as "cell wall loosening" (Yamamoto *et al.* 1970, Cleland 1971). Degradation or breakage of some load-bearing linkages in the cell wall was considered to be the chemical basis for mediation of the rheological properties. Thus, in exploring the cell wall change responsible for cell wall loosening, the degradation process of cell wall components was investigated from the viscoelastic point of view (see references cited in Sakurai 1990, Hoson 1993).

By use of pressure microprobe and pressure block techniques, Cosgrove (1985, 1987) measured the stress-relaxation process of the cell wall in growing intact tissues. The *in vivo* relaxation was interpreted as evidence that the cell wall structure relaxes as a consequence of chemical creepage of load-bearing linkages in the cell wall network structure (Cosgrove 1993b). Cosgrove designated this a chemorheological process. In support of the chemorheological view of wall expansion, Cosgrove and his colleagues demonstrated the presence of an enzyme activity capable of inducing wall creep in frozen-thawed wall specimens (Cosgrove 1989). The protein responsible for the creep activity, designated as expansin, was purified and characterized recently (McQueen-Mason *et al.* 1992). It is claimed that expansin causes cell wall creep by mediating slippage between cellulose microfibrils and the matrix polymers by still unknown mechanism (McQueen-Mason and Cosgrove 1994).

What are, then, the molecular process that are responsible for the cell wall loosening or the chemorheological process? In order to gain insight into the nature of the biochemical event, two lines of investigation were conducted, namely, 1) a search for the load-bearing molecules in the cell wall architecture and 2) purification of enzymes responsible for wall metabolism which leads to cell wall extension. In early studies, breakdown of several matrix polymers was considered as candidates for the key process responsible for cell wall loosening: xyloglucans (Labavitch and Ray 1974, Nishitani and Masuda 1981), β -1, 3; β -1, 4 glucans (Loesher and Nevins 1972, Sakurai *et al.* 1979), galactans (Nishitani and Masuda 1979, 1980, 1982a, Nishitani *et al.* 1979) and xylans (Carpita 1984, Nishitani and Nevins 1988, 1989, 1990). Using polysaccharide-specific antibodies and lectins as specific inhibitors for the wall metabolism, Hoson and his colleagues clarified that metabolism of xyloglucans in dicotyledons and β -(1-3), (1-4)-glucans in Poaceae is essential, at least, for auxin-induced wall loosening in excised tissue sections (Hoson and Masuda 1987, 1991, Hoson and Nevins 1989, Hoson *et al.* 1993).

In pea epicotyl sections, auxin (Labavitch and Ray 1974) and hydrogen ions (Jacobs and Ray 1975), two major endogenous signals capable of inducing cell expansion

growth, promoted metabolic turnover of xyloglucans in the cell wall as studied by a tracer experiment. In epicotyl sections of *Vigna angularis*, auxin caused a decrease in the mass average Mr of xyloglucans in the cell wall when the hormone induced cell extension growth (Nishitani and Masuda 1981, 1983). The decrease in the Mr of xyloglucans was also induced by acidic pH (Nishitani and Masuda 1982a). The effect of acid pH on the xyloglucan degradation was completely reversed by subsequent incubation in medium at neutral pH (Nishitani and Masuda 1982a). Similar Mr changes in xyloglucans were observed in *Avena sativa* (Inouhe *et al.* 1984), *Oryza sativa* (Revilla and Zorra 1987), *Pinus pinaster* (Lorences and Zorra 1987) and *Cucurbita maxima* (Wakabayashi *et al.* 1991). Recently, Talbott and Pickard (1994) reported differential changes in Mr distribution of xyloglucans in cell walls on the upper and lower sides of gravitropically responding epicotyls of *Pisum sativum* and claimed that the Mr changes were related to changes in extensibility of the epidermal cell walls. Furthermore, inhibition of xyloglucan breakdown in epicotyls of *V. angularis* by fucose-binding lectin and xyloglucan specific antibodies resulted

in inhibition of the auxin-induced cell wall expansion (Hoson and Masuda 1991, Hoson *et al.* 1993). Since plant tissues contain cellulase, which can hydrolyze xyloglucans (Verma *et al.* 1975, Koyama *et al.* 1981, McDougall and Fry 1990), the degradation of xyloglucan was considered as a hydrolase-mediated process. These observations were interpreted as evidence for a "cleavage model" for the cell wall loosening, which states that the splitting of load-bearing xyloglucan molecules by hydrolase is the key step controlling architectural changes which lead to cell wall extension, and hence cell expansion growth (Cosgrove 1989, Fry 1989, Hayashi 1989, Hoson 1993, Nishitani and Masuda 1981) (Fig. 1A).

The "cleavage model" has long been accepted and the breakage of load-bearing molecules was considered to be prerequisite for the stress-relaxation process of the growing cell wall. This hypothesis, however, does not account for depositions of cellulose microfibrils and other polysaccharide. As Ray (1967) demonstrated by means of electron microscope radioautography, the new cell wall material is deposited within the preexisting wall structure as well as at the cell surface. Experiments using meta-

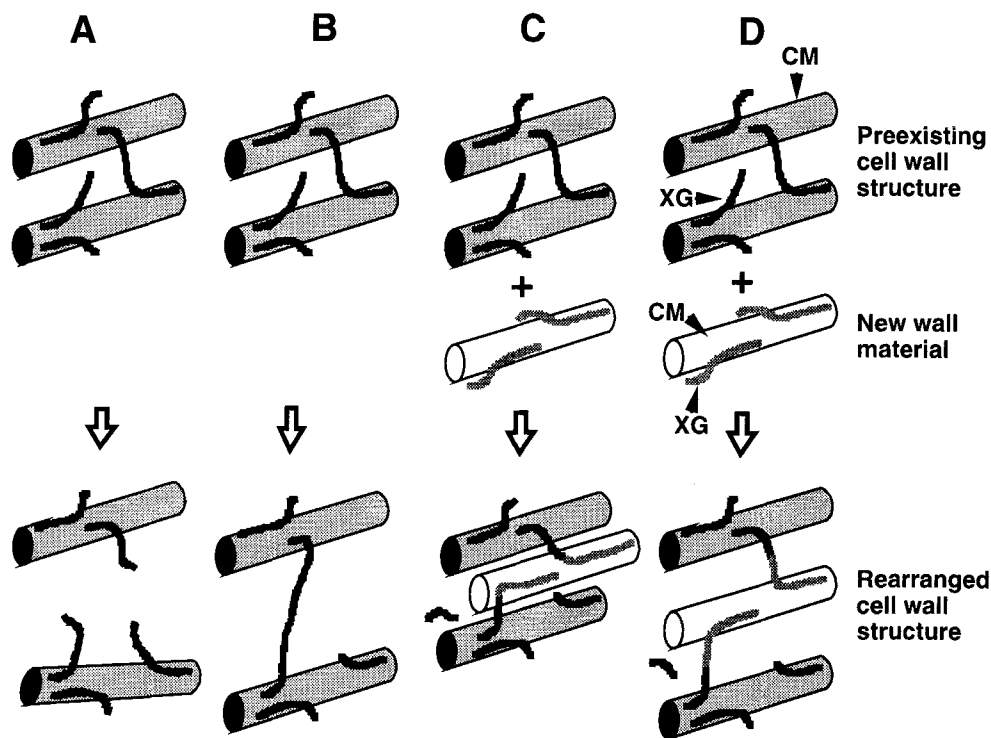


Fig. 1. Four possible unit processes involved in architectural changes in the plant cell wall during cell growth and differentiation. (A) Cell wall expansion by simple cleavage of a load-bearing xyloglucan cross-link as catalyzed by hydrolase. (B) Cell wall expansion by molecular grafting between xyloglucan cross-links in the preexisting cell wall as catalyzed by transferase. (C) Cell wall deposition without wall expansion catalyzed by transferase. Molecular grafting between a xyloglucan molecule in the preexisting wall and one in a newly synthesized wall component results in integration of the new component into the cell wall. (D) Cell wall expansion with wall deposition catalyzed by transferase. Incorporation of the new wall component into the preexisting cell wall structure by the molecular grafting is coupled with cell wall expansion. XG; xyloglucan, CM; cellulose microfibril.

bolic inhibitors of synthesis of cell wall polysaccharides showed that synthesis of matrix polymers is essential for auxin-induced cell wall extension (Inouhe *et al.* 1986, Inouhe and Yamamoto 1991, Hoson and Masuda 1992). Thus, the cell wall extension process is not fully explained merely by the cleavage model, which takes no account of the fact that cell wall expansion ordinarily must involve the deposition of new wall polymer (Nishitani and Masuda 1980, 1982b) (Fig. 1).

3) *Endoxyloglucan transferase as a key enzyme for molecular grafting between xyloglucan cross-links*

If the wall expansion process is not achieved by the cleavage of load-bearing linkages alone, what is its nature? The alternative is that certain load-bearing molecules between cellulose microfibrils are split and reconnected to another molecule in the wall network so as to allow both movement of cellulose microfibrils relative to one another and integration of new wall material into the cell wall. The archetype of this hypothesis was originally advanced by Albersheim as early as the mid-1970s (Albersheim 1976), although neither the load-bearing molecule nor the enzymatic process was not specified at that time. This model was subsequently modified by several researchers (Cleland 1989) and is now accepted as "chemical-creep hypothesis" (Cosgrove 1993a, 1993c, Carpita and Gibeaut 1993). The major difference between the chemical creep model and the hydrolytic cleavage model is that the latter identifies hydrolases as mediating the reaction, whereas in the former the catalytic role is played by transferase capable of mediating molecular grafting between cross-links in the cell wall, particularly between xyloglucans (Fig. 1B, D).

Analysis of Mr distributions of xyloglucans in the cell walls of *Vigna angularis* epicotyls by gel permeation chromatography revealed that the Mr distribution pattern was precisely controlled during cell growth (Nishitani and Masuda 1981, 1982a, 1983). By ^3H -fucose labeling procedures, Talbott and Ray (1992b) determined Mr distribution profiles of newly synthesized xyloglucans in *Pisum sativum* cell walls during cell expansion growth. Their labeling experiment showed that xyloglucans underwent a dynamic change that led to both a decrease and an increase in its Mr during cell growth. These facts implied the presence *in muro* of a certain regulatory machinery controlling molecular weight distribution of xyloglucans.

Incubation of xyloglucans with an average Mr of 420 kDa in solution containing enzyme preparation derived from the apoplastic or cell wall space of *Vigna angularis* epicotyl resulted in production of both higher Mr (820 kDa) and lower Mr (149 kDa) xyloglucans, which could be separated based on their solubility in water (Nishitani and Tominaga 1991). This observation led us to consider that the apoplastic enzyme preparation mediated transfer of a large portion of xyloglucan segment from one xyloglucan molecule to another, a reaction whereby splitting and reconnection of xyloglucan cross-links in the cell wall could be catalyzed. Simultaneously but independently,

Fry and his colleagues also noticed a similar transglycosylation reaction in plant tissues from a tracer labeling experiment. When a ^3H -labeled xyloglucan oligomer was incubated with suspension cultured spinach cells, a portion of the radioactivity was incorporated into extracellular polymers of the cultured cell (Smith and Fry 1991). They also detected similar enzyme activity in crude cell-free extracts of several plant tissues including *Pisum sativum* (Fry *et al.* 1992). Within a couple of years similar enzyme activities were reported from two other research groups (Farkas *et al.* 1992, Fanutti *et al.* 1993, Maclachlan and Brady 1994). There was every evidence in favor of the presence in plant tissues of the transferase capable of catalyzing segment transfer from one xyloglucan molecule to another.

Purification and characterization of the enzyme responsible for the molecular grafting between xyloglucans were made possible by development of two distinct assay systems: (1) direct measurement of disproportioning of the Mr distribution of xyloglucan molecules and (2) measurement of transfer of a label from the acceptor xyloglucan molecules to the donor molecule. The first method (Fig. 2A) used a xyloglucan preparation with defined Mr distribution as the substrate. Since transfer of a portion of xyloglucan segment from one molecule to another molecule will result in generation of higher and lower Mr products, one can quantify the transferase activity by measuring the degree of disproportioning of the Mr distribution of xyloglucan. The Mr distribution was analyzed by a GPC system equipped with a pulsed amperometric detector, which specifically detected carbohydrates in alkaline solution. In the second method (Fig. 2B), purified xyloglucan polymers with defined Mr distribution were used as a donor substrate and fluorescent derivatives of xyloglucan oligomers (pyridylamino xyloglucan oligomers) as an acceptor substrate. In this method, transfer of a xyloglucan segment from the donor to the acceptor molecule can be quantified by monitoring production of high Mr labeled xyloglucans by use of the GPC system equipped with both a pulsed amperometric detector and a fluorescence spectrophotometer (Nishitani 1992).

With the aid of these two detection procedures, the enzyme responsible for the molecular grafting between xyloglucans was finally purified from the apoplastic solution of epicotyl sections of *V. angularis*. The enzyme was a glycoprotein with a molecular mass of about 33 kDa. Based on its molecular grafting activity the new enzyme was designated as endo-xyloglucan transferase (EXT) (Nishitani and Tominaga 1992). For their part, Fry's group used the term, "xyloglucan endo-transglycosylase (XET) activity" to designate the transferase activity in the crude tissue extract (Fry *et al.* 1992). Since the protein for the XET activity was not isolated or purified, it was not demonstrated whether the XET activity was attributable to the EXT protein or not (Nishitani and Tominaga 1992).

On the other hand, Reid's group (Fanutti *et al.* 1993) reported that purified sample of *Tropaeolum majus* glucanase, which had been identified as a hydrolase that

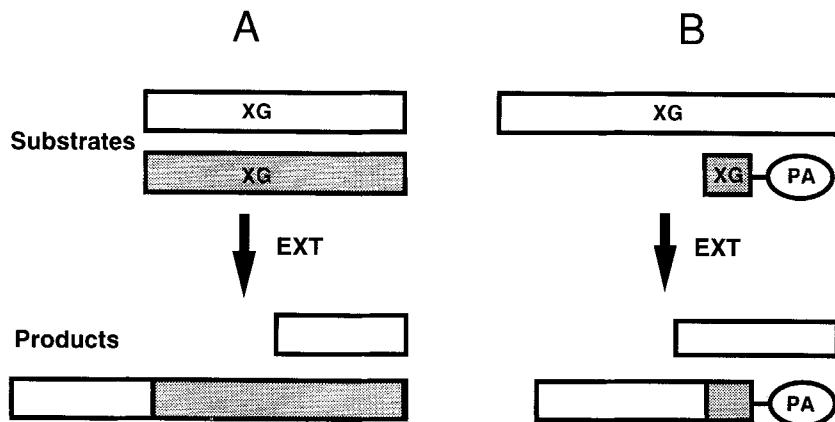


Fig. 2. Two assay systems for detection and quantification of endoxyloglucan transferase activity. (A) Unlabeled xyloglucan polymers (XG) with uniform Mr distribution are used as the substrate. Degree of disproportioning of the Mr distribution is monitored by GPC equipped with pulsed amperometric detector which responds specifically to carbohydrates. (B) An unlabeled xyloglucan polymer (XG) with defined Mr distribution and a xyloglucan oligomer tagged with a fluorescent group (pyridylamino group) (PA) were used as the donor and acceptor substrates, respectively. The quantity of high Mr fluorescent xyloglucan is measured by GPC equipped with a fluorescence spectrofluorometer.

specifically hydrolyze xyloglucans (Edwards 1986), exhibited an endo-transglycosylase activity in addition to its hydrolytic activity. The term xyloglucan endo-transglycosylase (XET) was also used for this *T. majus* glucanase. Although the enzymic reaction of *T. majus* glucanase (or XET) looks like that of *V. angularis* EXT, they are different because of two major reasons as follows: 1) *T. majus* glucanase (or XET) hydrolyzes xyloglucans, whereas *V. angularis* EXT does not exhibit any hydrolytic activity. 2) *T. majus* glucanase (or XET) catalyzes endo-transglycosylation between xyloglucan oligomers, whereas *V. angularis* EXT exhibits a strict donor substrate specificity for high Mr xyloglucan polymers and does not act on low Mr xyloglucans and xyloglucan oligomers (*cf.* Nishitani and Tominaga 1992, Fanutti *et al.* 1993). Very recently deSilva *et al.* (1994) purified a "XET" protein from homogenate of *L. esculentum* fruits by use of the XET assay as developed by Fry's group. Sequencing of the purified protein has shown that the N-terminal amino acid sequence of the *L. esculentum* "XET" is identical to the predicted amino acid sequence of *L. esculentum* EXT, the cDNA of which had been cloned and identified as the *L. esculentum* counterpart for the *V. angularis* EXT (Okazawa *et al.* 1993, also see Section 5). This result indicates that EXT protein is responsible for the XET activity.

4) Mode of action of endo-xyloglucan transferase

Donor substrate specificity of EXT was investigated using various polysaccharides including three xyloglucans with different side chains and several other polysaccharides, such as carboxymethyl cellulose and β -(1-3) (1-4)-glucan, as the donor substrate and a pyridylamino xyloglucan oligomer as the acceptor substrate. EXT exclu-

sively acted on three xyloglucans derived from *Vigna angularis*, *Tamarindus indica* and *Tropaeolum majus* equally, but not on other glucans at all. The donor substrate activity depended on the Mr of the xyloglucans: EXT exhibited higher reaction rates when higher Mr xyloglucans were used as donor substrates. Xyloglucans smaller than 10 kDa showed little or no donor substrate activity. Thus, EXT strictly exhibits donor substrate specificity for high Mr xyloglucans (Nishitani and Tominaga 1992).

The molecular size of transferred segment depended on the size of the donor molecule. The average Mr of transferred segment was found to be about 50-60% of the average Mr of the donor substrate, regardless of their Mrs. This means that the donor substrates are cleaved in an endo-type fashion to produce transferred segments with an average Mr that equals nearly half (50-60%) of the donor size. This is the first direct evidence that EXT actually mediates endo-type transglycosylation (Nishitani and Tominaga 1992).

Acceptor substrate activities were analyzed using various pyridylamino oligosaccharides as the acceptor substrate, and xyloglucan polymers as the donor substrate. EXT mediated the transfer of large segments of xyloglucan from donor xyloglucans to the three pyridylamino xyloglucan oligomers equally, irrespective of the presence of galactosyl or fucosyl side chains in the oligomers. On the other hand, pyridylamino celohexaose or laminarihexaose did not act as acceptor substrates. Furthermore, deletion of the terminal xylosyl side chain of xyloglucan oligomers completely abolished its acceptor substrate activity. Thus, β -(1-4) glucosyl residue of the non-reducing terminus with α -(1-6)-xylosyl substitution is required for the acceptor activity, suggesting that the EXT

protein recognizes the acceptor substrate in an exo-type fashion (Nishitani and Tominaga 1992).

EXT-mediated transglycosylation caused no change in glycosidic linkage compositions of xyloglucans as studied by $^1\text{H-NMR}$ spectrometry and the methylation procedure followed by gas-chromatography mass-spectrometry (Nishitani and Tominaga 1991, 1992). These observations indicate that a single type of glycosidic linkage, i.e. β -(1-4)-glucosyl linkage, is involved in both cleavage and reconnection reactions (Nishitani and Tominaga 1992). Based on these findings it was concluded that EXT catalyzes (1) endo-type splitting of a β -(1-4)-glucosyl linkage adjacent to the unsubstituted glucosyl residue of xyloglucan backbone and (2) linking of a newly generated reducing terminus of the xyloglucan to the non-reducing terminus of another xyloglucan, and thereby mediates molecular grafting between xyloglucans.

It should be noticed that the average Mr of xyloglucans remained constant during EXT-mediated molecular grafting reactions. This fact indicates that the cleaved segment of the donor molecule is exclusively transferred to the acceptor xyloglucan molecule but not to water molecule. One possible explanation for this unique reaction is that the formation of acceptor substrate-enzyme complex is prerequisite for the onset of the cleavage of the β -(1-4)-glucosyl linkage in the donor substrate. Alternatively, the enzyme might have certain structural features which allow the catalytic site to exclude water molecules, thereby avoiding transfer of the cleaved segment to water molecule. Clearly, the EXT-catalyzed xyloglucan grafting reaction is distinct from ordinary reverse reactions of glycosidase or glycanase, and seems to fit into no existing category of transglycosylation. Elucidation by X-ray crystallography of the topology of its catalytic domain, including binding sites for donor and acceptor molecules, will offer the opportunity for revealing both the determinant of substrate preference for xyloglucan molecule and the unique transglycosylation mechanism whereby a segment of xyloglucan molecules is exactly grafted to another xyloglucan molecule.

5) Structure of EXT proteins

The primary structure of *Vigna angularis* EXT protein was predicted from the open reading frame of *Vigna angularis* EXT-cDNA, which had been cloned based on the NH_2 -terminal amino acid sequence information of purified EXT (Okazawa *et al.* 1993). The deduced amino sequence indicated the presence of a signal peptide chiefly composed of hydrophobic amino acid residues, a characteristic signal peptide destined for initial transfer to the endoplasmic reticulum. The mature EXT protein is probably transported via vesicles derived from the Golgi apparatus and secreted into the apoplast or cell wall space by exocytosis. The deduced sequence also indicated the presence of one potential attachment site for an N-linked sugar chain. This result is consistent with the fact that EXT is glycosylated and secreted into the apoplast or cell wall space of the epicotyls of *V. angularis*.

The *V. angularis* cDNA was used as a probe to isolate corresponding EXT cDNAs from seedlings of *G. max*, *A. thaliana*, *L. esculentum* and *T. aestivum* (Okazawa *et al.* 1993). The predicted amino acid sequences of the four EXT proteins are 70-90% identical to the *V. angularis* EXT protein. The homology is distributed throughout the sequence, and the four cysteine residues located in the COOH-terminal region, which could be involved in two disulfide linkages, are all conserved in the five plant species. Thus, the secondary structure, as well as the primary structure of the EXT protein is highly conserved among the five plant species, indicating ubiquitous presence of the EXT protein at least among angiosperms.

6) Roles of EXT in cell wall construction

Although little is known of the molecular events by which the cell wall network is constructed and rearranged during cell wall expansion, it is apparent that the events act through molecular mechanisms that have certain prerequisites. Firstly, cell wall expansion is closely correlated with cell wall deposition so as to allow integration of new wall material, particularly xyloglucans and cellulose microfibrils, into the cell wall network structure, a process formerly called intususception. This process is essential in that it makes it possible for the cell wall to conserve its thickness and mechanical properties during cell growth. Secondly, the cellulose microfibrils are synthesized at the plasma membrane (Delmer 1987), whereas xyloglucans are polymerized in the Golgi apparatus and are discharged into wall space (Ray 1980, Hayashi and Matsuda 1981). These two sorts of component are then integrated into the preexisting network structure of the cell wall thereby remodeling the cell wall architecture during the wall deposition process. Molecular grafting reactions between xyloglucan cross-links are required for the integration process. Evidence strongly suggests that EXT is likely to play a role as the catalyst in charge of construction and rearrangement of the cell wall network structure during cell growth. Biochemical studies as to EXT actions in cell wall space will provide valuable clues.

Fry's group compared the XET activity with capacity of the cell wall to extend in several plant tissues. Along the root of *Zea mays* seedling, distribution pattern of XET activity was closely related to that of the degree of "cell wall loosening" as estimated by simple comparison between extension growth rate and turgor pressure in individual zones along the root (Prichard *et al.* 1993). Repeated application of GA to seedlings of a GA-responsive dwarf variety pea (*P. sativum* L. var Feltham) enhanced elongation growth of the fifth internode and concomitant elevation of the XET activity in the internode tissue (Potter and Fry 1993). In hypocotyls of intact cucumber seedlings, IAA-induced elongation growth was correlated with an increase in the XET activity (Potter and Fry 1994). Based on these findings Fry and his colleagues conclude that XET activity is responsible for cell wall loosening during cell extension growth, and claim that XET activity represents the wall-loosening enzyme in plants (Fry *et al.*

1992, Potter and Fry 1994).

A crude XET preparation derived from cucumber hypocotyls was assayed for its ability to induce "cell wall loosening" by means of "extension reconstitution assay", a procedure primarily developed by Cosgrove (1989). This assay system was based on measurement of *in vitro* extension of wall specimens which had been prepared by inactivating tissue sections by freezing and thawing followed by boiling for 15s. The extension analysis revealed that the XET fraction did not induce any *in vitro* extension of isolated wall specimens derived from cucumber hypocotyls under the conditions examined (McQueen-Mason *et al.* 1993). Thus, XET activity is not sufficient for the *in vitro* wall loosening. This negative result, however, does not exclude the possibility that EXT protein takes part in the cell wall rearrangement during cell wall deposition in living cells, and is not inconsistent with our current idea that EXT protein is responsible for cell wall construction during cell growth and differentiation.

Very recently, we quantified EXT-RNA levels in various organs of *V. angularis* by RNA gel blot analyses, and found that EXT-mRNA was extensively transcribed in growing epicotyls of *V. angularis* plants, particularly in tissues which exhibited high activities for the cell wall construction (Nishitani *et al.* 1994). The RNA gel blot analysis also indicated that Gibberellic acid and indole-3-acetic acid substantially increased EXT-mRNA levels in tissue sections excised from the epicotyls. Moreover, 50 mM sucrose, which caused cell wall deposition in the excised sections, significantly increased EXT-mRNA levels. These data clearly indicate that the gene expression for EXT protein is closely correlated with cell wall deposition as well as cell expansion growth, strongly suggesting important roles of the EXT protein in both cell wall extension and cell wall deposition.

7) Xyloglucan related proteins (XRP)

The GenBank/EMBL data base search reveals that the EXT proteins are homologous to *Tropaeolum majus* endo- β -(1-4)-glucanase (de Silva *et al.* 1993) and four proteins with unknown functions, e.g. *A. thaliana* Meri-5 protein (Medford *et al.* 1991), *Glycine max* BRU-1 protein (Zurek and Clouse 1994), *Zea mays* wus1 1005 gene product (Pesckke and Sachs, 1994) and *L. esculentum* XET-1 protein (Arrowsmith and de Silva 1994, de Silva *et al.* 1994). Alignment of the ten proteins shown in Fig. 3 indicates that they share several blocks of amino acid sequences, the homology being distributed throughout the sequence. The dendrogram based on the UPGMA method (Fig. 4) indicates that the ten proteins are tentatively classified into three clusters: EXT group, MER-BRU-XET-WUS group and NXG.

T. majus glucanase specifically hydrolyzes xyloglucans (Edwards *et al.* 1986) and is distinct from other glucanases classified as cellulases (Tucker and Milligan 1991). As described before *T. majus* glucanase mediates the transglycosylation reaction between xyloglucan oligomers as a side reaction (Fanutti *et al.* 1993), and is now called

xyloglucan endo-transglycosylase (XET). Although *V. angularis* EXT does not exhibit hydrolysis activity, the EXT-mediated transglycosylation reaction involves cleavage of a β -(1-4)-glucosyl linkage in the xyloglucan backbone. Thus, the EXT and *T. majus* glucanase (XET) share a common molecular process in their catalytic processes. In support of this idea the conserved amino acid sequence DEID-EFLG in the EXTs and *T. majus* glucanase as indicated by asterisks in Fig. 4 is also conserved among several *Bacillus* β -(1-3), (1-4)-glucanases which catalyze hydrolysis of β -(1, 4)-glucosyl linkage (Borriss *et al.* 1990). Furthermore, the sequence is also partially homologous to the sequence at the active site of phage T4 lysozyme. Thus, it seems quite likely that the conserved sequence serves as a catalytic center in both glucanases and EXTs.

A. thaliana Meri-5 protein was identified as a meristem specific gene by a differential screening (Medford *et al.* 1991). Analysis of expression profiles of Meri-5 promoter-GUS fusion gene in transgenic *N. tabacum* and *A. thaliana* showed that the gene is specifically expressed in the meristematic dome and branching points in the shoot and root. The BRU-1 protein was isolated as a brassinosteroid-regulated gene product from *G. max* epicotyls (Zurek *et al.* 1994, Zurek and Clouse 1994). RNase protection assays showed that BRU1 mRNA levels were preferentially high in both hypocotyls and epicotyls of soybean plants, and were enhanced by application of brassinosteroid. The wus11005 clone was isolated from *Z. mays* as an oxygen-deprivation inducible gene (Pesckke and Sachs 1994). The clone for *L. esculentum* XET-1 was screened from a tomato fruit (breaker stage) cDNA library using a DNA fragment with sequence homologous to *T. majus* glucanase as a probe. The recombinant XET-1 protein produced in *E. coli* was shown to exhibit xyloglucan endo-transglycosylase activity following purification and refolding (de Silva *et al.* 1994).

Although the function of the first three proteins is still unknown, their structural similarity to the *L. esculentum* XET-1 protein and *T. majus* glucanase (XET) as well as EXT proteins, strongly suggest their enzymatic functions are related to xyloglucans. As shown in the dendrogram in Fig. 4, there is every evidence that the ten proteins belong to a single family. For this family we propose tentatively the term "xyloglucan related proteins", to be abbreviated as XRPs. It is likely that XRPs with slightly different catalytic activities plays specific roles in cell wall construction, a complex process composed of several different types of reactions as shown in Fig. 1.

Gene expression of *T. majus* glucanase (de Silva *et al.* 1993), *L. esculentum* XET (Arrowsmith and de Silva 1994, de Silva *et al.* 1994), *V. angularis* EXT (Nishitani *et al.* 1994) and *A. thaliana* Meri-5 (Medford *et al.* 1991) are all strictly regulated spatially and temporally: *T. majus* glucanase is extensively expressed in cotyledons, *L. esculentum* XET-1 in ripening fruit, *V. angularis* EXT in growing epicotyls and *A. thaliana* meri-5 in meristematic tissues. Also *G. max* BRU-1 and *Z. mays* wus11005 are controlled by brassinos-


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EXT-A.t.  -MTVSSSPWALMALFLMVSSTMVMAIPP-----RKAIDVPFG RNYVPTWAFDHQKQFN
EXT-V.a.  ---MGSSLWTCILLLSLASASFAANP-----RTPIDVPFG RNYVPTWAFDHKIKYLN
EXT-L.e.  ---MGIKGVLFVIVLNLVSLVVFQGY-----RRPVDVPFW KNYEFSWASHHIKFLN
EXT-G.m.  --IPVFSVWTVCVILASLASAALCANP-----RRPVDVQFG RNYVPTWAFDHKIKYFN
EXT-T.a.  --MKATAGALLAVVAVLLRGVAAP-----RKPVDVPFD RNYVPTWAFDHKIHVFN
MER-A.t.  -----MSPFKIFFFTLLVAA-----FSVSAADFN IDVNVAVGNGRGRKILN
BRU-G.m.  ---MAPSSAHNNGFVYVLMVGVVSTM-----VATCAGSFY QDFDLTWGGDRAKIFN
XET-L.e.  -----MLLQQLSVLALLLL-----CPVWADNFY QDATVTFGDQRAQIQD
WUS-Z.m.  -----MKALILAAVLLQLHGG-----AASAGGNFY QVDITWGDGRGKILD
NXG-T.m.  MPPNILSIFLHLLPILMFSSSCLGQGGPPSPGYYPSSQITSLGFD QGYTNLWGPQHRVDDQ

EXT-A.t.  GGSELOLILDKYRGTGFQSKGSYLFGHFSMHIKLPA GD TAGVVTAF YLS STN---NEH DE
EXT-V.a.  GGSEIQHLHLDKYRGTGFQSKGSYLFGHFSMYIKLVPGD SAGT VTA F YLS STN---AEH DE
EXT-L.e.  GGTTDDLILDRSSGACFQSKKS YLFGHFSMKMRLVGD SAGV VTA F YLS SNN---AEH DE
EXT-G.m.  GSDIQLHLHLDKYRGTGFQSKGSYLFGHFSMYIKMVP GD SAGT VTA F YLS SQN---AEH DE
EXT-T.a.  GGREVQLSLDKYRGTGFQTRGSYLFGHFSMHIKLVG CD SAGT VTA F YLS SQN---SEH DE
MER-A.t.  NGQLLTLSLDKSSGSGFQSKTEYLFGHIDMQIKLVPGN SAGT VTA F YLK SEG---STW DE
BRU-G.m.  GGQLLSLSDKVS GSCEKSKKEYLFGRIDMQLKLVAGN SAGT VTA Y YLS SQG---PTH DE
XET-L.e.  GGRLLALS LDKISGSGFQSKNEYLFGRIDMQLKLVPGN SAGT VTA F YLS SQG---AGH DE
WUS-Z.m.  NGQLLTLSDR SS GSGFQSKAQYLYGRFDMQLKLVPGD SAGT VTA F YLS SQG---SQH DE
NXG-T.m.  GS--LTLWLDSTSGSCEK SINR YRSGYFGANIKLLOS GYTAGV LTFE YLS MNQDYPGKH DE

** ****
EXT-A.t.  IDFEFLGNRTGQPA ILQTNVFTGKKG----NREQRIYLWFDPS KAYHTYS ILWNMYQILV
EXT-V.a.  IDFEFLGNRTGQPY ILQTNVFTGKKG----DREQRIYLWFDPT QYHRYSLVWNMYQITV
EXT-L.e.  IDFEFLGNRTGQPY ILQTNVFTGKKG----NREQRIYLWFDPT KGYHRYSLVWNLYLITV
EXT-G.m.  IDFEFLGNRTGQPY ILQTNVFTGKKG----DREQRIYLWFDPT KHYHRYSLVWNLYQITV
EXT-T.a.  IDFEFLGNRTGQPY ILQTNVFTGKKG----DREQRIYLWFDPT KDYHRYSLVWNLYMIA
MER-A.t.  IDFEFLGNMSGQPY TLHTNVYTGKKG----DKEQQFHLWFDPT ANFHTYS ILWNPQRII
BRU-G.m.  IDFEFLGNLSGQPY TLHTNI FTQKKG----NREQQFYLWFDPT TRNFHTYS IIWK PQHII
XET-L.e.  IDFEFLGNSSGQPY TVHTNVYSQKKG----NKEQCFRLWFDPT SFFHTYS IVWN SQRII
WUS-Z.m.  IDFEFLCNASGQPY TVHTNVYSQKKG----GREQCFRMWFDPT AAFHTYS ILWNPAHVIV
NXG-T.m.  IDIEFLGTLIPGQPY TLQTNVFTGKSGD YNIIGREMRHLWFDPT QDYHRYSLVWNLYWT PSEII

EXT-A.t.  FFVDDYPIRVEFNKNDLGVKFPFNQPMKLYSSLWNADDWATR GGL EKTWNANA PFFVAS YK
EXT-V.a.  FFVDDYPIRVEFNKNDLGVKFPFNQPMKLYSSLWNADDWATR GGL EKTOWSKAPFIAS YK
EXT-L.e.  IFVDDVPIRVEFNKNDLGVKFPFNQPMKLYSSLWADDWATR GGL EKTWNANA PFTAS YK
EXT-G.m.  FFVDDVPIRVEFNKNDLGVKFPFDQPMKLYSSLWNADDWATR GGL EKTOWSKAPFI AA YK
EXT-T.a.  FFVDDTPIRVEFNKNDLGVRYPFQPMKLYSSLWNADDWATR GGR EKTOWSKAPFVAS YR
MER-A.t.  LTVDDTPIRVEFNKYESLGVLPKKNPMRMYSSLWNADDWATR GGL VKTOWSKAPFVAS YR
BRU-G.m.  FLVDDTPIRVEFNKAEPLGVLPKKNPMRMYSSLWNADDWATR GGL VKTOWSKAPFTAY YR
XET-L.e.  FLVDDNIPIRVEFNHEKLGVAFPKNQAMRVYSSLWNADDWATR GGR VKTOWSMA PFTAS YR
WUS-Z.m.  FFVDDVPIRVEFRRRGDGTVPFPPTSQPMRVYSSVWDAE EWAT QGR VRTOWSKAPFVAS YR
NXG-T.m.  FFVDDVPIRVEFRKSD--ATFPLR-PLWVYSSVWDAE SWATE NGK KADYRYQPFVCK YK

EXT-A.t.  GFHIDGCQAS--VEAKYCATQGRMWD-----QKEFRDLDAEQWRRLLKWRV MKWTFI YNY
EXT-V.a.  GFHIDGCEAS--VNAKFCDTQGRMWD-----QPEFRDLDAEQWQKFLAWVRNKYTI YNY
EXT-L.e.  SFHVDGCEAATPEVQVCNTKGMKMW-----QKAFQDLDALQYRRLRWRVROKYTV YNY
EXT-G.m.  GFHIDGCEAS--VNAKFCDTQGRMWD-----QPEFRDLDAEQWRRLLRWRVROKYTI YNY
EXT-T.a.  GFHVDGCEAS--AEAKFCATQGARWWD-----QPEFQDLDAEQYRRLAWVR KEHTI YNY
MER-A.t.  NI-----KIDSKPNSN-----WYTQEMDSTSQARILKWRVKNYMI YNY
BRU-G.m.  NF--KAIEFS--SKSSISNSG-----AEYEANELDAYSRRLRWVOKYFMI YNY
XET-L.e.  NFNTNACVWS--AASSTSSCGSKTDSVNNDQAWQTQELNGNDRNRLRWVOKYMI YNY
WUS-Z.m.  GYAAAGCTAP--DAAACARSN-----GAWMSQELDSAGQEQLRRRAQASYMI YNY
NXG-T.m.  DFKLGSCTVE--AASSCNPAS-----VSPYGO LSSQQQVAAMEWVOKNYM YNY

EXT-A.t.  CTDRTRFVMDAECKRDRDA
EXT-V.a.  CTDRKRYSQV--PEECTRDRDI
EXT-L.e.  CTDKARYFVP--PEECTRDRDI
EXT-G.m.  CTDTKRYPHISEPECKRDRDI
EXT-T.a.  CTDHDRYAA--APECKRDRDV
MER-A.t.  CTDHRRFPQGFPEECTTSS--
BRU-G.m.  CSDLKRF PQGLPAECKR---
XET-L.e.  CADAKRF SQGLSPECKRSRF-
WUS-Z.m.  CTDKYRF PQGFPEECS SPAK-
NXG-T.m.  CDDP TRDHTL--TPEG-----

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Fig. 3. Alignment of EXT proteins and five related proteins. The deduced amino acid sequences of EXT proteins and five gene products with the most homologous amino acid sequences were aligned using the multiple alignment program on CLUSTAL W Multiple sequence alignments software package (version 1.4). Sequences were obtained from GenBank. Abbreviations and accession numbers for the ten gene products are as follows: EXT-A.t.: *A. thaliana* EXT, D16454; EXT-V.a.: *V. angularis* EXT, D16458; EXT-L.e. *L. esculentum*-EXT, D16456; EXT-G.m.: *Glycine max* EXT, D16455; EXT-T.a.: *T. aestivum* EXT, D16457; MER-A.t.: *A. thaliana* meri-5, X82683; BRU-G.m.: *G. max* BRU-1, L22162; XET-L.e. *L. esculentum* XET1, X82685; WUS-Z.m.: *Zea mays* wush1005, U15781; NXG-T.m.: *T. majus* NXG-1. Of these, the residues that are identical among the ten sequences are shown in reverse contrast, whereas similar amino acid residues are enclosed by squares. Similar residues are defined as those that belong to the same group: group I: A, S, T, P, G; group II: N, D, E, Q; group III: H, R, K; group IV: L, M, I; group V: F, Y, W. Asterisks indicate sequence conserved in several *Bacillus* β -(1-3), (1-4) glucanases.

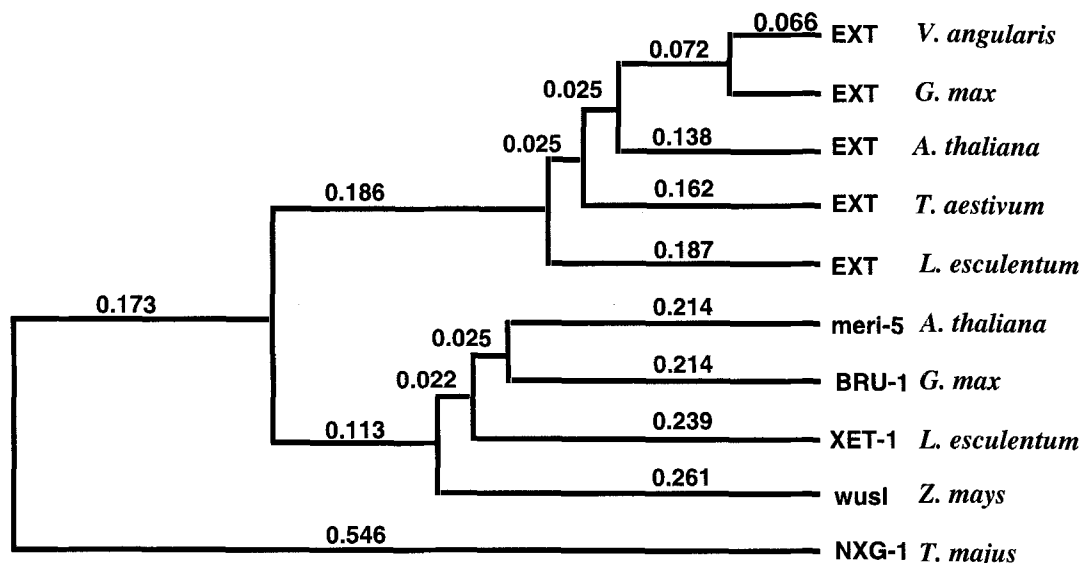


Fig. 4. Possible evolutionary relationship among the EXT proteins and five related proteins. A UPGMA dendrogram showing the genetic distances estimated based on comparisons of deduced amino acid sequences corresponding to mature protein domains using a multiple alignment program on Gene Works software packaging is shown. The ten proteins can be classified tentatively into three groups, EXT-family, MER-BRU-XET-WUS family and NXG (also see Fig. 3).

teroid and oxygen depletion, respectively. The versatile responsiveness of the XRP-family genes to developmental and environmental signals might reflect the fact that cell growth and differentiation, in which XRPs take part, is regulated by a wide spectrum of signals of different types.

In conclusion, the discovery of endo-xyloglucan transferase (EXT) is a substantial clue to the elucidation of the molecular machinery by which cell wall expansion and deposition processes are controlled. This discovery has also disclosed the presence of a group of xyloglucan related proteins (XRPs), some of which exhibit transferase activity. The evidence strongly suggests the usefulness of XRP genes as promising probes to analyze the mechanism through which the dynamic architecture of the cell wall is regulated by hormones and environmental signals. Thus, further investigations of gene expression for XRPs will offer the opportunity for a unique approach for exploring the molecular processes responsible for cell wall expansion and deposition, and hence cell growth and differentiation.

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References

- Albersheim, P. 1976. The primary cell wall. In J. Bonner and J.E. Varner, eds., *Plant Biochemistry*, Academic Press, New York, pp. 225-274.
- Arrowsmith, D. and de Silva, J. 1994. Molecular cloning of cDNAs encoding enzymes with xyloglucan endo transglycosylase (XET) activity from ripening tomato fruit. Abstract of 4th International Congress of Plant Molecular Biology. Abstract No. 537. Amsterdam.
- Bauer, W.D., Talmadge, K.W., Keegstra, K. and Albersheim, P. 1973. The structure plant cell walls II. The hemicellulose of suspension cultured sycamore cells. *Plant Physiol.* **51**: 174-187.
- Borriss, R., Buettner, K. and Maentsaelae, P. 1990. Structure of the beta-1,3-1,4-glucanase gene of *Bacillus macerans*: Homologies to other beta-glucanases. *Mol. Gen. Genet.* **222**: 278-283.
- Carpita N.C. 1984. Cell wall development in maize coleoptiles. *Plant Physiol.* **76**: 202-212.
- Carpita, N.C. and Gibeaut, D.M. 1993. Structural models of primary cell walls in flowering plants. *Plant J.* **3**: 1-30.
- Cleland, R. 1971. Cell wall extension. *Ann. Rev. Plant Physiol.* **22**: 197-222.
- Cleland, R.E. 1989. The mechanism of wall loosening and wall extension, In D.J. Cosgrove and D.P. Knievel, eds., *Physiology of Cell Expansion During Plant Growth*, The American Society of Plant Physiologist, Rockville, pp. 18-27.
- Cleland, R.E. and Haughton P.M. 1971. The effect of auxin on relaxation in isolated *Avena* coleoptiles. *Plant Physiol.* **47**: 812-815.
- Cosgrove, D.J. 1985. Cell wall yield properties of growing tissue. *Plant Physiol.* **78**: 347-356.
- Cosgrove, D.J. 1987. Wall relaxation in growing in growing stems: comparison of four species and assess-

- ment of measurement techniques. *Planta* **171**: 266-278.
- Cosgrove, D.J.** 1989. Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. *Planta* **177**: 121-130.
- Cosgrove, D.J.** 1993a. How do plant cell walls extend? *Plant Physiol.* **102**: 1-6.
- Cosgrove, D.J.** 1993b. Water uptake by growing cells. *Int. J. Plant Sci.* **154**: 10-21.
- Cosgrove, D.J.** 1993c. Wall extensibility: its nature, measurement and relationship to plant cell growth. *New Phytol.* **124**: 1-23.
- Darvill, J.E., McNeil, M., Darvill, A.G. and Albersheim, P.** 1980. Structure of plant cell walls. XI. Glucuronarabinoxylan, a second hemicellulose in the primary walls of suspension-cultured sycamore cells. *Plant Physiol.* **66**: 1135-1139.
- Delmer, D.P.** 1987. Cellulose biosynthesis. *Annu. Rev. Plant Physiol.* **38**: 259-290.
- de Silva, J., Arrowsmith, D., Hellyer, A., Whiteman, S. and Robinson, S.** 1994. Xyloglucan endotransglycosylase and plant growth. *J. Exp. Bot.* **45**: Special Issue 1693-1701.
- de Silva, J., Jarman, C.D., Arrowsmith, D.A., Stronach, M.S., Chengappa, S., Sidebottom, C. and Reid, J.S.G.** 1993. Molecular characterization of a xyloglucan-specific endo-(1-4)- β -D-glucanase (xyloglucan endo-transglycosylase) from nasturtium seeds. *Plant J.* **3**: 701-711.
- Edwards, M., Dea, I.C.M., Bulpin, P.V. and Reid, J.S.G.** 1986. Purification and properties of a novel xyloglucan-specific endo-(1-4)- β -D-glucanase from germinated nasturtium seeds (*Tropaeolum majus* L.). *J. Biol. Chem.* **261**: 9489-9494.
- English, P.D., Maglothlin, A., Keegstra, K. and Albersheim, P.** 1972. A cell-wall degrading endopolygalacturonase secreted by *Colletotichum lindemuthianum*. *Plant Physiol.* **49**: 293-297.
- Fanutti, C., Gidley, M.J. and Reid, J.S.G.** 1993. Action of a pure xyloglucan endo-transglycosylase (formerly called xyloglucan-specific endo-(1-4)- β -D-glucanase) from the cotyledons of germinated nasturtium seeds. *Plant J.* **3**: 691-700.
- Farkas, V., Sulova, Z., Stratilova, E., Hanna, R. and Maclachlan, G.** 1992. Cleavage of xyloglucan by nasturtium seed xyloglucanase and transglycosylation to xyloglucan subunit oligosaccharides. *Arch. Biochem. Biophys.* **298**: 365-370.
- Fry, S.C.** 1989. Cellulose, hemicelluloses and auxin-stimulated growth: a possible relationship. *Physiol. Plant.* **75**: 532-536.
- Fry, S.C., Smith, R.C., Renwick, K.F., Martin, D.J., Hodge, S.K. and Matthews, J.M.** 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochem. J.* **282**: 821-828.
- Hayashi, T.** 1989. Xyloglucans in the primary cell wall. *Annu. Rev. Plant Physiol. Mol. Biol.* **40**: 139-168.
- Hayashi, T. and Matsuda, K.** 1981. Biosynthesis of xyloglucan in suspension cultured soybean cells: occurrence and some properties of xyloglucan 4- β -D-glucosyltransferase and 6- α -D-xylosyltransferase. *J. Biol. Chem.* **256**: 11117-11122.
- Hoson, T.** 1993. Regulation of polysaccharide breakdown during auxin-induced cell wall loosening. *Plant Res.* **106**: 369-381.
- Hoson, T. and Masuda, Y.** 1987. Effect of lectins on auxin-induced elongation and wall loosening in oat coleoptile and azuki bean epicotyl segments. *Physiol. Plant.* **71**: 1-8.
- Hoson, T. and Masuda, Y.** 1991. Inhibition of auxin-induced elongation and xyloglucan breakdown in azuki bean epicotyl segments by fucose-binding lectins. *Physiol. Plant.* **82**: 41-47.
- Hoson, T. and Masuda, Y.** 1992. Relationship between polysaccharide synthesis and cell wall loosening in auxin-induced elongation of rice coleoptile segments. *Plant Science* **83**: 149-154.
- Hoson, T. and Nevins, D.J.** 1989. β -Glucan antibodies inhibit auxin-induced cell elongation and changes in the wall of *Zea* coleoptile segments. *Plant Physiol.* **90**: 1353-1358.
- Hoson, T., Sone, Y., Misaki, A. and Masuda, Y.** 1993. Role of xyloglucan breakdown in epidermal cell walls for auxin-induced elongation of azuki bean epicotyl segments. *Physiol. Plant.* **87**: 142-147.
- Inouhe, M. and Yamamoto, R.** 1991. Effects of 2-deoxygalactose on auxin-induced growth and levels of UDP-sugars in higher plants. *Plant Cell Physiol.* **32**: 433-438.
- Inouhe, M., Yamamoto, R. and Masuda, Y.** 1984. Auxin-induced changes in the molecular weight distribution of cell wall xyloglucans in *Avena* coleoptiles. *Plant Cell Physiol.* **25**: 1341-1351.
- Inouhe, M., Yamamoto, R. and Masuda, Y.** 1986. Inhibition of IAA-induced cell elongation in *Avena* coleoptile segments by galactose: its effect of UDP-glucose formation. *Physiol. Plant.* **66**: 370-376.
- Inouhe, M., Yamamoto, R. and Masuda, Y.** 1987. UDP-Glucose level as a limiting factor for IAA-induced cell elongation in *Avena* coleoptile segments. *Physiol. Plant.* **69**: 49-54.
- Jacobs, M. and Ray, P.M.** 1975. Promotion of xyloglucan metabolism by acid pH. *Plant Physiol.* **56**: 373-376.
- Kato, Y. and Matsuda, K.** 1976. Presence of a xyloglucan in the cell wall of *Phaseolus aureus* hypocotyls. *Plant Cell Physiol.* **17**: 1185-1198.
- Keegstra, K., Talmadge, K.W., Bauer, W.D. and Albersheim, P.** 1973. Structure of plant cell walls. III A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Physiol.* **51**: 188-197.
- Koyama, T., Hayashi, T., Kato, Y. and Matsuda, K.** 1981. Occurrence of xyloglucan-degrading enzymes in soybean cell wall. *Plant Cell Physiol.* **22**: 1191-1198.
- Labavitch, J.M. and Ray, P.M.** 1974. Turnover of cell wall polysaccharides in elongating pea stem segments. *Plant Physiol.* **53**: 669-673.
- Loesher, W. and Nevins, D.J.** 1972. Auxin-induced changes in *Avena* coleoptile cell wall composition. *Plant Physiol.* **50**: 556-563.
- Lorences, E.P. and Zarra, I.** 1987. Auxin-induced growth

- in hypocotyl segments of *Pinus pinaster* Aiton. *J. Exp. Bot.* **191**: 960-967.
- Maclachlan, G. and Brady, C.** 1994. Endo-1-4-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. *Plant Physiol.* **105**: 965-974.
- McDougall, G.J. and Fry, S.C.** 1990. Xyloglucan oligosaccharides promote growth and activate cellulase: evidence for a role of cellulase in cell expansion. *Plant Physiol.* **93**: 1042-1048.
- Masuda, Y.** 1990. Auxin-induced cell elongation and cell wall changes. *Bot. Mag. Tokyo* **103**: 345-370.
- Medford, J.I., Elmer, J.S. and Klee, H.J.** 1991. Molecular cloning and characterization of gene expressed in shoot apical meristems. *Plant Cell* **3**: 359-370.
- McCann, M.C. and Roberts, K.** 1990. Direct visualization of cross-links in the primary plant cell wall. *J. Cell Sci.* **96**: 3213-324.
- McCann, M.C., Wells, B. and Roberts, K.** 1991. Complexity in the spatial localization and length distribution of plant cell-wall matrix polysaccharides. *J. Microscopy* **166**: 123-136.
- McQueen-Mason, S. and Cosgrove, D.J.** 1994. Distribution of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. *Proc. Natl. Acad. Sci. USA* **91**: 6574-6578.
- McQueen-Mason, S. Durachko, D.M. and Cosgrove, D.J.** 1992. Two endogenous proteins that induce cell wall extension in plants. *Plant Cell* **4**: 1425-1433.
- McQueen-Mason, S. Fry, S.C., Durachko, D.M. and Cosgrove, D.J.** 1993. The relationship between xyloglucan endotransglycosylase and *in-vitro* cell wall extension in cucumber hypocotyls. *Planta* **190**: 327-331.
- Nishitani, K.** 1992. A novel method for detection of endo-xyloglucan transferase. *Plant Cell Physiol.* **33**: 1159-1164.
- Nishitani, K., Okamoto, S. and Tomita, E.** 1994. Functions of endo-xyloglucan transferase (EXT) in the cell wall construction in plants. *In Abstract of the 4th International Congress of Plant Molecular Biology, Amsterdam, No. 577.*
- Nishitani, K. and Masuda, Y.** 1979. Growth and cell wall changes in azuki bean epicotyls I. Changes in wall polysaccharides during intact growth. *Plant Cell Physiol.* **20**: 63-74.
- Nishitani, K. and Masuda, Y.** 1980. Modifications of cell wall polysaccharides during auxin-induced growth in azuki bean epicotyl segments. *Plant Cell Physiol.* **21**: 169-181.
- Nishitani, K. and Masuda, Y.** 1981. Auxin induced changes in the cell wall structure: changes in the sugar compositions, intrinsic viscosity and molecular weight distributions of matrix polysaccharides of the epicotyl cell wall of *Vigna angularis*. *Physiol. Plant.* **52**: 482-494.
- Nishitani, K. and Masuda, Y.** 1982a. Acid pH-induced structural changes in cell wall xyloglucans in *Vigna angularis* epicotyl segments. *Plant Sci. Lett.* **28**: 87-94.
- Nishitani, K. and Masuda, K.** 1982b. Roles of auxin and gibberellic acid in growth and maturation of epicotyls of *Vigna angularis*: Cell wall changes. *Physiol. Plant.* **56**: 38-45.
- Nishitani, K. and Masuda, Y.** 1983. Auxin-induced changes in the cell wall xyloglucans: effects of auxin on the two different subfractions of xyloglucans in the epicotyl cell wall of *Vigna angularis*. *Plant Cell Physiol.* **24**: 345-355.
- Nishitani, K. and Nevins, D.J.** 1988. Enzymic analysis of feruloylated arabinoxylans (feraxan) derived from *Zea mays* cell wall I. *Plant Physiol.* **87**: 883-890.
- Nishitani, K. and Nevins, D.J.** 1989. Enzymic analysis of feruloylated arabinoxylans (feraxan) derived from *Zea mays* cell walls II. *Plant Physiol.* **91**: 242-248.
- Nishitani, K. and Nevins, D.J.** 1990. Enzymic analysis of feruloylated arabinoxylans (feraxan) derived from *Zea mays* cell walls III. *Plant Physiol.* **93**: 396-402.
- Nishitani, K. and Nevins, D.J.** 1991. Glucuronoxylan xylanohydrolase, a unique xylanase with the requirement for appendant glucuronosyl units. *J. Biol. Chem.* **256**: 6539-6543.
- Nishitani, K., Shibaoka, H. and Masuda, Y.** 1979. Growth and cell wall changes in azuki bean epicotyls II. Changes in wall polysaccharides during auxin-induced growth of excised segments. *Plant Cell Physiol.* **20**: 463-472.
- Nishitani, K. and Tominaga, R.** 1991. *In vitro* molecular weight increase in xyloglucans by an apoplastic enzyme preparation from epicotyls of *Vigna angularis*. *Physiol. Plant.* **82**: 490-497.
- Nishitani, K. and Tominaga, R.** 1992. Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. *J. Biol. Chem.* **267**: 21058-21064.
- Okazawa, K., Sato, Y., Nakagawa, T., Asada, K., Kato, I., Tomita, E. and Nishitani, K.** 1993. Molecular cloning and cDNA sequencing of endo-xyloglucan transferase, a novel class of glycosyltransferase that mediates molecular grafting between matrix polysaccharides in plant cell walls. *J. Biol. Chem.* **268**: 25364-25368.
- Peschke, V.M. and Sachs, M.M.** 1994. Characterization and expression of transcripts induced by oxygen deprivation in maize (*Zea mays* L.). *Plant Physiol.* **104**: 387-394.
- Pfeffer, W.** 1900. *In the Physiology of Plants.* p. 480-485.
- Potter, I. and Fry, S.C.** 1993. Xyloglucan endotransglycosylase activity in pea internodes. *Plant Physiol.* **103**: 235-241.
- Potter, I. and Fry, S.C.** 1994. Changes in xyloglucan endotransglycosylase (XET) activity during hormone-induced growth in lettuce and cucumber hypocotyls and spinach cell suspension cultures. *J. Exp. Bot.* **45**: Special Issue 1703-1710.
- Preston, R.D.** 1974. *Physical Biology of Plant Cell Walls.* Chapman and Hall Ltd. London.
- Pritchard, J., Hetherington, P.R., Fry, S.C. and Tomos, A.D.** 1993. Xyloglucan endotransglycosylase activity, microsomal orientation and the profiles of cell wall

- properties along growing regions of maize roots. *J. Exp. Bot.* **44**: 1281-1289.
- Ray, P.M.** 1967. Radioautographic study of cell wall deposition in growing plant cells. *J. Cell Biol.* **35**: 659-674.
- Ray, P.M.** 1980. Cooperative action of β -glucan synthetase and UDP-xylose xylosyl transferase of Golgi membranes in the synthesis of xyloglucan like-polysaccharides. *Biochim. Biophys. Acta.* **629**: 431-444.
- Revilla, G. and Zarra, I.** 1987. Changes in the molecular weight distribution of the hemicellulosic polysaccharides from rice coleoptiles growing under different conditions. *J. Exp. Bot.* **38**: 1818-1825.
- Sakurai, N.** 1990. Cell wall functions in growth and development—a physical and chemical point of view. *Bot. Mag. Tokyo* **104**: 235-251.
- Sakurai, N., Nishitani, K. and Masuda, Y.** 1979. Auxin-induced changes in the molecular weight of hemicellulosic polysaccharides of *Avena* coleoptile cell wall. *Plant Cell Physiol.* **20**: 1349-1357.
- Schulze, E.** 1891. Zur Kenntniss der chemischen Zusammensetzung der pflanzlichen Zellmembranen. *Berichte der Deutsche Chemische Gesellschaft.* **24**: 2277-2287.
- Smith, R.C. and Fry, S.C.** 1991. Endotransglycosylation of xyloglucans in plant cell suspension cultures. *Biochem. J.* **279**: 529-535.
- Talbott, L.D. and Pickard, B.G.** 1994. Differential changes in size distribution of xyloglucan in the cell walls of gravitropically responding *Pisum sativum* epicotyls. *Plant Physiol.* **106**: 755-761.
- Talbott, L.D. and Ray, P.M.** 1992a. Molecular size and separability features of pea cell wall polysaccharides. *Plant Physiol.* **98**: 357-368.
- Talbott, R.D. and Ray, P.M.** 1992b. Changes in molecular size of previously deposited and newly synthesized pea cell wall matrix polysaccharides. *Plant Physiol.* **98**: 369-379.
- Talmadge, K., Keegstra, K., Bauer, W.D. and Albersheim, P.** 1973. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. *Plant Physiol.* **51**: 158-173.
- Tucker, M.L. and Milligan, S.B.** 1991. Sequence analysis and comparison of avocado fruit and bean abscission cellulases. *Plant Physiol.* **95**: 928-933.
- Verma, D.P.S., MacIachlan, G.A., Byrne, H. and Ewing, D.** 1975. Regulation and *in vitro* translation of messenger ribonucleic acid for cellulase from auxin-treated pea epicotyls. *J. Biol. Chem.* **250**: 1019-1026.
- Waeghe, T.J., Darvill, A.G., McNeil, M. and Albersheim, P.** 1983. Determination, by methylation analysis, of the glycosyl-linkage compositions of microgram quantities of complex carbohydrates. *Carbohydr. Res.* **123**: 281-304.
- Wakabayashi, K., Sakurai, N. and Kuraishi, S.** 1991. Differential effect of auxin on molecular weight distributions of xyloglucans in cell walls of outer and inner tissues from segments of dark grown squash (*Cucurbita maxima* Duch.) hypocotyls. *Plant Physiol.* **95**: 1070-1076.
- Yamamoto, R. and Masuda, Y.** 1971. Stress-relaxation properties of *Avena* coleoptile cell wall. *Physiol. Plant.* **25**: 330-335.
- Yamamoto, R., Shinozaki, K. and Masuda, Y.** 1970. Stress-relaxation properties of plant cell walls with special reference to auxin action. *Plant Cell Physiol.* **11**: 947-956.
- Zurek, D.M. and Clouse, S.D.** 1994. Molecular cloning and characterization of a brassinosteroid-regulated gene from elongating soybean (*Glycine max* L.) epicotyls. *Plant Physiol.* **104**: 161-170.
- Zurek, D.M., Rayle, D.L., McMorris, T.C. and Clouse, S.D.** 1994. Investigation of gene expression, growth kinetics, and wall extensibility during brassinosteroid-regulated stem elongation. *Plant Physiol.* **104**: 505-513.

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