

Mini Review

The XTH Family of Enzymes Involved in Xyloglucan Endotransglucosylation and Endohydrolysis: Current Perspectives and a New Unifying Nomenclature

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The polysaccharide xyloglucan is thought to play an important structural role in the primary cell wall of dicotyledons. Accordingly, there is considerable interest in understanding the biochemical basis and regulation of xyloglucan metabolism, and research over the last 16 years has identified a large family of cell wall proteins that specifically catalyze xyloglucan endohydrolysis and/or endotransglucosylation. However, a confusing and contradictory series of nomenclatures has emerged in the literature, of which xyloglucan endotransglycosylases (XETs) and endoxyloglucan transferases (EXGTs) are just two examples, to describe members of essentially the same class of genes/proteins. The completion of the first plant genome sequencing projects has revealed the full extent of this gene family and so this is an opportune time to resolve the many discrepancies in the database that include different names being assigned to the same gene. Following consultation with members of the scientific community involved in plant cell wall research, we propose a new unifying nomenclature that conveys an accurate description of the spectrum of biochemical activities that cumulative research has shown are catalyzed by these enzymes. Thus, a member of this class of genes/proteins will be referred to as a xyloglucan endotransglucosylase/hydrolase (XTH). The two known activities of XTH proteins are referred to enzymologically as xyloglucan endotransglucosylase (XET, which is hereby re-defined) activity and xyloglucan endohydrolase (XEH) activity. This review provides a summary of the biochemical and functional diversity of XTHs, including an overview of the structure and organization of the *Arabidopsis* XTH gene family, and highlights the potentially important roles that XTHs appear to play in numerous examples of plant growth and development.

Keywords: Cell expansion — Cell wall — Hydrolase — Endotransglucosylase — Xyloglucan.

Abbreviations: Ara, arabinose; EXT or EXGT, endo-xyloglucan transferase (one of the previous nomenclatures used for an XTH); Fuc,

fucose; Gal, galactose; Glc, glucose; M_r , relative molecular mass; O→O, oligosaccharide-to-oligosaccharide (endotransglucosylation); P→O, polysaccharide-to-oligosaccharide (endotransglucosylation); P→P, polysaccharide-to-polysaccharide (endotransglucosylation); XEH, xyloglucan endohydrolase (name of one of the two enzyme activities exhibited by XTHs); XET, formerly xyloglucan endotransglycosylase (one of the previous nomenclatures used for an XTH) — here re-defined as xyloglucan endotransglucosylase (name of one of the two enzyme activities exhibited by XTHs); XGO, xyloglucan oligosaccharide (general); XTH, xyloglucan endotransglucosylase/hydrolase (name of protein or gene); XTR, XET-related (one of the previous nomenclatures used for an XTH); XXXG, XXLG, XLLG, XXFG, XXG, XG, etc., specific XGOs (for nomenclature, see Fry et al. (1993); Xyl, xylose. The suffix “-ol” refers to XGOs that have been reduced.

Introduction

The plant primary cell wall is a dynamic structure that exhibits considerable spatial and temporal variability in terms of composition and organization, reflecting a balance between wall synthesis, deposition, reorganization and selective disassembly. This is achieved through the coordinated action of a battery of wall synthesizing and modifying enzymes that collectively provide a mechanism for regulating cell size, shape and cell-cell adhesion. Thus, selective modification of wall architecture is an integral part of processes as diverse as cellular growth, fruit softening, organ abscission, vascular differentiation and responses to pathogens (reviewed in Carpita and McCann 2000).

Studies over the last four decades suggest that land plants have evolved a fundamentally similar primary wall structure: layers of cellulose microfibrils enmeshed in matrices of structurally varied hemicellulosic and pectic polysaccharides, with additional quantitatively minor components such as structural proteins (Carpita and Gibeau 1993). While compositional analysis can confirm the presence of different classes of polysaccharides and proteins, it is much more difficult to establish how they are associated with each other in a mature wall.

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However, there is evidence that one of the key interactions in the primary wall of dicotyledons is formed between cellulose and the hemicellulose xyloglucan, which together typically comprise about two thirds of the dry wall mass. Xyloglucan binds non-covalently to cellulose, coating and cross-linking adjacent cellulose microfibrils (Hayashi 1989, McCann et al. 1990) and the resulting extensive xyloglucan-cellulose network is thought to act as the major tension-bearing structure in the primary wall. Xyloglucan metabolizing enzymes therefore represent potentially important agents for controlling wall strength and extensibility, for example through the modification of load-bearing xyloglucan tethers (Fry 1989).

Cleavage of load-bearing xyloglucan chains by hydrolytic enzymes might be a means of achieving rapid wall loosening; however, in the absence of significant wall synthesis and reinforcement, the net result would be a reduction in wall tensile strength, with an increased potential for disastrous structural failure. Thinning of the wall as cell expansion proceeded would further exacerbate this weakening. An alternative mechanism for cleaving structurally important xyloglucans emerged from an early hypothesis that cell growth involves "...an endotransglucosylase that transfers a portion of a polysaccharide to itself" (Albersheim 1976).

Two independent research groups originally described evidence of such enzymes, now referred to by the Enzyme Commission as EC 2.4.1.207, capable of splitting and reconnecting xyloglucan molecules in rapidly growing plant tissues. Fry and colleagues named the enzyme corresponding to this activity xyloglucan endotransglucosylase (XET; Smith and Fry 1991), while Nishitani and Tominaga (1992) described the purification of a protein, with a similar activity, that they termed endoxyloglucan transferase (EXT, later redesignated EXGT). At the same time, and as part of a third independent line of research, a "xyloglucan-specific endo- β -1,4-glucanase", or "xyloglucanase", from nasturtium seeds, involved in depolymerization of storage xyloglucans after germination, was shown to exhibit xyloglucan endotransglucosylase activity under certain conditions *in vitro* (Farkaš et al. 1992, Fanutti et al. 1993). This contrasted with its known xyloglucan endohydrolase activity and exemplifies an interesting distinction between transglycosylase or hydrolase activities detected *in vitro*, and enzymic modes of action *in vivo*, as is discussed in more detail later in this review.

The first cloning of genes encoding proteins with xyloglucan endotransglucosylase activity from different plant species (Okazawa et al. 1993) revealed sequence homology with the gene corresponding to the nasturtium xyloglucanase (de Silva et al. 1993), suggesting that they belong to the same class of proteins. Over the last 10 years, a large number of XET/EXGT-related genes have been cloned from a range of plant species, where they comprise substantial gene families that are typically present in each species as three or four phylogenetically distinct subgroups (Nishitani 1997, Schünmann et al. 1997, Campbell and Braam 1999a, Catalá et al. 2000, Uozu et al.

2000, Yokoyama and Nishitani 2001b). Furthermore, purification and characterization of the enzymic activity of XETs/EXGTs, or recombinant proteins derived from *XET/EXGT* genes, suggests that a spectrum of activities exists, from isozymes that act exclusively as xyloglucan endotransglucosylases to those that function as xyloglucan endohydrolases (Tabuchi et al. 1997, Schröder et al. 1998, Tabuchi et al. 2001).

This review provides a summary of the structural, biochemical and functional diversity of members of this class of proteins/genes and includes a proposal to resolve the increasingly confusing nomenclature that has evolved over the last 10 years.

Outline of a new unifying nomenclature

The proliferation of research in this area has unfortunately also led to the adoption of many overlapping and contradictory nomenclatures and classifications for the same sets of genes/proteins. Worse still, some XET/EXGT genes have been assigned more than one name in the public databases. Now that the genomes of a monocotyledon (rice) and a dicotyledon (*Arabidopsis*) have been sequenced and the extensive nature of this class of genes has been revealed, it is an appropriate time to develop a systematic and logical approach to naming new and existing XET/EXGT genes. This will become increasingly important as the databases expand and additional large-scale plant genome sequencing initiatives progress. With this in mind, a discussion was initiated among researchers in the field through the Cell Wall Newsgroup (cellwall@listserv.nodak.edu), culminating in an open discussion forum at the 9th International Cell Wall Meeting, Toulouse, France in September 2001. It was noted that two of the talks in the main meeting presented data related to the expression and regulation of the same XET/EXGT gene, but used different gene names, thus making potentially important comparisons of the results impossible for those in the audience who were unaware of the naming discrepancy. It was unanimously agreed that a standardized nomenclature is urgently needed and, after some discussion, a consensus was reached that a member of this class of genes/proteins should be termed a xyloglucan endotransglucosylase/hydrolase (XTH). This name was selected to describe accurately the range of enzymic activities encoded by divergent XTH family members, the specificity of the substrate, and the identity of the non-terminal glycosyl residues (Glc) that are cleaved during the transglycosylation or hydrolysis reaction. The adoption of a three-letter abbreviation also conforms to the convention for naming gene families (<http://mbclserver.rutgers.edu/CPGN/Guide.html>).

We further make the following proposals.

(1) The XTH nomenclature should be adopted universally when naming a newly identified gene that, based on sequence homology, would previously have been designated as an XET or EXGT. The availability of the entire genome sequences of rice and *Arabidopsis* should make the identification of *XTH* orthologs relatively easy.

| | New Gene Name | Previous Gene Name(s) | AGI ID | Gene Structure |
|---------|-----------------|--------------------------------|-----------|----------------|
| Group 1 | <i>At-XTH1</i> | | At4g13080 | |
| | <i>At-XTH2</i> | | At4g13090 | |
| | <i>At-XTH3</i> | | At3g25050 | |
| | <i>At-XTH4</i> | <i>EXGT-A1</i> or <i>EXT</i> | At2g06850 | |
| | <i>At-XTH5</i> | <i>EXGT-A4</i> or <i>XTR12</i> | At5g13870 | |
| | <i>At-XTH6</i> | <i>XTR10</i> | At5g65730 | |
| | <i>At-XTH7</i> | <i>XTR15</i> | At4g37800 | |
| | <i>At-XTH8</i> | | At1g11545 | |
| | <i>At-XTH9</i> | <i>EXGT-A6</i> or <i>XTR16</i> | At4g03210 | |
| | <i>At-XTH10</i> | <i>XTR14</i> | At2g14620 | |
| | <i>At-XTH11</i> | | At3g48580 | |
| Group 2 | <i>At-XTH12</i> | | At5g57530 | |
| | <i>At-XTH13</i> | | At5g57540 | |
| | <i>At-XTH14</i> | <i>XTR9</i> | At4g25820 | |
| | <i>At-XTH15</i> | <i>XTR7</i> | At4g14130 | |
| | <i>At-XTH16</i> | | At3g23730 | |
| | <i>At-XTH17</i> | <i>XTR1</i> | At1g65310 | |
| | <i>At-XTH18</i> | | At4g30280 | |
| | <i>At-XTH19</i> | | At4g30290 | |
| | <i>At-XTH20</i> | | At5g48070 | |
| | <i>At-XTH21</i> | <i>XTR17</i> | At2g18800 | |
| | <i>At-XTH22</i> | <i>TCH4</i> | At5g57560 | |
| | <i>At-XTH23</i> | <i>XTR6</i> | At4g25810 | |
| | <i>At-XTH24</i> | <i>Mer15</i> | At4g30270 | |
| | <i>At-XTH25</i> | <i>EXGT-A5</i> or <i>XTR3</i> | At5g57550 | |
| | <i>At-XTH26</i> | <i>XTR18</i> | At4g28850 | |
| Group 3 | <i>At-XTH27</i> | <i>EXGT-A3</i> | At2g01850 | |
| | <i>At-XTH28</i> | <i>EXGT-A2</i> or <i>XTR2</i> | At1g14720 | |
| | <i>At-XTH29</i> | <i>XTR13</i> | At4g18990 | |
| | <i>At-XTH30</i> | <i>XTR4</i> | At1g32170 | |
| | <i>At-XTH31</i> | <i>ATXG</i> or <i>AtXTR8</i> | At3g44990 | |
| | <i>At-XTH32</i> | | At2g36870 | |
| | <i>At-XTH33</i> | | At1g10550 | |

Fig. 1 Systematic nomenclature and gene structure of the *Arabidopsis XTH* gene family. The family of 33 *Arabidopsis XTH* genes is shown divided into three major groups (see also Fig. 2). Each gene has the new systematic XTH nomenclature, the previously used common names, the designated identification number assigned by the Arabidopsis Genome Initiative (AGI ID; Arabidopsis Genome Initiative 2000) and a schematic diagram of the corresponding gene structure. Exons are shown as open boxes and introns as lines. The crosshatched boxes indicate a putative signal peptide directing secretion of the polypeptide into the cell wall and the filled boxes represent the DEIDFEFLG motif that is thought to correspond to the XTH catalytic site.

(2) Newly identified *XTH* genes should be named with the first letter of the genus and the species, followed by a hyphen and then the XTH abbreviation and a number; thus we have designated the members of the *Arabidopsis thaliana XTH* gene family *At-XTH1*, *At-XTH2* etc.

(3) To facilitate the naming of newly identified *XTH* genes, and to address any queries, a website (www.plantbio.cornell.edu/XTH) will be established at Cornell University with responsibility for listing existing *XTH* genes that are currently in the database, using the proposed standardized nomenclature. Researchers with newly identified *XTH* genes are encouraged to refer to the website when naming the genes and to inform the website administrator of the new gene name and accession number. The website will contain additional XTH-related information and resources. Researchers who have already named

and published *XET/EXGT* gene sequences are encouraged to contact the website administrator to allow a new XTH designation to be assigned. The databases may then be updated accordingly so that each XTH in the database has a reference to both the old and the new nomenclature.

(4) In studies of enzymic activity or polysaccharide metabolism, where it is necessary to specify whether the reaction being discussed is a transglycosylation or a hydrolysis, the terms xyloglucan endotransglucosylase (activity) and xyloglucan endohydrolase (activity) should be used. These activities should be abbreviated as XET (which is hereby re-defined) and XEH, respectively. In the rest of this present paper, we use 'XET' in this new sense: XET and XEH should be used to refer to enzyme activities, and not for naming genes or proteins.

As an example of this approach, this review includes a

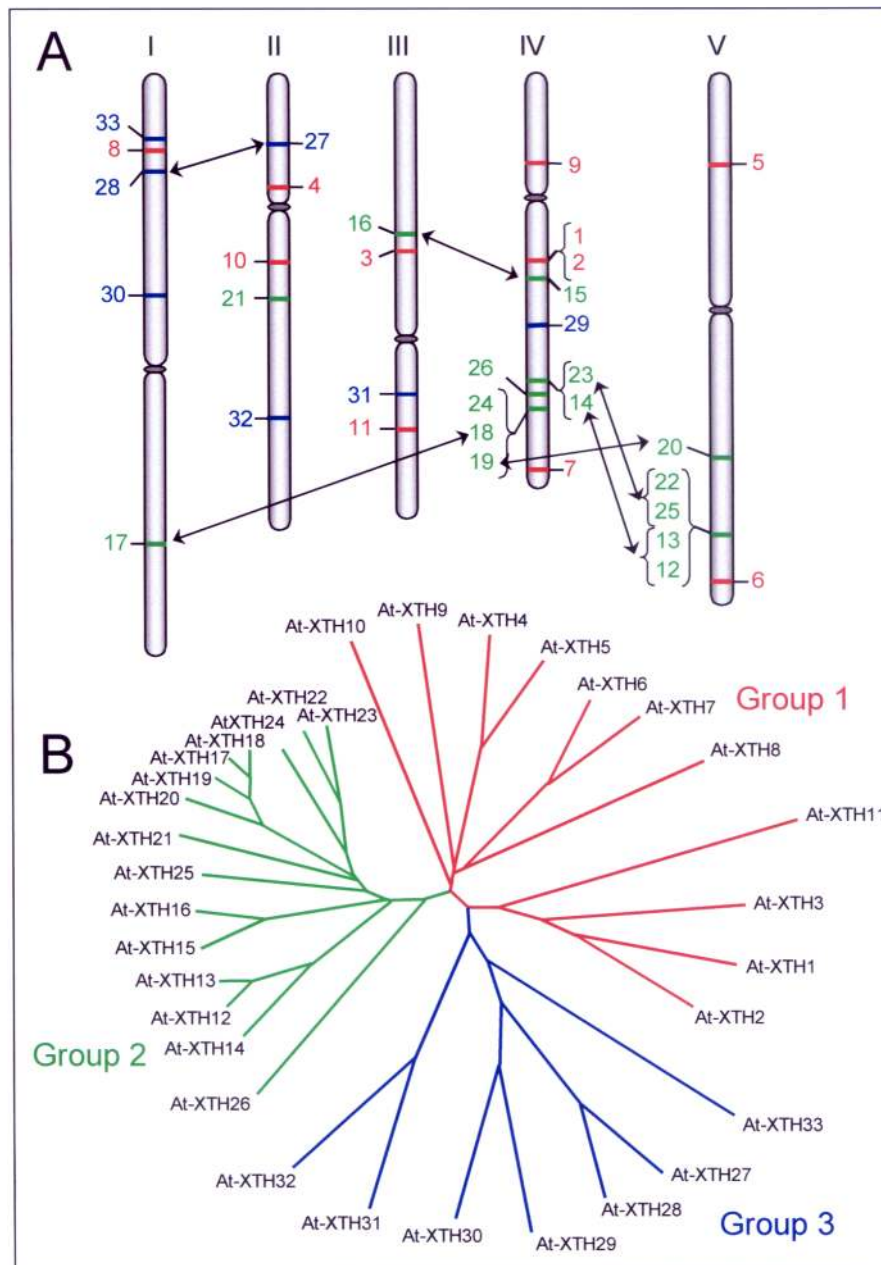


Fig. 2 Genomic structure and phylogenetic relationship between the members of the *Arabidopsis XTH* gene family. (A) Physical map showing the distribution of the *Arabidopsis XTH* genes (see also Fig. 1) among the five chromosomes with arrows indicating duplicated genes. (B) Dendrogram generated using CLUSTALW (<http://spiral.genes.nig.ac.jp/homology>) and TreeViewPPC software, based on the predicted amino acid sequences of all 33 *Arabidopsis XTH* genes. For both parts A and B of the figure, genes from groups 1, 2 and 3 are shown in red, green and blue, respectively. The physical map and dendrogram are revised from versions published in Yokoyama and Nishitani (2001b).

description of the *Arabidopsis XTH* gene family and a list of genes with both the new nomenclature and all previously used names (Fig. 1).

The Arabidopsis XTH gene family and its possible evolutionary origins

In *A. thaliana* Columbia ecotype, 33 open reading frames

(ORFs) potentially encoding XTH proteins have been identified based on the genome sequence database (*Arabidopsis* Genome Initiative 2000) and the cDNA sequence data (Asamizu et al. 2000). Transcripts derived from each of the 33 genes have been identified by RT-PCR using gene-specific primer-sets (Yokoyama and Nishitani 2001b), indicating that all 33 XTH ORFs represent functional genes. Thus, there are

good grounds for a systematic genome-based nomenclature for the *Arabidopsis XTH* gene family (Fig. 1).

The 33 *XTH* genes are dispersed across all five chromosomes of *Arabidopsis*, with one third of the genes occurring as clusters consisting of two to four members (Fig. 2A and Yokoyama and Nishitani 2001b), probably resulting from genome duplication and gene reshuffling (Blanc et al. 2000). For example, *At-XTH28* located on chromosome I and *At-XTH27* on chromosome II share one ancestral gene, while *At-XTH16* on chromosome III and *XTH15* on chromosome IV also share a common ancestor. The cluster of *At-XTH14* and *At-XTH23* on chromosome IV corresponds to the cluster of *At-XTH12*, *At-XTH13*, *At-XTH25*, and *At-XTH22* on chromosome V. Based on the order and orientation of these six genes, *At-XTH14* on chromosome IV may correspond to an ancestral gene that was further duplicated to generate *At-XTH12* and *At-XTH13* on chromosome V. Similarly, *At-XTH23*, located next to *At-XTH14* on chromosome IV, may correspond to the ancestral gene for *At-XTH22* and *At-XTH25* on chromosome V (Yokoyama and Nishitani 2001b). These gene duplications are believed to have resulted from large-segment gene duplication (*Arabidopsis* Genome Initiative 2000). However, in addition, two pairs of solitary *XTH* genes have been identified as having been duplicated, possibly by transposition: *At-XTH17* and *At-XTH18* share almost identical sequences in their promoter regions, while *At-XTH19* and *At-XTH20* are identified as duplicates by phylogenetic analysis (Fig. 2B).

The *Arabidopsis XTH* gene family can be divided into three major phylogenetic groups, or subfamilies (Fig. 2B and Yokoyama and Nishitani 2001b), in good agreement with other phylogenetic studies of *XTH* genes from a broad range of plant species (Nishitani 1997, Schünmann et al. 1997, Campbell and Braam 1999b, Catalá et al. 2000, Uozu et al. 2000). This classification is based not only on the dendrogram shown in Fig. 2B, but also on the structure and organization of individual genes, as outlined in Fig. 1. Most members of Group 1 contain four exons, whereas *XTHs* in Group 2 have two or three exons, with the exception of *At-XTH26*, which has four exons. On the other hand, members of Group 3 are composed of four or five exons and possess characteristic amino acid sequences, particularly in their C-terminal regions. Many *XTHs* share a conserved DEIDFEFLG sequence, a motif that is considered to function as catalytic site for both hydrolase and transferase activity (Okazawa et al. 1993, Campbell and Braam 1998). This motif is located in the third exon in Group 1 and 3, while in Group 2 it is located in the second exon, again with the exception of *At-XTH26*. Another structural feature common to the *XTH* family is the sequence in the beginning of the first exon, encoding a potential signal peptide that has been shown to direct secretion into the apoplast (Yokoyama and Nishitani 2001b). As an apparent exception, the current database sequence for *At-XTH6* does not include a predicted signal peptide, although it remains to be confirmed that this sequence is actually absent in the full-length *At-XTH26* mRNA.

The group numbering used here is consistent with that in previous studies, where the closely related *XTH* genes described in Okazawa et al. (1993) belong to Group 1 and the nasturtium *XTH*-encoding *NXG1* aligns most closely with Group 3 (Campbell and Braam 1999b). It should be noted that some of these previous reports identified a fourth phylogenetic group when *XTH* sequences from monocotyledons such as barley (*Hordeum vulgare*) were included. An obvious consideration is that this phylogenetic divergence reflects the evolution of *XTH* subgroups with different biochemical mechanisms of action, such as transglycosylation versus hydrolysis. Indeed, individual members of Groups 1 and 2, including *At-XTH4* (Okazawa et al. 1993), and *At-XTH22* (Xu et al. 1995), respectively, have been demonstrated to mediate exclusively transglycosylation between xyloglucans in vitro (Nishitani and Tominaga 1992, Xu et al. 1996, Campbell and Braam 1999a), while members of Group 3, as represented by *At-XTH31* (Aubert and Herzog 1996) have been shown to catalyze xyloglucan endohydrolysis (Fanutti et al. 1993, de Silva et al. 1993, Tabuchi et al. 2001). Thus, the *XTH* gene family has apparently undergone diversification into two groups in terms of acceptor-substrate specificity. However, while this suggests a trend, it is important to note that the phylogenetic grouping has not been definitively shown to reflect any particular biochemical characteristics or mechanism of action and exceptions have been reported (Schröder et al. 1998). Indeed, not all *At-XTH* genes have yet been shown to encode catalytically active enzymes.

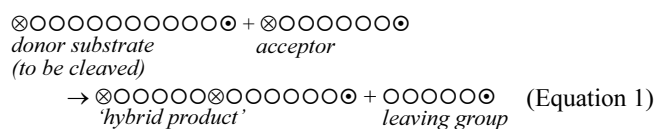
Furthermore, within each *XTH* subfamily, no clear diversification is found with respect to enzyme action (Campbell and Braam 1999a). The presence of multiple genes encoding similar enzymic activities raises the question of whether individual members, particularly those in each subfamily, are redundant in terms of their physiological roles, or whether there is simply a functional distinction that has not yet been discerned. To address this question, a comprehensive expression analysis of the *Arabidopsis XTH* gene family was conducted, using real-time PCR to quantify mRNA levels accurately, and to distinguish between gene products with similar nucleotide sequences (Yokoyama and Nishitani 2001b). This analysis has revealed that many members of the *XTH* gene family exhibit distinct organ- or tissue-specific expression profiles. For example, within Group 2, *At-XTH17*, *-18*, *-19*, and *-20* are predominantly expressed in roots, *At-XTH21* and *At-XTH22* in siliques and *At-XTH24* in stems. Furthermore, individual *XTH* genes respond differently to plant hormones: *At-XTH17*, *-18*, *-19*, and *-20*, which exhibit the same organ-specific expression profile, are differentially regulated by auxin, gibberellins, brassinolide and abscisic acid (Yokoyama and Nishitani 2001b). These results are consistent with those reported by Xu et al. (1996) and Akamatsu et al. (1999) using RNA blot analyses, and similar studies demonstrated differential regulation of *XTH* mRNA expression in response to environmental stimuli (Xu et al. 1996). In conclusion, despite the

limited range of regulatory factors and spatial and temporal variables that have been tested experimentally, it is clear that there is considerable variability in gene expression among members of the *Arabidopsis XTH* family. Therefore, functional redundancy is not likely to be common: each *XTH* gene is likely to have a unique “fingerprint” of expression and regulation, reflecting a unique physiological function, which will be revealed as the degree of experimental sophistication and complexity increases.

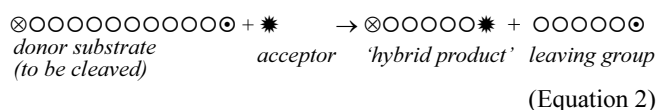
Assay of the activities of XTHs in vitro

As described above, divergent XTHs exhibit one or both of the two distinct biochemical activities in vitro: xyloglucan endotransglucosylation (XET) and xyloglucan hydrolysis (XEH). The following section provides an overview of the biochemical diversity that has been observed and methods of detecting and characterizing that diversity.

(a) *Xyloglucan endotransglucosylase activity*—The transglucosylation reaction catalyzed by XTHs (EC 2.4.1.207) can be represented as:



Each circle represents a Glc₄-based xyloglucan oligosaccharide (XGO) and the reducing end is shown to the right. The substrates and products do not necessarily differ from each other chemically, so in-vitro assays are designed to lead to a size-change. Xyloglucan ($M_r \approx 10^5$ – 10^6) is used as a donor substrate and the acceptor is a single Glc₄-based XGO (M_r 1,062–1,386). The catalyzed reaction is then



where * is an XGO.

If the acceptor is ³H-labeled (e.g. [³H]XLLGol) the ‘hybrid’ product is then easily assayed because, unlike the donor, it is radioactive and, unlike the acceptor, it is of high- M_r (Fry et al. 1992a). The reaction mixture is dried on to filter paper, then washed in water: the hybrid product is the only ³H-labeled material that stays attached to the paper and is assayed by scintillation-counting. The assay is quantitative and highly sensitive.

Alternatively, the XGO (Equation 2) is fluorescently labeled with a pyridylamino (Nishitani and Tominaga 1992), sulphorhodamine (Fry 1997) or fluorescein group (Ito and Nishitani 1999). In this case, since the hybrid product is fluorescently rather than radioactively labeled, and has a high M_r , acceptor substrates such as XGO–sulphorhodamine can be used in a high-throughput screen for XET activity (Fry 1997). Enzyme extracts are spotted on to, and incubated with, filter paper impregnated with xyloglucan plus XGO–sulphorhodam-

ine. The paper is then washed free of XGO, leaving fluorescent spots of hybrid product bound to the paper. The assay is suitable for crude plant extracts and tissue-prints, and for blotting gel electrophoretograms to make zymograms (Iannetta and Fry 1999).

Another assay for XET activity uses non-labeled XGOs, which cause a reduction in mean M_r of the polysaccharide, detectable by colorimetry (Sulová et al. 1995) or viscometry (Farkaš et al. 1992).

(b) *Xyloglucan endohydrolase activity*—XEH, the other enzyme activity of some XTHs, is assayed as the production of new reducing termini when xyloglucan is incubated in the absence of XGOs (Tabuchi et al. 2001).

Potential repertoire of XTH action observed in vitro

The simplified assays given above do not necessarily display the enzymes’ full repertoires. We discuss here the ability of XTHs to catalyze diverse reactions in vitro. Transglucosylation (Equation 1) is the breaking of one glucosyl bond and making of another. We will describe transglucosylations in the format ‘X→Y’, where ‘X’ is the donor and ‘Y’ the acceptor.

(i) *Polysaccharide-to-oligosaccharide (P→O) endotransglucosylation*—The first reported XTH-catalyzed transglucosylations were P→O. Baydoun and Fry (1989) found that an activity from cultured spinach cells attached [*Xyl*-³H]XXFG or [*Fuc*-³H]XXFG to high- M_r xyloglucan, apparently by transglucosylation. When [*reducing-terminal-Glc*-1-³H]XXFG was used, [³H]xyloglucan was still formed (Smith and Fry 1989), and within this product the [³H]Glc moiety remained at the reducing terminus (reducible to [³H]glucitol by NaBH₄) (Smith and Fry 1991), as required by Equation 1. This shows that the oligosaccharide was the acceptor substrate, since if it had been the donor the ³H would have been released as part of a leaving group that was even smaller than the initial [³H]XGO. Extracts from diverse land plants contained an enzyme activity that catalyzed P→O transglucosylation (Equation 2) (Fry et al. 1992a). The [³H]XGO was releasable, intact, from the hybrid product by cellulase, showing that the xyloglucan was linked to the [³H]XGO by a β-(1→4) bond.

Independently, Nishitani and Tominaga (1991) identified an enzyme activity from the apoplast of azuki bean stems that catalyzed molecular grafting between xyloglucans, and subsequently purified a 33-kDa enzyme from the same tissue that catalyzed P→O transglycosylation with pyridylamino XXXG as the acceptor (Nishitani and Tominaga 1992). NMR spectra indicated that the only detectable Glc→Glc bonds, both before and after enzyme action, were β-(1→4). Thus, the enzyme broke β-Glc-(1→4)-Glc bonds and made new β-Glc-(1→4)-Glc bonds. Also independently, nasturtium seed enzymes were shown to catalyze P→O transglucosylation in addition to endohydrolysis (Farkaš et al. 1992, Fanutti et al. 1993).

(ii) *Polysaccharide-to-polysaccharide (P→P) endotransglucosylation*—Sudden increases in the M_r of wall-bound xyloglucans had been noted several times in vivo (Nishitani

and Masuda 1982, Talbott and Ray 1992), and these were speculated to be due to P→P transglucosylation (Talbott and Ray 1992). P→P transglucosylation was catalyzed in vitro by an enzyme activity from pea stem that was erroneously called ‘cellulase’: in a mixture of moderate- M_r , ^3H -labeled xyloglucan plus high- M_r , ^1H -xyloglucan, it caused some convergence of M_r , yielding high- M_r , ^3H -labeled products (McDougall and Fry 1990). Nishitani and Tominaga (1991) obtained complementary evidence for P→P transglucosylation catalyzed by an activity from azuki bean stems. An initially uniform population of xyloglucans with a mean M_r of 420,000 diverged to give two populations with mean M_r of about 149,000 and 820,000, the latter being insoluble. In addition, a purified azuki bean XTH catalyzed the transfer of ~130,000- M_r segments of xyloglucan from a donor substrate of M_r 230,000 to a fluorescently labeled xyloglucan (of M_r 15,000) that acted as acceptor substrate (Nishitani and Tominaga 1992).

Equation 1 shows that P→P transglucosylation does not cause any change in the mean M_r of the xyloglucan population. Nevertheless, an increase in measured mean M_r could come about either (a) if the donor substrate were always cleaved near its reducing terminus and the very small leaving group were overlooked, e.g. because it was lost by dialysis, or (b) if products over a certain M_r became resistant to further enzymic cleavage, e.g. owing to their low solubility.

P→P transglucosylation, independent of any change in M_r distribution, was demonstrated in a mixture of high- and low-density xyloglucans, i.e. xyloglucans with and without ^{13}C -labeling, respectively, which thereby converged with respect to their buoyant densities (Thompson et al. 1997).

(iii) *Oligosaccharide-to-oligosaccharide (O→O) endotransglucosylation*—Fanutti et al. (Fanutti et al. 1993, Fanutti et al. 1996) showed that a nasturtium seed XTH could catalyze O→O endotransglucosylation with donors as small as the Glc₈-based XXXGXXXG:



However, most XTHs do not use such small donors.

(iv) *Polysaccharide endohydrolysis*—The XEH action of XTHs is effectively a variant of Equation 2, in which the acceptor is H₂O instead of an XGO. While some XTHs cannot catalyze xyloglucan endohydrolysis at measurable rates (Nishitani and Tominaga 1992), XEH activity has been reported in nasturtium seeds (Fanutti et al. 1993), pea stems (Matsumoto et al. 1997), azuki bean stems (Tabuchi et al. 1997), ripening tomato fruit (Maclachlan and Brady 1994) and kiwifruit cores (Schröder et al. 1998). The activity is particularly prevalent in vitro when few acceptor molecules are present; however, an XTH from azuki bean stems exhibited XEH, but not XET, activity even in the presence of XGOs (Tabuchi et al. 2001).

It should be noted that plants also have endo-β-1,4-glucanases, also termed “cellulases”, some of which can hydrolyze xyloglucan and other glucans, such as carboxymethylcellulose (Ohmiya et al. 1995, Rose and Bennett 1999). However, the

amino acid sequences of these enzymes are unrelated to those of XTHs and are not considered here (they are reviewed elsewhere in this volume).

Substrate specificity

(a) *Donor (or hydrolyzable) substrate*—The only known donor molecules for XTHs are xyloglucans and, in one case, Glc₈-based XGOs. Some XTHs prefer certain chemical features in the donor: for example, some show greater (Nishitani and Tominaga 1992, Campbell and Braam 1999a) or lesser (Fry et al. 1992a, Rose et al. 1996, Campbell and Braam 1999a) reaction rates with xyloglucans containing Fuc residues. Similarly, a tomato XTH showed a preference for solanaceous xyloglucan, which has no Fuc, over xyloglucan from other sources (Catalá et al. 2001). Some XTHs appear to require relatively high- M_r donor substrates, e.g. $M_r > 10,000$ (Nishitani and Tominaga 1992) or $> 60,000$ (Tabuchi et al. 1997), while others prefer (Schröder et al. 1998), or at least tolerate (Fanutti et al. 1996), lower M_r donor substrates.

The K_m of an XTH for its donor substrate may be difficult to determine because the non-reducing terminus of the ‘donor’ can also act as an acceptor, competing with the labeled XGO used in the assay. Minimizing such competition by using a high concentration of ^3H -acceptor, Purugganan et al. (1997) estimated the K_m of the *Arabidopsis* XTH, At-XTH22, to be 1.8 and 0.62 mg ml⁻¹ for non-fucosylated and fucosylated xyloglucan donors, respectively. However, it should be noted that these values were determined using recombinant XTH enzyme that was generated in *E. coli*, and so may not correspond exactly to the values for the native enzyme due to the lack of co- and post-translational modifications (Campbell and Braam 1998). K_m for donors is quoted in mg ml⁻¹, not μM, because XTHs can ‘see’ any segment of the polysaccharide chain (Steele et al. 2001) — not just one site per molecule, as is the case with the acceptor.

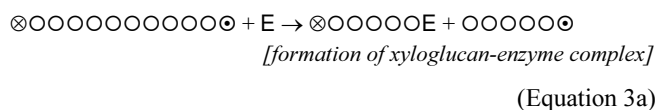
(b) *Acceptor substrate*—All known XTHs that show XET activity can use Glc₄-based XGOs as acceptors (Equation 2). The reducing terminus is not essential, since NaBH₄-reduced XGOs have been shown to act as acceptors for XET activities from several tissues: carrot embryos (Hetherington and Fry 1993); maize roots (Pritchard et al. 1993); kiwifruits (Redgwell and Fry 1993); and pea stems (Potter and Fry 1993). The Xyl/Glc-rich backbone is essential for acceptor function, though XTHs differ in their susceptibility to the shortening or de-xylosylation of this core (Farkaš et al. 1992, Fry et al. 1992a, Lorences and Fry 1993, Fanutti et al. 1996, Steele and Fry 2000). Neither Gal nor Fuc residues are necessary for acceptor function (Fry et al. 1992a, Nishitani and Tominaga 1992), though they may influence the interaction with the enzyme. Characterization of the affinities of an XET activity from pea stems for a range of acceptor substrates revealed a structural preference of XLLG (K_m 19 μM) > XXXG (33 μM) > XXFG (50 μM) > XXG (~200 μM) (Fry et al. 1992a, Lorences and Fry 1993). Similar studies showed that

native XTHs from cauliflower florets had lower affinities for XLLGol as an acceptor (K_m 70–130 μM) than those from mung bean hypocotyls (K_m 16–35 μM) (Steele and Fry 2000). This may be related to the main roles of XTHs in the two tissues: integration of new xyloglucan into the walls of the densely cytoplasmic florets, or restructuring of existing wall material in the rapidly vacuolating hypocotyls. Some XTHs prefer Glc₄-based acceptors (Takeda et al. 1996, Steele and Fry 2000), while others prefer larger XGOs (Steele and Fry 2000) or xyloglucans. For example, At-XTH22 has a much higher affinity for high- M_r xyloglucan (K_m 0.3 μM) than for XLLGol (K_m 73 μM) (Purugganan et al. 1997). Again, it should be noted that these affinity values were calculated using recombinant XTH protein.

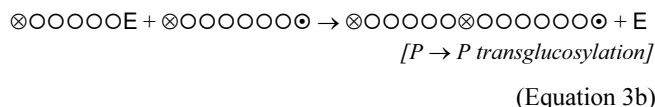
Enzymic mechanisms

XTHs are principally apoplasmic proteins, with appropriate pH optima for this location: typically between 5 and 6 (Campbell and Braam 1999a). The active sites of XTHs are proposed to be DEIDFEFLG or DEIDIEFLG (Okazawa et al. 1993). In agreement with this, a Glu \rightarrow Gln substitution in At-XTH22, converting DEIDFEFL to DQIDFEFL, abolished XET activity (Campbell and Braam 1998). The essential role of an acidic amino acid, presumed to be the first E in DEIDFEFL, was supported by direct observations of XTH–xyloglucan interactions (Sulová et al. 1998). A high- M_r xyloglucan–XTH complex was formed, which could bind both filter paper via the xyloglucan component, and anion-exchange resins via the XTH, unlike either XTH or xyloglucan alone. The complex was stable for min or h, but broke down when an XGO was added, which enabled transglucosylation to be completed. The xyloglucan–XTH linkage was proposed to be a glycosyl ester bond, presumably between the potentially reducing end of part of the donor substrate and the side-chain of glutamate (Sulová et al. 1998).

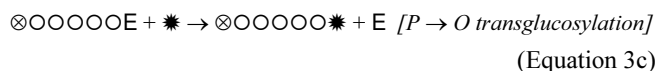
The data suggest that XTH acts in two independent steps. Thus, Equations 1 and 2 can be re-written in more complete form:



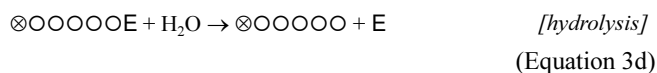
followed by either



or



or



where E = enzyme.

The longevity of the polysaccharide–XTH complex suggests that, in vivo, it can spend some considerable time in the cell wall ‘seeking’ an acceptor with which to consummate the transglucosylation (Fry et al. 1992b). Hydrolysis (Equation 3d) can thus be delayed for long periods, despite the high concentration of H₂O. The existence of xyloglucan–XTH complexes in vivo was supported by the leaching of XTHs from the wall upon addition of XGOs (Sulová et al. 2001).

Kinetic evidence for the double-displacement reaction mechanism proposed above has been provided in the case of purified nasturtium seed XTH (Sulová and Farkaš 1998, Baran et al. 2000), and this is the mechanism normally expected (Laidler 1958) of any transferase that retains the anomeric configuration (β -linkages in both substrate and product). In contrast, it has been suggested, on the basis of kinetic data obtained with an extract containing poplar XET activities, that the donor and acceptor substrates can bind the enzyme independently of each other (Takeda et al. 1996). However, there is still no direct evidence for bonding of an acceptor substrate (e.g. [³H]XGO) to an XTH in the absence of donor substrate; this is in contrast to the direct evidence for bonding of a portion of the donor substrate to the enzyme in the absence of acceptor substrate (Sulová et al. 1998).

Studies of 10 diverse XTHs indicated that none had any tendency to attack a single donor molecule repeatedly (Steele et al. 2001). Thus, after the completion of XET action, the products leave the enzyme’s active site. The ability to form long-lived xyloglucan–XTH complexes provides a simple, mechanism-based procedure for the purification of non-hydrolytic XTHs (Steele and Fry 1999, Sulová and Farkaš 1999).

Site of cleavage of the donor substrate

XTHs could in principle cleave the donor substrate (a) randomly, (b) a prescribed distance from the reducing end, or (c) a prescribed distance from the non-reducing end. Preferential cleavage near the reducing terminus of a xyloglucan would enable the transfer of most of the donor chain onto the acceptor molecule and would generate higher- M_r products. However, cleavage a prescribed distance from the non-reducing terminus would result in the construction of xyloglucan chains with a step-wise distribution of chain-lengths. XTHs that cleave xyloglucan with patterns (a) and (c) have been identified. As an example of the first scenario, an XTH from azuki bean stems, that exhibited only XET activity, catalyzed transglucosylation of polymeric xyloglucan with fluorescent XGOs to give fluorescent products with a mean M_r about 60% of that of the original donor, regardless of the M_r of the latter, within the range 10,900–158,000 (Nishitani and Tominaga 1992). This showed that the enzyme cleaved the donor essentially ran-

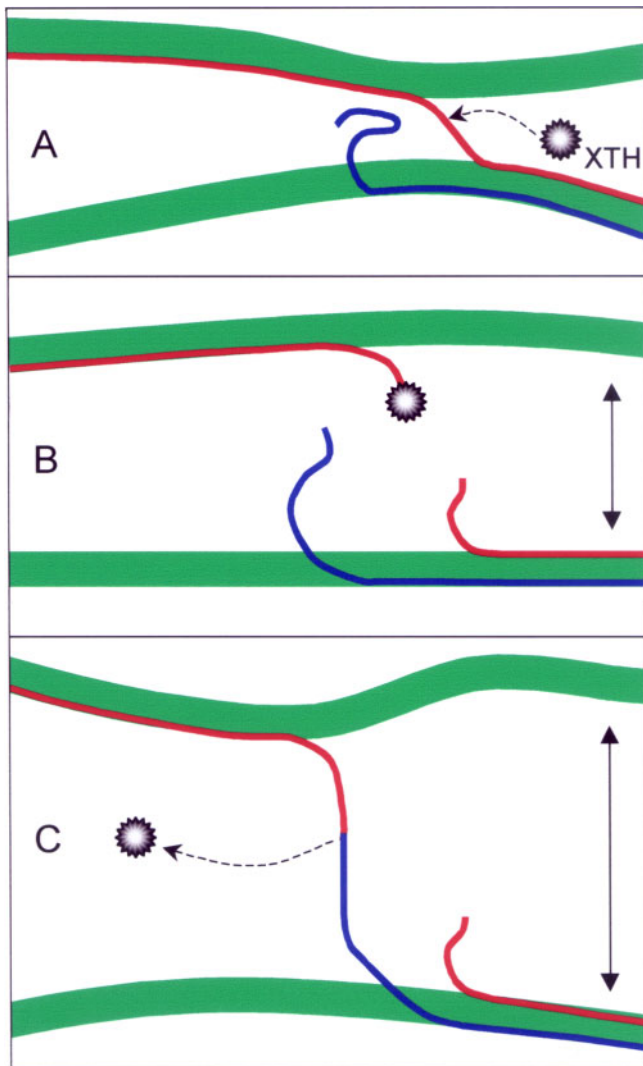


Fig. 3 Polysaccharide-to-polysaccharide (P→P) transglucosylation of xyloglucan in the cell wall showing restructuring-type transglucosylation. (Step 1; panels A to B) XTH cleaves a xyloglucan chain (red line), which is acting as a tether between two neighboring microfibrils. This xyloglucan chain is broken and a xyloglucan–XTH complex is formed. If the cell is turgid, the microfibrils can now move further apart, as indicated by the unbroken arrows. (Step 2; panels B to C) The xyloglucan–XTH complex is now out of reach of the new non-reducing terminus but within reach of the non-reducing terminus of an adjacent xyloglucan chain (blue line). The latter acts as an acceptor substrate and a tether is thereby re-formed between the two microfibrils. The reducing ends of the xyloglucan chains are to the right. Revised from Thompson and Fry (2001).

domly, generating products of, on average, about half the initial length. Conversely, another XTH from azuki bean epicotyls, that showed both XET and XEH activities, hydrolyzed high- M_r xyloglucans to give products with M_r 50,000, but did not degrade the xyloglucan further; xyloglucans of M_r 60,000 were not hydrolyzed (Tabuchi et al. 1997). When the XET activity of the same XTH was observed, 50,000- M_r units of

xyloglucan were transferred from high- M_r donors to fluorescent XGOs. Thus, this XTH, whether exhibiting XET or XEH activity, cleaves xyloglucan at ~ 50 kDa (≈ 80 nm chain length) from the non-reducing terminus. It is not known how a 32-kDa enzyme (~ 5 nm in diameter) might ‘measure out’ a distance of 80 nm from the non-reducing terminus of its substrate, but the result could lead to a step-wise distribution of xyloglucan chain-lengths, as observed by McCann et al. (1992). Another possibility is that the enzyme recognizes specific structural ‘motifs’, corresponding perhaps to variations in side chain abundance or structure, that occur at ~ 50 -kDa intervals along the xyloglucan polymer, although there is no evidence for this.

It appears that most XTHs follow the ‘random cleavage’ pattern described by Nishitani and Tominaga (1992). Steele et al. (2001) incubated each of ten diverse XTHs with xyloglucan donors (21 μ M) whose mean M_r was 205,000, plus 0.6 μ M [3 H]XLLGol as the acceptor. The size profiles of the 3 H-products indicated the positions of cleavage relative to the non-reducing terminus of the donor (Equation 2). All ten profiles fitted a model in which the enzyme cuts its donor substrate, once only, at a random distance from the terminus.

An important point to bear in mind is that all the above assays were performed *in vitro* using soluble substrates. *In vivo*, the substrates are largely insoluble and wall-bound, and the repertoire of enzyme action may be significantly different.

XTH action observed in vivo

Two main roles have been proposed for XTHs in growing cells.

(a) Wall restructuring (Fig. 3). XTH may reversibly (XET) or irreversibly (XEH) loosen existing wall material, enabling cell expansion (Fry et al. 1992a, Fry et al. 1992b, Nishitani and Tominaga 1992, Nishitani 1997, Nishitani 1998). In favor of this hypothesis, XET activity is often correlated with growth rate (Fry et al. 1992a, Hetherington and Fry 1993, Pritchard et al. 1993, Potter and Fry 1994, Xu et al. 1995, Palmer and Davies 1996, Antosiewicz et al. 1997). Moreover, xyloglucan turnover is associated with rapid cell expansion, such as that which occurs during auxin-induced elongation (Labavitch and Ray 1974, Nishitani and Masuda 1982, Talbot and Ray 1992).

(b) Integration/wall assembly (Fig. 4). XTHs may also catalyze the integration of newly synthesized xyloglucans into the cell wall through XET activity (Nishitani and Tominaga 1992, Nishitani 1997, Nishitani 1998, Xu et al. 1996, Thompson et al. 1997). Such integration is necessary for wall synthesis in meristems and also usually accompanies vacuolation since wall thickness is usually maintained during cell expansion.

Enzymological ‘activity’ is measured, usually as V_{max} , under artificially optimized conditions. The detection of XTH activity, protein, or mRNA in plant cells is not proof of ‘action’. For instance, the enzyme may not be able to access its donor or acceptor substrate, or a natural inhibitor could be present. It is therefore important to document XTH action *in vivo*. However, since P→P transglucosylation need not cause

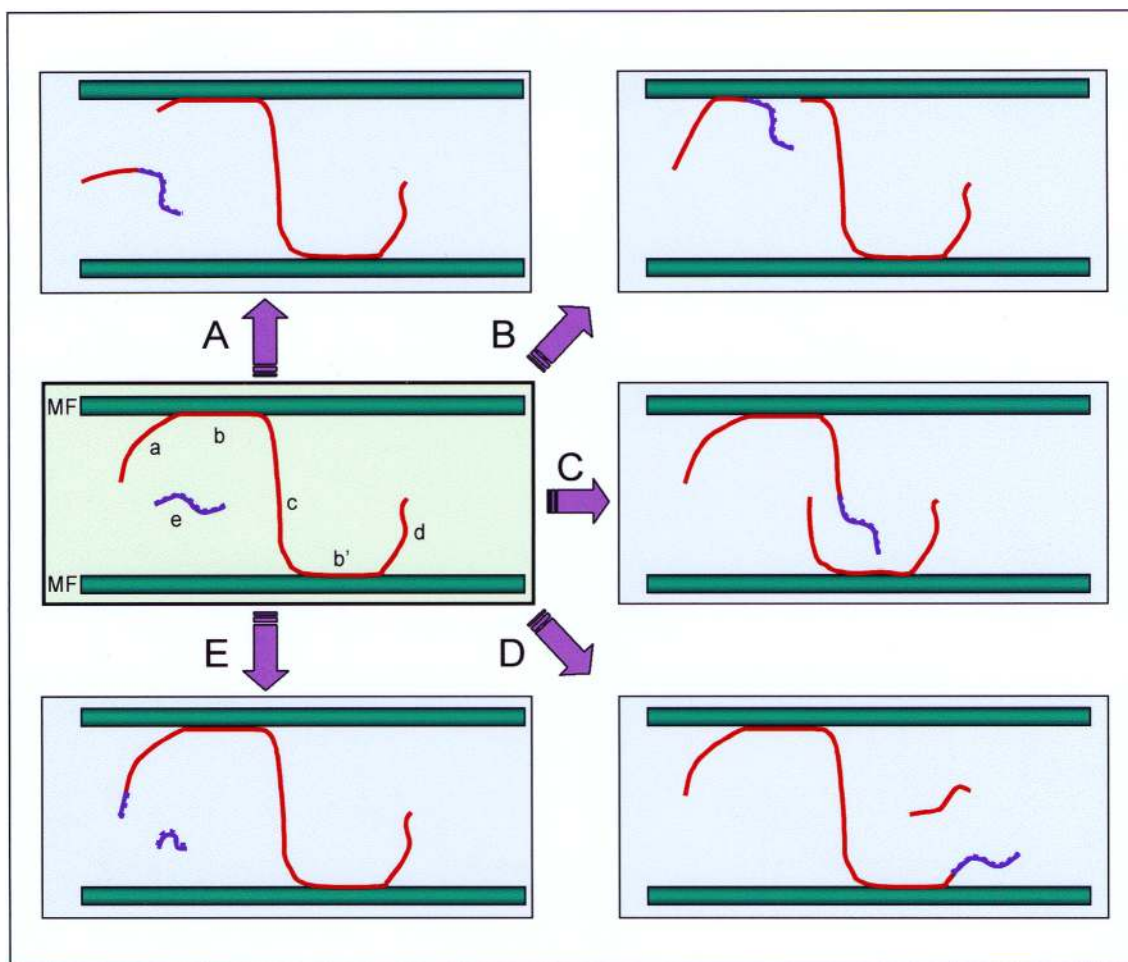


Fig. 4 Polysaccharide-to-polysaccharide (P→P) transglucosylation of xyloglucan in the cell wall showing integrational transglucosylation. There are five possible modes of integrational endotransglucosylation between a newly secreted xyloglucan (blue line) and a previously wall-bound xyloglucan chain (red line). The reducing terminus is the right-hand end of each chain. In the initial state (green shaded left center panel), four different segments of the wall-bound chain are distinguished: (a) a non-reducing loose end, (b, b') regions anchored to cellulose microfibrils (MF) by hydrogen bonding, (c) a tether between the microfibrils, (d) a reducing loose end. Arrows (A⇒ to D⇒) indicate what happens when XTH cleaves the wall-bound chain at sites a to d, respectively, and the newly secreted chain acts as acceptor. Arrow E⇒ shows what happens if the XTH attacks the newly secreted chain at site e and the previously wall-bound chain acts as acceptor. Revised from Thompson and Fry (2001).

any chemical change in the substrates, monitoring xyloglucan endotransglucosylation *in vivo* is an interesting challenge, requiring observations of the behavior of endogenous polysaccharide substrates in the walls of living cells. Two approaches have been developed to allow such insights.

(i) *Isotopic labeling of endogenous substrates*—Thompson et al. (1997) demonstrated integrational transglucosylation using cultured rose cells grown in media with two different isotopes. Following extraction from the cells, xyloglucan populations of different densities were separated by isopycnic centrifugation in CsTFA. Thus, when raised on [^{13}C]glucose, the cells made 'heavy' xyloglucan, that was distinguishable from unlabeled xyloglucan. The cells were then switched to [^{12}C]glucose medium and a trace of L-[^3H]Ara (a precursor for the xylose residues of xyloglucan [Fry 2000]) was added. Seg-

ments of new ('light') [^3H]xyloglucan became grafted to portions of old ('heavy') xyloglucan to form 'moderately-heavy' [^3H]xyloglucan. This indicated P→P transglucosylation, using only endogenous substrates, *in vivo*. The density-shift in the [^3H]xyloglucan was very rapid, indicating that transglucosylation accompanied, and possibly caused, the integration of newly secreted xyloglucans into the wall (Thompson et al. 1997).

Other isotope experiments confirmed 'integrational' transglucosylation and also detected 'restructuring' transglucosylation, in which one previously wall-bound xyloglucan reacts with another (Thompson and Fry 2001). 'Heavy' rose cells were shifted into 'light' medium, then given a short (2-h) pulse of L-[^3H]Ara. This would result in 'light' [^3H]xyloglucans being secreted into 'heavy', non-radioactive walls. Two h after

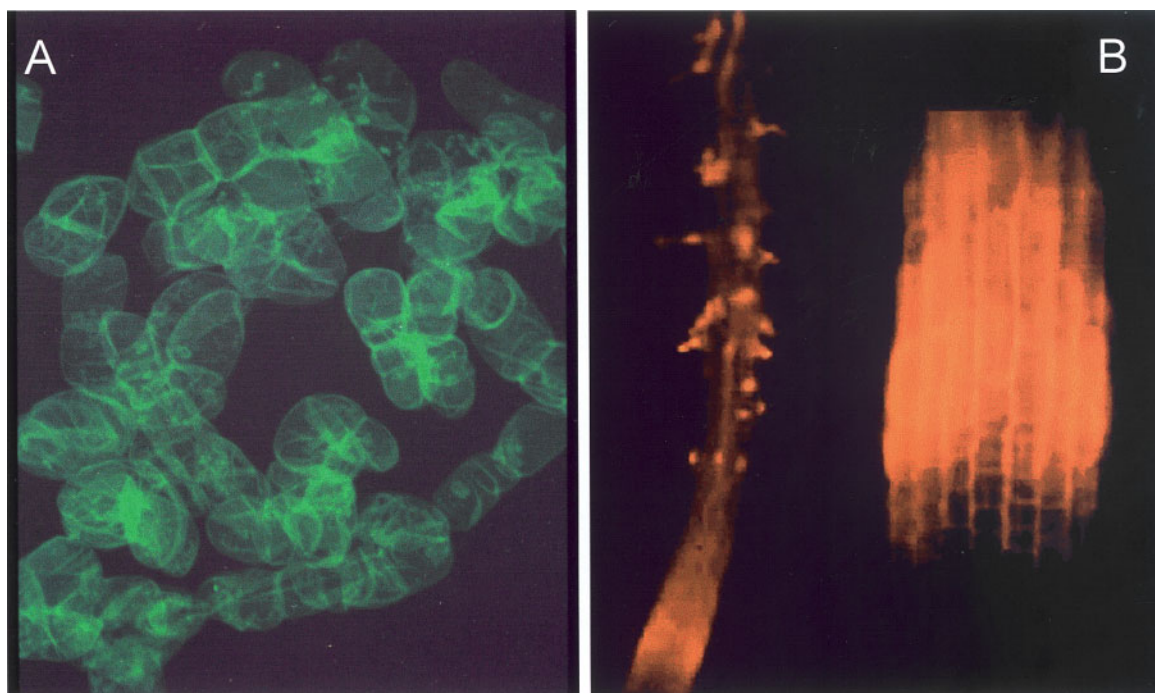


Fig. 5 Distribution of the action of endogenous XTHs exhibiting xyloglucan endotransglucosylase activity with endogenous donor substrates. Fluorescently labeled plant tissues, following infiltration of XGO–fluorophore conjugates, which act as acceptor substrates for XTHs, while endogenous xyloglucan acts as the donor substrate. After removal of unreacted XGO–fluorophore conjugates by washing, any remaining fluorescence in the cell wall is due to xyloglucan–fluorophore conjugates formed by transglucosylation. (A) Fluorescent labeling of tobacco BY-2 cells over-expressing an XTH, after incubation with a fluoresceinyl xyloglucan heptasaccharide (see also Ito and Nishitani 1999). The photograph was taken at a magnification of $\times 400$. (B) *Arabidopsis* roots exhibiting a characteristic distribution pattern of XET action on the donor substrate: high transglucosylase activity is limited to the root cell elongation zone and to the initiating root hairs. *Left image*: low-power view of an *Arabidopsis* root, showing xyloglucan endotransglucosylation in the zone of elongation and in young root hairs (K. Vissenberg, J.-P. Verbelen and S.C. Fry, unpublished). *Right image*: close-up of elongation zone — note that the cell size increases along the length of the fluorescent zone, confirming that it is the zone of elongation (Vissenberg et al. 2000). In both images, the root tip is pointing downwards and the root diameters in the zone of elongation are $\sim 100\ \mu\text{m}$.

radiolabeling, segments of ‘light’ [^3H]xyloglucan had become covalently bonded to ‘heavy’ xyloglucan, forming hybrid molecules that were on average 29% heavy, as expected for integrational transglucosylation. The buoyant density of the [^3H]xyloglucan then gradually increased further until, by 11 h, the hybrids were 38% heavy. Since negligible new [^3H]xyloglucan was secreted between 2 and 11 h, and the only new non-radioactive xyloglucans being secreted were ‘light’, the increase in density demonstrated that ‘restructuring’ reactions occurred between the existing (29% heavy) [^3H]xyloglucan and other mainly ‘heavy’ (i.e. older) wall-bound non-radioactive xyloglucans (Thompson and Fry 2001). Brefeldin A (BFA), a fungal toxin that disrupts the normal functioning of the Golgi apparatus, and that has been shown to inhibit the secretion of cell wall polysaccharides and proteins (Schindler et al. 1994), inhibited [^3H]xyloglucan secretion but, when added 2.5 h after the [^3H]arabinose, did not prevent an increase in density of the [^3H]xyloglucan from occurring between 2 and 11 h (Thompson and Fry 2001). Thus, wall-bound [^3H]xyloglucans continued to undergo endotransglucosylation reactions with the

limited pool of existing ‘heavy’ wall-bound xyloglucans. Such transglucosylation must have been of the restructuring type since the BFA had blocked xyloglucan secretion, which is necessary for integrational transglucosylation.

In vivo experiments with ^3H - and ^{13}C -labeled substrates have the advantage that all substrates are endogenous and chemically normal. These experiments provided the first direct evidence for P→P transglucosylase action in vivo.

(ii) *Fluorescent labeling of exogenous substrates*—Isotopic labeling does not easily permit exploration of the cellular distribution of XTH action. To obtain such information, an exogenous fluorescent XGO can be fed to tissues and the transglycosylation products visualized microscopically. This approach provides excellent spatial information but has its own disadvantages: (a) P→O transglucosylation is studied, rather than the P→P type, which in many cases may be more biologically relevant; (b) the acceptor substrate is chemically abnormal since it has a fluorophore attached; and (c) the acceptor substrate is exogenous and so may not reach the correct sub-cellular location.

Localized formation of fluorescent xyloglucan indicates the action of endogenous XET activity on endogenous donor molecules (Equation 3a) at that location. Although this demonstrates XTH action, not just activity, there is no proof that transglucosylation (Equation 3b) would normally be completed *in vivo*, since it is not possible to know whether or not any endogenous acceptor substrate is usually accessible at the site of fluorescence. Nevertheless, valuable data on the co-occurrence of XTH and its donor substrate have been obtained by this method. Ito and Nishitani (1999) used XXXG-fluorescein to visualize the transglucosylase action of wall-bound XTH on endogenous xyloglucan in cultured tobacco cells. Enzyme action, detectable by wall-bound green fluorescence (Fig. 5A), was reduced in transgenic cells in which XTH expression had been suppressed by antisense technology, and was enhanced in transgenic lines that over-expressed XTH. The resulting fluorescent polysaccharide was not extractable by 0.6 M NaOH, indicating that it was firmly integrated in the cellulose-xyloglucan framework. Vissenberg et al. (2000) used a similar approach, with XGO-sulphorhodamine conjugates (XGO-SRs) infiltrated into pieces of living plant tissue, and high- M_r transglucosylation products were detected by an orange fluorescence (Fig. 5B). In young celery petioles, XET action was particularly high in the thick-walled collenchyma cells and action was prominent in the zone of cell elongation in roots of both *Arabidopsis* and tobacco (Fig. 5B and Vissenberg et al. 2000). Thus, high levels of XET action were correlated with cell expansion or wall restructuring. Somewhat further back from the root tip, the forming root hairs also exhibited fluorescence (Fig. 5B). Hair initiation, which involves a highly localized bulging of the epidermal wall, was accompanied by similarly localized XET action on the donor substrate, suggesting an important role of XTHs in the early stages of root hair formation. Older root hairs showed a uniform fluorescence over the hair wall, possibly reflecting the integration of new xyloglucan for wall strengthening (Vissenberg et al. 2001).

Functional Diversity of XTHs

The large and complex families of genes that encode XTHs in various plant species predict that the encoded products have physiological functions that are critical for a broad spectrum of plant developmental processes. Insight into the potential physiological roles of XTHs can be gained from investigating where and when XTHs appear to be recruited for action; that is, expression patterns of *XTH* RNA, protein or enzymic activity or action may indicate the physiological processes in which specific XTHs participate.

Positive correlations between XTH protein, activity or action levels and elongation growth have been demonstrated in diverse species, including *Arabidopsis* (Antosiewicz et al. 1997, Vissenberg et al. 2000), tobacco (Vissenberg et al. 2000), tomato (de Silva et al. 1994), rice (Uozu et al. 2000), carrot (Hetherington and Fry 1993), pea (Potter and Fry 1993), barley (Smith et al. 1996), maize (Pritchard et al. 1993) and lettuce

and cucumber (Potter and Fry 1994). In addition, *XTH* gene expression, monitored by RNA blot hybridization, *in situ* hybridization and/or reporter gene fusions, is also often found at sites of rapid cell expansion or cell division (Medford et al. 1991, Zurek and Clouse 1994, Nishitani 1995, Xu et al. 1995, Catalá et al. 1997, Catalá et al. 2000, Oh et al. 1998, Akamatsu et al. 1999). However, there are examples in which the patterns of elevated XTH activity and growth do not correlate (Pritchard et al. 1993, Palmer and Davies 1996, Smith et al. 1996), and in some cases specific *XTH* genes are down regulated during rapid growth (Catalá et al. 2001), indicating that interpretations should not be over simplified. Overall, however, these correlations between XTH presence, activity, action or gene expression and cellular expansion strongly argue for a functional role for XTHs in cell enlargement.

It has been reported that XET activities do not induce wall extension using *in vitro* assays (McQueen-Mason et al. 1993). However, while these results are valuable in distinguishing XET activity from that of the wall-loosening proteins expansins (see the appropriate review in this journal issue), they should be interpreted cautiously when used to assess XTH function. Boiled plant tissue was used as a substrate in the assay and this treatment would have eliminated cellular turgor and any active process, including secretion of xyloglucan into the wall, in addition to potentially causing drastic changes in the apoplastic environment, such as XTH acceptor or donor concentration; these are all factors that may be essential for XTH action *in vivo*. Another important consideration is that McQueen-Mason et al. (1993) focused on the XET activity in their protein extracts, whereas a more recent report described a purified XTH, with exclusively XEH activity that indeed increased cell wall extensibility in similar *in vitro* assays (Kaku et al. 2002).

Growth-promoting effects of exogenous XGOs have previously been reported (McDougall and Fry 1990, Cutillas-Iturralde and Lorences 1997) and their wall loosening action attributed to P→O endotransglycosylation between endogenous wall-bound xyloglucans and the exogenous XGOs acting as acceptor substrates. Even clearer evidence was recently published in a report supporting the view that the XET action of XTHs results in a change in the mechanical properties of the cell wall (Takeda et al. 2002). The authors demonstrated that incubation of 50,000- M_r xyloglucan with pea stems resulted in a decrease in wall extensibility, while similar incubations with XGOs induced wall loosening. This effect was ascribed to the XET action of XTHs in the tissue. Transglucosylation between wall-bound xyloglucan and exogenous XGO acceptors promoted cell elongation by effectively cleaving wall-bound chains, while transglucosylation with exogenous, high- M_r xyloglucan suppressed elongation, presumably by enhancing the formation of inter-microfibrillar tethers. Endogenous XGO concentrations in the apoplast are very low (Fry 1986), and the reported effects of exogenous XGOs may be 'pharmacological' rather than accurately representing any physiologically

normal wall-loosening process. Nevertheless, these reports underscore the importance of the differences in biochemical activities catalyzed by divergent members of the XTH family, both in terms of XET versus XEH action, and the nature of the acceptor and donor molecules.

It may be questioned whether the grafting of 50,000- M_r pieces of exogenous xyloglucan (Takeda et al. 2002) on to endogenous pea stem xyloglucans by P→P transglucosylation could cause enough of an increase in mean M_r to bring about any appreciable wall tightening. However, 50,000 is a relatively high M_r compared with some of the M_r values reported for the endogenous xyloglucans of pea stems [e.g. peak M_r varying from 9,000 to 300,000 (Talbot and Ray 1992)]. In addition, the key effect of XTH action in the experiments of Takeda et al. (2002) might be the grafting of new lengths of xyloglucan on to the termini of existing microfibril-bonded xyloglucan chains, thus giving them 'sticky ends' through which to adhere to additional neighboring microfibrils, creating new tethers.

XTHs appear to be involved in many physiological processes in addition to elongation growth. Expression of at least one XTH, *Arabidopsis At-XTH22*, is strongly induced in response to mechanical perturbations such as wind or touch (Braam and Davis 1990, Xu et al. 1995). In this case, *At-XTH22* expression correlates with thigmomorphogenesis, a strong reduction in elongation growth, resulting in shorter, often stockier plant phenotypes (Biddington 1986, Braam and Davis 1990). Thigmomorphogenesis is likely to have evolved to enable plants to acclimate to windy environments and acclimation may include changes in the biomechanical properties of the affected tissues (Biddington 1986). Since the cellulose microfibril/xyloglucan network is thought to contribute to the mechanical properties of primary cell walls, XTHs may be recruited to alter tissue tensile strength, or flexibility, enabling adaptation to mechanically stressful environments. XTH expression, XET and XEH in situ activities, and xyloglucan metabolism also correlate with mechanical stresses that occur during development. The *Arabidopsis XTH* gene, *At-XTH24*, was originally identified in a screen for meristem-specific genes; however, one of the most conspicuous sites of *At-XTH24::reporter* gene expression is at organ/tissue branch points (Medford et al. 1991). Thus, *At-XTH24* may be regulated during development to modify the walls of cells under mechanical stress. In addition, *At-XTH24-* and *At-XTH22-*reporter genes are expressed and corresponding proteins accumulate at sites of emerging lateral roots (Medford et al. 1991, Xu et al. 1995, Antosiewicz et al. 1997). Similarly, XET action is detected along the primary root where lateral root primordia are forming (Vissenberg et al. 2000). One possibility is that the mechanical force generated by growth of the pericycle may induce XTH expression at these sites, and XET action may aid either wall loosening required for cellular emergence, or wall repair as the nascent lateral root pushes through the cortex and epidermis. As previously mentioned, XET activity and sub-

strates co-localize in collenchyma cells, vascular bundles and the epidermis of celery petioles (Vissenberg et al. 2000). These cells provide mechanical strength and increased XET action at these sites may indicate that XTH-mediated wall modification contributes to the structural integrity of the plant body. Alterations in xyloglucan size may also influence differential wall extensibility during shoot curvature. For example, gravitropism of pea epicotyls correlates with rapid and transient changes in xyloglucan distribution: xyloglucans extracted from the upper side of the reoriented epicotyls were found to have a greater M_r than those from the lower surface (Talbot and Pickard 1994). Thus, the degree of polymerization of xyloglucan was inversely correlated with growth. The mechanism of this potentially gravity-induced differential metabolism of xyloglucan is unclear.

XTH activities and/or levels of XTH expression also increase during a number of developmental processes that involve net cell wall degradation, including fruit ripening (Redgwell and Fry 1993, Cutillas-Iturralde et al. 1994, Maclachlan and Brady 1994, Arrowsmith and de Silva 1995, Schröder et al. 1998, Rose and Bennett 1999), mobilization of seed reserves (Farkaš et al. 1992, Fanutti et al. 1993, Chen et al. 2002) and formation of root aerenchyma (Saab and Sachs 1996), mesophyll air spaces (Antosiewicz et al. 1997), abscission zones (Xu et al. 1995) and vascular tissue (Medford et al. 1991, Xu et al. 1995, Antosiewicz et al. 1997, Oh et al. 1998, Vissenberg et al. 2000), suggesting that, besides their apparent involvement in cell expansion, XTHs may have an important function catalyzing xyloglucan depolymerization and/or solubilization during cell wall disassembly.

In accordance with their multifunctional roles, and in addition to showing different tissue- and organ-specific expression patterns, divergent XTH genes show a broad range of responses to different hormones (Zurek and Clouse 1994, Xu et al. 1995, Xu et al. 1996, Aubert and Herzog 1996, Saab and Sachs 1996, Catalá et al. 1997, Catalá et al. 2000, Nishitani 1997, Yokoyama and Nishitani 2001b, Chen et al. 2002) and biotic and abiotic environmental stimuli (Braam and Davis 1990, Peschke and Sachs 1994, Xu et al. 1995, Xu et al. 1996, Saab and Sachs 1996, Van Buuren et al. 1999, Burstin 2000, Ma et al. 2001). Thus, gene duplications, followed by evolutionarily selected diversification, appear to have resulted in products with not only differing enzymic properties, but also with distinct patterns of regulation (Xu et al. 1996, Akamatsu et al. 1999, Burstin 2000, Uozu et al. 2000, Catalá et al. 2001, Yokoyama and Nishitani 2001b). The complexity of this regulation probably reflects the functional diversity of the gene family.

The problem of defining physiological roles for XTHs is further complicated by the multifunctional nature of transglucosylation/hydrolysis and because of the potential for divergent functions evolving among the extensive gene family members. Elucidation of physiological roles for XTHs will be helped by the application of reverse genetic approaches. One report (Ver-

ica and Medford 1997) described the developmental effects of altering the expression of *At-XTH24* in transgenic *Arabidopsis*; however, because the described phenotypes were dependent upon growth on the antibiotic kanamycin, one cannot conclude that these defects were due specifically to the alterations in *At-XTH24* expression, rather than possible detrimental effects of the antibiotic. More recently, transgenic tobacco plants with reduced XET activity were shown to accumulate xyloglucan with a M_r at least 20% greater than that of wild-type plants (Herbers et al. 2001). The consequences of these alterations for wall properties and plant fitness are not yet clear. Additional generation and characterization of transgenic lines and mutants with altered expression of specific XTHs, as well as further metabolic studies on the nature of the reactions occurring in vivo (Fig. 4), should help reveal the undoubtedly complex and critical roles that XTHs play in growth and development.

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