Plant Physiol. (1998) 117: 337–343

Update on Cell Walls

Polygalacturonases: Many Genes in Search of a Function[1]

Kristen A. Hadfield and Alan B. Bennett*

Mann Laboratory, Department of Vegetable Crops, University of California, Davis, California 95616

Pectins are a major component of the plant cell wall and comprise one of the two major coextensive networks in which cellulose microfibrils are embedded (Carpita and Gibeaut, 1993). Pectic polysaccharides exist in the cell wall as either "smooth" regions of a linear copolymer of α-(1–4)-linked GalUA or "hairy" regions that have attached α-(1–2)-linked rhamnosyl residues that may be substituted with Ara- and Gal-rich side chains. The pectin structure is further elaborated by divalent cation cross-linkages and possible esterification to other cell wall polymers. Because of the contribution of both ionic and covalent linkages, the structure of pectin may be modified by the ionic strength of the apoplast, by enzymes that modify the charge of the GalUA residues, or by enzymes that cleave either the α-(1–4)-linked GalUA backbone or side chains of the hairy pectin regions. Because plant cells undergo dramatic changes in shape and developmentally regulated episodes of cell separation, in which the pectin network is systematically disassembled, pectin metabolism is critical to many developmental processes.

A wide range of enzymes are known to catalyze aspects of pectin modification and disassembly. The best characterized are *exo*- and *endo*-PGs, pectate lyase, pectin methylesterase, and β-galactosidase, which has been proposed to have the capacity to reduce the apparent molecular size of pectic polymers by cleaving neutral side chain residues (De Veau et al., 1993). In addition, it is likely that there are as-yet-undiscovered enzymes that may play critically important roles in cleaving covalent cross-linkages that tether pectins to other structural networks within the cell wall. Because of the extensive study of PG-mediated pectin disassembly, this review summarizes what is known about the complexity and structure of genes encoding plant PGs and their role in developmental processes.

PGs were first identified over 35 years ago and have been suggested to be involved in the disassembly of pectin that accompanies many stages of plant development, particularly those that require cell separation. For example, PG activity has been shown to be associated with organ abscission (Taylor et al., 1990; Bonghi et al., 1992), pod and anther dehiscence (Meakin and Roberts, 1991), and pollen grain maturation and pollen tube growth (Pressey and Reger, 1989; Pressey, 1991). PG activity has also been detected in rapidly growing tissues, indicating that it may be involved in cell expansion (Pressey and Avants, 1977; Sitrit et al., 1996). Although it is clear that PG participates in many plant developmental processes, the majority of research has focused on PG in ripening fruit, abscission zones, or pollen. Molecular cloning and the modification of PG gene expression in transgenic plants have provided new insights into the physiological function of this enzyme. In addition, it is now clear that PGs are encoded by relatively large gene families in plants and that they are expressed in a wider range of developmental contexts than previously appreciated.

PG AND PECTIN DISASSEMBLY IN RIPENING FRUIT

Changes in cell wall structure are thought to underlie fruit softening, and ripening-associated cell wall disassembly has been examined in a number of fruit species, especially tomato (*Lycopersicon esculentum*) (Fischer and Bennett, 1991). Pectins, hemicelluloses, and possibly the amorphous regions of cellulose undergo structural modifications during ripening (Huber, 1983, 1984; O'Donoghue et al., 1994). Pectin disassembly is particularly extensive and is associated with the later stages of ripening and with fruit deterioration in the overripe stages (Dawson et al., 1992; Huber and O'Donoghue, 1993).

We recently described the temporal sequence of cell wall polymer disassembly in very rapidly ripening melon (*Cucumis melo* cv Charentais). In this ripening system, the initiation and early stages of fruit softening were accompanied by a decrease in the molecular size of hemicellulosic polysaccharides, most notably of a tightly bound fraction of xyloglucan. Later in ripening, pectin disassembly was found to be extensive in the deteriorative overripe stages (Rose et al., 1998). These results suggest that fruit softening of Charentais melon is the consequence of the disassembly of two distinct networks, with the initiation of xyloglucan disassembly occurring earlier in ripening.

Late softening and tissue deterioration are associated with extensive pectin disassembly. In addition, disassembly of pectins may increase the pore size of this network, resulting in the cell wall swelling that is seen in many fruit during the late stages of softening (Redgwell et al., 1997), or in increased accessibility of the substrate to enzymic action. Correlated with the solubilization and depolymer-

[1] This research was supported in part by Zeneca Plant Science, Jealotts's Hill, UK.

* Corresponding author; e-mail abbennett@ucdavis.edu; fax 1–530–752–4554.

Abbreviations: EGase, *endo*-glucanase; PG, polygalacturonase; UA, uronic acid.

ization of pectins in cv Charentais melon was an increase in pectin-degrading enzyme activity and the appearance of three PG mRNAs, one of which was demonstrated by heterologous expression in *Aspergillus oryzae* to encode an *endo*-PG (Hadfield et al., 1998). Overall, these results suggest that PG-mediated pectin disassembly occurs after the early stages of fruit softening and is likely to contribute significantly to the overripe stages of softening and deterioration.

PG ACTIVITY IS NOT NECESSARY OR SUFFICIENT FOR FRUIT SOFTENING

PG-dependent pectin disassembly has been most extensively studied in ripening tomato, and the development of molecular genetic techniques has provided a direct means of determining the contribution of PG activity to ripening-associated fruit softening. The suppression of PG gene expression in wild-type tomato and the ectopic expression of PG in the ripening-impaired pleiotropic mutant *ripening inhibitor* (*rin*) showed that PG-mediated pectin depolymerization was not necessary for normal ripening and softening (Sheehy et al., 1988; Smith et al., 1988; Giovannoni et al., 1989).

In transgenic fruit in which PG mRNA accumulation was suppressed 99% by the expression of an antisense PG transgene, the solubility of pectins remained at wild-type levels, but depolymerization of solubilized pectins was suppressed (Smith et al., 1990). These same transgenic fruit ripened normally, demonstrating that high levels of PG activity are not necessary for normal ripening of tomato. Softening of transgenic antisense PG fruit was also comparable to wild-type fruit, with only a slight attenuation of softening noted (Fig. 1A) (Langley et al., 1994). In spite of the very modest effect of PG suppression on fruit softening, an improvement in horticulturally important traits, such as storage life in the overripe stages and enhanced viscosity of processed tomato products, was observed (Schuch et al., 1991; Kramer et al., 1992; Brummell and Labavitch, 1997).

In a converse experiment, a chimeric gene consisting of the PG structural gene under control of the ethylene-inducible promoter E8 was introduced into mutant *rin* tomato fruit that normally fail to ripen or soften and that do not express PG. In the transgenic fruit, PG gene expression, enzyme activity, and pectin solubilization and depolymerization were restored to near wild-type levels. However, these fruit did not soften and were not altered in other ripening parameters such as pigment accumulation or ethylene production, indicating that PG-mediated pectin disassembly is not sufficient for normal ripening or softening to occur (Fig. 1B) (Giovannoni et al., 1989). However, the PG-complemented *rin* fruit were more susceptible to pathogen attack than were control fruit (Bennett et al., 1993). Similarly, PG antisense fruit showed a decrease in pathogen susceptibility compared with control fruit (Kramer et al., 1992).

Collectively, the results obtained with transgenic tomatoes having altered PG levels are consistent with the hypothesis that PG-mediated pectin disassembly does not contribute to early fruit softening but contributes significantly to tissue deterioration in the late stages of fruit ripening. The effect of PG suppression is particularly evident in a comparison of control and transgenic antisense PG fruit allowed to reach the overripe stage (Fig. 2).

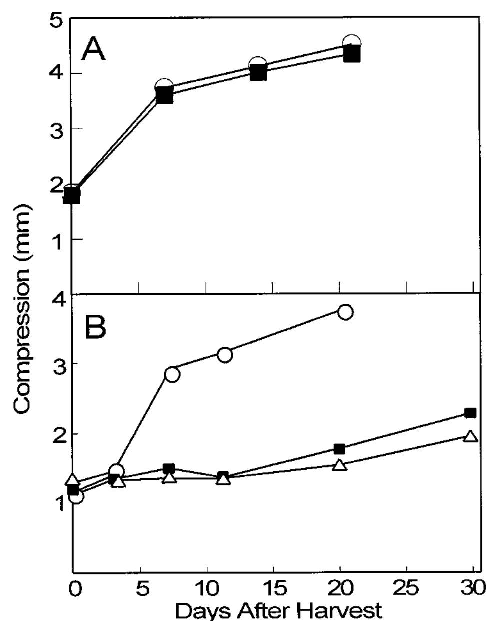

Figure 1. Compressibility of transgenic tomato fruit with altered levels of PG gene expression. A, Compressibility of PG antisense (■) and control (○) tomato fruits. Fruit were harvested at the breaker stage and the compressibility was determined at the time points indicated. The difference in compressibility is statistically significant at $P < 0.001$. Data are from Langley et al. (1994). B, Compressibility of wild-type (○), *rin* (△), and transgenic *rin* expressing PG under control of the E8 promoter (■) tomato fruit. Fruit were harvested 35 d after anthesis and exposed to propylene to induce the transgene, and compressibility was determined at the indicated time points. Graph was redrawn from Giovannoni et al. (1989).

Analysis of cell walls from transgenic fruit with altered levels of PG activity have also elucidated the mechanisms of pectin disassembly in vivo. The suppression of pectin depolymerization but not of pectin solubilization in transgenic PG antisense fruit indicates that these two processes have distinct enzymic determinants, with depolymerization but not solubilization being primarily due to the action of PG. In contrast, pectins in PG-complemented *rin* fruit were both solubilized and depolymerized. Taken together, the data suggest that PG activity is both necessary and sufficient for pectin depolymerization but that it may be one of multiple, redundant pectin-solubilizing activities, all of which are suppressed in the *rin* mutant.

PG ACTIVITY IN THE FRUIT OF OTHER PLANT SPECIES

PG activity is also high in fruit other than tomato, such as avocado (*Persea americana*) and peach (*Prunus persica*). In peach three distinct activities have been identified, two of which cleave the substrate by an *exo* mode of action, and one that hydrolyzes the pectin backbone in an *endo* fashion (Downs et al., 1992). In contrast to tomato, in which *exo*-PG

Figure 2. Effect of antisense PG gene expression on overripe tomato fruit. Left, Control fruit expressing normal levels of PG; right, PG antisense fruit expressing < 1% wild-type level of PG. Control and antisense fruit were harvested on the same day and stored until control fruit advanced to the overripe stage and showed visible signs of deterioration. Photo courtesy of Calgene, Inc.

activity is present during early fruit development and remains constant during ripening, *exo*-PG activities in peach are ripening regulated (Pressey, 1987; Downs et al., 1992). *Endo*-PG activity in peach is associated with the freestone character, and a genetic linkage between freestone and *endo*-PG has recently been identified (Lester et al., 1996).

Avocado fruit have high levels of PG activity that are temporally correlated to solubilization and depolymerization of polyuronides during fruit ripening (Huber and O'Donoghue, 1993). In contrast, a number of fruit have been reported to lack *endo*-PG activity, including strawberry (*Fragaria ananassa*) (Huber, 1984), apple (*Malus domestica*) (Bartley, 1978), persimmon (*Diospyros kaki*) (Cutillas-Iturralde et al., 1993), and melon (McCollum et al., 1989). In many of these fruit, ripening-associated pectin depolymerization does not occur, and pectin solubilization may be catalyzed by other enzymes. Alternatively, PG activity may be present but at as-yet-undetectable levels. For example, apple pectins are disassembled at the late stages of ripening (Knee, 1973), and recently, under rigorous examination, *endo*-PG activity and PG mRNA accumulation have been detected but the levels are much lower than that observed in tomato (Wu et al., 1993).

In strawberry, three different PG activities were detected and partially purified, two of which were *exo*-PGs and one of which was *endo* acting (Nogata et al., 1993). In melon (McCollum et al., 1989; Rose et al., 1998) and persimmon (Cutillas-Iturralde et al., 1993), pectins are extensively depolymerized during ripening but PG activity is low or undetectable. It has been suggested that in melon, pectin depolymerization results from β-galactosidase activity because cell wall pectins extracted from preripe fruit undergo a downshift in M_r when treated with a partially purified β-galactosidase extract (Ranwala et al., 1992). However, the characterization of ripening-regulated mRNAs that encode functional PGs in melon suggests that pectin disassembly during the late stages of fruit softening may be at least in part PG dependent (Hadfield et al., 1998).

It is important to note that even very low levels of PG may be sufficient to catalyze extensive pectin disassembly. This became apparent in transgenic antisense tomato fruit, in which reductions in PG activity of 80% had little impact on pectin structure, indicating that in tomato PG is present in at least 5-fold excess (Smith et al., 1990). It is therefore possible that the disassembly of pectin is PG dependent, even in fruit with very low levels of PG activity.

PG IN CELL-SEPARATION ZONES

During plant development various organs undergo programmed senescence and are shed from the parent plant (Hadfield and Bennett, 1997). Abscission is the consequence of the dissolution of cell walls between adjacent cells in the separation layer consisting of 5 to 50 rows of small, isodiametric cells, with the fracture usually occurring in the plane of the middle lamella. The cells flanking the fracture plane remain largely intact after abscission has taken place, except for the nonliving cells of the vascular system, which are ruptured due to physical forces (Sexton and Roberts, 1982).

During separation pectins are solubilized, the middle lamella swells, fenestrates, and disappears, and the microfibrillar network becomes disorganized. During abscission there is an increase in cell secretion, presumably to accommodate the increase in secretion of cell wall-modifying enzymes. Inhibitors of protein and RNA synthesis markedly delay abscission, implying that de novo protein and RNA synthesis are required for cell separation (Sexton and Roberts, 1982).

EGase activity increases dramatically during abscission, and multiple EGase genes are expressed in the abscission region (Lashbrook et al., 1994; Del Campillo and Bennett, 1996). Both the activity of EGase and the rate of abscission are suppressed by auxin and accelerated by ethylene, supporting a role for EGase in the abscission process. Antisense suppression of one EGase isoform resulted in a partial reduction in flower abscission (Lashbrook et al., 1998). PG activity also increases during abscission of both tomato flower and *Sambucus nigra* leaf explants (Tucker et al., 1984; Taylor et al., 1993).

In contrast to EGase activity, which is distributed throughout the abscission zone, PG activity is restricted to the distal portion of the zone tissue (Roberts et al., 1989). PG activity has also been detected in flower and fruit abscission zones in a variety of species, but the relative predominance of EGase or PG varies depending on the subtending organ. For example, in peach EGase activity is high in leaf-abscission zones but much lower in fruit-abscission zones, whereas PG activity is not detected in leaf-abscission zones but is present at very high levels in fruit-abscission zones (Bonghi et al., 1992). The difference in hydrolytic activities between the two types of abscission zones is of potential practical use because it may allow genetic alteration of abscission of specific plant organs.

In tomato a fruit PG-specific antibody and a cDNA corresponding to the ripening-regulated gene did not react with protein or mRNA isolated from leaf-abscission zones. In addition, PG activity in abscission zones was unaffected by the presence of an antisense fruit PG gene that suppressed PG activity in fruit by 99% (Taylor et al., 1990). These results strongly suggested that the PG in leaf-abscission zones is distinct from the fruit-ripening-associated PG. This has been confirmed by the recent identification of three PG genes that are expressed in both tomato leaf- and flower-abscission zones, and that are divergent from the fruit-ripening-associated PG gene (Kalaitzis et al., 1997). The three abscission PGs differ from each other in sequence and in their temporal patterns of expression. TAPG4 is divergent from and expressed much earlier than either TAPG1 or TAPG2. It is possible that the divergent PGs have different substrate specificities or a different mode of action, i.e. *endo*- versus *exo*-cleavage, or they may correspond to redundant activities under the control of different regulatory factors.

Pod dehiscence is similar to abscission in that it occurs at a precise location in the pod and results from cell wall disassembly and separation of a specialized layer of unlignified cells called the dehiscence zone. Like abscission, the middle lamella is broken down and pectin is lost during cell separation, and the process is accompanied by an increase in EGase activity (Meakin and Roberts, 1991). Although PG enzyme activity has not been definitively demonstrated in dehiscence zones, cDNAs have recently been identified that encode putative PGs expressed specifically in the dehiscence zone during the cell-separation process, suggesting a role for PG in pod dehiscence (Jenkins et al., 1996; Petersen et al., 1996).

PG IN POLLEN AND POLLINATION

Germination and growth of the pollen tube through the pistil occurs rapidly, and many of the biochemical events that occur during pollen maturation prepare the pollen for this coming developmental event (Mascarenhas, 1990). High levels of *exo*-PG activity have been detected in the pollen of many species, including maize (*Zea mays*) and other grasses (Pressey and Reger, 1989) and a large number of trees (Pressey, 1991). The activity measured in pollen appears to be exclusively *exo*-PG; the pollen of some species contain extraordinarily high levels of *exo*-PG activity, with Eastern cottonwood (*Populus deltoides*) pollen having levels of PG activity 12 times higher than that of tomato fruit (Pressey, 1991). The functional role of PG in pollen tubes may be to degrade the walls of the stylar cells to allow penetration of the pollen tube or to provide wall precursors for tube growth.

Alternatively, pollen tube PG may be acting on its own wall to facilitate growth (Brown and Crouch, 1990). The addition of pectinase to the growth medium of pollen tubes germinating in vitro stimulates their growth (Mascarenhas, 1990). It is possible that oligogalacturonide signaling is involved in the guidance of the pollen tube to the ovule, and that *exo*-PG may facilitate the rapid turnover of oligogalacturonide-signaling molecules by trimming active oligomers to a size that is inactive, as suggested for other systems (Garcia-Romera and Fry, 1995). In purified cottonwood pollen, PG reaction rates were highest for substrates with a degree of polymerization of 13 (Pressey, 1991), which is in the range of sizes for oligouronides that act as elicitors in other systems (Darvill et al., 1992).

A number of genes are expressed in pollen and have been classified based on the timing of expression. Early genes are expressed before the first mitosis and late genes are expressed thereafter. The proteins encoded by early genes are presumed to be cytoskeletal proteins and proteins involved in cell wall synthesis and starch accumulation, whereas late genes may encode proteins needed for pollen maturation and pollen tube growth (Mascarenhas, 1990). In many species, including maize, *Oenothera organensis*, tobacco (*Nicotiana tabacum*), *Brassica napus*, and others, cDNAs with sequence homology to PG have been identified and classified as late genes (Brown and Crouch, 1990; Allen and Lonsdale, 1992; Robert et al., 1993; Tebbutt et al., 1994).

The mRNA abundance of pollen PGs is greatest in mature pollen, germinating pollen, and growing pollen tubes. Within the anther wall, PG expression was detected close to the stomium, suggesting its involvement in anther dehiscence (Dubald et al., 1993). In situ hybridization also detected the presence of PG mRNA in the tapetum and in some cells of the stamen filament prior to pollen formation, and in the microspores, anther wall, and endothecium of maize at a later stage of development. Immunolocalization of PG in maize using antiserum raised to maize *exo*-PG revealed the presence of *exo*-PG in the walls of cells undergoing modifications such as lysis in the tapetum or expansion in the growing pollen tubes (Dubald et al., 1993), indicating potentially widespread roles of *exo*-PG in pollen development and in pollination.

PG IN GROWING TISSUES: A ROLE IN XYLOGENESIS?

Although the expression and activity of PG is quite high in pollen and fruit, it is also found throughout the plant, suggesting that PG may have a generalized function. For example, PG activity and mRNA accumulation have been detected in germinating seeds and seedlings (Pressey and Avants, 1977; Sitrit et al., 1996). In oat seedlings the level of *exo*-PG activity was highest during the most rapid period of elongation in the most rapidly growing region of the seed-

ling. PG activity has also been detected in other growing plant tissues, including bean hypocotyls, corn and pea seedlings, tomato and oat stems, and the roots of asparagus, turnip, and beet (Pressey and Avants, 1977). In germinating tomato seeds, an mRNA encoding a novel PG was localized to the elongating portions of the embryo, predominantly in the developing vascular tissue of the radicle tip (Sitrit et al., 1996). The localization of PG mRNA and protein to the developing vascular system in a number of young, growing tissues (Allen and Lonsdale, 1992; Dubald et al., 1993; Sitrit et al., 1996) suggests that PG may be involved in xylogenesis and disassembly of the xylem vessel primary cell wall.

SEQUENCE DIVERSITY OF PG GENE FAMILIES

PG activities associated with distinct phases of plant development are encoded by multigene families with members differentially regulated in terms of their spatial and temporal expression. It is also possible that divergent PG gene family members encode enzymes that may differ in their biochemical properties, such as mode of hydrolysis or substrate specificity. Multiple genes encoding PG have been described in a number of species, including tomato (Grierson et al., 1986; Kalaitzis et al., 1997), melon (Hadfield et al., 1998), *B. napus* (Robert et al., 1993; Jenkins et al., 1996), maize (Allen and Lonsdale, 1992), peach (Lester et al., 1994), *O. organensis* (Brown and Crouch, 1990), and tobacco (Tebbutt et al., 1994), suggesting that multigene families encoding PGs are ubiquitous in the plant kingdom.

The deduced amino acid sequences of PG gene family members are relatively divergent from one another. For example, the amino acid sequence of the tomato fruit ripening-related PG is only 41% identical to a tomato-abscission zone PG, but 60% identical to a melon fruit PG (MPG3), indicating that the sequence divergence of the PG gene families occurred prior to the divergence of the major angiosperm families. Even though many of the PG sequences show a relatively high level of divergence over their entire length, there are regions that are highly conserved among all of the sequences cloned from plants and microbial sources, particularly in the carboxy-terminal portion of the enzyme, and catalytic functions have been ascribed to some highly conserved residues within this region (Scott-Craig et al., 1990; Caprari et al., 1996; Stratilova et al., 1996). Some of these conserved sequence domains have served as the basis for designing degenerate oligonucleotides to clone PG gene families from several plant species by reverse-transcriptase PCR.

The presence of three conserved domains in the carboxyl part of PG have proved extremely useful by allowing the amplification of a PCR product that includes a domain that is highly conserved among all PGs cloned to date and can be used diagnostically to determine if the sequence encodes a PG (Lester et al., 1994; Jenkins et al., 1996; Petersen et al., 1996; Hadfield et al., 1998). This approach has resulted in the identification of multigene families with members showing distinct temporal and spatial patterns of gene expression.

Multiple PG genes are typically expressed in pollen and their mRNAs are highly homologous to each other. In maize, pollen PGs are more than 99% identical to each other, with differences occurring primarily in the untranslated regions (Allen and Lonsdale, 1992), suggesting that the catalytic activities of PGs expressed in maize pollen are redundant. Pollen PG genes presumably encode *exo*-PGs, although evidence for this is mainly correlative and is based on the ability to detect only *exo*-PG activity in pollen extracts. A number of features of the deduced amino acid sequences are conserved among pollen PGs, and it has been suggested that these features reflect a functional distinction of an *exo* mode of PG action (Tebbutt et al., 1994). Although it is tempting to use these sequence characteristics to define PGs as *exo*- or *endo*-acting enzymes, caution must be used until more rigorous biochemical evidence is available to define the activity corresponding to PGs of defined amino acid sequence. This will require heterologous expression of individual plant PG cDNAs in a host that does not express endogenous PGs.

The PG protein from tomato fruit undergoes extensive co- and posttranslational modifications, including sequential processing of the hydrophobic N-terminal signal peptide and an acidic propeptide immediately adjacent to the signal sequence, differential glycosylation (DellaPenna and Bennett, 1988), and processing at the C terminus (Sheehy et al., 1987). All of the PGs cloned to date have a predicted N-terminal hydrophobic signal sequence that targets the protein to the lumen of the ER and presumably to the cell wall. The presence of an N-terminal prosequence, however, appears to be present in only one subgroup. The function of the prosequence is not known but may keep the protein in an inactive state while it is being transported to its ultimate destination, or it may target the protein to a specific location within the cell wall (DellaPenna and Bennett, 1988).

A phylogenetic tree generated from an alignment of deduced amino acid sequences from a number of cloned plant PGs groups them into three major clades (Hadfield et al., 1998) (Fig. 3). Clade A is comprised of genes expressed

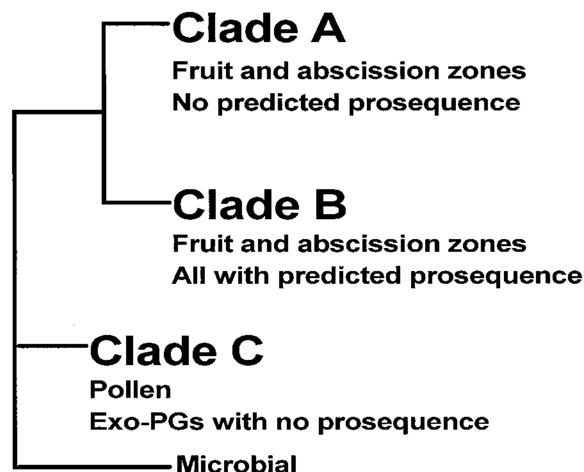

Figure 3. Diagrammatic representation of the evolutionary relationships of the three major PG clades. (From Hadfield et al., 1998.)

in nonpollen tissues that encode proteins lacking a predicted prosequence; clade B is comprised of all of the cloned genes that encode PGs with predicted prosequences, including the tomato fruit-ripening PG; and clade C is comprised entirely of genes expressed in pollen. Members of clade C are thought to encode *exo*-PGs and may reflect a functional divergence of PGs based on mode of action. The biochemical action pattern of most of the cloned PGs is not known, however, and it is possible that *exo*-PGs may be members of clades A or B as well.

Clades A and B cannot be distinguished based on expression patterns because both include genes that are expressed during fruit ripening and during abscission or dehiscence. The presence of a predicted prosequence does, however, set these two groups apart, with all members of clade B having a predicted prosequence. No members of clade A are predicted to encode a prosequence. Alignment of partial-length sequences truncated to omit the prosequence results in the generation of an identical phylogenetic tree with the same three clades, indicating that the prosequence is not the basis for the divergence of these sequences into their own clade. The functional significance of the grouping of PGs into three major clades is intriguing and as more information is obtained regarding the biochemistry and expression of PGs in a variety of developmental contexts, the functional significance of these groupings should become clear.

CONCLUSIONS

Although PGs have been studied mostly in relation to fruit ripening, the existence of large multigene families encoding PGs that are expressed in a wide range of different tissues and developmental stages implicate them as much more than fruit-ripening enzymes. Cell wall modifications are associated with almost every stage of development, and it is becoming evident that there is a common suite of cell wall-localized enzymes that are expressed in a number of these stages, including fruit ripening, abscission/dehiscence, pathogenesis, and cell expansion. The existence of multiple genes to carry out similar functions in each developmental context provides the basis for complex regulation of gene expression by a number of developmental and environmental signals and for specialization of the biochemical function of each distinct gene product.

ACKNOWLEDGMENTS

We are grateful to Dr. Jocelyn K.C. Rose for critical reading of this manuscript and to Dr. William Hiatt for providing Figure 2.

Received January 23, 1998; accepted February 4, 1998.
Copyright Clearance Center: 0032–0889/98/117/0337/07.

LITERATURE CITED

Allen RL, Lonsdale DM (1992) Sequence analysis of three members of the maize polygalacturonase gene family expressed during pollen development. Plant Mol Biol **20:** 343–345

Bartley IM (1978) *Exo*-polygalacturonase of apple. Phytochemistry **12:** 213–216

Bennett AB, Chetelat R, Klann E, Lashbrook C, Martin R, Gilchrist D (1993) Physiologically directed molecular approaches to plant improvement. Trans Malaysian Soc Plant Physiol **3:** 200–209

Bonghi C, Rascio N, Ramina A, Casadoro G (1992) Cellulase and polygalacturonase involvement in the abscission of leaf and fruit explants of peach. Plant Mol Biol **20:** 839–848

Brown SM, Crouch ML (1990) Characterization of a gene family abundantly expressed in *Oenothera organensis* pollen that shows sequence similarity to polygalacturonase. Plant Cell **2:** 263–274

Brummell DA, Labavitch JM (1997) Effect of antisense suppression of endopolygalacturonase activity on polyuronide molecular weight in ripening tomato fruit and in fruit homogenates. Plant Physiol **115:** 717–725

Caprari C, Mattei B, Basile ML, Salvi G, Crescenzi V, Delorenzo G, Cervone F (1996) Mutagenesis of endopolygalacturonase from *Fusarium moniliforme*: histidine residue 234 is critical for enzymatic and macerating activities and not for binding to polygalacturonase-inhibiting protein (PGIP). Mol Plant Microb Interact **9:** 617–624

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J **3:** 1–30

Cutillas-Iturralde A, Zarrra I, Lorences EP (1993) Metabolism of cell wall polysaccharides from persimmon fruit: pectin solubilization during fruit ripening occurs in apparent absence of polygalacturonase activity. Physiol Plant **89:** 369–375

Darvill A, Augur C, Bergmann C, Carlson RW, Cheong J-J, Eberhard S, Hahn MG, Lo V-M, Marfa V, Meyer B, and others (1992) Oligosaccharins: oligosaccharides that regulate growth, development and defense responses in plants. Glycobiology **2:** 181–198

Dawson DM, Melton LD, Watkins CB (1992) Cell wall changes in nectarines (*Prunus persica*). Solubilization and depolymerization of pectic and neutral polymers during ripening and in mealy fruit. Plant Physiol **100:** 1203–1210

Del Campillo E, Bennett AB (1996) Pedicel breakstrength and cellulase gene expression during tomato flower abscission. Plant Physiol **111:** 813–820

DellaPenna D, Bennett AB (1988) *In vitro* synthesis and processing of tomato fruit polygalacturonase. Plant Physiol **86:** 1057–1063

De Veau EJI, Gross KC, Huber DJ, Watada AE (1993) Degradation and solubilization of pectin by β-galactosidases purified from avocado mesocarp. Physiol Plant **87:** 279–285

Downs CG, Brady CJ, Gooley A (1992) Exopolygalacturonase protein accumulates late in peach fruit ripening. Physiol Plant **85:** 133–140

Dubald M, Barakate A, Mandaron P, Mache R (1993) The ubiquitous presence of exopolygalacturonase in maize suggests a fundamental cellular function for this enzyme. Plant J **4:** 781–791

Fischer RL, Bennett AB (1991) Role of cell wall hydrolases in fruit ripening. Annu Rev Plant Physiol Plant Mol Biol **42:** 675–703

Garcia-Romera I, Fry SC (1995) The longevity of biologically-active oligogalacturonides in rose cell cultures: degradation by exo-polygalacturonase. J Exp Bot **46:** 1853–1857

Giovannoni JJ, DellaPenna D, Bennett AB, Fischer RL (1989) Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. Plant Cell **1:** 53–63

Grierson D, Tucker GA, Keen J, Ray J, Bird CR, Schuch W (1986) Sequencing and identification of a cDNA clone for tomato polygalacturonase. Nucleic Acids Res **14:** 8595–8601

Hadfield KA, Bennett AB (1997) Programmed senescence of plant organs. Cell Death Differ **4:** 662–670

Hadfield KA, Rose JKC, Yaver DS, Berka RM, Bennett AB (1998) Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. Plant Physiol **117:** 363–373

Huber DJ (1983) Polyuronide degradation and hemicellulose modifications in ripening tomato fruit. J Am Soc Hortic Sci **108:** 405–409

Huber DJ (1984) Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. J Food Sci **49:** 1310–1315

Huber DJ, O'Donoghue EM (1993) Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol **102:** 473–480

Jenkins ES, Paul W, Coupe SA, Bell SJ, Davies EC, Roberts JA (1996) Characterization of an mRNA encoding a polygalacturonase expressed during pod development in oilseed rape (*Brassica napus* L.). J Exp Bot **47:** 111–115

Kalaitzis P, Solomos T, Tucker ML (1997) Three different polygalacturonases are expressed in tomato leaf and flower abscission, each with a different temporal expression pattern. Plant Physiol **113:** 1303–1308

Knee M (1973) Polysaccharide changes in cell walls of ripening apples. Phytochemistry **12:** 1543–1549

Kramer M, Sanders R, Bolkan H, Waters C, Sheehy RE, Hiatt WR (1992) Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. Postharvest Biol Tech **1:** 241–255

Langley KR, Martin A, Stenning R, Murray AJ, Hobson GE, Schuch WW, Bird CR (1994) Mechanical and optical assessment of the ripening of tomato fruit with reduced polygalacturonase activity. J Sci Food Agric **66:** 547–554

Lashbrook CC, Giovannoni JJ, Hall BD, Fischer RL, Bennett AB (1998) Transgenic analysis of tomato endo-β-1,4-glucanase gene function: role of *Cel1* in floral abscission. Plant J **13:** 303–310

Lashbrook CC, Gonzalez-Bosch C, Bennett AB (1994) Two divergent endo-β-1,4-glucanase genes exhibit overlapping expression in ripening fruit and abscising flowers. Plant Cell **6:** 1485–1493

Lester DR, Sherman WB, Atwell BJ (1996) Endopolygalacturonase and the *Melting Flesh* (*M*) locus in peach. J Am Soc Hortic Sci **121:** 231–235

Lester DR, Speirs J, Orr G, Brady CJ (1994) Peach (*Prunus persica*) endopolygalacturonase cDNA isolation and mRNA analysis in melting and nonmelting peach cultivars. Plant Physiol **105:** 225–231

Mascarenhas JP (1990) Gene activity during pollen development. Annu Rev Plant Physiol Plant Mol Biol **41:** 317–338

McCollum TG, Huber DJ, Cantliffe DJ (1989) Modification of polyuronides and hemicelluloses during muskmelon fruit softening. Physiol Plant **76:** 303–308

Meakin PJ, Roberts JA (1991) Anatomical and biochemical changes associated with the induction of oilseed rape (*Brassica napus*) pod dehiscence by *Dasineura brassicae* (Winn.). Ann Bot **67:** 193–197

Nogata Y, Ohta H, Voragen AGJ (1993) Polygalacturonase in strawberry fruit. Phytochemistry **34:** 617–620

O'Donoghue EM, Huber DJ, Timpa JD, Erdos GW, Brecht JK (1994) Influence of avocado (*Persea americana*) C*x*-cellulase on the structural features of avocado cellulose. Planta **194**: 573–584

Petersen M, Sander L, Child R, Van Onckelen H, Ulvskov P, Borkhardt B (1996) Isolation and characterisation of a pod dehiscence zone-specific polygalacturonase from *Brassica napus*. Plant Mol Biol **31:** 517–527

Pressey R (1987) Exopolygalacturonase in tomato fruit. Phytochemistry **26:** 1867–1870

Pressey R (1991) Polygalacturonase in tree pollens. Phytochemistry **30:** 1753–1755

Pressey R, Avants JK (1977) Occurrence and properties of polygalacturonase in *Avena* and other plants. Plant Physiol **60:** 548–553

Pressey R, Reger BJ (1989) Polygalacturonase in pollen from corn and other grasses. Plant Sci **59:** 57–62

Ranwala AP, Suematsu C, Masuda H (1992) The role of β-galactosidases in the modification of cell wall components during muskmelon fruit ripening. Plant Physiol **100:** 1318–1325

Redgwell RJ, MacRae E, Hallett I, Fischer M, Perry J, Harker R (1997) In vivo and in vitro swelling of cell walls during fruit ripening. Planta **203:** 162–173

Robert LS, Allard S, Gerster JL, Cass L, Simmonds J (1993) Isolation and characterization of a polygalacturonase gene highly expressed in *Brassica napus* pollen. Plant Mol Biol **23:** 1273–1278

Roberts JA, Taylor JE, Laslett YV, Tucker GA (1989) Changes in gene expression during ethylene-induced leaf abscission. *In* DJ Osborne, MB Jackson, eds, Cell Separation in Plants, Vol H 35. Springer-Verlag, Berlin, pp 61–68

Rose JKC, Hadfield KA, Labavitch JM, Bennett AB (1998) Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol **117:** 345–361

Schuch W, Kanczler J, Robertson D, Hobson G, Tucker G, Grierson D, Bright S, Bird C (1991) Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. HortScience **26:** 1517–1520

Scott-Craig JS, Panaccione DG, Cervone F, Walton JD (1990) Endopolygalacturonase is not required for pathogenicity of *Cochliobolus carbonum* on maize. Plant Cell **2:** 1191–1200

Sexton R, Roberts JA (1982) Cell biology of abscission. Annu Rev Plant Physiol **33:** 133–162

Sheehy RE, Kramer M, Hiatt WR (1988) Reduction of polygalacturonase activity in tomato fruit by antisense RNA. Proc Natl Acad Sci USA **85:** 8805–8809

Sheehy RE, Pearson J, Brady CJ, Hiatt WR (1987) Molecular characterization of tomato fruit polygalacturonase. Mol Gen Genet **208:** 30–36

Sitrit Y, Downie B, Bennett AB, Bradford KJ (1996) A novel *exo*-polygalacturonase is associated with radicle protrusion in tomato (*Lycopersicon esculentum*) seeds. (abstract no. 752). Plant Physiol **111:** S-161

Smith CJS, Watson CF, Morris PC, Bird CR, Seymour GB, Gray JE, Arnold C, Tucker GA, Schuch W, Harding S, and others (1990) Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. Plant Mol Biol **14:** 369–379

Smith CJS, Watson CF, Ray J, Bird CR, Morris PC, Schuch W, Grierson D (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature **334:** 724–726

Stratilova E, Dzurova M, Markovic O, Jornvall H (1996) An essential tyrosine residue of *Aspergillus* polygalacturonase. FEBS Letts **382:** 164–166

Taylor JE, Tucker GA, Lasslett Y, Smith CJS, Arnold CM, Watson CF, Schuch W, Grierson D, Roberts JA (1990) Polygalacturonase expression during leaf abscission of normal and transgenic plants. Planta **183:** 133–138

Taylor JE, Webb STJ, Coupe SA, Tucker GA, Roberts JA (1993) Changes in polygalacturonase activity and solubility of polyuronides during ethylene-stimulated leaf abscission in *Sambucus nigra*. J Exp Bot **44:** 93–98

Tebbutt SJ, Rogers HJ, Lonsdale DM (1994) Characterization of a tobacco gene encoding a pollen-specific polygalacturonase. Plant Mol Biol **25:** 283–297

Tucker GA, Schindler BC, Roberts JA (1984) Flower abscission in mutant tomato plants. Planta **160:** 164–167

Wu Q, Szakacs-Dobozi M, Hemmat M, Hrazdina G (1993) Endopolygalacturonase in apples (*Malus domestica*) and its expression during fruit ripening. Plant Physiol **102:** 219–225