

# ASSEMBLY AND ENLARGEMENT OF THE PRIMARY CELL WALL IN PLANTS

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## ABSTRACT

Growing plant cells are shaped by an extensible wall that is a complex amalgam of cellulose microfibrils bonded noncovalently to a matrix of hemicelluloses, pectins, and structural proteins. Cellulose is synthesized by complexes in the plasma membrane and is extruded as a self-assembling microfibril, whereas the matrix polymers are secreted by the Golgi apparatus and become integrated into the wall network by poorly understood mechanisms. The growing wall is under high tensile stress from cell turgor and is able to enlarge by a combination of stress relaxation and polymer creep. A pH-dependent mechanism of wall loosening, known as acid growth, is characteristic of growing walls and is mediated by a group of unusual wall proteins called expansins. Expansins appear to disrupt the noncovalent bonding of matrix hemicelluloses to the microfibril, thereby allowing the wall to yield to the mechanical forces generated by cell turgor. Other wall enzymes, such as (1 → 4) β-glucanases and pectinases, may make the wall more responsive to expansin-mediated wall creep, whereas pectin methylesterases and peroxidases may alter the wall so as to make it resistant to expansin-mediated creep.

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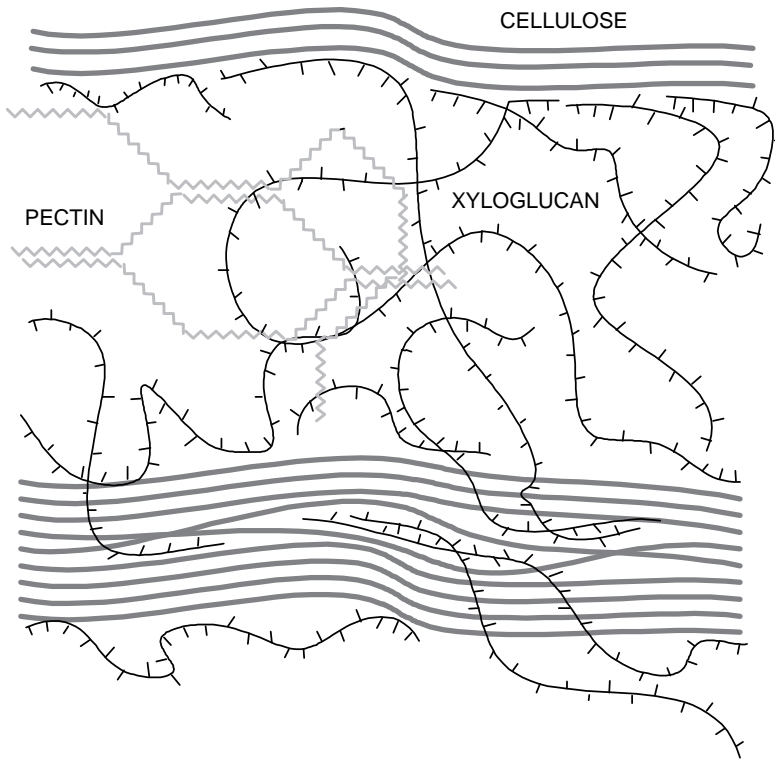
## INTRODUCTION

Plant cells are encapsulated within a complex, fibrous wall whose properties are crucial to the form and function of plants. As the strongest mechanical component of the cell, the wall acts as an exoskeleton to give the plant cell its shape and to allow high turgor pressures. As the skin of the plant cell, the wall participates in adhesion, cell-cell signaling, defense, and numerous growth and differentiation processes. As a natural product, the plant cell wall is used in the manufacture of paper, textiles, lumber, and various processed polymers that go into plastics, films, coatings, adhesives, and thickeners in a staggering variety of products (Lapasin & Priel 1995). As the most abundant reservoir of organic carbon in nature, the plant cell wall greatly impacts numerous ecological processes.

This review focuses on selected topics concerning the growing (primary) plant cell wall, which has unique rheological properties. Although knowledge of the structure and chemistry of the plant cell wall is extensive and detailed, many issues remain unresolved about how the wall is put together and how it expands during plant cell enlargement. These questions comprise the focus of this review. Other aspects of the primary plant cell wall may be found in reviews concerning its chemistry (McNeil et al 1984, Fry 1988, Carpita & Gibeaut 1993) and ultrastructure (Roland & Vian 1979, McCann et al 1990).

## ARCHITECTURE OF PRIMARY WALLS

The general picture of the primary wall to emerge in the last two decades is that of a composite polymeric structure in which crystalline cellulose microfibrils are embedded in a complex, highly hydrated, and less ordered polysaccharide matrix, with smaller quantities of structural protein intercalated in the matrix (Figure 1). Such a picture of the wall is based largely on biochemical analysis of



*Figure 1* Model of the primary plant cell wall, showing major structural polymers and their likely arrangement in the wall. Cellulose microfibrils contain noncrystalline regions that may be formed by entrapment of hemicelluloses such as xyloglucan. Xyloglucan can also bond to the surface of cellulose and may link two microfibrils together. Although the side chains of xyloglucan interfere with bonding of the glucan backbone to other glucans, they may twist such that short regions of the backbone form a planar configuration suitable for bonding to cellulose. Pectins form a space-filled hydrophilic gel between microfibrils.

homogeneous suspension cultures and on microscopy of limited cell types and has generally been assumed to hold true for all cell types. As discussed below, there is some divergence of thought about the extent of covalent cross-linking between the matrix polymers. Also, it is good to keep in mind that primary walls vary in thickness and morphology, depending on source. McCann et al (1990) emphasized the thinness (100 nm) and architectural simplicity of the primary wall of the onion bulb. This contrasts with the thicker, multilayered, helicoidal walls of the bean hypocotyl and other growing tissues (Roland & Vian 1979,

Vian & Roland 1987). These studies thus convey very different impressions of primary wall ultrastructure.

The wall of enlarging plant cells is composed of approximately 30% cellulose, 30% hemicellulose, and 35% pectin, with perhaps 1–5% structural protein, on a dry weight basis. Substantial deviations from these values may be found, notably in the grasses (Carpita 1996), where, for example, the walls of growing maize coleoptiles consist of  $\approx 55\%$  hemicellulose, 25% cellulose, and only 10% pectin. Given its structure (see below), cellulose obviously plays a major role in determining the strength and structural bias of the cell wall. Hemicelluloses such as xyloglucan bind to the surface of cellulose, perhaps forming tethers that bind cellulose microfibrils together or acting as a lubricating coating to prevent direct microfibril-microfibril contact. Pectins form a gel phase in which the cellulose-hemicellulose network is embedded. Pectin may act as a hydrophilic filler to prevent aggregation and collapse of the cellulose network (Jarvis 1992) and to modulate the porosity of the cell wall to macromolecules (Baron-Epel et al 1988). The precise role of the structural proteins remains a matter of speculation.

The most abundant, but often overlooked, component of the wall is water, which makes up about two thirds of the wall mass in growing tissues. This water is located mainly in the matrix ( $\approx 75\text{--}80\%$  water), which suggests that the matrix has the properties of a relatively dense hydrogel. Localized phase changes or melting of the matrix may be important for wall expansion (Lin et al 1991). Edelmann (1995) found that the extension properties of isolated cell walls were quite sensitive to wall dehydration. Dehydration of the wall matrix may also play a direct role in the growth inhibition by water deficits (Chazen & Neumann 1994, Passioura 1994). Closer study of the state of water in the cell wall may yield valuable insights into the special rheological properties of the growing wall.

New cell walls originate from the cell plate formed by the phragmoplast during cytokinesis of plant cells. Early stages of cell plate formation have been reviewed recently (Samuels et al 1995, Verma & Gu 1996), but the later transition from a labile cell plate into a stable wall has drawn less attention. This may be the same process by which wall polymers are added to and integrated into existing growing walls, conceptually shown as synthesis  $\rightarrow$  secretion  $\rightarrow$  integration and assembly  $\rightarrow$  stress relaxation and polymer creep.

To this may be added a possible fifth stage, in which wall polymers may become cross-linked, leading to a loss of the wall's ability to expand.

From microscopy studies it has long been known that cellulose is synthesized at the plasma membrane (Brown et al 1996), whereas the matrix polysaccharides are synthesized by the Golgi apparatus and secreted in vesicles

to the wall (Staehein & Moore 1995). However, the membrane-bound enzymes responsible for these synthetic activities have proven very difficult to characterize. The following sections briefly review the structure and interaction of the major wall polysaccharides.

### *Cellulose*

This is a tightly packed aggregate of linear polymers of 1 → 4 linked β-D-glucopyranose. The aggregates take the form of long microfibrils of indeterminate length that vary in width and in degree of order, depending on source. Cellulose microfibrils in plants appear to be 4–10 nm wide under the electron microscope (Emons 1988, McCann et al 1990), although those formed by algae may be up to 30 nm wide and more crystalline (Preston 1974). The cellulose microfibril has a substructure consisting of highly organized crystalline domains linked together by less organized amorphous regions. The individual glucans of cellulose are composed of 2000 to >25,000 monomers and assume an extended ribbon conformation (Brown et al 1996). They are long enough (i.e. ≈1–5 microns in length) to extend into multiple crystalline subdomains and amorphous regions of a microfibril.

The tight noncovalent bonding between adjacent glucans within a cellulose microfibril gives this structure remarkable properties. Cellulose has very high tensile strength—equivalent to steel (Wainwright et al 1976)—and is insoluble, chemically stable, and relatively immune to enzymatic attack. Microbial cellulases have cellulose-binding domains that apparently assist the separation of the individual glucans from the microfibril (Din et al 1991, Hall et al 1995), whereas plant glucanases lack these domains and do not hydrolyze crystalline cellulose effectively; they are more effective on matrix glucans.

**SYNTHESIS** The microfibril appears to be extruded by large, ordered complexes, called rosettes or terminal complexes, embedded in the plasma membrane (Brown et al 1996). Sucrose synthase may be complexed with cellulose synthase and may act as a metabolic channel to transfer glucose from sucrose via uridine diphosphate glucose (UDPG) to the growing glucan chain (Amor et al 1995). Although cellulose synthase of land plants has long eluded definitive characterization in vitro (Delmer & Amor 1995, Haigler & Blanton 1996), homologues (*celA*) of bacterial cellulose synthase genes were recently identified in cotton (Pear et al 1996). *celA* contains UDP-glucose binding domains characteristic of glucosyl transferase catalytic sites, and its pattern of expression during cotton fiber development coincides with the pattern of cellulose deposition. Although it has not been shown directly that the protein product of *celA* can synthesize cellulose, this report has stimulated much speculation about the

enzymatic machinery for synthesis of cellulose and related wall polysaccharides (Carpita et al 1996). Glycosyltransferases of this type are likely to have two active sites for transferring glycosyl residues from their nucleotide donors to the nascent polymer acceptor (Saxena et al 1995), an arrangement that could explain the disaccharide-repeating unit found in the backbones of many wall polysaccharides (Albersheim et al 1997).

The formation of cellulose involves not only the synthesis of the glucan, but also the crystallization of multiple glucans into a microfibril. Little is known about the control of this process, except that the direction of microfibril deposit may be guided by microtubules adjacent to the membrane (Emons et al 1992) and that xyloglucan and perhaps other hemicelluloses may alter microfibril structure. Binding studies (Hayashi et al 1987, Hayashi 1989) suggest that xyloglucan does not only bind to the surface of the microfibril, but may also become physically entrapped within the microfibril during its formation. Much of the xyloglucan can be released only by harsh extractions that cause microfibril swelling (Hayashi & Maclachlan 1984, Chambat et al 1984). A model system to study microfibril formation is *Acetobacter xylinum*, which normally forms large, highly ordered cellulose microfibrils. When *Acetobacter* makes cellulose in the presence of materials that bind to  $\beta$ -1,4-glucans, the resulting microfibrils are altered in shape, i.e. more fragmented and frayed (Haigler et al 1982, Hayashi et al 1987), and with X-ray diffraction patterns that appear to resemble more closely those of microfibrils synthesized by land plants (Atalla et al 1993). Such results suggest that hemicellulose entrapment in muro significantly alters microfibril morphology. This is consistent with the fact that cotton fibers have low hemicellulose content and highly crystalline cellulose.

### *Hemicelluloses*

This is a practical term for a heterogeneous group of noncrystalline glycans that are rather tightly bound in the wall. Typically they are solubilized from de-pectinated walls by the use of strong alkali (0.1–4 M NaOH), although they may also be found in soluble form in the medium of cell cultures. In the primary wall of dicotyledons, the most abundant and best studied hemicellulose is xyloglucan, a branched polymer consisting of a backbone of 1  $\rightarrow$  4 linked  $\beta$ -D-glucopyranose residues with short side chains containing xylose, galactose and, often, a terminal fucose (McNeil et al 1984, Fry 1989b). The molecular mass of xyloglucans from growing pea stems was estimated to be about 30 kDa, with a minor component of  $\approx$ 300 kDa (Talbot & Ray 1992a); in other plants, smaller and larger xyloglucans have been reported (Fry 1989b). Because xyloglucans are longer ( $\approx$ 50–500 nm) than the spacing between cellulose microfibrils (20–40 nm), they have the potential to link microfibrils together.

Other polymers may be found in the hemicellulose fraction of the wall, depending on developmental state and species (Carpita & Gibeau 1993, Brett &

Waldron 1996). In pea stems, the major hemicellulose was a large ( $\approx 1000$  kDa) arabinan/galactan, a component that is usually extracted in the pectin fraction. Whereas grass walls contain some xyloglucan (Labavitch & Ray 1978), other hemicelluloses are more abundant, e.g. mixed-linked (1  $\rightarrow$  3) (1  $\rightarrow$  4)  $\beta$ -glucan and arabinoxylan, a (1  $\rightarrow$  4)  $\beta$ -xylan backbone with short sidechains of arabinose and glucuronic acid (Carpita 1984).

Although the hemicelluloses are not organized into crystalline arrays like cellulose, neither are they randomly arranged in the wall, but appear from spectroscopy studies to lie in a preferred orientation parallel to the cellulose microfibrils (Morikawa et al 1978, McCann et al 1992a, Séné et al 1994). This is consistent with the conclusions of binding studies showing that xyloglucan can bind tightly to the surface of the cellulose microfibril (Hayashi 1989). From molecular modeling and in vitro studies of xyloglucan/cellulose binding (Hayashi et al 1994, Levy et al 1997), it appears that the terminal L-fucose on the side chains stabilizes a planar configuration of the xyloglucan backbone that is essential for optimal cellulose binding. In the *mur1* mutant of *Arabidopsis*, L-galactose is substituted for the terminal L-fucose in xyloglucan branches (Zablackis et al 1996), and the reduced tensile strength of the *mur1* stems may reflect weaker xyloglucan/cellulose binding (Reiter et al 1993).

In addition to xyloglucan, other hemicelluloses may bind to cellulose, e.g. xylans, arabinoxylans, and mannans. In grasses, the branched arabinoxylans and mixed-link  $\beta$ -glucans are hypothesized to serve the role that xyloglucans play in dicotyledons (Carpita & Gibeau 1993).

### *Pectins*

Pectins are the most soluble of the wall polysaccharides and can be extracted with hot water or with calcium chelators. Like the hemicelluloses, pectins also constitute a heterogeneous group of polysaccharides, characteristically containing acidic sugars such as glucuronic acid and galacturonic acid. Some pectins have a relatively simple primary structure such as homogalacturonan, a linear polymer of (1  $\rightarrow$  4)  $\beta$  galacturonic acid, with occasional rhamnosyl residues that put a kink in the chain. Rhamnogalacturonan I (RG I) has repeating subunits of 1  $\rightarrow$  2)  $\alpha$ -L-rhamnosyl-(1  $\rightarrow$  2)  $\alpha$ -D-galacturonyl disaccharides, with long side chains of arabinans and arabinogalactans. The size of RG I in growing pea internodes is reported to range from 500 to 2000 kDa (Talbot & Ray 1992a). Of lesser abundance is rhamnogalacturonan II, a highly complex carbohydrate containing diverse sugars in varying linkages (Darvill et al 1978).

### *Bonding Between Wall Polysaccharides*

Unlike the bacterial peptidoglycan wall, which is conceived of as a giant, covalently linked macromolecule, the plant wall is thought to be more like a polymer composite held together primarily by noncovalent bonds. In this model,

cellulose is associated with the hemicelluloses via a mixture of hydrogen and hydrophobic bonding and physical entrapment. Pectins form a gel around the cellulose-hemicellulose network. This model is based on the fact that these wall components can be extracted and separated from one another under conditions unlikely to break most covalent bonds (Talbot & Ray 1992a, Carpita & Gibeau 1993).

However, there are uncertainties in the evidence for this model of the wall. For example, it is not feasible to extract all of the hemicelluloses and pectins from cellulose; small amounts of matrix polysaccharides remain attached or entrapped (Chambat et al 1984, McCann et al 1990). There is always a concern that labile covalent bonds, e.g. ester bonds, could be broken during extraction. The influential model of the plant cell wall by Keegstra et al (1973) originally proposed that the pectins and hemicelluloses were covalently linked, but this notion was later discarded when the linkage could not be confirmed. A recent analysis of pea internode walls (Talbot & Ray 1992a) found that the major matrix polysaccharides are not covalently bound to each other. In contrast, in cotton suspension cell walls (Fu & Mort 1996), some xyloglucan is covalently linked to rhamnogalacturonan. Other studies likewise suggest at least some covalent linkage between pectins and xyloglucans (Chambat et al 1984). Given the technical difficulty of wall extraction without labile bond breakage or artifactual polymer aggregation, and the likelihood that polysaccharide cross-linking may change during wall maturation, an open mind is required on this important issue. If we look to the yeast cell wall for analogy, most (1 → 3)  $\beta$ -glucans and (1 → 6)  $\beta$ -glucans are initially secreted in an alkali-soluble form and subsequently become linked to chitin, thereby becoming alkali insoluble (Klis 1994). Such cross-linking may contribute to cessation of wall expansion.

Pectins are subject to a number of modifications that alter their conformation and linkage in the wall. Many of the acidic residues in pectins are esterified with methyl, acetyl, and other unidentified groups (Kim & Carpita 1992, McCann et al 1994). Such esterification occurs during biosynthesis in the Golgi and may be removed by esterases in the wall. By creating carboxyl groups, de-esterification increases the charge density in the wall, which may influence the activity of wall enzymes (Moustacas et al 1991). De-esterification also greatly affects the physical properties of pectin, causing pectin chains to assemble into expanded, highly hydrated gel networks linked by  $\text{Ca}^{2+}$  (Lapasin & Pricl 1995). Such  $\text{Ca}^{2+}$  bridging exhibits high cooperativity, and the processivity of pectin methyl esterases is likely an important property of these enzymes (Bordenave 1996).

In addition to calcium bridging, pectins may be linked to each other by various covalent bonds, including ester linkages through phenolic dimers such as diferulic acid (Fry 1983, Wallace & Fry 1994). It has long been known that



boron, an essential element for plants, is complexed in the wall, and recently borate diesters of rhamnogalacturonan II were identified (Kobayashi et al 1996, O'Neill et al 1996, Ishii & Matsunaga 1997). Wall elasticity was reported to be sensitive to borate status, indicating that such borate esters likely affect cell wall mechanics (Findelee & Goldbach 1996).

### *Structural Proteins*

In addition to the major polysaccharides described above, the growing plant cell wall also contains structural proteins (Showalter 1993). Several classes of wall structural proteins have been described in the plant kingdom, classified according to their predominant amino acid composition, e.g. hydroxyproline-rich glycoprotein (HRGP), glycine-rich protein (GRP), proline-rich protein (PRP), and so on. The distinction between these proteins is becoming blurred as proteins with sequences characteristic of more than one class are found (Showalter 1993). Many of these proteins are highly glycosylated. Typically, these proteins have highly repetitive primary structures and become insolubilized in the cell wall during cell maturation or upon wounding. The biochemical nature of the insolubilization is uncertain, although intermolecular diphenylether linkages between tyrosines have been suggested (Fry 1986, Wallace & Fry 1994, Brady et al 1996). In tobacco stems, PRPs become rapidly insolubilized upon wounding, and this is associated with an H<sub>2</sub>O<sub>2</sub> burst and mechanical stiffening of the cell walls (Bradley et al 1992, Brisson et al 1994). Peroxidase-mediated phenolic cross-linking has been hypothesized.

It is notable that structural proteins vary greatly in their abundance, depending on cell type, maturation, and previous stimulation. Wounding, pathogen attack, and treatment with elicitors increase expression of many of these proteins, and wall structural proteins are often localized to specific cell and tissues type (Showalter 1993). For example, HRGPs are mostly associated with phloem, cambium, and sclerenchyma; GRPs and PRPs are most often localized to the xylem. Thus these wall components may be more characteristic of the differentiated state of the plant cell than of the growing state.

HRGPs are the best-studied class of wall structural protein in plants and comprise a large family of proteins (Kieliszewski & Lamport 1994). They are related to the major structural protein of the noncellulosic wall found in *Chlamydomonas* and other volvocine species of green algae (Woessner & Goodenough 1994). A subfamily of plant HRGPs with the repeating pentapeptide Ser-Hyp<sub>4</sub> was originally called extensin because it was thought to determine the extensibility of the cell wall, but evidence for this function has not materialized. Perhaps this misnomer should be dropped in favor of the more descriptive term HRGP. Although the precise functions of HRGPs are unknown, expression patterns suggest that they are involved in protection against pathogens and desiccation

and perhaps in structural strengthening of walls (Cooper et al 1987, Tiré et al 1994).

Unlike the structural proteins listed above, arabinogalactan proteins (AGPs) are soluble and heavily glycosylated (Fincher et al 1983, Showalter 1993). Multiple AGP forms are found in plant tissues, either in the wall or associated with the plasma membrane, and they display tissue- and cell-specific expression patterns (Pennell et al 1989, Serpe & Nothnagel 1995). cDNAs encoding the protein cores of AGPs have recently been cloned (Du et al 1994, Chen et al 1994, Davies & Pogson 1995, Li & Showalter 1996). The protein structure deduced from these cDNAs contains an N-terminal secretion signal, a central domain rich in Pro (Hyp) residues, and a potential membrane anchor at the C terminus. Because AGPs have a sticky quality and show specific binding to pectins, they are thought to be involved in cell adhesion. They are not a major structural component of the wall, however. For example, only about 0.1% of the wall dry mass in rose suspension cells is made up of AGPs (Serpe & Nothnagel 1995). In maize coleoptiles, AGPs appear to turn over rapidly, and their abundance in Golgi vesicles has led to the speculation that they are "shuttle molecules that bind to polymers to render them soluble in Golgi vesicles until they arrive at their proper sites of assembly at the cell surface" (Gibeaut & Carpita 1991). Treatment of suspension cultures with exogenous AGPs or with Yariv phenylglucosides that specifically bind AGPs is reported to influence cell proliferation and embryogenesis (Kreuger & Van Holst 1993, Serpe & Nothnagel 1994); however, the presence of wall AGP epitopes did not coincide with the embryogenic potential of carrot suspension cells (Toonen et al 1996). AGPs are also implicated in the growth, nutrition, and guidance of pollen tubes through stylar tissues (Gane et al 1994, Cheung et al 1995, Wu et al 1995, Jauh & Lord 1996, Gerster et al 1996), as well as other developmental processes (Pennell & Roberts 1990).

### *Wall Enzymes*

Numerous enzymes may be found associated with cell walls (Cassab & Varner 1988, Fry 1995). Some can modify the major polysaccharides of the plant wall, e.g. endoglucanases, xylosidases, pectinases, pectin methyl esterases, and xyloglucan endotransglycosylases. Others can act on the wall of potential bacterial and fungal pathogens, e.g. chitinases and (1 → 3)  $\beta$ -glucanases, or modify other substrates in the wall, e.g. invertase, peroxidases, phosphatases, various dehydrogenases. The precise function of many of these wall enzymes has yet to be determined, but they are doubtless involved in numerous processes, from wall modification to metabolite transport and cell signaling.

This partial listing of enzymes suggests that the wall is a metabolically active compartment of the cell. Although it is conventional to picture the plant cell wall

as an extracellular compartment, it is also true that most cell walls in the plant body (with the exception of the outer epidermal wall) are essentially contained by and in intimate contact with the plasma membrane of closely packed cells. As the wall constitutes a mere 5% of the volume of growing tissues (Cosgrove & Cleland 1983), it is easy to imagine that small fluxes of materials across the plasma membrane could greatly alter wall pH, redox potential,  $H_2O_2$ , and other conditions, which in turn may modulate the activity of wall enzymes.

## WALL ASSEMBLY

After their secretion into the extracellular space, the wall polymers must be assembled into a cohesive structure, with the arrangement and bonding relationships characteristic of the wall. The prime candidates for this process are self-assembly and enzyme-mediated assembly.

### *Self-Assembly*

Self-assembly is attractive because of its mechanistic simplicity and because of the marked tendency of wall polysaccharides to aggregate into organized structures (Roland & Vian 1979, Lapasin & Pricl 1995). When cellulose is regenerated *in vitro*, it spontaneously forms fibers, better known as rayon. Likewise, when the hemicellulose fraction of the wall was dissolved and subsequently precipitated, it spontaneously aggregated into concentric, ordered networks that ultrastructurally resembled the native wall (Roland et al 1977). Pectins, in contrast, formed much more dispersed, isotropic networks. Some hemicelluloses are noted for their tendency to aggregate and to come out of solution (Wada & Ray 1978), making them difficult to fractionate by many conventional means.

Although xyloglucan is normally tightly bound in the wall, once it is solubilized it does not readily self-aggregate and the complexes formed with cellulose *in vitro* are less stable than those in the native wall (Hayashi 1989, Hayashi et al 1994). Xyloglucan/cellulose complexes reconstituted *in vitro* contained only 7% of the xyloglucan found in the native complexes (Hayashi et al 1987). This figure raises some doubt about the meaning of the binding of xyloglucan to cellulose *in vitro* and implies that special physical conditions may be required to form the native wall structure, perhaps assisted by chaperones that aid the folding and interaction of matrix polysaccharides in the correct order.

An alternative interpretation, discussed above, is that xyloglucan is woven into the microfibril during cellulose synthesis (Hayashi et al 1994). This idea has recently been extended by Whitney (1996), who found that the cellulose network formed by *Acetobacter* cultures is substantially altered by the presence of xyloglucan in the medium. Cross bridges between microfibrils were observed, with lengths approximating those observed in preparations of plant

walls (McCann et al 1992b), and the networks exhibited a greater degree of order than normally found in *Acetobacter* pellicles. Also of interest were mechanical measurements of the bacterial pellicles, showing that incorporation of xyloglucan made the networks more pliant than the native networks. This weakening effect, which seems at odds with the observed formation of xyloglucan cross bridges and with the notion that such cross bridges strengthen the network, might result from a reduction of sticky cellulose-cellulose contacts by a nonsticky xyloglucan coat. One might also infer that the xyloglucan bridges formed in such networks are not mechanically strong, but precise experiments on this point have not yet been made. Even in the native plant cell wall, the mechanical significance of hypothetical xyloglucan cross links has not been tested directly, even though the common view is that such cross links “are the principal tension-bearing molecules in the longitudinal axis of an elongating cell” (Carpita & Gibeaut 1993). Direct evidence is needed to substantiate this view.

### *Enzyme-Mediated Assembly*

There are few examples in which specific wall enzymes have been shown to aid the assembly of polymers into the wall structure. Potential candidates for this function include xyloglucan endotransglycosylase (XET), esterases, oxidases, and de-branching enzymes such as xylosidases.

XET has drawn considerable interest since its recent discovery (Smith & Fry 1991, Nishitani & Tominaga 1991, Fry et al 1992). This enzyme has the ability to cut the backbone of xyloglucans and to join the newly formed reducing end to the nonreducing end of an acceptor xyloglucan. Originally it was proposed that such transglycosylation might be a means of loosening the wall in a limited and controlled fashion. In support of this view, XET activity and growth are approximately correlated in various systems (Pritchard et al 1993, Potter & Fry 1993, Wu et al 1994, Xu et al 1995). In vitro experiments, however, failed to detect loosening or extension of the wall by XET (McQueen-Mason et al 1993).

An alternative role for XET might be to integrate newly synthesized xyloglucans into the wall (Nishitani & Tominaga 1991, Okazawa et al 1993). Because newly synthesized xyloglucans are much smaller than the bulk xyloglucans in the wall (Talbot & Ray 1992b), there is evidently some way that these polymers can be “stitched” together, end to end. Moreover, xyloglucan size distributions in pea stem segments were reported to change rapidly and reversibly in response to auxin, cutting, and turgor changes (Nishitani & Masuda 1981, 1983, Talbot & Ray 1992b). Although it is tempting to speculate that XET mediates these changes in xyloglucan size (Nishitani & Tominaga 1991), there is a problem with this idea. In solution, XET appears to cut xyloglucan at random locations along the backbone, thus randomizing the xyloglucan size distribution, rather

than causing a progressive increase in size. An increase in average xyloglucan size could occur if XET selectively cut at the end of xyloglucan molecule. Perhaps conditions in the cell wall, such as xyloglucan conformation or the electrostatic environment of the wall, modulate the action of this enzyme to produce larger or smaller xyloglucans.

## EXPANSION OF THE WALL

Cell expansion patterns may be highly localized, as in tip-growing cells, or more evenly distributed over the wall surface, as in cells with diffuse growth. However, even in cells with diffuse growth, different walls may enlarge at different rates or in different directions. This may be a matter of structural variations of specific walls (Freshour et al 1996) or of variations in the stresses borne by different walls (Lintilhac 1987).

Plants cells may expand enormously in volume before reaching maturity, e.g. a meristematic cell, measuring  $5\ \mu\text{m}$  on a side ( $\approx 125\ \mu\text{m}^3$ ), may give rise to a xylem vessel element, measuring  $50\ \mu\text{m}$  in diameter and  $300\ \mu\text{m}$  in length ( $\approx 2,355,000\ \mu\text{m}^3$ ). The cell wall accommodates this enormous expansion without losing mechanical integrity and generally without becoming thinner. This implies an effective means of integrating new polymers into the wall without destabilizing the load-bearing network.

Diffuse growth and tip growth are often said to take place by distinct mechanisms, yet analogous, if not identical, processes of polymer integration, stress relaxation, and creep must occur in both types of wall expansion. Some fungi such as *Achlya* and *Saprolegnia* are able to soften their walls to such an extreme extent that they may continue to grow at negligible turgor pressure, albeit with an altered morphology (Kaminskyj et al 1992, Money & Harold 1992, 1993). Such greatly softened walls are easily burst by an osmotic influx of water, and their biophysical properties are probably more like those of the animal extracellular matrix than of turgor-resistant walls.

### *Special Properties of Growing Cell Walls*

The walls of growing plant cells are characterized by high rates of synthesis and selective turnover of wall polysaccharides and by a form of stress relaxation that confers on the wall its ability to expand. Growing plant walls also display a pH-dependent form of wall extension (acid growth; Rayle & Cleland 1992), which is strongly promoted by wall acidification, such as that induced by the fungal toxin fusicoccin (Kutschera & Schopfer 1985).

**WALL SYNTHESIS** Cell wall expansion is generally well coordinated with wall polymer synthesis and secretion. Thus the rate of wall deposition in young stems and roots is highest in the zone of maximal cell expansion (Silk et al

1984, Schmalstig & Cosgrove 1990). Likewise, induction of cell elongation by auxin or by fusicoccin leads to increased rates of wall polysaccharide biosynthesis after 30 to 120 min of faster growth (Brummell & Hall 1983, Edelmann et al 1989). However, wall thinning may occur in growing cells under unusual conditions. For example, when pea stem segments were starved of sugars, yet stimulated to elongate by auxin (Bret-Harte et al 1991), the epidermal walls became thinner (a 25% loss of mass/area in 4 h). Bret-Harte et al (1991) cite other examples where wall thinning occurs. Inhibitor studies (Brummell & Hall 1984, Edelmann et al 1989, Hoson & Masuda 1992) indicate that cellulose synthesis is not essential for growth stimulation by auxin or fusicoccin, whereas Golgi secretion is necessary for auxin-induced growth, but not for fusicoccin-induced growth. Evidently, wall expansion may be stimulated without additional wall polysaccharide biosynthesis, implying a mechanism of wall expansion that does not necessarily entail concomitant secretion of wall polysaccharides. The necessity of Golgi secretion for auxin action may have to do with delivery of essential matrix polysaccharides or of growth-limiting wall proteins (Edelmann & Schopfer 1989) or of membranes containing  $H^+$ -ATPase for wall acidification (Frías et al 1996).

**WALL TURNOVER** Growing walls exhibit substantial turnover of certain matrix polysaccharides, and this has supported the common view that wall expansion requires structural weakening of the wall by hydrolytic enzymes (Fry 1989a, Carpita & Gibeaut 1993). Auxin promotion of cell elongation, in particular, has often been associated with wall autolysis (Hoson 1993). Hydrolysis of xyloglucan has drawn particular attention because it is specifically enhanced during auxin stimulation of cell elongation (Labavitch & Ray 1974b, Nishitani & Masuda 1981). In grass coleoptiles, the  $\beta$ -1,3-1,4-glucan likewise exhibits turnover and net loss during elongation (Carpita 1984), and wall glucanases are implicated in auxin promotion of coleoptile growth (Fry 1989a, Inouhe & Nevins 1991).

One should not conclude from this, however, that wall enlargement necessarily entails wholesale turnover of the wall. Pulse-chase experiments with pea stem segments indicate that most wall polymers are relatively stable, even during auxin-stimulated growth (Labavitch & Ray 1974a). The highest turnover was found in galactose residues attached to rhamnogalacturonan (50% turnover in 7 h), but this turnover was unaffected by auxin promotion of growth. The auxin-induced turnover of xyloglucan in these experiments was rather small (<5% of total autolysis).

Does the breakdown of matrix polysaccharides reflect an essential process for wall loosening and expansion? The answer is not fully resolved. For the majority of wall hydrolytic activities in pea stems, the answer seems to be negative (Labavitch & Ray 1974a). In isolated cucumber walls, long-term extension was

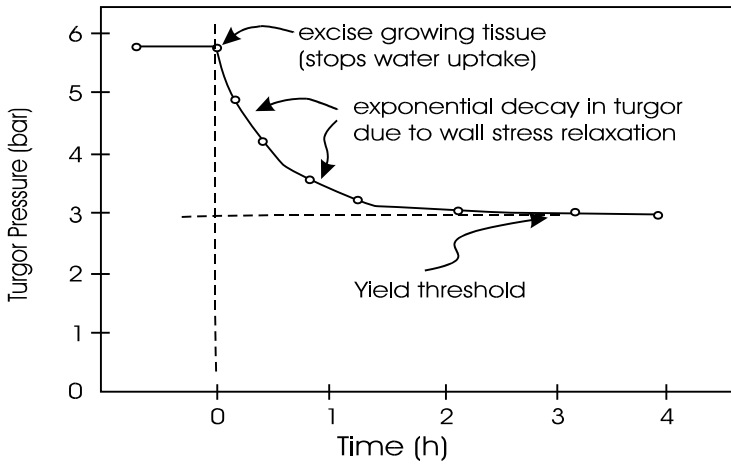
independent of bulk autolytic activity (Cosgrove & Durachko 1994). Other reports likewise indicate that much of the wall hydrolysis in growing tissues may be independent of wall expansion (Goldberg 1980, Pierrot et al 1982). Hence, wall autolysis may serve some other function(s). Perhaps matrix polysaccharides also function as mobilizable storage polysaccharides. This may be an unconventional notion for the walls of growing cells, yet there are other examples, e.g. in seed storage tissues (Meier & Reid 1984), where wall polysaccharides clearly serve this role. Support for this idea may also be found in studies of growing pea stems, where wall breakdown was stimulated by a combination of sugar starvation and fast growth (Bret-Harte et al 1991), whereas much less turnover was noted when sugars were plentiful (Labavitch & Ray 1974a).

**STRESS RELAXATION AND EXTENSIBILITY** As a complex, hydrated polymeric material, the plant cell wall—from both growing and nongrowing cells—has inherent viscoelastic and rheological (flow) properties. Growing cell walls are generally found to be more pliant than walls from nongrowing cells, and under appropriate conditions they exhibit a long-term irreversible yielding, which is a type of polymer creep that is lacking or nearly lacking in nonexpanding walls (Cosgrove 1993b).

Stress relaxation is a crucial biophysical property of growing cell walls (Cosgrove 1986, 1993a,b). Stress is used here in the mechanical sense as a force per unit area, and wall stresses arise as an inevitable consequence of cell turgor. Turgor pressure in growing plant cells is typically in the range of 0.3 to 1 MPa (Cosgrove 1986) and, because the wall is so thin relative to the cross section of the cell, wall stresses are calculated to be very large, in the range of 30 to 100 MPa (equivalent to 300 to 1000 atm). Wall stress relaxation is crucial because it is the means by which plant cells reduce their turgor and water potential, thereby enabling them to absorb water and to expand. Without stress relaxation, wall synthesis would only thicken the wall, not expand it.

It is a general rule for polymeric materials that the rate of stress relaxation is a function of the stress (the higher the stress, the greater the tendency to relax). Walls from growing tissues likewise exhibit this phenomenon (Figure 2) (Yamamoto et al 1970, Cosgrove et al 1984, Cosgrove 1985, 1987). Consistent with this stress relaxation behavior, the rate of wall enlargement also depends on the force applied to the walls, either from endogenous turgor or from externally applied forces (reviewed in Cosgrove 1993b). Growing walls usually exhibit a threshold or minimum wall stress needed for extension, and this threshold may change according to the growth state of the cell (Cosgrove 1987, Okamoto et al 1990, Nakahori et al 1991).

The term extensibility generally refers to the ability of the wall to expand or extend irreversibly during growth. Unfortunately, some confusion in the exact meaning of this term has arisen because of multiple technical definitions and



*Figure 2* Time course for in vivo stress relaxation of growing plant cell walls. If growing cells are removed from an external water supply, wall relaxation continues and reduces cell turgor pressure until the yield threshold is attained. The exponential decay in turgor is consistent with the general principle that relaxation rate is a function of stress. These results are adapted from Cosgrove (1985, 1987).

methods for measuring wall extensibility, each measuring a somewhat different property of the wall. This topic has been reviewed in detail elsewhere (Cosgrove 1993b), and here I will only briefly summarize. Some mechanical techniques, such as the Instron tensile tester, measure the stress/strain properties of the wall and are useful indicators of major structural changes in the wall. However, because wall stress relaxation depends not only on wall structure, but also on the rate of wall loosening processes, mechanical assays of the wall do not always give a faithful estimate of the wall extensibility that is most pertinent to growth, at least in part because ephemeral conditions in the wall space such as pH do not survive wall preparation. Other techniques, such as in vivo stress relaxation methods (Cosgrove 1987), give much more direct measures of wall-yielding properties related to growth, and the results can be directly related to the theory of growth biophysics. Methods in which a growing tissue is temporarily stretched with an applied force or in which turgor pressure is transiently altered have also proven to be useful indicators of wall extensibility (Okamoto et al 1989, Nakahori et al 1991, Chazen & Neumann 1994, Cramer & Schmidt 1995).

### *Molecular Mechanisms of Wall Expansion*

Two major themes concerning the molecular basis of wall expansion may be found in the relevant literature (Taiz 1984). One concept is that wall yielding



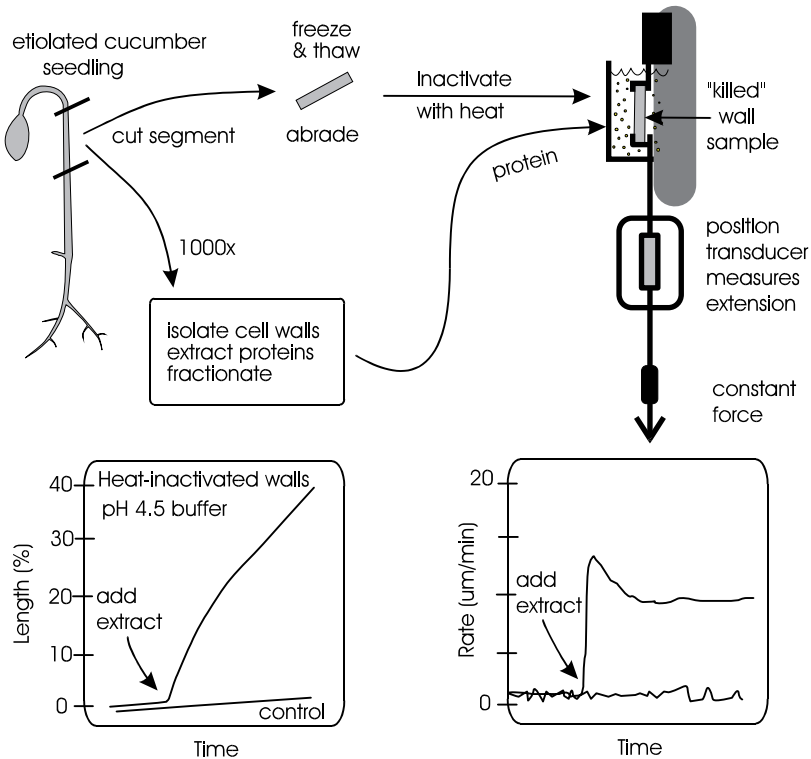
results from a biochemical loosening of the wall to permit turgor-driven extension of the wall polymer network. This idea draws support from a wide range of studies, but it neglects the ultimate need for integration of new polymers into the expanding wall.

A second idea considers wall expansion to be the direct or indirect result of wall polymer synthesis and secretion. This view accounts for the coupling of wall synthesis and wall expansion but fails to explain how the secretion of polysaccharides might lead to the wall stress relaxation essential for water uptake and expansion of the turgid cell. How might this come about? Effectively, the newly secreted polymer would need to insert itself into the load-bearing network, causing a (transient) relaxation in wall stress and thereby a reduction in cell turgor. The cell would then take up water, expanding the wall and restoring wall stress, in the process of which the new polymer would begin to bear some of the wall stress. There is little experimental evidence, however, that wall stress relaxation can be induced directly by secretion of wall structural polymers. In contrast, the concept of wall loosening has considerable experimental support and is considered below.

### *Expansins and Acid Growth*

Recent work has shown that the acid growth response of plant cell walls is mediated by an unusual class of proteins we have named expansins (Cosgrove 1996). Acid growth refers to the rapid cell enlargement induced by acidic solutions (pH <5.5) or by fusicoccin (Cleland 1976, Rayle & Cleland 1992, Kutschera 1994). Isolated walls possess this acid-growth property, which is lost upon protein denaturation (Rayle & Cleland 1972, Tepfer & Cleland 1979, Cosgrove 1989). Such results indicate that acid growth is an inherent property of the cell wall; it is not simply due to the physical chemistry of the wall (e.g. a weakening of the pectin gel) but is catalyzed by one or more wall proteins. This idea was directly verified by reconstitution experiments (McQueen-Mason et al 1992) in which heat-inactivated walls were restored to nearly full acid-growth responsiveness by addition of proteins extracted from growing walls (Figure 3). Fractionation of the active components from cucumber hypocotyls led to the identification of two related wall proteins of about 29 kDa, as estimated by SDS-PAGE. Subsequent work indicates 25–27 kDa as better size estimates for expansins (Keller & Cosgrove 1995, Cho & Kende 1997a,b; D Cosgrove & D Durachko, unpublished data).

Expansins exert remarkable effects on the pH-dependent rheology of isolated walls. Not only is acid-induced extension restored to walls by addition of catalytic amounts of expansins ( $\approx 1$  part protein per 5000 parts wall, by dry weight; McQueen-Mason et al 1992), but wall relaxation is also increased. Native walls from growing cucumber hypocotyls relax faster when clamped at acidic pH



*Figure 3* Schematic diagram of in vitro reconstitution of acid-induced extension of growing cell walls. (*Top*) The growing region of the cucumber hypocotyl is prepared so as to kill the cells, but leave wall enzymes intact, and is clamped in constant tension in an extensometer. Proteins may be extracted from walls and added to the cuvette surrounding the clamped wall. (*Bottom*) When protein fractions containing expansins are added, the wall samples quickly begin expanding (*left*, increase in length; *right*, change in extension rate).

compared with neutral pH (Cosgrove 1989). This pH effect on stress relaxation is eliminated by a brief heat treatment that inactivates expansins, and it may be largely restored by addition of purified expansins to the heat-treated walls (McQueen-Mason & Cosgrove 1995).

The molecular basis for expansin action on wall rheology is still uncertain, but the weight of published evidence indicates that expansins cause wall creep by loosening noncovalent associations between wall polysaccharides. Polysaccharide hydrolase or transglycosylase activity has not been detected in purified expansin preparations (McQueen-Mason et al 1992, 1993, McQueen-Mason & Cosgrove 1995). Expansins weakened pure cellulose paper—a hydrogen-

bonded network of cellulose fibrils—without detectable hydrolysis of cellulose and caused a significant increase in the rate of stress relaxation in the paper, an effect that cellulase did not mimic (McQueen-Mason & Cosgrove 1994). High concentrations of chaotropes such as urea also enhanced stress relaxation and were found to act synergistically with expansins to enhance creep of plant cell walls.

Binding studies suggest that expansins act at the interface between cellulose and one or more hemicelluloses (McQueen-Mason & Cosgrove 1995). Given the conventional model of the plant cell wall described above, the binding between cellulose and xyloglucan would seem a logical target for expansin action. However, the results indicate that some other hemicellulose-cellulose complex is the site for expansin binding and presumed action. The identity of the hemicellulose remains to be established.

The work described above was based largely on expansins from growing cucumber hypocotyls. Expansin proteins have also been identified in other growing tissues such as tomato leaves (Keller & Cosgrove 1995) and oat coleoptiles (Li et al 1993, Cosgrove & Li 1993). Recently, Cho & Kende (1997a,b) identified expansin proteins in deepwater rice internodes and showed that growth induction by submergence was correlated with expansin-mediated changes in wall extension properties.

### *The Growing Expansin Gene Family*

At the nucleotide level, expansins cDNAs were first identified in cucumber, and then homologues were found in the rice and *Arabidopsis* EST (Expressed Sequence Tag) collections (Shcherban et al 1995). An expansin cDNA from pea petals has also been characterized (Michael 1996), as has an expansin cDNA in ripening tomato fruit (Rose et al 1997) and two auxin-induced expansin cDNAs from the hypocotyls of pine seedlings (K Hutchison, personal communication). The proteins predicted from these cDNAs are highly conserved, with sequence similarities in the range of 70 to 90% (excluding the N-terminal signal peptide, which is cleaved to form the mature protein). This conservation is much greater than that found, for example, in the multigene families for cellulases, pectin methyl esterases, and HRGPs. Considering that gymnosperms and angiosperms diverged  $\approx 400$  million years ago, it is remarkable that hypocotyl expansins from cucumber and pine share  $>90\%$  amino acid sequence similarity. This implies rigid constraints on the structure of the protein and its interaction with wall components. Because acid growth has been observed in angiosperms, gymnosperms, ferns, mosses, and even in some green algae with cellulosic walls, it is likely that expansins will be found in all of these groups. This mechanism of wall expansion may be deeply embedded in the evolution of land plants.

In *Arabidopsis*, 12 distinct expansin cDNAs or genes have been identified so far (D Cosgrove, unpublished results), and more are likely to be found. This naturally raises questions about the function of each of these genes. Because the number of distinct expansin cDNAs increases with the complexity of the cDNA library (Cosgrove 1996), different expansin genes are probably expressed in different organs and cell types. As a test for this idea, we cloned two expansins expressed in the cucumber root and found them to be distinct from the cucumber hypocotyl expansins (B Link & DJ Cosgrove, manuscript in preparation). Likewise, different expansin genes are expressed in the roots and shoots of rice seedlings (H Kende, personal communication). Moreover, an expansin gene that is highly expressed in ripening tomato fruit shows negligible expression in other organs (Rose et al 1997). These observations indicate that the multiplicity of expansins has to do with regulated gene expression in different organs and cell types. The duplication and evolution of the expansin gene family may have more to do with evolution of the promoter for more complex control of gene expression than with the evolution of the protein itself.

On the other hand, the two expansins from the cucumber hypocotyl were found to vary in subtle biochemical ways, such as pH dependence, stress relaxation effects, and resistance to methanol boiling (McQueen-Mason & Cosgrove 1995). It is possible that expansins also vary in substrate specificity and act preferentially on different components of the wall.

The presence of expansins in snail digestive juices (Cosgrove & Durachko 1994) indicates that expansins may, in some situations, assist the degradation of the plant cell wall. High abundance of an expansin mRNA in ripening fruit likewise suggests that expansins may also assist wall disassembly (Rose et al 1997). If these inferences prove correct, then some expansins may also function in developmental processes where cell separation or dissolution are important, e.g. abscission, intercellular air space formation, and seed pod dehiscence (Cosgrove 1997).

**A SECOND FAMILY OF EXPANSINS** Shcherban et al (1995) noted a remote sequence similarity between expansins and a group of proteins previously identified as the major allergens in grass pollen, the so-called group I allergens (Knox & Suphioglu 1996). Follow-up work showed that the group I allergen from maize pollen indeed has potent expansin-like activity on maize silk walls (Cosgrove et al 1997). Because the group I allergens are highly soluble and copiously secreted by grass pollen as they germinate, it is likely that these proteins aid pollen tube invasion of the stigma and style by softening the walls of these maternal tissues. Furthermore, the presence of vegetative homologues of the group I allergens in rice, *Arabidopsis*, and soybean implicates these proteins in cell wall loosening processes outside the stigma and style. Cosgrove et al (1997)

proposed that the group I allergens and their vegetative homologues comprise a second family of expansins ( $\beta$  expansins) that act on a different set of wall components than does the original family of  $\alpha$  expansins.

### *(1 $\rightarrow$ 4) $\beta$ -Glucanases*

There is a large body of literature, dating back at least three decades, that implicates (1  $\rightarrow$  4)  $\beta$ -glucanases in cell wall loosening processes, especially during auxin-induced cell elongation (Taiz 1984, Fry 1989a, Hoson 1993). Matrix glucans show enhanced turnover in excised segments upon growth stimulation by auxin, as described above. Interference with this hydrolytic activity by use of antibodies or lectins reduces growth in excised segments (Huber & Nevins 1981, Hoson & Nevins 1989, Inouhe & Nevins 1991, Hoson et al 1991, 1992, Hoson & Masuda 1995). Expression of (1  $\rightarrow$  4)  $\beta$ -glucanases has been associated with growing tissues (Verma et al 1975, Labrador & Nicolas 1984, Hayashi et al 1984, Brummell et al 1997), and exogenous glucanases have sometimes been found to give a modest growth stimulation (Labrador & Nevins 1989). Such results support the concept that wall stress relaxation and expansion are the direct result of xyloglucan hydrolysis in dicotyledons or hydrolysis of (1  $\rightarrow$  3) (1  $\rightarrow$  4)  $\beta$ -glucans in grass walls.

On the other hand, the most direct evidence for this idea is lacking. When various wall hydrolases were applied to isolated heat-inactivated walls clamped in an extensometer, the walls either exhibited little extension or broke (Cosgrove & Durachko 1994). Although these hydrolases could not cause wall creep by themselves, brief pretreatment of walls with (1  $\rightarrow$  4)  $\beta$ -glucanases or with pectinases substantially enhanced the subsequent extension response to expansins. It is plausible, then, that endogenous wall hydrolases or transglycosylases might enhance wall expansion by making the wall more sensitive to expansin-induced creep. Related to this, pretreatment of pea cell walls with xyloglucan oligosaccharides increased the responsiveness of the wall to subsequent acid-induced extension (Cutillas-Iturralde & Lorences 1997), possibly by stimulating endogenous (1  $\rightarrow$  4)  $\beta$ -glucanase activity in the wall (Farkas & Maclachlan 1988, McDougall & Fry 1990). These results suggest that wall hydrolytic enzymes such as (1  $\rightarrow$  4)  $\beta$ -glucanase may not be the prime movers in the wall expansion process, as formerly envisioned, but may act indirectly by modulating expansin-mediated polymer creep.

## CESSATION OF WALL EXPANSION

Growth cessation during cell maturation is generally irreversible and is typically accompanied by a reduction in wall extensibility, as measured by various biophysical methods (Kutschera 1996). These physical changes in the wall might

come about by (a) a reduction in wall loosening processes, (b) an increase in wall cross-linking, or (c) an alteration in the composition of the wall, making for a more rigid structure or one less susceptible to wall loosening. There is some evidence for each of these ideas. Growth cessation during maturation of bean leaves is coincident with loss in capacity for acid-induced extension (Van Volkenburgh et al 1985). In cucumber hypocotyls, nongrowing walls likewise lose their ability for acid-induced extension (Cosgrove 1989), and this cannot be restored by addition of exogenous expansin (McQueen-Mason et al 1992, McQueen-Mason 1995). This shows that the mature wall does not simply lack expansins, but it is also cross-linked or otherwise made unresponsive to expansins. On the other hand, expansin mRNA abundance correlated precisely with *in vivo* patterns for cell elongation and acid-induced extension along the cucumber hypocotyl (M Shieh, J Shi & DJ Cosgrove, manuscript in preparation). Thus growth cessation in this organ involves both loss of expansin expression and an increased rigidification of the wall, making it unresponsive to expansin. In oat coleoptiles, growth cessation was likewise associated with both wall rigidification (measured as loss in susceptibility to expansin action) and loss of expansin activity in the wall (Cosgrove & Li 1993).

Several modifications of the maturing wall may contribute to wall rigidification. Newly secreted matrix polysaccharides may be altered in structure, so as to form tighter complexes with cellulose or other wall polymers, or they may be resistant to wall-loosening activities. An example is the arabinoxylan of maize coleoptiles, which becomes less branched as the coleoptile matures and may form tighter complexes with cellulose (Carpita 1984). Removal of mixed-link  $\beta$ -D-glucans is also coincident with growth cessation in these walls. De-esterification of pectins, leading to more rigid pectin gels, is similarly associated with growth cessation in both grasses and dicotyledons (Yamaoka et al 1983, Yamaoka & Chiba 1983, Goldberg 1984, Kim & Carpita 1992, McCann et al 1994). Cross-linking of phenolic groups in the wall, e.g. HRGPs, ferulate residues attached to pectins, and lignin, generally coincides with wall maturation (Tan et al 1991) and is believed to be mediated by peroxidase, a putative wall rigidification enzyme (Goldberg et al 1987, Macadam et al 1992, Shinkle et al 1992, Schopfer 1996). It is evident that many structural changes occur in the wall during and after cessation of growth, and it has not yet been possible to dissect out the significance of individual processes for cessation of wall expansion. Mutants with genetic lesions in specific wall maturation processes may be useful to address this question.

Another potential approach is the use of isolated walls to examine the influence of specific chemical modifications on wall creep properties. Schopfer (1996) treated maize coleoptile walls with hydrogen peroxide and noted a consequent mechanical stiffening. When incubated at neutral pH, isolated cucumber

walls lost their ability for subsequent acid-induced extension, with a half time of about 40 min (Cosgrove 1989). This stiffening, however, was not the result of peroxidative cross-linking, because treatment with 10  $\mu$ M cyanide inhibited peroxidase activity but did not reduce the loss of creep activity. Extractable expansin activity was not reduced during this stiffening, which seems instead to be the result of an enzymatic change in the wall—possibly de-esterification of pectin—making the wall less sensitive to expansin action (S McQueen-Mason & DJ Cosgrove, manuscript in preparation). By use of such wall assays it may be possible to test other hypotheses of wall rigidification.

The pollen tube presents an intriguing example of a cell wall that makes the transition from a highly extensible state to a nonextensible state in a very brief time (Heslop-Harrison 1987, Derksen et al 1995). Surface expansion is maximal at the top of the apical dome, which is the site of polysaccharide deposition; as the wall is displaced to the flanks of the apical dome, the wall gradually ceases to expand. The side wall does not elongate, although deposition of cellulose, (1  $\rightarrow$  3)  $\beta$ -glucan, and other polymers causes wall thickening and probably stabilizes the wall against further enlargement. Because fast-growing lily pollen tubes elongate approximately 1 tube diameter per min (Pierson et al 1996), the transition from extensible to nonextensible wall must also occur quickly—on the order of one minute. Cytological observations indicate that this transition is correlated with de-esterification of pectins. Pectins are secreted at the tip in a highly esterified form, and as they are displaced down the flanks of the apical dome, they become de-esterified (Li et al 1994, Jauh & Lord 1996). Because pollen tubes express pectin methyl esterases (Mu et al 1994), it is likely that these enzymes are secreted with pectins at the tip and that they progressively promote pectin gelation. This process ought to be delicately controlled, since the tip-growth morphology is regulated by the tip-to-flank gradient in the rate of wall surface expansion (Green 1969). Control is not perfect, however, as spontaneous oscillations in pollen tube growth rate do occur, and they leave behind waves of pectin in varying esterification states (Li et al 1996). Because pectin methyl esterase activity is sensitive to pH (Bordenave & Goldberg 1993, Bordenave et al 1996), fluctuations in  $H^+$  currents at the tip may underlie these fluctuations in growth and pectin esterification. Growth rate oscillations are also intimately connected with the tip-focused cytoplasmic calcium gradient, which likely regulates exocytotic secretion of wall polyaccharides and enzymes (Li et al 1996). Although pollen tube elongation is inhibited by secretion inhibitors, the spontaneous fluctuations in growth rate do not seem to be the consequence of fluctuations in delivery of Golgi vesicles to the wall (Geitmann et al 1996). Rather, the rate of wall expansion appears to fluctuate independently of Golgi secretion, leading to walls of varying thickness (Li et al 1996). Wall secretion and expansion thus seem to be distinct, but coupled, processes in the pollen tube.

## CONCLUDING REMARKS

The plant cell wall is a dynamic structure whose physical and chemical properties are finely tuned to its many functions. One of these functions is enlargement, and it appears that the biochemical machinery underlying wall assembly and expansion may be analyzed with some success by a combination of wall fractionation, reconstitution, and rheological analysis. Such studies are still in their infancy, and we can look forward to further insights into this subject as this approach is extended. Genetics has as yet contributed little to our understanding of the plant wall, but this will soon change as more wall-related genes are identified in the plant genome projects and as more efficient schemes for mutant identification become commonplace. The ability to re-engineer plant cell walls may not only uncover further mechanisms of wall assembly and enlargement, but may yield plant walls with more useful properties for commercial uses.

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