

Relaxation in a High-Stress Environment: The Molecular Bases of Extensible Cell Walls and Cell Enlargement

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INTRODUCTION

Before they mature, plant cells usually enlarge 10- to 1000-fold in volume by a process that entails massive vacuolation and irreversible cell wall expansion. This increase in volume is accomplished mostly by uptake of water and requires relatively little increase in the amount of cytoplasm. In terms of energy and material investment, this is an economical way to grow and enables some plants, such as conifers, to attain sizes unmatched by any other organisms on Earth.

It is clear from histological analyses that plant cell enlargement is regulated in a highly cell-specific manner. For example, meristematic cells can give rise to wide xylem vessels that form immediately adjacent to much smaller parenchyma cells. Cell expansion is also coordinately regulated at the whole-organ level by external stimuli such as light, temperature, gravity, and water availability as well as by internal factors such as gibberellins, auxins, and other growth hormones.

The control of cell expansion is essential for all morphogenetic processes in plants because the morphology of plant organs is determined by the size, shape, and number of the individual cells. Cell division patterns that are initiated in the embryo and in meristems are subsequently amplified and modified by cell enlargement, consequently forming organs of substantial size and with distinct shapes.

There are many interrelated aspects of plant cell expansion and its control (Figure 1). These include wall mechanics, cell hydraulics, numerous cellular and biochemical processes that directly or indirectly influence these properties of the cell, and, of course, regulated expression of the genes that encode the proteins taking part in all of these processes.

In physical terms, cell shape and size are governed by the mechanics of the cell wall, which is a thin, fibrous layer strong enough to withstand the high physical stresses generated by cell turgor pressure (P). Given that P of growing cells is typically in the range of 0.3 to 1 MPa and that wall stress is approximately $P \times$ cell radius/wall thickness, one may calculate that wall stresses are on the order of 10 to 100 MPa. These pressures are much higher than can be sustained by unsupported membranes or by the cytoskeleton.

As an inevitable consequence of this physical situation, the critical physical event required for cell enlargement is

wall stress relaxation (Cosgrove, 1993a). This is the means by which growing cells simultaneously loosen their walls and reduce their turgor and water potential, thereby enabling them to take up water and to expand physically. Although, in principle, water uptake could be partially limiting for cell enlargement, most experimental studies have concluded that water transport is too rapid to be a major growth limitation (Cosgrove, 1993b). When hormones or other agents modulate the rate of cell expansion, they usually do so by influencing cell wall properties. Indeed, the essential difference between growing and nongrowing cells resides in the cell wall's ability to undergo stress relaxation and polymer creep. This review focuses on wall relaxation and its molecular basis as the key controls of cell enlargement.

SPECIAL RHEOLOGICAL PROPERTIES OF GROWING CELL WALLS: STRESS RELAXATION AND POLYMER CREEP

The walls of growing cells have unique rheological properties that underlie their ability to expand. These properties may be measured in various ways (Cosgrove, 1993a), but the clearest distinctions between growing and nongrowing walls may be seen by *in vivo* stress relaxation of living tissues and by long-term creep of isolated walls.

The principle behind *in vivo* stress relaxation is simple: if growing cells are physically prevented from taking up water, then wall relaxation progressively reduces cell turgor (the Newtonian counterforce to wall stress), and this may be detected, for example, by turgor measurements with a pressure probe or by water potential measurements with a psychrometer or a pressure chamber (Cosgrove, 1987).

The stress relaxation time course derived from these experiments demonstrates two common properties of growing cells: wall stress relaxation exhibits a yield threshold; and above the yield threshold, the rate of relaxation depends on wall stress (or turgor pressure). These characteristics are directly related to the often-observed turgor dependence of cell enlargement, which also exhibits a yield threshold or minimum turgor required for growth, and a dependence of growth rate upon the turgor in excess of the yield threshold.

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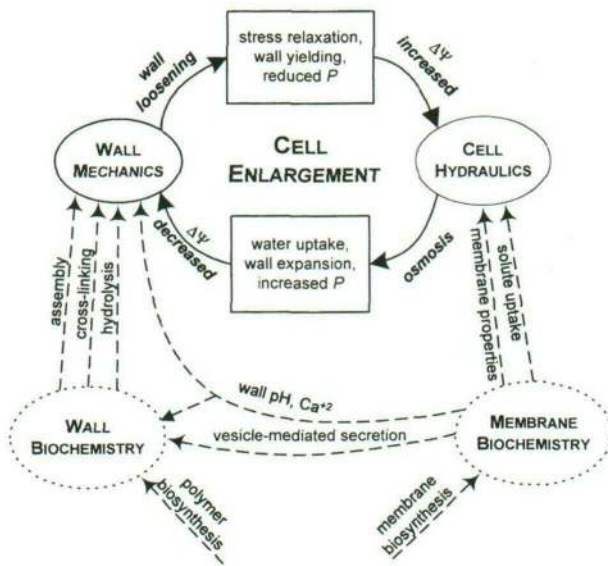


Figure 1. Schematic Diagram of Processes Involved in Cell Enlargement.

The biophysical processes (e.g., water uptake and cell wall expansion) that directly lead to cell enlargement are indicated with solid lines. The biochemical processes that support these biophysical processes are indicated with broken lines. $\Delta\Psi$, the water potential difference driving cellular water uptake.

The slope of this growth–turgor relation is usually called wall extensibility (ϕ). Experimental studies indicate that wall extensibility and yield threshold may be modulated by hormones and growing conditions (see, e.g., Nakahori et al., 1991; Cramer, 1992; Frensch and Hsiao, 1995). As expected, nongrowing cells exhibit negligible *in vivo* wall relaxation.

Although it is possible to measure wall relaxation in isolated wall specimens (Yamamoto et al., 1970), the distinction between growing and nongrowing walls is most readily seen in extensometer assays of pH-dependent wall creep. Creep refers to a time-dependent irreversible extension, typically due to slippage of the wall polymers relative to one another. When growing walls are clamped and put under constant tension at neutral pH, the rate of wall extension falls quickly. When put in an acidic buffer ($\text{pH} \leq 5$), the wall extends rapidly, in some instances continuing for many hours (Cosgrove, 1989). This acid-induced creep, or acid growth, is characteristic of walls from growing cells but is not typical of nongrowing walls. The change in pH appears to convert the growing wall from a viscoelastic solid to a viscoelastic liquid, implying the weakening of one or more key bonds linking wall polymers together.

Just as is observed for the growth and relaxation of living cells, creep of isolated walls also requires a minimum stress, and the creep rate depends on the amount by which stress exceeds this threshold (Okamoto and Okamoto, 1995). In living cells, acid growth is also evident when growing cells

are treated with acid buffers or with fusicoccin, which induces wall acidification by activating an H^+ -ATPase in the plasma membrane. There has been some debate about whether the acidification caused by auxin is sufficient to account for growth induction by this hormone (Rayle and Cleland, 1992; Luethen and Boettger, 1993; Schopfer, 1993; Kutschera, 1994). However, we should not let this controversy obscure the more revealing fact that walls of growing cells possess a pH-dependent mechanism of wall extension. As described below, this extension mechanism is mediated by a protein family that is highly conserved in the evolution of flowering plants.

CELL WALL STRUCTURE AND POTENTIAL “LOOSENING” MECHANISMS

The wall of an enlarging plant cell is a heterogeneous polymeric structure in which cellulose microfibrils are embedded in a complex, less ordered polysaccharide matrix, with smaller quantities of structural protein intercalated in the matrix (Figure 2). In dicotyledons, the growing wall contains $\sim 30\%$ cellulose, 30% hemicellulose, and 35% pectin, with

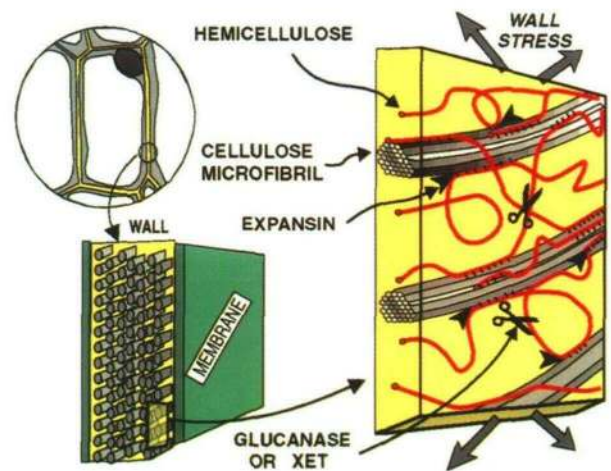


Figure 2. Primary Wall Architecture and Potential Mechanisms for Stress Relaxation and Creep of Cell Walls.

At right is a simplified sketch of the growing wall, represented as crystalline microfibrils of cellulose coated with hemicelluloses (red lines). For clarity, only a few of the hemicelluloses are shown. These long, flexible polymers are thought to act as tethers that link the cellulose microfibrils into a network. Expansins (black arrowheads) may act to weaken the noncovalent bonds between the cellulose and hemicellulose (small black rods), with the result that the polymers move (creep) in response to the wall stress, which is generated by cell turgor. Glucanases and xyloglucan endotransglycosylase (XET; black scissors) can modify the backbone of the hemicelluloses. Pectins, which make up approximately half of the matrix and form a second, interlaced network around the microfibrils, are not shown.

perhaps 1 to 5% structural protein, on a dry-weight basis. This polymeric network is stuck together by various non-covalent adhesions and entanglements. There is some divergence of thought regarding the extent of covalent cross-linking between matrix polysaccharides and structural proteins. Earlier views that the hemicelluloses, pectins, and structural proteins were covalently linked to each other (Keegstra et al., 1973) have shifted in favor of models in which the majority of these polymers are separable although physically intertwined (McCann et al., 1990; Talbott and Ray, 1992a; Carpita and Gibeaut, 1993). Cross-linking between these polymers may become more common as the wall ceases to expand.

Although often overlooked, water is an important wall component that significantly affects wall rheology. Because the matrix is ~75% water by mass, it resembles a very dense aqueous gel. Localized phase changes or "melting" of the matrix may be an important aspect of wall rheology in growing cells (Lin et al., 1991). Indeed, Edelmann (1995) found that the extension properties of isolated cell walls were sensitive to wall dehydration. Dehydration of the wall matrix may also play a direct role in the growth inhibition by water deficits (Chazen and Neumann, 1994; Passioura, 1994). Moreover, as a solvent and a lubricant, water reduces physical interactions between wall polymers, thus facilitating cell wall expansion.

Major Structural Polymers of the Cell Wall

Cellulose consists of long, unbranched 1→4-linked β-D-glucans tightly packed into crystalline microfibrils of indeterminate length and of varying width and degree of order, depending on species. Electron micrographs show that microfibrils are synthesized and/or assembled by protein arrays (terminal complexes) that are embedded in the plasma membrane (Brown et al., 1996). These complexes are associated with sucrose synthase, which may act as a metabolic channel to transfer glucose from sucrose via uridine 5'-diphosphoglucose to the growing glucan chain (Amor et al., 1995). Homologs of bacterial cellulose synthase genes were recently identified in higher plants (Pear et al., 1996), and the proteins encoded by these genes are probably contained in the terminal complexes.

The cellulose microfibril has a substructure consisting of highly organized crystalline domains linked together by less crystalline "amorphous" regions. Unlike the matrix polysaccharides, the cellulose microfibril is remarkably stable and usually undergoes negligible turnover or breakdown during cell wall growth. Given its crystalline structure, cellulose obviously plays a major role in determining the strength and structural bias of the cell wall. Nevertheless, there is evidence (see below) that hemicelluloses may influence microfibril structure.

Hemicelluloses are a heterogeneous group of mixed-linked polysaccharides that are synthesized in the Golgi ap-

paratus and secreted in vesicles to the wall, where they are believed to coat the surface of cellulose microfibrils. They may also form tethers that bind microfibrils together and perhaps act as a lubricant to prevent direct microfibril-microfibril contact (Carpita and Gibeaut, 1993). In the primary wall of dicots, the most abundant hemicellulose is xyloglucan, a branched polymer consisting of a 1→4-linked β-D-glucan backbone with short side chains containing xylose, galactose, and often, although not always, a terminal fucose (McNeil et al., 1984; Fry, 1989a). Although less abundant, other hemicelluloses are found tightly bound to cellulose, and they may also help anchor the microfibril to the matrix (reviewed in Cosgrove, 1997).

Binding studies (Hayashi, 1989) suggest that xyloglucan does not simply bind to the microfibril surface, but much of it may also be entrapped within the microfibril during its formation and can be released only by extractions that cause microfibril swelling (Chambat et al., 1984; Hayashi and MacLachlan, 1984). Cellulose microfibrils made by the bacterium *Acetobacter xylinum* are altered when they are synthesized in the presence of hemicelluloses; they become fragmented and more closely resemble microfibrils synthesized by land plants (Haigler et al., 1982; Hayashi et al., 1987). Thus, hemicellulose entrapment in the plant cell wall may alter microfibril morphology and help to anchor the microfibril to the matrix.

Like hemicelluloses, pectins are a heterogeneous group of polysaccharides that are secreted by Golgi bodies and make up a major fraction of the wall matrix. Pectins are acidic polysaccharides that form an independent gel phase in which the cellulose-hemicellulose network is embedded. They may also act as a hydrophilic filler that prevents the aggregation and collapse of the cellulose network (Jarvis, 1992) and modulates the porosity of the cell wall to macromolecules (Baron-Epel et al., 1988).

Pectins are subject to a number of modifications that alter their conformation and linkage in the wall. Many of the acidic residues are esterified with methyl, acetyl, and other unidentified groups during biosynthesis in the Golgi apparatus, and these groups may be removed subsequently by esterases in the wall (Kim and Carpita, 1992; McCann et al., 1994). By creating free carboxyl groups, deesterification increases the charge density in the wall, which in turn may influence the activities of wall enzymes (Moustakas et al., 1991). Deesterification also has a large effect on the physical properties of pectin. Calcium is readily bound by the free carboxyl groups and links pectin chains together into expanded, highly hydrated gel networks (reviewed in Lapasin and Pricl, 1995).

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 and Fry, 1994). Recently, borate diesters of rhamnogalacturonan II were identified (Kobayashi et al., 1996; O'Neill et al., 1996), and wall elasticity was reported to be sensitive to borate status, indicating that such borate esters may affect cell wall mechanics (Findekle and Goldbach, 1996).

Potential Wall Loosening Mechanisms

Two major concepts have been advanced to account for the molecular basis of wall expansion (reviewed in Taiz, 1984; Cosgrove, 1997). One hypothesis is that wall expansion results from the synthesis and secretion of wall polymers. This view accounts for the coupling of wall synthesis to wall expansion, but it fails to explain how the secretion of polysaccharides may lead to the wall stress relaxation that is essential for water uptake and expansion of the turgid cell. A second 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 long-term need for integration of new polymers into the expanding wall. Ultimately, the rheological properties of the growing wall must be explained in terms of its structure and biochemistry.

Given the model of the wall described above, several potential mechanisms of stress relaxation and expansion are evident (Figure 2). The hemicelluloses that anchor microfibrils to the matrix and perhaps to each other may be cut by an enzyme that transfers the newly formed reducing end to water (i.e., a hydrolysis) or to the nonreducing end of another hemicellulose (i.e., a transglycosylation). Alternatively, wall loosening agents may weaken the noncovalent associations of matrix polymers to microfibrils or to other polymers in the matrix. Evidence for each of these possibilities is reviewed in the following sections.

EXPANSINS AND ACID GROWTH

Biochemical dissection of the acid-growth mechanism led to the discovery of a novel family of proteins (named expansins) that mediate this rheological behavior of growing walls (reviewed in Cosgrove, 1996). The observation that proteases and various protein-denaturing treatments eliminated the acid-extension response (Tepfer and Cleland, 1979; Cosgrove, 1989) suggested that wall proteins act as catalysts of this extension mechanism. This idea was directly verified by reconstitution experiments in which a crude protein extract from growing walls was able to restore acid-induced creep to denatured walls; fractionation of the active components led to the identification of two related wall proteins of ~29 kD, as estimated by SDS-PAGE (McQueen-Mason et al., 1992). Subsequent work indicates that 25 to 27 kD is a better size estimate for expansins (Keller and Cosgrove, 1995; Shcherban et al., 1995; Cho and Kende, 1997a).

It is remarkable and perhaps surprising that the acid-extension behavior of isolated plant walls depends only on the action of expansins. Each of the cucumber expansins by itself was sufficient to induce wall extension. The extension of cucumber hypocotyl walls does not appear to require

other proteins, although other proteins may modulate expansin action (see below).

Expansin activity was associated with the growing region of the cucumber hypocotyl and was lacking in the basal (nongrowing) part of the hypocotyl (McQueen-Mason et al., 1992). Similar results were obtained with the coleoptile of oat seedlings (Cosgrove and Li, 1993) and the internode of deepwater rice (Cho and Kende, 1997b). These results support the idea that expansins facilitate wall expansion in growing cells *in vivo* as well as wall expansion *in vitro*.

Addition of catalytic amounts of expansins (~1 part protein per 5000 parts wall, by dry weight) not only restores acid-induced creep to cell walls but also enhances wall relaxation (McQueen-Mason and Cosgrove, 1995). This amount of expansin is comparable to the amount found endogenously in growing cucumber hypocotyl walls. Native walls from growing cucumber hypocotyls relax faster when clamped at acidic pH 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 restored largely by addition of purified expansins to the heat-treated walls. Thus, the pH-dependent rheology of isolated walls appears to be controlled principally by expansins.

However, not all walls in the plant are susceptible to the action of expansins. Walls from the nongrowing part of the plant do not creep when treated with expansins, and the living cells likewise lack an acid-growth response (McQueen-Mason et al., 1992; Cosgrove and Li, 1993). Evidently, as the cells mature, their walls lose susceptibility to expansin action. The basis for this change is considered later.

The molecular basis for expansin action on wall rheology is still uncertain, but most published evidence indicates that expansins cause wall creep by loosening noncovalent associations between wall polysaccharides. Expansins weakened pure cellulose paper (a hydrogen-bonded network of cellulose fibrils) without detectable hydrolysis of cellulose (McQueen-Mason and Cosgrove, 1994) and caused a significant increase in the rate of stress relaxation in the paper—an effect that cellulase did not mimic. Polysaccharide hydrolyase or transglycosylase activities have not been detected in purified expansin preparations (McQueen-Mason et al., 1992, 1993; McQueen-Mason and Cosgrove, 1995). By contrast to these experimental results, sequence analysis shows that the most highly conserved regions of the expansin protein have significant although limited similarity to the catalytic site of a small group of fungal glycosyl hydrolases (family-45 glycanases; see below). However, these fungal glycanases do not catalyze expansin-like wall creep (D.J. Cosgrove, unpublished results), so the action of expansin remains a puzzle.

Binding studies suggest that expansins act at the interface between cellulose and one or more hemicelluloses (McQueen-Mason and Cosgrove, 1995). Given the conventional model of the plant cell wall described above, cellulose-xyloglucan bonds would seem to be a logical target for expansin action. However, the results indicate that some

other cellulose-hemicellulose complex is probably the site for expansin binding and presumed action. The identity of this hemicellulose remains uncertain.

At present, there remain two plausible explanations for the profound action of expansin on the rheology of the growing wall: it may act by a novel biochemical mechanism to disrupt polysaccharide associations within the wall, or it may possess an unusual substrate specificity for a cryptic glycosyl transferase activity. These mechanisms are not mutually exclusive.

The Expansin Gene Family

Expansin cDNAs have been cloned from cucumber, rice, and Arabidopsis seedlings (Shcherban et al., 1995), pea petals (Michael, 1996), ripening tomato fruit (Rose et al., 1997), and pine hypocotyls (K. Hutchison, personal communication). The predicted proteins are highly conserved, with sequence similarities in the range of 70 to 90%. The proteins encoded by these cDNAs have an N-terminal signal peptide, which is cleaved to form the mature protein of ~25 to 26 kD. Motif searches using the PROSITE and BLOCKS programs have not identified functional domains that might account for expansin's action on the cell wall. Major structural features of the conserved expansin protein are illustrated in Figure 3.

In Arabidopsis, 11 distinct expansin cDNAs and one unique genomic clone have been identified to date (D.J.

Cosgrove, unpublished results), and a number of observations suggest that different expansin genes are expressed in different organs and cell types. For example, two expansins expressed in the cucumber root are distinct from those expressed in the hypocotyl (B. Link and D.J. Cosgrove, unpublished results). In deepwater rice, expansin genes are differentially expressed in the roots and shoots (H.-T. Cho and H. Kende, personal communication), and an expansin cDNA expressed in ripening tomato fruit shows negligible expression in other organs (Rose et al., 1997). In Arabidopsis, the nucleotide sequences coding for the expansin proteins are much more conserved than are the flanking untranslated regions and the promoter regions (D.J. Cosgrove, unpublished results). Thus, the size of the expansin gene family may have more to do with duplication and evolution of the promoter for more complex control of gene expression than with evolution of the protein itself.

However, it is also possible that expansins vary in substrate specificity and act preferentially on different components of the wall. In support of this idea, the two expansins from the cucumber hypocotyl differ in their pH dependence, stress relaxation effects, and resistance to denaturation in methanol (McQueen-Mason and Cosgrove, 1995). The presence of expansins in snail digestive juices (Cosgrove and Durachko, 1994) indicates that expansins may, in some situations, assist the degradation of the plant cell wall, and the high abundance of an expansin mRNA in ripening fruit hints

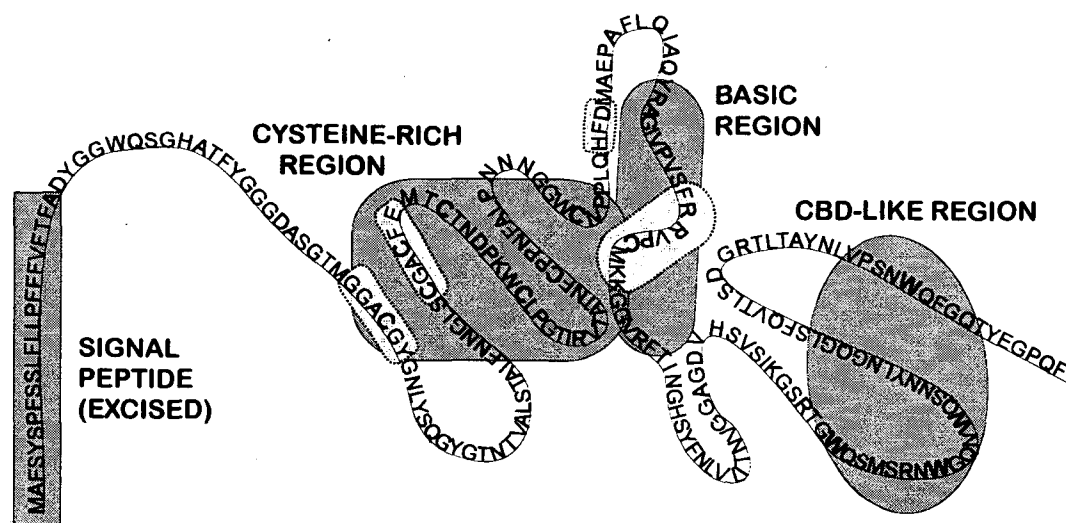


Figure 3. Structural Features of the Expansin Protein.

The amino acid sequence of the cucumber S1 expansin (CS-EXP1) is illustrated using the single-letter code. The following features are highlighted: a hydrophobic signal peptide at the N terminus; a central region containing eight conserved cysteines (C; boldface), some of which likely form intramolecular bridges; a region containing several conserved basic residues (K and R); and a C terminus containing four conserved tryptophans (W; boldface) with spacing similar to that found in some cellulose-binding domains (CBD). The short stretches of amino acids outlined in dotted boxes are sequences conserved between α expansins and β expansins (group I pollen allergens). The HFD domain and conserved cysteines also exhibit similarity with the catalytic domain of family-45 glycosyl hydrolases. Reproduced from Cosgrove (1996), with the permission of ICSU Press, Inc.

that expansins may also function in cell wall disassembly (Rose et al., 1997).

A Second Family of Expansins

Expansins share 20 to 25% amino acid similarity with a group of proteins previously identified as the major allergens in grass pollen (Shcherban et al., 1995), the so-called group I allergens (Knox and Suphioglu, 1996). Recent work showed that the group I allergen from maize pollen indeed has potent expansin-like activity when tested on grass cell walls, whereas it has only a small effect on dicot walls (Cosgrove et al., 1997). Unlike classical expansins, the group I allergens are very soluble and are copiously secreted by grass pollen as they germinate. These characteristics suggest that the expansin-like allergens may function during pollen tube invasion of the stigma and style by softening the walls of these maternal tissues. Moreover, the presence of vegetative homologs 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) have proposed that the group I allergens and their vegetative homologs comprise a second family of expansins (β expansins) that act on different wall components than do members of the original family of α expansins.

Several structural features are conserved between these two families of expansins (Figure 3), and these are likely to form the active sites for expansin action. Of particular interest is the similarity of the cysteine-rich region and the HFD domain (for His-Phe-Asp) to the active site of family-45 glycosyl hydrolases (D.J. Cosgrove, unpublished result). Once an effective protein expression system for expansins is developed, site-directed mutagenesis should help to test whether these conserved residues are critical for expansin activity.

GLUCANASES AND XYLOGLUCAN ENDOTRANSGLYCOSYLASES

A large body of literature implicates (1 \rightarrow 4) β -glucanases in cell wall loosening processes, and matrix glucans show enhanced turnover upon growth stimulation by auxin (reviewed in Taiz, 1984; Fry, 1989b; Hoson, 1993). Antibodies or lectins that interfere with this hydrolytic activity reduce the growth stimulation by auxin in excised segments (Hoson and Nevins, 1989; Hoson and Masuda, 1995). Moreover, the expression of (1 \rightarrow 4) β -glucanases is associated with growing tissues (Hayashi et al., 1984; Brummell et al., 1997), and exogenous glucanases have been reported to give a modest growth stimulation (Labrador and Nevins, 1989). Such results support the concept that wall stress relaxation and expansion are the direct result of xyloglucan hydrolysis or (1 \rightarrow 3) (1 \rightarrow 4) β -glucan hydrolysis in dicot and grass cell walls, respectively.

However, the most direct evidence for this idea is lacking. When various cell wall hydrolases were applied to isolated heat-inactivated walls clamped in an extensometer, the walls either failed to extend or broke suddenly without a prolonged period of wall extension (Cosgrove and Durachko, 1994). Although these enzymes did not cause wall creep by themselves, brief pretreatment of walls with these hydrolases substantially enhanced the subsequent extension response to expansins. It is plausible then that endogenous wall hydrolases may enhance wall expansion by making the wall more sensitive to expansin-induced creep (Cosgrove and Durachko, 1994). Pretreatment of pea cell walls with xyloglucan oligosaccharides increased the responsiveness of the wall to subsequent acid-induced extension (Cutillas-Isturralde and Lorences, 1997), possibly by stimulating endogenous (1 \rightarrow 4) β -glucanase activity in the wall (Farkas and Maclachlan, 1988). These results suggest that cell wall hydrolytic enzymes, such as (1 \rightarrow 4) β -glucanase, may not be the prime movers in the wall expansion process, as was formerly envisioned, but may act indirectly to modulate expansin-mediated polymer creep.

Xyloglucan endotransglycosylase (XET) is another wall enzyme with a proposed role in wall loosening (Smith and Fry, 1991; Fry et al., 1992; Nishitani and Tominaga, 1992). This enzyme has the ability to cut the backbone of xyloglucans and to join the newly formed reducing end to the non-reducing end of an acceptor xyloglucan. Originally, it was proposed that such transglycosylation may be a means of loosening the wall in a limited and controlled fashion. In support of this view, XET activity and growth rate are approximately correlated in various systems (Potter and Fry, 1993; Pritchard et al., 1993; Wu et al., 1994; Xu et al., 1995). However, *in vitro* experiments failed to detect loosening or extension of the wall by XET (McQueen-Mason et al., 1993).

An alternative role for XET may be to integrate newly synthesized xyloglucans into the wall (Nishitani and Tominaga, 1991). Because newly synthesized xyloglucans were found to be much smaller than were the bulk xyloglucans in the wall (Talbot and Ray, 1992a), there is evidently some way that these polymers can be assembled end to end in the cell wall. Moreover, xyloglucan size distributions in pea stem segments were reported to change rapidly and reversibly in response to auxin, cutting, and turgor changes (Nishitani and Masuda, 1981, 1983; Talbot and Ray, 1992b). The mechanism underlying such changes has not been determined, but xyloglucan conformation or the electrostatic environment of the wall may modulate the action of XET so as to produce larger or smaller xyloglucans.

CELL WALL SYNTHESIS: ANOTHER BRICK IN THE WALL?

Cell walls cannot expand indefinitely without losing mechanical integrity, which is why wall synthesis must occur before

or concomitant with prolonged wall enlargement. However, this is not as simple as adding a brick to a wall, because the integration of new material into a stressed network is tricky. It is conceivable that the secretion of specific polysaccharides may lead directly to the wall stress relaxation that is essential for cell enlargement. To function, such a polymer would need the ability to insert itself into the load-bearing network, perhaps with the aid of a helper protein, a hypothetical "wall chaperonin." The resulting relaxation in wall stress would enable the cell to take up water and thereby restore the wall stress. During this time, the new polymer would begin to bear some of the wall stress. However, there is little experimental support for this idea.

Many studies indicate that cell wall expansion is well coordinated with wall synthesis, so that walls rarely become thinner as cells enlarge. For example, wall deposition is highest in the zone of maximal cell expansion in young stems and roots (Silk et al., 1984; Schmalstig and Cosgrove, 1990). Stimulation of cell elongation by auxin or by fusicoccin leads to increased rates of wall synthesis after 30 to 120 min of growth stimulation (Brummell and Hall, 1983; Edelmann et al., 1989). However, wall thinning may occur in growing cells under unusual conditions. For example, in auxin-treated pea stem segments that were starved of sugars, the epidermal wall lost 25% of its mass/area in 4 hr (Bret-Harte et al., 1991).

Inhibitor studies indicate that cellulose biosynthesis is not essential for the initial growth stimulation by auxin or fusicoccin, whereas Golgi-derived secretion seems to be important for auxin-induced growth but not for fusicoccin-induced growth (Brummell and Hall, 1984; Edelmann et al., 1989; Hoson and Masuda, 1992). The requirement of Golgi-derived secretion for auxin action may have to do with delivery of essential matrix polysaccharides, undefined growth-limiting wall proteins (Edelmann and Schopfer, 1989), or membranes containing H⁺-ATPase for wall acidification (Frias et al., 1996). In the case of fusicoccin-induced growth, the activation of the plasma membrane H⁺-ATPase and consequent acidification of the wall space are evidently sufficient to cause cell wall expansion.

RIGIDIFYING THE CELL WALL

The growth cessation that occurs during cell maturation is generally irreversible and is accompanied by rigidification of the cell wall (Kutschera, 1996). These physical changes in the wall may come about by a reduction in wall loosening processes, an increase in wall cross-linking, or an alteration in the structure and composition of the wall so as to make the wall more rigid and/or less susceptible to wall loosening. There is evidence to support each of these hypotheses, and each may operate to some degree at the same time.

During maturation of bean leaves, growth cessation coincides with a loss in the capacity for acid-induced extension

(Van Volkenburgh et al., 1985). Similarly, in cucumber hypocotyls, walls lose their ability to undergo acid-induced extension when they leave the elongation zone (Cosgrove, 1989), and this ability cannot be restored by the addition of exogenous expansin (McQueen-Mason et al., 1992; McQueen-Mason, 1995). This result shows that the nongrowing wall does not simply lack expansins but that it is somehow made unresponsive to expansins, perhaps by wall polymer cross-linking or other modifications of the wall. An analysis of expansin mRNA abundance along the cucumber hypocotyl found a close correlation between expansin transcript levels, growth rate, and the ability for acid-induced extension of isolated walls (M. Shieh, J. Shi, and D.J. Cosgrove, unpublished results). Thus, it appears that growth cessation in this organ entails both loss of expansin expression and an increased "rigidification" of the wall. In oat coleoptiles, growth cessation was also associated with both wall rigidification (measured as loss in susceptibility to expansin action) and loss of expansin activity (Cosgrove and Li, 1993).

Various structural modifications may contribute to wall rigidification. For example, hemicelluloses may become less branched and thereby form tighter complexes with cellulose or with other wall polymers. Alternatively, they may be altered in other ways to become resistant to wall loosening activities. Indeed, the arabinoxylan of maize coleoptiles becomes less branched as the coleoptile matures and may form tighter complexes with cellulose (Carpita, 1984). The disappearance of mixed-link β -D-glucans also coincides with growth cessation in these walls. Furthermore, pectin deesterification is associated with growth cessation in both grasses and dicotyledons and may contribute to wall rigidification by strengthening pectin-calcium networks (Yamaoka and Chiba, 1983; Yamaoka et al., 1983; Goldberg, 1984). Cross-linking of phenolic groups in the wall, such as those on structural proteins, lignin, and pectins, generally coincides with wall maturation and is believed to be mediated by peroxidase, a putative wall rigidification enzyme (Goldberg et al., 1987; Tan et al., 1991).

It is evident that cell maturation involves many changes in wall structure. The complexity of these changes has made it difficult to determine the significance of the individual processes that participate in the cessation of wall expansion. One useful way to address this question would be to isolate mutants with genetic lesions in specific wall maturation processes. Isolated walls may also be used to examine the influence of specific chemical modifications on wall creep properties. For example, treatment of maize coleoptile walls with hydrogen peroxide causes a mechanical stiffening (Schopfer, 1996). Cucumber hypocotyl walls show a pH-dependent loss in their ability to undergo acid-induced extension, with a half-time of \sim 40 min (Cosgrove, 1989). Extractable expansin activity was not reduced during this rigidification, which seems instead to be the result of an enzymatic change in the wall—possibly deesterification of pectin—making the wall less sensitive to expansin action (S. McQueen-Mason and D.J. Cosgrove, unpublished results).

By use of such wall assays, it may be possible to test other hypotheses of wall rigidification.

SUMMARY AND PERSPECTIVE

The enlargement of plant cells involves the coordinate control of wall synthesis and expansion, solute and water transport, membrane synthesis, Golgi secretion, ion transport, and many other processes. In this review, I have focused on the wall because it is the major control point for cell enlargement. Some of the key processes that may be involved in wall enlargement are summarized in Figure 4.

I offer the following speculative picture as a tentative working model for the control of wall expansion. The primary wall is initially secreted and assembled in a form that is mechanically tough yet has "hot spots" where expansin can weaken microfibril-matrix adhesion. Expansin activity, which is modulated both by secretion of the protein to the wall and by changes in the pH and redox potential of the wall, induces the stress relaxation and polymer creep needed for wall enlargement and water uptake by the cell. By altering

the viscosity of the matrix, glucanases, other wall hydrolases, and XET affect the amount of wall enlargement that results from expansin activity. These enzymes may control the yield threshold by modulating the size of the polysaccharide cluster that is dragged along by expansin-induced creep. Xylosidases and related "debranching" enzymes remove side chains from xyloglucan and other hemicelluloses, promoting their binding to cellulose. Pectin methylesterases, peroxidases, and other potential cross-linking enzymes eventually lock up the wall during its maturation, preventing further wall creep.

This model of cell enlargement has many interacting components, yet it is almost certainly oversimplified because the complexity of wall polysaccharides has been greatly reduced for this discussion.

Many of the developmental processes discussed in other reviews in this special issue are ultimately expressed via control of the rate, duration, and directionality of cell expansion; these in turn depend on the structure of the cell wall and the activity of wall proteins. Unraveling the details of this control remains a challenge for the future. However, the cell wall also serves many other functions besides control of cell enlargement, and additional roles such as adhesion (see

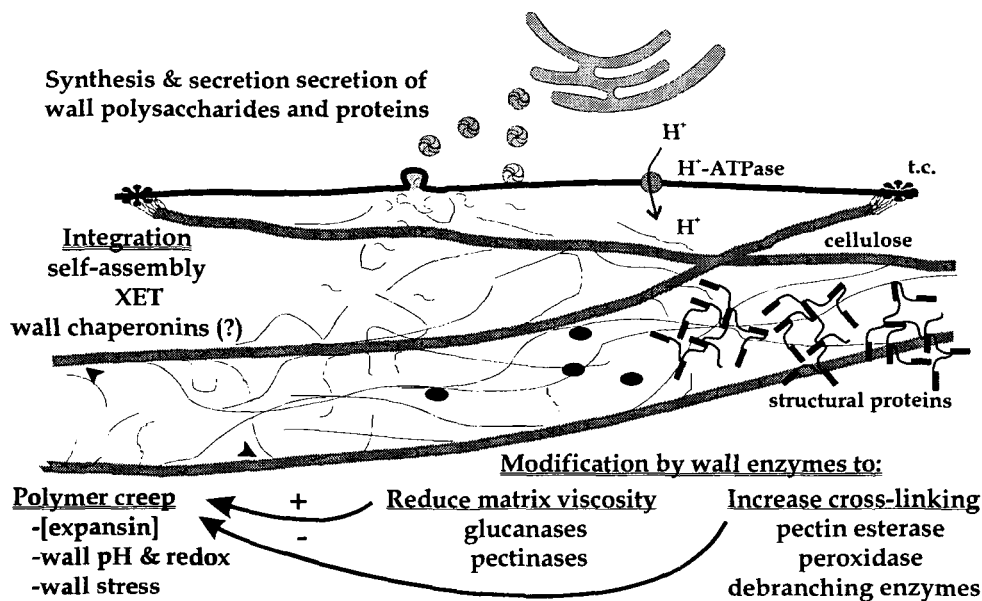


Figure 4. Processes Involved in Cell Wall Assembly, Expansion, and Rigidification.

Cellulose microfibrils are extruded into the wall by terminal complexes (t.c.). Matrix polymers, secreted by Golgi-derived vesicles, bind to the microfibrils and to themselves to form a mechanically tough network. The details of this integration process are not known but may involve self-assembly, enzymes such as XET, and hypothetical polysaccharide chaperonins to aid in wall assembly. When this network is put in mechanical stress by cell turgor, the microfibrils and matrix polymers may extend by an expansin-mediated creep. This process is sensitive to the amount of expansin, its activity (a function of wall pH and redox potential), and wall stress. Wall pH is determined principally by the activity of the plasma membrane H^+ -ATPase. Polymer creep may also be indirectly modified by cell wall enzymes that change the structure of the wall, for example, by reducing the viscosity of the matrix polymers or by enhancing cross-linking among the various matrix polymers and structural proteins. Enhancement and suppression of expansin-mediated creep by other wall enzymes are denoted by (+) and (-), respectively.

Kropf, 1997, in this issue), cell-cell signaling (see McLean et al., 1997, in this issue), cell differentiation, and defense against pathogens must also be incorporated into our concept of the wall. At the rate of current advances in cell wall research (Carpita et al., 1996), we anticipate that our view of the cell wall and its role in plant development will continue to evolve rapidly.

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