

Molecular Regulation of Plant Cell Wall Extensibility

Daniel J. Cosgrove

Department of Biology, 208 Mueller Lab, Pennsylvania State University, University Park, PA

ABSTRACT

Gravity responses in plants often involve spatial and temporal changes in cell growth, which is regulated primarily by controlling the ability of the cell wall to extend. The wall is thought to be a cellulose-hemicellulose network embedded in a hydrated matrix of complex polysaccharides and a small amount of structural protein. The wall extends by a form of polymer creep, which is mediated by expansins, a novel group of wall-loosening proteins. Expansins were discovered during a molecular dissection of the "acid growth" behavior of cell walls. Expansin alters the rheology of plant walls in profound ways, yet its molecular mechanism of action is still uncertain. It lacks detectable hydrolytic activity against the major components of the wall, but it is able to disrupt noncovalent adhesion between wall polysaccharides. The discovery of a second family of expansins (β -expansins) sheds light on the biological role of a major group of pollen allergens and implies that expansins have evolved for diverse developmental functions. Finally, the contribution of other processes to wall extensibility is briefly summarized.

INTRODUCTION

Plants possess a remarkable developmental plasticity that enables them to modify their form and the progression of their development to suit environmental conditions. Such plasticity entails a perception or detection of the environment and an ensuing set of responses leading to altered development. Of the various environmental conditions sensed by plants, a few are generally recognized to be the most influential, specifically: light, water and

nutrient availability, temperature, and gravity.

Gravity modulates plant morphogenesis in several ways (Halstead and Dutcher, 1987). **Gravitropism** is probably the most notable, if only because it has been investigated for so long and in such detail. The gravity sensing mechanism for gravitropism is believed to involve buoyancy effects, particularly via amyloplast sedimentation (Caspar and Pickard, 1989, Kiss et al., 1989a, Kiss and Sack, 1989b, Sievers et al., 1989), which somehow alters the transport and metabolism of growth hormones such as auxin and other substances (Bandurski et al., 1984, Iino, 1991, MacDonald and Hart, 1987, Migliaccio and Rayle, 1989, Pengelly et al., 1981, Trewavas, 1992). The end result is a growth asymmetry and bending, as illustrated in Figure 1 (Cosgrove, 1990a, Cosgrove, 1990b, Iwami and Masuda, 1974, Iwami and Masuda, 1976, Shen-Miller and Masuda, 1973). Because the cell wall acts as a tough, yet pliant, sheath surrounding the cell, the growth asymmetry during gravitropism requires an asymmetry in the processes underlying cell wall yielding and extension. Asymmetries in wall pH and acid growth have also been reported in gravitroping organs (Collings et al., 1992, Evans and Mulkey, 1982, Migliaccio and Rayle, 1984, Mulkey et al., 1981, Pickard, 1985, Wright and Rayle, 1983) and these are thought to underlie, at least in part, the asymmetry in wall extensibility and consequent changes in organ shape. Other gravity-sensitive morphogenetic processes include the development of reaction wood, the formation of the apical hook in dark-grown seedlings (MacDonald et al., 1983), and the development of a "peg" or "foot" in

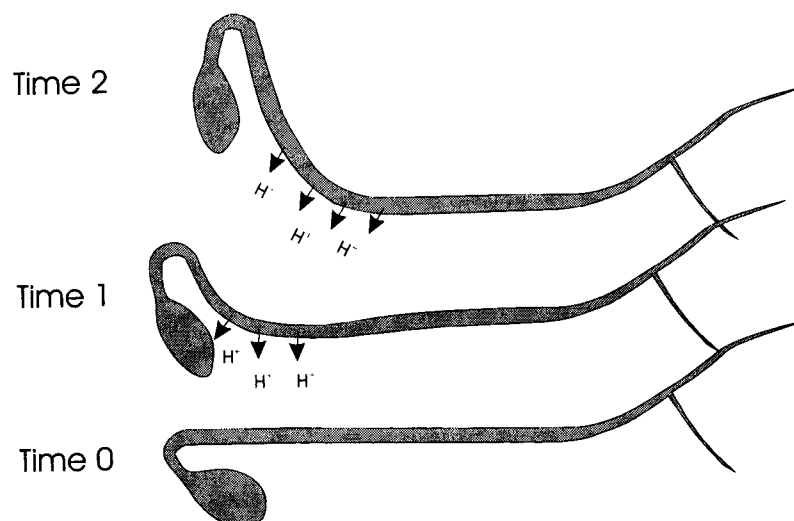


Figure 1. Gravitropic Bending of Stems is Associated with Asymmetries in Apoplastic pH, Wall Extensibility, and Growth on the Upper and Lower Surfaces of the Stem. This figure illustrates the change in shape of a cucumber hypocotyl at three points in time after horizontal placement. The lower surface of the hypocotyl grows faster and becomes acidified through an asymmetry in proton extrusion.

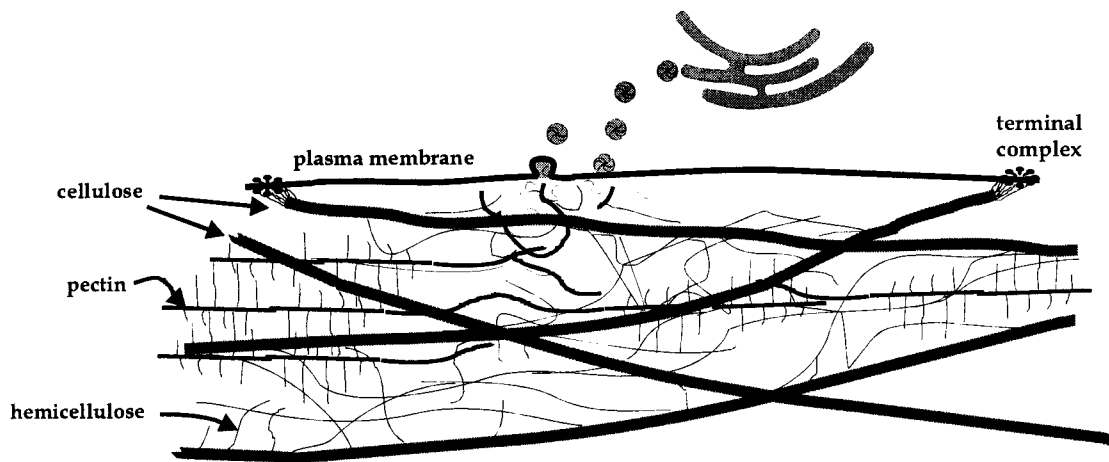


Figure 2. Diagram of the Major Structural Components of the Cell Wall. *Cellulose microfibrils are assembled at the surface of the plasma membrane through the action of putative synthase complexes called terminal complexes. Hemicelluloses and pectins are secreted in Golgi-derived vesicles.*

germinating seedlings of certain species (Takahashi and Suge, 1988, Witztum and Gersani, 1975).

All of these morphogenetic effects of gravity involve alterations in the rate and directionality of cell expansion, which in turn are largely controlled by cell wall properties, specifically the ability of the cell wall to extend or yield irreversibly (Cosgrove, 1997b). This review will summarize and put into context some recent advances in our understanding of how cell wall extensibility is regulated at the molecular level. It is a summary of an invited lecture presented to the 1997 Annual Meeting of the American Society for Gravitational and Space Biology.

STRUCTURE AND EXTENSIBILITY OF THE GROWING CELL WALL

Growing plant cells secrete a complex, highly hydrated, fibrous wall that encases the protoplast, resists the mechanical forces of cell turgor, and serves other roles in cell adhesion, cell-cell communication, and defense (Carpita and Gibeaut, 1993, Cosgrove, 1997a). The wall consists principally of three classes of polysaccharides and a small amount of structural protein. The three major classes of wall polysaccharides are cellulose, hemicellulose and pectin (Figure 2). **Cellulose** is extruded as an ordered microfibril (~5 nm wide) from membrane synthesis complexes (Brown, Jr. et al., 1996). **Hemicellulose** consists of various branched and mixed-linked glycans, such as xyloglucans and xylans, typically containing short side chains or mixed linkages that interfere with the alignment and inter-chain bonding between neighboring chains; as a result, hemicellulose is not so well ordered as is cellulose. Hemicellulose chains are synthesized in the Golgi apparatus and secreted to the wall via vesicles. They characteristically bind to the surface of cellulose and may also

become physically entrapped in the cellulose microfibril during its formation; such surface adhesion and entrapment is believed to tether cellulose microfibrils to each other while at the same time preventing direct microfibril-microfibril contact. Like hemicellulose, **pectin** consists of complex polysaccharides that are synthesized in the Golgi apparatus. Pectin forms the major part of the hydrated matrix in which the cellulose-hemicellulose network is embedded. **Structural proteins** make up a minor fraction of the matrix in growing cell walls. These proteins often consist of repeats of short motifs and in some cases may be covalently linked with other structural proteins or with matrix polysaccharides (Showalter, 1993).

Compared with the wall from mature plant cells, the wall of growing plant cells is relatively malleable and is able to undergo irreversible deformation by a form of polymer "creep" in which the load-bearing wall network expands at the same time that newly secreted wall polysaccharides are integrated into the wall. Wall "extensibility" refers to the ability of the wall to yield (extend irreversibly) to mechanical tension, consequently increasing in surface area. In living cells, such tension is normally supplied by cell turgor pressure, which is typically 3 to 8 bars in growing cells. Turgor generates wall stresses in the range of hundreds of bars. Wall stresses are so large because the walls are thin relative to the diameter of the cell, resulting in a large force (due to turgor pressure) concentrated in a small area (the wall). Growing cells have the challenge of producing a wall that is strong enough to resist the mechanical forces of turgor pressure, yet has sufficient extensibility to enlarge many-fold before growth ceases.

Wall extensibility in growing cells has been estimated by a variety of physical and physiological

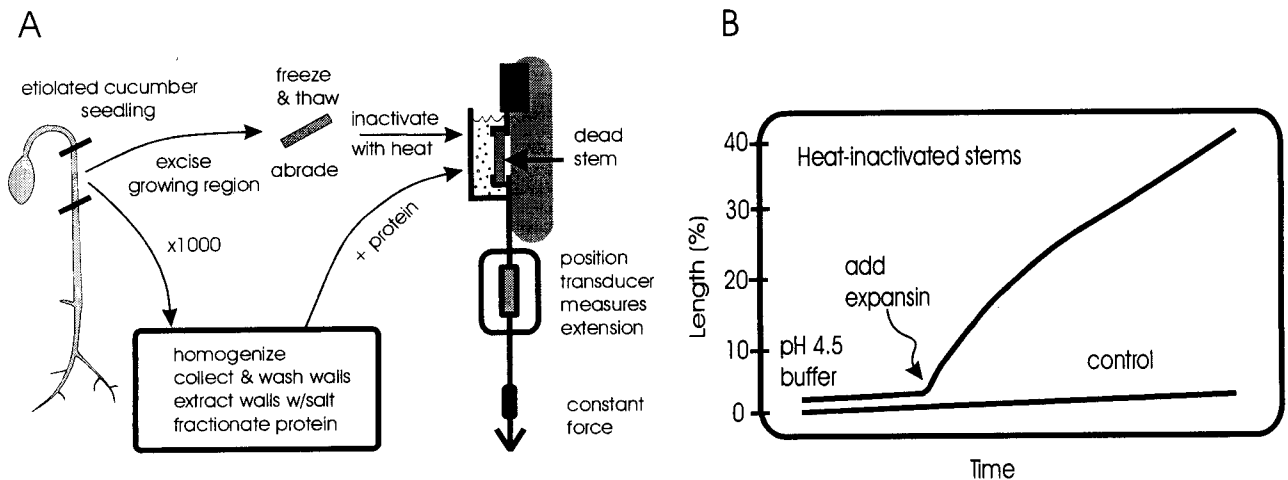


Figure 3. Diagram of the Reconstitution Assay Used to Identify Proteins with Wall Extension Activity. *A: Cell walls from growing seedlings are prepared for extensometer assay by a freeze/thaw treatment (to kill the cells), light abrasion (to permeabilize the cuticle), and a brief heat treatment (to inactivate endogenous wall extension activity). Proteins extracted from active walls may be added to the buffer in which the walls are suspended, to test for extension activity (creep activity). B: Diagram of a typical extension curve obtained when expansin protein is added to inactivated wall specimens.*

techniques. Each of these techniques measures a somewhat different rheological property of the wall, and thus results obtained by one technique do not always agree in detail with results obtained by another method; this technical aspect of wall extensibility was discussed in detail in a previous review (Cosgrove, 1993). This technical complication notwithstanding, most methods show that growing walls are more extensible than nongrowing walls.

Is wall extensibility an inherent viscoelastic property of the wall, i.e. simply due to the bonding arrangements of the wall polysaccharides, or is it controlled by more complex processes? The answer seems to be that wall extensibility is partly viscoelastic and partly controlled in a rapid and reversible way by cellular processes. The influence of wall viscoelasticity is most readily seen in maturing cell walls, where thickening of the wall and cross linking of its components make the wall less extensible (Kutschera, 1996). One also sees changes in wall viscoelasticity after stimulation of growth by auxin (Cleland, 1967, Masuda, 1990). On the other hand, growth may also be rapidly and reversibly controlled without detectable changes in wall viscoelasticity. For example, blue light quickly suppresses cell wall extension and cell growth without a major change in wall structure or viscoelasticity (Cosgrove and Green, 1981). Various authors have suggested that wall extension is regulated via the activity of wall "loosening" enzymes (Cleland and Rayle, 1978, Fry, 1989) and this concept was shown to have some validity with the discovery of "expansins", the major focus of this review.

EXPANSIN DISCOVERY AND ITS BIOCHEMICAL ACTION

In 1992 we reported the isolation and characterization of two related proteins with the ability to catalyze cell wall extension (McQueen-Mason et al., 1992). We used a simple reconstitution approach (outlined in Figure 3) to identify wall proteins that could induce prolonged wall extension in vitro. The characteristics of these proteins, subsequently named expansins, indicate that they are responsible for most, if not all, of the "acid growth" mechanism of plant cell walls. Acid growth refers to the cell enlargement and wall extension that occurs when living cells or isolated walls are placed in acidic buffers ($\text{pH} < 5.5$) (Cleland, 1992, Cleland et al., 1991, Luethen and Boettger, 1993, Rayle and Cleland, 1992, Schopfer, 1993). Because acid growth responses are found in dicots, monocots, gymnosperms, ferns, mosses and some algae, we suspect that acid growth, and therefore expansins, may be universal to plants with cellulosic walls. Expansin-mediated wall extension appears to be a fundamental mechanism of wall extension in land plants. In contrast, algae have many more diverse mechanisms of cell wall enlargement, which may be controlled by other mechanisms (Pickett-Heaps, 1975). Although expansins were originally discovered in cucumber hypocotyls, we now have additional evidence that they exist and are functional in many other plant species. Table 1 lists the species in which expansins have been identified at the protein or nucleotide sequence level. To date, the only proteins that have been identified with wall extension activity in the reconstitution assay are expansins. For example, when proteins from the growing cell walls of deep water rice plants were extracted and assayed for wall extension activity, two proteins were identified with properties very similar to cucumber expansins (Cho and Kende, 1997b).

Table 1. List of Species Known to Express Expansins.

PLANT MATERIAL	REFERENCES
<u>Based on protein data:</u>	
cucumber hypocotyls	(McQueen-Mason et al., 1992)
oat coleoptiles	(Cosgrove and Li, 1993)
maize roots	(Wu et al., 1996)
tomato leaves	(Keller and Cosgrove, 1995)
rice internodes	(Cho and Kende, 1997a, Cho and Kende, 1997b)
celery stalks	Unpublished (D.J. Cosgrove, D.M. Durachko)
grass pollen*	(Cosgrove et al., 1997c)

Based on cDNA sequences:

cucumber hypocotyls	(Shcherban et al., 1995)
pine seedlings	K. Hutchinson (unpublished data)
<i>Arabidopsis</i> plants	(Shcherban et al., 1995)
grasses	(Cosgrove et al., 1997c, Shcherban et al., 1995)
cotton trichomes	(Shimizu et al., 1997)
ripening tomato fruit	(Rose et al., 1997)
tomato shoot meristems	(Fleming et al., 1997)
soybean suspension cells*	(Crowell, 1994)

*denotes *beta expansin*

There are other reports of wall extension activity in vitro (Okamoto and Okamoto, 1995), but the active proteins have not yet been identified and it remains to be seen whether the activity is due to expansins or some novel class of wall protein.

What is unique about expansin is how it affects the rheology (flow behavior) of plant cell walls. Expansins - alone of all wall enzymes tested to date - induce irreversible extension in cell walls that are clamped in tension in an extensometer and bathed in an acidic buffer. When various wall hydrolases were tested for their ability to cause wall extension, we found that they could weaken the wall, but not cause significant creep of the wall polymers. The uniqueness of expansin's rheological effect may also be seen in stress relaxation analyses of walls. Expansins enhance wall stress relaxation over a wide time spectrum, whereas cellulases lack this action (McQueen-Mason and Cosgrove, 1994). It is evident from such results that hydrolysis of the matrix components is not sufficient to cause wall extension. The concept of wall "loosening", as it pertains to cell expansive growth, is thus not as simple as it once seemed (Cleland, 1976, Fry, 1989).

With regard to its biochemical mechanism of action, expansin does not fit the usual idea of a wall "loosening" enzyme because it lacks detectable hydrolase activity and transglycosylase activity against the main constituents of the wall (McQueen-Mason and Cosgrove, 1995, McQueen-Mason et al., 1993). Instead, these proteins appear to dissolve the adhesion between matrix

hemicelluloses and cellulose microfibrils, at least in part by disrupting hydrogen bonding between these two major components of the wall (McQueen-Mason and Cosgrove, 1994).

Binding studies indicate that expansins do not bind to the major components of the matrix, but instead bind to cellulose, or perhaps to a complex between cellulose and a tightly-bound hemicellulose which is not xyloglucan. More work is needed to define the exact molecular mechanism by which expansins make cell walls more extensible.

EXPANSIN SEQUENCE ANALYSIS

Originally, we cloned and sequenced two different expansin cDNAs expressed in the growing cucumber hypocotyl (Shcherban et al., 1995). These cDNAs let us identify homologs from *Arabidopsis* and rice. In brief, the sequence analysis reveals that:

- ▶ Expansins are novel proteins without previously known functional motifs.
- ▶ Expansins constitute a multigene family (14 expansin genes have been identified in *Arabidopsis* so far; this is likely an incomplete count).
- ▶ Expansins are highly conserved evolutionarily (80% identity between a rice expansin and a cucumber expansin, at the amino acid level).

Figure 4 diagrams the structure of the typical expansin protein, as predicted from the cDNA sequences. The primary translation product contains a signal peptide of



Number, *Arabidopsis*, and

extracted proteins from non-stressed pollen. The extension activity, as assayed in the pollen, was not affected by the stress relaxation as the extension activity of the pollen extract was not affected. The activity was due, at least in part, to the presence of a 11 kDa allergen of maize pollen. The results suggest that the effects that classify it as a pollen allergen are not a function of *Zea m1*.

yle. In maize, the pollen
reaching the ovule. The n

Several observations are likely. Germinating pollen release large amounts of the protein. The protein binds tightly to cell walls of the cucumber hypocotyl. Pollen grains have low solubility, and, thus, the allergen protein secreted in pollen is secreted to loosen the pollen grain rather than as a protein on the grain whose expanding surface area of adhesion is supported by the loosening effect on maize cell walls. The stigma of the maize flower must push its way between the cell walls (Heslop-Harrison 1988) as a diffusible wall loosening agent for the invasion through the stigma. The proteins secreted by most

To test this idea, we extracted proteins from non-pollen and tested for wall extension activity. The extract proved to have potent expansin activity, as assayed in the wall extension assays and the stress relaxation assay (Cosgrove et al., 1997c). Fractionation of the pollen extract showed that the wall extension activity was due, at least in part, to Zea m1, the group-1 allergen of maize pollen. Thus, Zea m1 has rheological effects that classify it as an expansin.

What is the biological function of Zea m1? We suggest that it is secreted to aid pollen tube penetration and through the stigma and style. In maize, the pollen must grow many cm before reaching the ovule. The n target of these proteins might be the pollen tube wall o walls of the stigma and style. Several observations r the latter possibility seem more likely. Germinating p grains secrete the allergen in large amounts. The prote very soluble and does not bind tightly to cell walls. In comparison, expansins in the cucumber hypocoty found in very low abundance, have low solubility, and bind very tightly to cell walls. Thus, the allergen protein se better suited as a mobile protein secreted to loosen the walls of the maternal tissues, rather than as a protein loosens the pollen tube wall, whose expanding surfa limited to the tip. This suggestion is supported by the fact that Zea m1 has a potent loosening effect on maize cell walls (the silk is the style and stigma of the maize flower). Another pertinent fact is that as the pollen tube grows through the stigma and style it must push its way betw cells and force apart adjacent cell walls (Heslop-Harrison et al., 1984). Secretion of a diffusible wall loosening protein may speed pollen tube invasion through the s

Are expansin-like proteins secreted by most

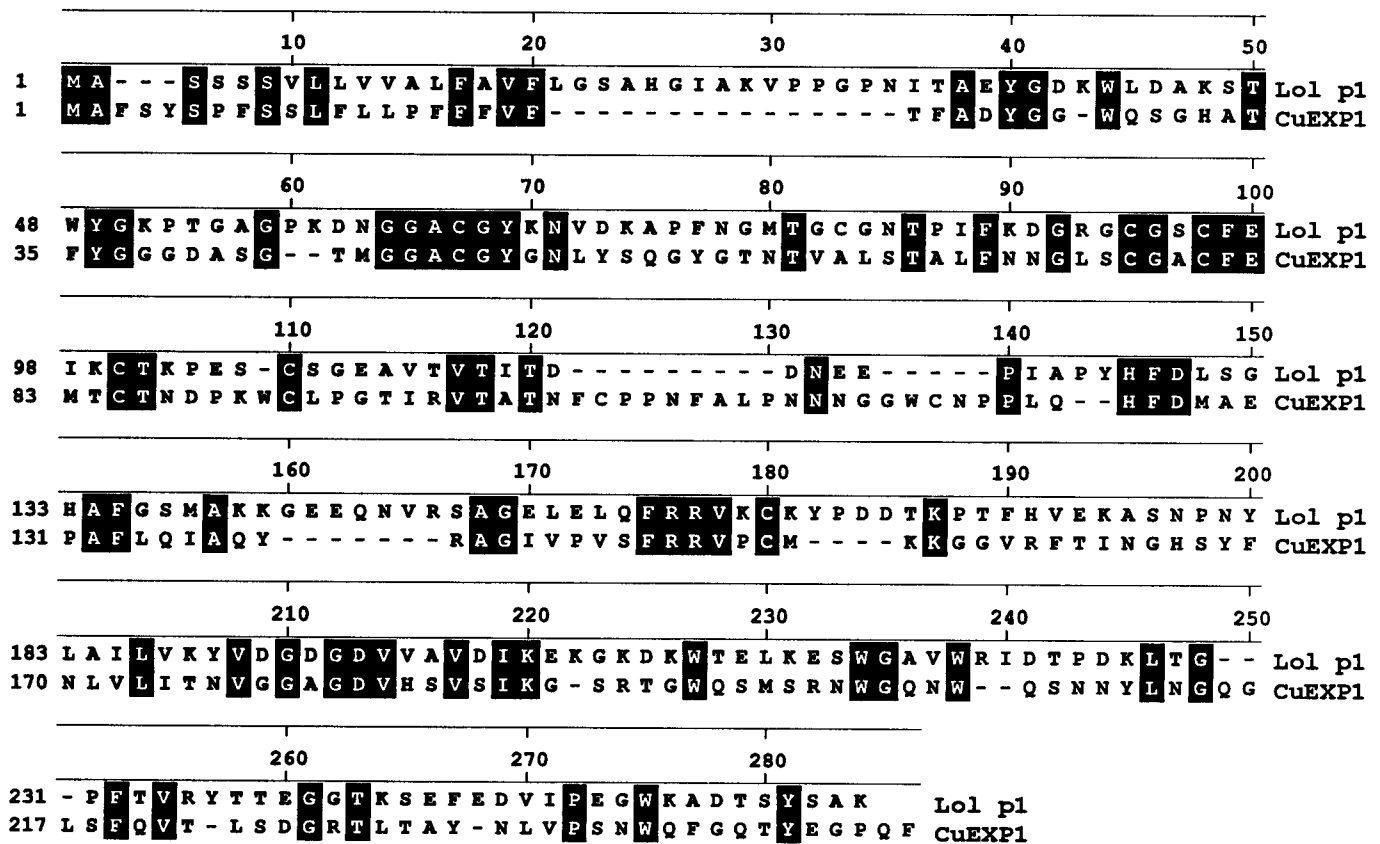


Figure 5. Alignment of a Cucumber Expansin (CuEXP1) with Lol p1, a Group-1 Allergen from Ryegrass Pollen. Gaps to allow best alignment are indicated with "-". Identical amino acid residues are shown in reverse color.

germinating pollen to loosen stigmatic surfaces? The answer seems to be, no. Although many allergenic proteins from pollen have been identified, cloned and sequenced, only the grasses have been shown to express proteins of this sequence class. The expansin-like group-1 allergens are not major allergens of ragweed pollen, for example. Furthermore, no wall extension activity was detected in pollen extracts from *Petunia* (a dicot) and *Lilium* (a monocot, but not a grass). Thus, the "trick" of having the pollen secrete an expansin-like loosening factor may have been learned only by the grasses. But perhaps in other species the cells of the style or stigma secrete such loosening agents. In a more speculative vein, if grass pollen use this method to aid pollen tube invasion, it is an easy step to imagine that some pathogenic fungi have learned a similar trick to assist their invasion of plant tissues. Snail digestive juices were found to contain expansin-like activity (Cosgrove and Durachko, 1994), so a screen of fungal cultures that invade wood or other plant tissues might uncover new expansin-like proteins.

Two other important conclusions stemmed from this study of Zea m1 (Cosgrove et al., 1997c). First, the rheological activity of Zea m1 was most effective when

tested on wall specimens from grasses, with only minor activity detected when assayed with dicot walls. Because grass cell walls differ in composition from dicot walls (Carpita, 1996), it seems likely that Zea m1 acts preferentially on one of the wall components specific to grass walls, e.g. mixed-link (-glucans or glucurono-arabinoxylans.

Second, an analysis of GenBank identified several entries that were homologs of the group-1 allergens, yet were expressed in vegetative tissues rather than in grass pollen. The rice EST (Expressed Sequence Tag) database showed 25 entries, which upon elimination of redundancies narrowed down to 7 distinct sequence classes. All of these cDNAs were from young seedlings, that is, well before formation of flowers and pollen. The Arabidopsis EST collection contains at least one homolog of the group-1 allergens. The soybean CIM1 gene likewise falls into this sequence class. CIM1 is expressed in suspension cultures and its message is induced by cytokinin (Crowell, 1994). On the basis of our biochemical results and sequence analysis, we proposed that these sequences define a second family of expansins, which we call β -expansins. β -expansins include the grass pollen group-1 allergens and

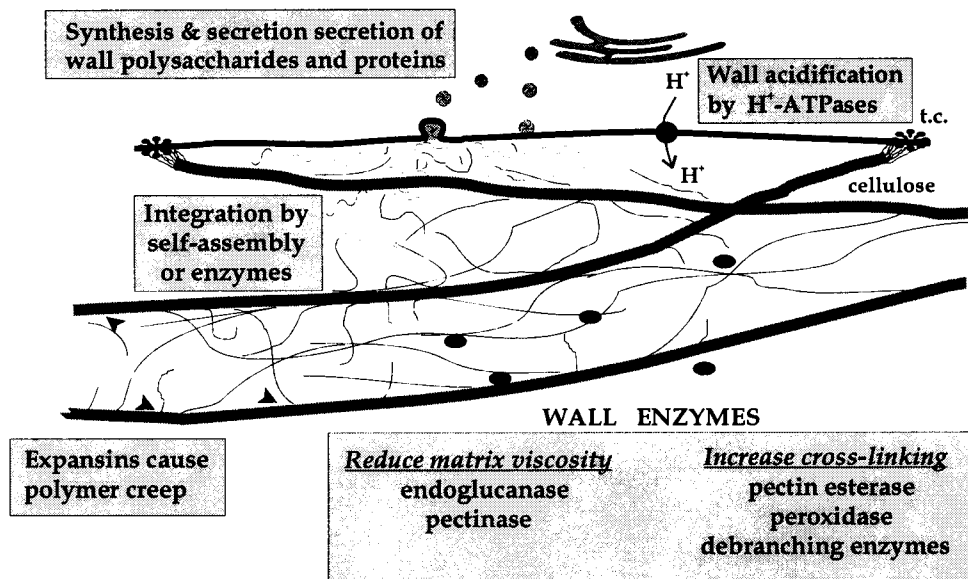


Figure 6. Processes that Contribute to Wall Extensibility. Adapted from Cosgrove, 1997b.

their vegetative homologs in rice, *Arabidopsis*, soybean and other plants. The β -expansins are found in both dicots and grasses, but in grasses they seem to have duplicated and diverged more than the α -expansins. One subclass of the β -expansins has acquired a special function in grass pollen, but a more general role in cell wall loosening is predicted from the fact that many β -expansins are also expressed in rice vegetative tissues.

OTHER WALL LOOSENING AND RIGIDIFYING PROCESSES

The focus of this review has been on the expanding family of expansins and their action to loosen walls and make them more extensible. It should be borne in mind that expansins function in the context of the wall, and other processes that alter either the environment of the wall or its structure may influence expansin activity. In Figure 6 I have summarized our current view of the processes that may be important molecular determinants of cell wall extensibility. They may be listed as follows:

- 1) synthesis and secretion of wall polysaccharides;
- 2) assembly and integration of the wall polysaccharides into a load-bearing network;
- 3) induction of wall polymer creep by expansins;
- 4) hydrolysis of matrix polymers by endoglucanases, pectinases and like enzymes;
- 5) cross-linking or network formation resulting from the action of peroxidases, pectin methyl esterases, and glycosidases that remove branches from matrix polymers;
- 6) control of wall pH, which may alter the gel/sol state of pectins and the activity of the various enzymes in the wall.

Synthesis and secretion of wall polysaccharides.

Addition of new material to the wall will make a more cohesive wall by increasing the thickness of the wall and, perhaps, the density of networked polymers (assuming the polysaccharides become integrated into the wall network). This will make the wall more difficult to stretch, but at the same time will enable the wall to enlarge to a greater extent before a point of physical failure is reached. For instance, isolated cucumber hypocotyl walls clamped in extensometers will creep for many hours, until they thin and break, typically at 40-100% extension. Such thinning and breakage does not occur in walls of living cells because of wall polymers are secreted as the wall extends. Also, in living cells the type and relative proportions of the three wall components (cellulose, hemicellulose, pectin) may vary, giving rise to walls of varying structural properties.

Assembly and integration of the wall components into a load-bearing network. We know relatively little about the mechanisms by which the wall polymers assemble into a cohesive network (Cosgrove, 1997a). Self assembly of the networks is likely, in view of the tendency of wall polysaccharide to aggregate and form ordered complexes (Roland et al., 1982, Roland et al., 1977). Also, enzymes such a xyloglucan endotransglycosylase may stitch newly synthesized xyloglucans into the wall, thereby making a tighter fabric (Nishitani, 1997).

Induction of wall polymer creep by expansins. The activity of expansins may be modulated by wall pH, by altering the rate of secretion of these proteins to the wall, or by altering the structure of the wall.

Hydrolysis of matrix polymers by endoglucanases, pectinases and related enzymes. The activity of these

enzymes may reduce the viscosity of the wall matrix, thereby sensitizing the wall to the action of expansins (Cosgrove and Durachko, 1994). In this model, these enzymes act as synergists with expansins.

Cross-linking or network formation. Opposing the "loosening" activity of wall hydrolases are potential cross linking enzymes, such as peroxidase, pectin methyl esterase, and glycosidases that remove branches from matrix polymers and thus permit strong polymer-polymer adhesion. These enzymes would tend to reduce the effectiveness of expansins, ultimately making the wall a non-extensible structure.

Control of wall pH. Alteration of the wall pH is likely to have complicated, time-dependent effects on the rheology of the growing wall. For example, an increase in wall pH from 5 to 7 would immediately decrease expansin activity and increase the gellation of pectins. It would also affect the activity of the various wall enzymes, leading to gradual changes in the structure of the wall. Changes in wall pH can be effected quickly and it is likely that rapid and reversible control of cell wall extension is controlled at least in part by this means.

Figure 6 illustrates that wall extensibility is a result of many contributory processes. Expansins seem to be crucial to the mechanism that allows the wall to extend during growth, but other factors may modulate expansin's effect on the wall.

Acknowledgements

This work was supported by grants from the National Science Foundation, the Department of Energy, and the National Aeronautics and Space Administration.

REFERENCES

- Bandurski, R.S., Schulze, A., Dayanandan, P. and Kaufman, P.B. 1984. Response to gravity by *Zea mays* seedlings. I. Time course of the response. *Plant Physiology* 74:282-288.
- Brown, R.M., Jr., Saxena, I.M. and Kudlicka, K. 1996. Cellulose biosynthesis in higher plants. *Trends in Plant Science* 1:149-155.
- Carpita, N.C. 1996. Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology* 47:445-476.
- Carpita, N.C. and Gibeaut, D.M. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal* 3:1-30.
- Caspar, T. and Pickard, B.G. 1989. Gravitropism in a starch-less mutant of *Arabidopsis*. Implications for the statolith theory of gravity sensing. *Planta* 177:185-197.
- Cho, H.T. and Kende, H. 1997a. Expansins and internodal growth of deepwater rice. *Plant Physiology* 113:1145-1151.
- Cho, H.T. and Kende, H. 1997b. Expansins in deepwater rice internodes. *Plant Physiology* 113:1137-1143.
- Cleland, R.E. 1967. Extensibility of isolated cell walls: measurement and changes during cell elongation. *Planta* 74:197-209.
- Cleland, R.E. 1976. The control of cell enlargement. *Symposium of the Society for Experimental Biology* 31:101-115.
- Cleland, R.E. 1992. Auxin-induced growth of *Avena* coleoptiles involves two mechanisms with different pH optima. *Plant Physiology* 99:1556-1161.
- Cleland, R.E., Buckley, G., Nowbar, S., Lew, N.M., Stinemwitz, C., Evans, M.L. and Rayle, D.L. 1991. The pH profile for acid-induced elongation of coleoptile and epicotyl sections is consistent with the acid-growth theory. *Planta* 186:70-74.
- Cleland, R.E. and Rayle, D.L. 1978. Auxin, H^+ -excretion and cell elongation. *Botanical Magazine Tokyo Special Issue* 1:125-139.
- Collings, D.A., White, R.G. and Overall, R.L. 1992. Ionic current changes associated with the gravity-induced bending response in roots of *Zea mays* L. *Plant Physiology* 100:1417-1426.
- Cosgrove, D.J. 1990a. Gravitropism of cucumber hypocotyls: biophysical mechanism of altered growth. *Plant Cell and Environment* 13:235-241.
- Cosgrove, D.J. 1990b. Rapid, bilateral changes in growth rate and curvature during gravitropism of cucumber hypocotyls: implications for mechanism of growth control. *Plant Cell and Environment* 13:227-234.
- Cosgrove, D.J. 1993. Wall extensibility: its nature, measurement, and relationship to plant cell growth. *New Phytologist* 124:1-23.
- Cosgrove, D.J. 1997a. Assembly and enlargement of the primary cell wall in plants. *Annual Review of Cell and Developmental Biology* 13:171-201.
- Cosgrove, D.J. 1997b. Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement. *Plant Cell* 9:1031-1041.
- Cosgrove, D.J., Bedinger, P.A. and Durachko, D.M. 1997c. Group I allergens of grass pollen as cell wall loosening agents. *Proceedings of the National Academy of Sciences, USA* 94:6559-6564.

- Cosgrove, D.J. and Durachko, D.M. 1994. Autolysis and extension of isolated walls from growing cucumber hypocotyls. *Journal of Experimental Botany* 45:1711-1719.
- Cosgrove, D.J. and Green, P.B. 1981. Rapid suppression of growth by blue light. Biophysical mechanism of action. *Plant Physiology* 68:1447-1453.
- Cosgrove, D.J. and Li, Z.-C. 1993. Role of expansin in developmental and light control of growth and wall extension in oat coleoptiles. *Plant Physiology* 103:1321-1328.
- Crowell, D.N. 1994. Cytokinin regulation of a soybean pollen allergen gene. *Plant Molecular Biology* 25:829-835.
- Davies, G.J., Tolley, S.P., Henrissat, B., Hjort, C. and Schulein, M. 1995. Structures of oligosaccharide-bound forms of the endoglucanase V from *Humicola insolens* at 1.9 Å resolution. *Biochemistry* 34:16210-16220.
- Evans ML, and Mulkey TJ. 1982. Comparative effects of auxin and abscisic acid on growth, hydrogen ion efflux and gravitropism in primary roots of maize. In: Wareing PF, editor. *Plant Growth Substances*. Academic Press:London. p 33-42.
- Fleming, A.J., McQueen-Mason, S., Mandel, T. and Kuhlemeier, C. 1997. Induction of leaf primordia by the cell wall protein expansin. *Science* 276:1415-1418.
- Fry, S.C. 1989. Cellulases, hemicelluloses and auxin-stimulated growth: A possible relationship. *Physiologia Plantarum* 75:532-536.
- Halstead, T.W. and Dutcher, F.R. 1987. Plants in space. *Annual Review of Plant Physiology* 38:317-345.
- Heslop-Harrison, Y., Reger, B.J. and Heslop-Harrison, J. 1984. The pollen-stigma interaction in the grasses. 5. Tissue organization and cytochemistry of the stigma ("silk") of *Zea mays* L. *Acta Botanica Neerlandica* 33:81-99.
- Iino, M. 1991. Mediation of tropisms by lateral translocation of endogenous indole-3-acetic acid in maize coleoptiles. *Plant Cell and Environment* 14:279-286.
- Iwami, S. and Masuda, Y. 1974. Geotropic response of cucumber hypocotyls. *Plant and Cell Physiology* 15:121-129.
- Iwami, S. and Masuda, Y. 1976. Distribution of labeled auxin in geotropically stimulated stems of cucumber and pea. *Plant and Cell Physiology* 17:227-237.
- Keller, E. and Cosgrove, D.J. 1995. Expansins in growing tomato leaves. *Plant Journal* 8:795-802.
- Kiss, J.Z., Hertel, R. and Sack, F.D. 1989a. Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. *Planta* 177:198-206.
- Kiss, J.Z. and Sack, F.D. 1989b. Reduced gravitropic sensitivity in roots of a starch-deficient mutant of *Nicotiana glauca*. *Planta* 180:123-130.
- Knox, B. and Suphioglu, C. 1996. Environmental and molecular biology of pollen allergens. *Trends in Plant Science* 1:156-164.
- Kutschera, U. 1996. Cessation of cell elongation in rye coleoptiles is accompanied by a loss of cell-wall plasticity. *Journal of Experimental Botany* 47:1387-1394.
- Luethen, H. and Boettger, M. 1993. The role of protons in the auxin-induced root growth inhibition - a critical reexamination. *Botanica Acta* 106:58-63.
- MacDonald, I.R., Gordon, D.C., Hart, J.W. and Maher, E.P. 1983. The positive hook: the role of gravity in the formation and opening of the apical hook. *Planta* 158:76-81.
- MacDonald, I.R. and Hart, J.W. 1987. New light on the Cholodny-Went theory. *Plant Physiology* 84:568-570.
- Masuda, Y. 1990. Auxin-induced cell elongation and cell wall changes. *Botanical Magazine (Tokyo)* 103:345-370.
- McQueen-Mason, S. and Cosgrove, D.J. 1994. Disruption of hydrogen bonding between wall polymers by proteins that induce plant wall extension. *Proceedings of the National Academy of Sciences, USA* 91:6574-6578.
- McQueen-Mason, S. and Cosgrove, D.J. 1995. Expansin mode of action on cell walls: Analysis of wall hydrolysis, stress relaxation, and binding. *Plant Physiology* 107:87-100.
- McQueen-Mason, S., Durachko, D.M. and Cosgrove, D.J. 1992. Two endogenous proteins that induce cell wall expansion in plants. *Plant Cell* 4:1425-1433.
- McQueen-Mason, S., Fry, S.C., Durachko, D.M. and Cosgrove, D.J. 1993. The relationship between xyloglucan endotransglycosylase and in vitro cell wall extension in cucumber hypocotyls. *Planta* 190:327-331.
- Migliaccio, F. and Rayle, D.L. 1984. Sequence of key events in shoot gravitropism. *Plant Physiology* 75:78-81.
- Migliaccio, F. and Rayle, D.L. 1989. Effect of asymmetric auxin application on *Helianthus* hypocotyl curvature. *Plant Physiology* 91:466-468.

- Mulkey, T.J., Kuzmanoff, K.M. and Evans, M.L. 1981. Correlations between proton-efflux patterns and growth patterns during geotropism and phototropism in maize and sunflower. *Planta* 152:239-241.
- Nishitani, K. 1997. The role of endoxyloglucan transferase in the organization of plant cell walls. *International Review of Cytology* 173:157-206.
- Okamoto, A. and Okamoto, H. 1995. Two proteins regulate the cell wall extensibility and the yield threshold in glycerinated hollow cylinders of cowpea hypocotyl. *Plant Cell and Environment* 18:827-830.
- Pengelly, W.L., Bandurski, R.S. and Schulze, A. 1981. Validation of a radioimmunoassay for indole-3-acetic acid using gas chromatography-selected ion monitoring mass spectroscopy. *Plant Physiology* 68:96-98.
- Pickard BG. 1985. Roles of hormones, protons and calcium in geotropism. In: Pharos RP, Reid DM, editors. *Encyclopedia of Plant Physiology, New Series. Hormonal Regulation of Development III. Role of Environmental Factors*. Springer-Verlag:Berlin. p 193-281.
- Pickett-Heaps JD. 1975. *Green Algae: Structure, Reproduction and Evolution in Selected Genera*. Sunderland, MA: Sinauer.
- Rayle, D.L. and Cleland, R.E. 1992. The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiology* 99:1271-1274.
- Roland, J.C., Reis, D., Mosiniak, M. and Vian, B. 1982. Cell wall texture along the growth gradient of the mung bean hypocotyl: ordered assembly and dissipative processes. *Journal of Cell Science* 56:303-318.
- Roland, J.C., Vian, B. and Reis, D. 1977. Further observations on cell wall morphogenesis and polysaccharide arrangement during plant growth. *Protoplasma* 91:125-141.
- Rose, J.K.C., Lee, H.H. and Bennett, A.B. 1997. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proceedings of the National Academy of Sciences, USA* 94:5955-5960.
- Schopfer, P. 1993. Determination of auxin-dependent pH changes in coleoptile cell walls by a null-point method. *Plant Physiology* 103:351-357.
- Shcherban, T.Y., Shi, J., Durachko, D.M., Guiltinan, M.J., McQueen-Mason, S., Shieh, M. and Cosgrove, D.J. 1995. Molecular cloning and sequence analysis of expansins - A highly conserved, multigene family of proteins that mediate cell wall extension in plants. *Proceedings of the National Academy of Sciences, USA* 92:9245-9249.
- Shen-Miller, J. and Masuda, Y. 1973. Kinetics of stress relaxation properties of oat coleoptile cell wall after geotropic stimulation. *Plant Physiology* 51:464-467.
- Shimizu, Y., Aotsuka, S., Hasegawa, O., Kawada, T., Sakuno, T., Sakai, F. and Hayashi, T. 1997. Changes in levels of mRNAs for cell wall-related enzymes in growing cotton fiber cells. *Plant and Cell Physiology* 38:375-378.
- Showalter, A.M. 1993. Structure and function of plant cell wall proteins. *Plant Cell* 5:9-23.
- Sievers, A., Kruse, S., Kuo-Huang, L.L. and Wendt, M. 1989. Statoliths and microfilaments in plant cells. *Planta* 179:275-278.
- Takahashi, H. and Suge, H. 1988. Involvement of ethylene in gravity-regulated peg development in cucumber seedling. *Plant and Cell Physiology* 29:313-320.
- Trewavas, A.J. 1992. What remains of the Cholodny-Went theory? Introduction. *Plant Cell and Environment* 15:761.
- Witztum, A. and Gersani, M. 1975. The role of polar movement of IAA in the development of the peg in *Cucumis sativus* L. *Botanical Gazette* 136:5-16.
- Wright, L.Z. and Rayle, D.L. 1983. Evidence for a relationship between H⁺ excretion and auxin in shoot gravitropism. *Plant Physiology* 72:99-104.
- Wu, Y., Sharp, R.E., Durachko, D.M. and Cosgrove, D.J. 1996. Growth maintenance of the maize primary root at low water potentials involves increases in cell wall extensibility, expansin activity and wall susceptibility to expansins. *Plant Physiology* 111:765-772.