

New genes and new biological roles for expansins

Daniel J Cosgrove

Expansins are extracellular proteins that loosen plant cell walls in novel ways. They are thought to function in cell enlargement, pollen tube invasion of the stigma (in grasses), wall disassembly during fruit ripening, abscission and other cell separation events. Expansins are encoded by two multigene families and each gene is often expressed in highly specific locations and cell types. Structural analysis indicates that one expansin region resembles the catalytic domain of family-45 endoglucanases but glucanase activity has not been detected. The genome projects have revealed numerous expansin-related sequences but their putative wall-loosening functions remain to be assessed.

Addresses

Department of Biology, 208 Mueller Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802, USA;
e-mail: dCosgrove@psu.edu

Current Opinion in Plant Biology 2000, **3**:73–78

1369-5266/00/\$ – see front matter © 2000 Elsevier Science Ltd.
All rights reserved.

Abbreviations

CIM1 cytokinin-induced mRNA 1
EST expressed sequence tag
HFD His–Phe–Asp

Introduction

As plant cells mature, they transform themselves into a variety of differentiated cell types with unique shapes, sizes and structural properties fitting to their final station in life. This transformation requires a major remodeling of the cell wall, in which new structural polymers are added to the old and the wall is reshaped by selective yielding to the mechanical forces generated by cell turgor pressure. Such yielding is mediated, at least in part, by expansins and occurs in a pattern that is distinctive for each cell type. This short review focuses on the latest insights concerning the biological functions and possible mechanism of action of expansins.

Recent reviews [1•,2] have detailed the discovery and properties of expansins, which were first identified in studies of wall extension [3,4]. What is characteristic and unique about these proteins is their ability to induce cell wall relaxation and extension in isolated cell walls. This is readily observed by adding expansin to walls that have been given a brief heat pretreatment to eliminate their endogenous extension ability. This so-called wall-loosening activity is maximal at acid pH and is thought to be the underlying basis for ‘acid growth’, that is, the stimulation of cell enlargement by acidic buffers, by fusicoccin, and, at least in part, by auxin. Recent work [5•] concludes that initiation of root hairs in *Arabidopsis* also requires a localized acidification of the cell wall and thus initiation is likely to

be mediated by local activation of expansins to cause the initial outgrowth of the outer epidermal cell wall.

At present we recognize two families of expansin genes, termed α -expansins and β -expansins. I will first discuss the α family and then move on to the newer and relatively unexplored family of β -expansins.

The α -expansins

Arabidopsis currently affords us the best inventory of expansin genes, where we have identified 22 distinct α -expansin genes and anticipate that the completed genome will disclose ~25 members of this family (see the expansin web site URL: <http://www.bio.psu.edu/expansins/>). In other species, there also appear to be many members of the α -expansin gene family. For instance, in tomato eleven α -expansin genes have been identified to date [6•,7••], in tobacco six are now identified [8], in *Rumex palustris* six α -expansins are identified in Genbank (Accession numbers AF167356–AF167364), and so on (see the expansin web site for detailed lists). No doubt the gene numbers for these species will increase as their genomes are revealed in greater depth.

Recent studies confirm the function of these proteins as wall-loosening agents and extend this idea. The α -expansin proteins extracted from rice internodes possess wall-loosening activities very similar to those extracted from cucumber [9,10] and expression of these genes is highest in growing and differentiating cells, particularly in cells with thickened walls that are likely to require higher loosening activity in order to expand [11]. Under conditions that stimulate rice internode elongation (e.g. submergence and gibberellin application), the mRNA for one of these genes (*Os-EXP4*) increases in abundance prior to onset of increased wall extensibility and faster growth [12]. These studies of deepwater rice thus support the role of α -expansin in cell elongation.

Other studies, although less detailed, also support this idea. In elongating cotton fibers, an expansin gene is expressed maximally during the time of fast cell elongation [13,14]. In *Zinnia*, three specific expansin mRNAs accumulate during xylem cell elongation and differentiation [15]. The presence of large numbers of expansin expressed sequence tag (EST) clones in a poplar cDNA library made from wood-forming tissue [16] likewise suggests that expansins play a prominent role in xylem development. Similarly, adventitious rooting in pine seedlings is associated with early induction of expression (by ~100-fold) of a specific α -expansin gene, which is inducible by auxin [17•]. It is notable that the protein sequence of this pine expansin is very similar to that of expansins from flowering plants, representing a period of evolutionary divergence of

at least 250 million years. The high conservation of expansin sequences found in the water fern *Marsilea* [18] and the liverwort *Marchantia* (R Carey, DJ Cosgrove, unpublished data) indicates that the process of expansin-mediated cell enlargement dates back to the earliest lineages of land plants. The yeast genome does not, however, contain any sequences related to expansin, and so this mechanism of wall expansion probably evolved in the algal lineage that gave rise to land plants. It will be of interest to see whether expansins can be found in the extant Characean line of green algae most closely related to the algal progenitors of all land plants.

In the shoot apical meristem of tomato, an α -expansin gene is specifically and locally expressed at the future site of leaf primordium emergence [7••]. This may be one of the earliest molecular markers identified so far for primordium initiation. This work is complemented by two reports showing that localized application of cucumber expansins to the incipient leaf primordium can induce premature outgrowth of the primordium and a reversal in phyllotaxy [19•,20]. These observations were interpreted as supportive of Green's hypothesis [21] that the pattern of leaf initiation on the surface of the meristem depends on physical stresses within the meristem that inevitably arise during the growth of older primordia. According to this idea, a cell's genetic machinery takes its cues about cellular position and developmental timing from the pattern of physical forces impinging on the meristem, which in turn is a consequence of growth that is regulated by the pattern of expressed genes. This is simply a regulatory feedback loop that is proposed to create a self-generating and stable pattern of spatial and temporal control of gene expression and growth that is characteristic of the shoot apical meristem. It is clear from newer work, however, that the primordium that is accelerated by expansin application is often abnormal in morphology [19•]. This might be because the spatial distribution of expansin, when applied exogenously, does not adequately mimic the normal pattern of expansin expression. It is also likely that growth perturbations induced by expansin application lead to confused chemical signaling between different parts of the meristem, for example via the CLAVATA1/CLAVATA3 signaling system [22]. A defect in either of these genes causes meristem enlargement and fasciation, apparently by disrupting the normal signaling between the tunica and the corpus.

The expression patterns for several other tomato α -expansin genes have been characterized during fruit development [6•]. In this material, several α -expansins are selectively expressed during the growth and early maturation of the fruit and are not expressed in other organs (except for one of the expansins, which also appears in stems). When the fruit reaches the later ripening stages, most of these growth-stage α -expansin genes turn off and another gene (*EXPI*) begins to be expressed at relatively high levels. Although the first report on the tomato *EXPI* gene [23] suggested that fruit

expansin genes might be divergent in sequence and have a specialized biochemical function, newer sequence data have not borne out this suggestion. The tomato *EXPI* sequence is not particularly divergent from many other expansin sequences that are now in Genbank, and other expansin genes that are expressed in ripening fruit (e.g. apricot, Genbank Accession number U93167) are equally unremarkable in terms of their sequence. Nonetheless, the expression of an expansin specifically during the stages of fruit softening and wall breakdown suggests that expansins might function in cell wall disassembly [24•].

In *Arabidopsis* we have begun to examine expression of the many expansin genes by using their promoters to drive expression of reporter genes, and so far the results are very intriguing ([25]; DJ Cosgrove, unpublished data). Judging from the eight genes assessed to date, expansin genes are expressed in specific cell types and at specific developmental stages: for example, the *AtEXPI* gene is expressed specifically in stomatal guard cells; another expansin gene is expressed specifically in emerging root hairs; two other expansins are restricted to the vascular bundles; another expansin is expressed in midvein cortical cells; while yet another is expressed in root caps, and in other specific places in the root. The expression results indicate that many expansin genes are expressed in growing cells but others are expressed in cells with unusual cell wall properties (e.g. guard cells) or in places where cell wall slippage or movement might be particularly important. A large number of expansin genes appear to be needed to regulate the growth of the many different cell types that make up the plant body and perhaps to participate in other wall loosening events for cell separation and disassembly.

The new family of β -expansins

When expansins were first cloned and sequenced, a distant sequence similarity to group-1 grass pollen allergens was noted [26]. These allergenic proteins had been studied intensively by immunologists but their native biological functions were unknown. Follow-up work showed that *Zea m1*, the group-1 allergen from maize pollen, induces wall extension and stimulates wall stress relaxation in a pH-dependent manner, that is, it has classical expansin activity [27]. *Zea m1* and orthologous group-1 pollen allergens in other grasses differ from 'classical' expansins (now called α -expansins) in several important ways: they are highly abundant proteins in pollen; they show weaker binding to cell walls; and, although they loosen grass cell walls they have little or no effect on walls from dicots and other species with 'Type 1' walls. In contrast, α -expansin proteins studied to date are expressed in very low abundance, they stick very tightly to cell walls, and they are less effective on grass ('Type 2') walls as compared with dicot cell walls. ('Type 1' walls are typical of most land plants except for grasses, which possess 'Type 2' walls that have a different makeup of matrix polymers; see [28].) This difference in selectivity suggests that *Zea m1* acts on a structure or polymer that is unique to grass cell walls, prime candidates being glucuronarbinoxylans

and β 1-3,1-4 glucan [28]. The properties and expression pattern for *Zea m1* make it seem likely that this protein functions to loosen the walls of the grass stigma and style, to aid pollen tube invasion of the maternal tissues.

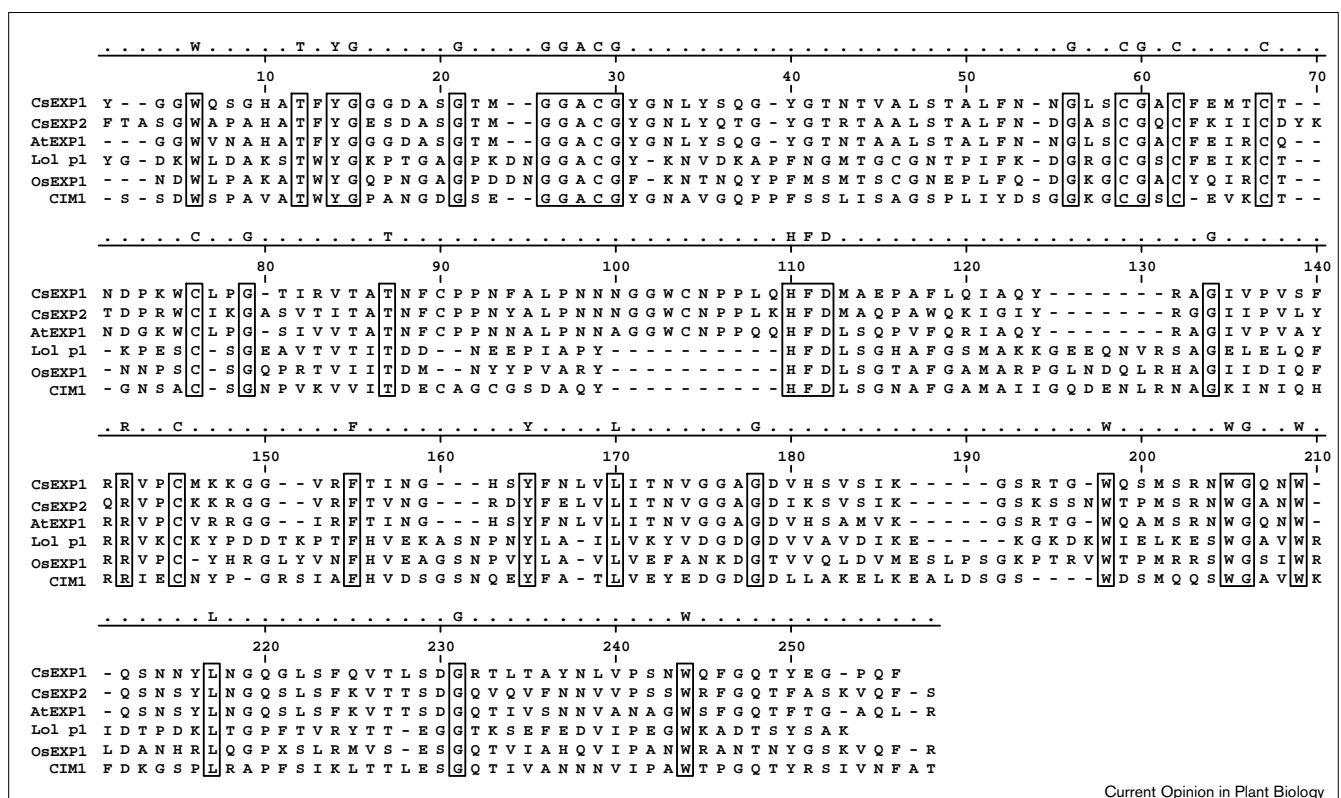
In addition to the rather unusual group-1 allergens, which are exclusively expressed in pollen, grasses also express a series of closely related genes in vegetative tissues [27]. Maize has at least eight β -expansin genes, most of which are expressed in vegetative tissues [29]. In the rice EST database, at least 10 distinct genes, represented by 75 EST entries, may be classified as β -expansins on the basis of sequence similarity (DJ Cosgrove, unpublished data). The proteins encoded by these genes have the signature motifs that we use to recognize expansins (Figure 1), and so we hypothesize that they have a wall-loosening function for vegetative tissues; however, studies of the proteins themselves are needed to establish this point experimentally and to contrast their wall-loosening activities with those of *Zea m1* and with α -expansins.

Although β -expansin genes are particularly numerous in grasses, they are also found in dicots, albeit in reduced numbers. In *Arabidopsis*, we have identified three

β -expansin genes, as compared with 22 α -expansin genes (DJ Cosgrove, unpublished data). Some β -expansins appear in the tomato EST database, but their function is unexplored. A soybean β -expansin was originally identified as a cytokinin-induced mRNA1 (CIM1) in cell cultures [30], and recent work shows that cytokinin increases CIM1 message stability [31]. It seems likely that expression of CIM1 is part of the mechanism by which cytokinin induces cell proliferation; however, the presumptive wall-loosening activity of CIM1 and other β -expansins needs to be confirmed by *in vitro* assays of the proteins.

A β -expansin mRNA (Genbank Accession number U91981) from barley roots was recently discovered during a study of genes that are upregulated by exposure to toxic levels of aluminum. Expression of this protein in yeast conferred aluminum tolerance to the yeast (E Delhaize, personal communication). In this context, it is interesting to note that early work on expansins found aluminum ions to be the most potent inhibitor of expansin activity [4,32]. Aluminum may inhibit root growth, in part, by poisoning expansins; however, the significance of this observation for barley root growth and its response to aluminum remains uncertain.

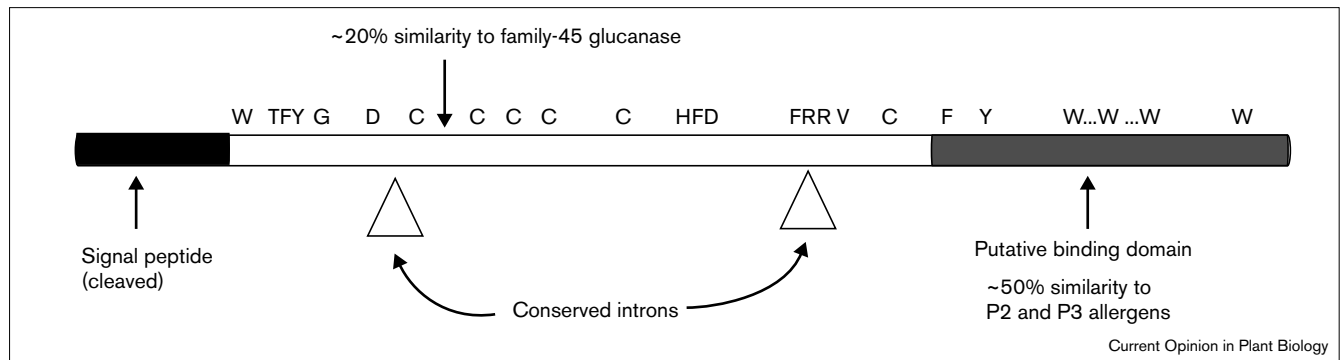
Figure 1



Alignment of α -expansins and β -expansins reveals the limited amino-acid residue sequence conservation characteristics of expansins (single letter code for amino acids used throughout). The top three sequences are α -expansins from cucumber (CsEXP1, CsEXP2) and *Arabidopsis* (AtEXP1); the bottom three sequences are β -expansins from rye grass (LO1 p1), rice (OsEXP1), and soybean (CIM1). Strictly

conserved residues are enclosed in boxes and listed at the top of the alignment. We use the conserved cysteines (C), tryptophans (W), and the HFD motif at position 110 as the key signatures of the expansin family. An FRRV motif or a closely related sequence, starting at position 140 in the alignment, is also typically found in most expansins, as is a T(F/W)YG motif starting at position 10.

Figure 2



Schematic diagram of the putative domain structure of expansins, with a signal peptide, a central domain that resembles family-45 glycosyl hydrolases (glucanase), and a carboxyl terminus with resemblance to cellulose-binding domains. Conserved residues are indicated in the

single letter code for amino acids. In the pollen allergen class of β -expansins, this carboxyl terminus also has sequence similarity to the group-2 (P2) and group-3 (P3) allergens. Also shown is the position of two introns conserved in most α -expansin and β -expansin genes.

In addition to the family of β -expansins, there are a number of other sequences in Genbank that are more distantly related to expansins, but they lack one or more of the signature motifs that are shared by α -expansins and β -expansins. We've identified five such 'expansin-related' sequences in *Arabidopsis*. Some expansin-related genes are expressed in tumors or in embryonic tissues [33], implying a possible role in cell proliferation, but whether they have wall-loosening functions characteristic of expansins needs to be determined experimentally.

How do expansins increase wall extensibility?

Current evidence indicates that wall loosening by expansin is not mediated by hydrolysis of wall polysaccharides [1•]. Instead, a novel mechanism of local disruption of polysaccharide adhesion is proposed as the means by which expansins induce the slow extension (creep) of cell walls. An inchworm-like movement is imagined, in which expansin enables the local sliding of wall polymers by reducing adhesion between adjacent wall polysaccharides (e.g. xyloglucan adhesion to the cellulose surface, or perhaps between matrix polymers not immediately in contact with the cellulose surface). A recent nuclear magnetic resonance (NMR) study of cucumber hypocotyl walls was unable to detect a difference in wall polymer mobility when expansins were active or inactive in the wall [34]. Two explanations are likely: first, the increased polymer mobility induced by expansin might be on a time scale that is out of range (longer times) than the NMR methods could evaluate; and second, the low abundance and local action of expansin in the wall means that at any given instant only a small fraction of the wall polysaccharides are in contact with expansin. It is presumably this minor fraction that has higher mobility, and its detection might be masked by the much larger fraction of polysaccharides that is not in contact with expansin (and is thus less mobile).

In a notable study, Grobe *et al.* [35] report that Phl p1, the group-1 allergen from timothy grass, might have protease

activity, and they suggest that such activity might account for expansin's wall loosening activity. They found that recombinant Phl p1, expressed in *Pichia*, was highly unstable and was rapidly degraded. In crude *Pichia* cultures expressing Phl p1, high protease activity was present that was suppressed by inhibitors of serine proteases and cysteine proteases. The authors make a case that the sequence for Phl p1 is highly similar to the C1 family of cysteine proteases; however, I have run a statistical test of the alignments presented by these authors, using the BLAST2 program for alignment of two sequences [36], and the results indicate that the alignment is not statistically significant. Orthologs of Phl p1 have been studied for many years (e.g. Lol p1 from rye grass pollen, and many others) and these proteins are noted for their stability. Our own experience with Zea m1, the maize ortholog of Phl p1, likewise indicates that this protein is highly stable. Grobe *et al.* [35] reported that protease activity of native Phl p1 was negligible but that activation with an overnight treatment at low pH under reducing conditions increased proteolytic activity. We have tested these conditions with purified Zea m1, but have not been able to detect proteolytic activity (L-C Li, DJ Cosgrove, unpublished data).

Another significant point is that several proteases were tested previously for expansin-like wall loosening activity, including papain (a C1 cysteine protease), and the results failed to give any indication that they caused wall extension [32]. Finally, expansins cause loosening action in pure cellulosic papers [37], a result that would be very hard to explain in terms of proteolytic activity. Thus, the provocative conclusions of Grobe *et al.* [35] need further examination.

Figure 2 shows our current model of the triple domain structure of the expansin protein. At the amino terminus is a signal peptide (~25 amino-acid residues) which is cleaved to form the mature protein of ~225 residues. The carboxyl terminus of the protein has some similarity to the cellulose-binding domains found in some microbial

cellulases — most notably in the spacing of the highly conserved tryptophans, which are particularly important in protein–carbohydrate binding [38]. We suspect that this region forms a distinct domain because in group-1 allergens this region has high sequence similarity to a group of small (11–13 kDa) proteins known as grass pollen group-2 allergens [39]. Their function is unknown, but a structure for one of them has been solved (PDB #1WHO) and I have been able to use this structure to model the carboxyl terminus of the *Zea m1* protein (DJ Cosgrove, unpublished data). The conserved tryptophans are on the surface of the protein and surfaces of this protein resemble polysaccharide-binding surfaces of cellulose-binding domains. This portion of the protein thus might form a distinct domain that is responsible for expansin binding to the cell wall.

The remaining portion of the protein, containing the highly conserved cysteines and the HFD (His–Phe–Asp) motif, has about 20% identity to the catalytic domain of an obscure *Trichoderma* endoglucanase [40], which is classified as a family-45 endoglucanase. Statistical tests with the BLAST2 program indicate highly significant sequence similarity between expansins and this endoglucanase (probability = 2×10^{-6}). Most notably, the two key aspartate residues thought to function in the enzyme's catalytic site are readily identified as conserved features of both α -expansins and β -expansins [41]. Recognition of this structural similarity gave us renewed impetus to look for glucanase activity in expansins but our results continue to indicate that the unique loosening activity of expansin is not due to glucanase activity [42]. The conservation of the catalytic site remains a tantalizing enigma.

In another vein, Ceccardi *et al.* [43] reported that a small (12 kDa), soluble protein (called p12), which accumulates in the leaves and stems of plants infected by citrus blight, has distant structural similarity to α -expansin. This protein is 29% identical to the amino terminus of *Arabidopsis* EXP1, but the conserved residues do not match those that are conserved between α -expansins and β -expansins (Figure 1) and do not match those shared between expansins and family-45 endoglucanases. Thus, p12 does not resemble the catalytic domain described above; moreover, in wall-extension assays p12 did not exhibit wall-loosening activity. Thus the relatedness of p12 to expansin function is uncertain. BLAST searches (DJ Cosgrove, unpublished data) indicate that *Arabidopsis* has p12-like genes and their sequence indicates they are extracellular—like the citrus p12 protein — but their biological role is still speculative.

Conclusions

The flood of gene sequences from *Arabidopsis*, rice, tomato, maize and other plants is providing lots of information about expansin genes and other sequences related to expansins. We can infer much from the sequence and from patterns of gene expression, but functional tests by *in vitro* assays and gene knockouts are needed to test

these educated inferences and to assign specific roles for members of this protein superfamily.

Note added in proof

Additional evidence of the role of expansin in tomato fruit softening is provided by a study [44] in which LeEXP1 gene expression in transgenic tomato plants was either increased or reduced. Transgenic plants that overexpressed LeEXP1 had softer fruit, whereas underexpressors had firmer fruit, than wild-type plants. Complex changes in wall polymer metabolism were also noted.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Cosgrove DJ: **Enzymes and other agents that enhance cell wall extensibility.** *Annu Rev Plant Physiol Plant Mol Biol* 1999, **50**:391-417.
- Many enzymes have the ability to digest components of the plant cell wall but is this activity synonymous with the ability to cause wall extension? This review assesses this question, which has implications for the structure of the wall.
2. Cosgrove DJ: **Cell wall loosening by expansins.** *Plant Physiol* 1998, **118**:333-339.
3. Li Z-C, Durachko DM, Cosgrove DJ: **An oat coleoptile wall protein that induces wall extension *in vitro* and that is antigenically related to a similar protein from cucumber hypocotyls.** *Planta* 1993, **191**:349-356.
4. McQueen-Mason S, Durachko DM, Cosgrove DJ: **Two endogenous proteins that induce cell wall expansion in plants.** *Plant Cell* 1992, **4**:1425-1433.
5. Bibikova TN, Jacob T, Dahse I, Gilroy S: **Localized changes in apoplastic and cytoplasmic pH are associated with root hair development in *Arabidopsis thaliana*.** *Development* 1998, **125**:2925-2934.
- With confocal microscopy, the pH of the wall at the future site of root hair emergence is shown to decrease to 4.5 – ideal for expansin activation.
6. Brummell DA, Harpster MH, Dunsmuir P: **Differential expression of expansin gene family members during growth and ripening of tomato fruit.** *Plant Mol Biol* 1999, **39**:161-169.
- At least six α -expansin genes are expressed in tomato fruits, with staggered and overlapping periods of expression.
7. Reinhardt D, Wittwer F, Mandel T, Kuhlmeier C: **Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem.** *Plant Cell* 1998, **10**:1427-1437.
- Using confocal microscopy the authors show that a specific α -expansin gene is expressed at a site in the shoot apical meristem that predicts the future emergence of a leaf primordium.
8. Link BM, Cosgrove DJ: **Acid-growth response and α -expansins in suspension cultures of bright yellow 2 tobacco.** *Plant Physiol* 1998, **118**:907-916.
9. Cho HT, Kende H: **Expansins in deepwater rice internodes.** *Plant Physiol* 1997, **113**:1137-1143.
10. Cho HT, Kende H: **Expansins and internodal growth of deepwater rice.** *Plant Physiol* 1997, **113**:1145-1151.
11. Cho HT, Kende H: **Tissue localization of expansins in rice.** *Plant J* 1998, **15**:805-812.
12. Cho HT, Kende H: **Expression of expansin genes is correlated with growth in deepwater rice.** *Plant Cell* 1997, **9**:1661-1671.
13. Orford SJ, Timmis JN: **Specific expression of an expansin gene during elongation of cotton fibres.** *Biochim Biophys Acta Gene Struct Expression* 1998, **1398**:342-346.
14. Shimizu Y, Aotsuka S, Hasegawa O, Kawada T, Sakuno T, Sakai F, Hayashi T: **Changes in levels of mRNAs for cell wall-related enzymes in growing cotton fiber cells.** *Plant Cell Physiol* 1997, **38**:375-378.

15. Im KH, Jones AM, Cosgrove DJ: **Expression of expansin genes during tracheary element differentiation in *Zinnia elegans*.** Abstract 190 of *Ann Meeting Amer Soc Plant Physiol*: 1999 July 24-28; Baltimore (available on-line at URL <http://www.rycomusa.com/aspp1999/public/>).
16. Sterky F, Regan S, Karlsson J, Hertzberg M, Rohde A, Holmberg A, Amini B, Bhalerao R, Larsson M, Villarreal R *et al.*: **Gene discovery in the wood-forming tissues of poplar: analysis of 5,692 expressed sequence tags.** *Proc Natl Acad Sci USA* 1998, **95**:13330-13335.
17. Hutchison KW, Singer PB, Diaz-Sala C, Greenwood MS: **Expansins are conserved in conifers and expressed in response to exogenous auxin.** *Plant Physiol* 1999, **120**:827-832.
Induction of adventitious rooting in pine seedlings leads to 100-fold increase in expression of an α -expansin that is highly conserved with angiosperm expansins.
18. Kim J-H, Cho HT, Kende H: **Presence and expression of an expansin gene in the fern *Marsilea quadrifolia*.** Abstract 994 of *Ann Meeting Amer Soc Plant Physiol*: 1999 July 24-28; Baltimore (available on-line at URL <http://www.rycomusa.com/aspp1999/public/>).
19. Fleming AJ, Caderas D, Wehri E, McQueen-Mason S, Kuhlemeier C: **Analysis of expansin-induced morphogenesis on the apical meristem of tomato.** *Planta* 1999, **208**:166-174.
When tiny beads pre-soaked in solutions containing cucumber α -expansins are placed on the shoot apical meristem of tomato at the site of future leaf primordium emergence, a premature outgrowth occurs in ~20% of the experiments, resulting in a reversal in phyllotaxy. The outgrowths are reminiscent of a primordium but have abnormal structure and do not form normal leaves.
20. Fleming AJ, McQueen-Mason S, Mandel T, Kuhlemeier C: **Induction of leaf primordia by the cell wall protein expansin.** *Science* 1997, **276**:1415-1418.
21. Green PB: **Expansin and morphology: a role for biophysics.** *Trends Plant Sci* 1997, **2**:365-366.
22. Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM: **Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems.** *Science* 1999, **283**:1911-1914.
23. Rose JKC, Lee HH, Bennett AB: **Expression of a divergent expansin gene is fruit-specific and ripening-regulated.** *Proc Natl Acad Sci USA* 1997, **94**:5955-5960.
24. Rose JKC, Bennett AB: **Cooperative disassembly of the cellulose xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening.** *Trends Plant Sci* 1999, **4**:176-183.
This review makes the case that much of the same enzymatic machinery involved in cell wall enlargement is also used in softening of fruit.
25. Durachko DM, Cosgrove DJ: **Expression patterns for selective expansin genes in *Arabidopsis*.** Abstract 56 of *Ann Meeting Amer Soc Plant Physiol*: 1999 July 24-28; Baltimore (available on-line at URL <http://www.rycomusa.com/aspp1999/public/>).
26. Shcherban TY, Shi J, Durachko DM, Guiltinan MJ, McQueen-Mason S, Shieh M, Cosgrove DJ: **Molecular cloning and sequence analysis of expansins – a highly conserved, multigene family of proteins that mediate cell wall extension in plants.** *Proc Natl Acad Sci USA* 1995, **92**:9245-9249.
27. Cosgrove DJ, Bedinger P, Durachko DM: **Group I allergens of grass pollen as cell wall-loosening agents.** *Proc Natl Acad Sci USA* 1997, **94**:6559-6564.
28. Carpita NC: **Structure and biogenesis of the cell walls of grasses.** *Annu Rev Plant Physiol Plant Mol Biol* 1996, **47**:445-476.
29. Wu Y, Cosgrove DJ: **Expression of expansin genes in drought-stressed maize roots. Part II: expression pattern of 14 expansins in well-watered maize plants.** Abstract 352 of *Ann Meeting Amer Soc Plant Physiol*: 1999 July 24-28; Baltimore (available on-line at URL <http://www.rycomusa.com/aspp1999/public/>).
30. Crowell DN: **Cytokinin regulation of a soybean pollen allergen gene.** *Plant Mol Biol* 1994, **25**:829-835.
31. Downes BP, Crowell DN: **Cytokinin regulates the expression of a soybean β -expansin gene by a post-transcriptional mechanism.** *Plant Mol Biol* 1998, **37**:437-444.
32. Cosgrove DJ: **Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls.** *Planta* 1989, **177**:121-130.
33. Michael AJ: **A cDNA from pea petals with sequence similarity to pollen allergen, cytokinin-induced and genetic tumour-specific genes: identification of a new family of related sequences.** *Plant Mol Biol* 1996, **30**:219-224.
34. Fenwick KM, Apperley DC, Cosgrove DJ, Jarvis MC: **Polymer mobility in cell walls of cucumber hypocotyls.** *Phytochem* 1999, **51**:17-22.
35. Grobe K, Becker WM, Petersen A: **Grass group I allergens (β -expansins) are novel, papain-related proteinases.** *Eur J Biochem* 1999, **263**:33-40.
36. Tatusova TA, Madden TL: **Blast 2 sequences - a new tool for comparing protein and nucleotide sequences.** *FEMS Microbiol Lett* 1999, **174**:247-250.
37. McQueen-Mason S, Cosgrove DJ: **Disruption of hydrogen bonding between wall polymers by proteins that induce plant wall extension.** *Proc Natl Acad Sci USA* 1994, **91**:6574-6578.
38. Linder M, Teeri TT: **The roles and function of cellulose-binding domains.** *J Biotechnol* 1997, **57**:15-28.
39. Dolecek C, Vrtala S, Laffer S, Steinberger P, Kraft D, Scheiner O, Valenta R: **Molecular characterization of Phl p II, a major timothy grass (*Phleum pratense*) pollen allergen.** *FEBS Lett* 1993, **335**:299-304.
40. Saloheimo A, Henrissat B, Hoffren AM, Teleman O, Penttilä M: **A novel, small endoglucanase gene, egl5, from *Trichoderma reesei* isolated by expression in yeast.** *Mol Microbiol* 1994, **13**:219-228.
41. Davies GJ, Tolley SP, Henrissat B, Hjort C, Schulein M: **Structures of oligosaccharide-bound forms of the endoglucanase V from *Humicola insolens* at 1.9 Å resolution.** *Biochemistry* 1995, **34**:16210-16220.
42. Cosgrove DJ, Durachko DM, Li L-C: **Expansins may have cryptic endoglucanase activity and can synergize the breakdown of cellulose by fungal cellulases.** Abstract 171 of *Ann Meeting Amer Soc Plant Physiol*: 1998 July 27-July 1; Vancouver, BC (available on-line at URL <http://www.sheridan.com/aspp98/abs/abs/32/0689.html>).
43. Ceccardi TL, Barthe GA, Derrick KS: **A novel protein associated with citrus blight has sequence similarities to expansin.** *Plant Mol Biol* 1998, **38**:775-783.
44. Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, Dunsmuir P: **Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening.** *Plant Cell* 1999, **11**:2203-2216.