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Update on Cell Growth

Cell Wall Loosening by Expansins[1]

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In his 1881 book, *The Power of Movement in Plants*, Darwin described a now classic experiment in which he directed a tiny shaft of sunlight onto the tip of a grass seedling. The region below the coleoptile tip subsequently curved toward the light, leading to the notion of a transmissible growth stimulus emanating from the tip. Two generations later, follow-up work by the Dutch plant physiologist Fritz Went and others led to the discovery of auxin. In the next decade, another Dutchman, A.J.N. Heyn, found that growing cells responded to auxin by making their cell walls more "plastic," that is, more extensible. This auxin effect was partly explained in the early 1970s by the discovery of "acid growth": Plant cells grow faster and their walls become more extensible at acidic pH. Auxin was hypothesized to stimulate growth, in part, by inducing plant cells to acidify their extracellular space. How an acidic pH makes walls more extensible was unclear until 1992, when the proteins that catalyze this process (later named "expansins") were identified. Expansins alone can induce cell walls to extend, but in living cells they probably act in concert with a variety of enzymes that cut and restructure the wall. In this *Update* I will discuss expansin in the context of plant growth and describe emerging concepts about its roles in plant development.

GROWING CELL WALLS ARE PLIANT AND EXTEND AT ACIDIC PH

Growing cell walls differ from mature walls in many ways. They are generally thinner, have a different polymer composition, are not highly cross-linked by covalent bonds, and are pliant and easily deformed by mechanical forces. Such wall pliancy is important for growing cells, because the wall surface must enlarge as the cell grows. The pliancy of growing walls is special in that it enables prolonged wall extension ("creep") and stress relaxation. Wall stress relaxation reduces cell turgor and thereby creates the driving forces needed for water uptake by growing cells. It is the key physical process limiting cell enlargement (Cosgrove, 1997).

Wall pliancy sounds simple, but its underlying molecular basis is complex. It seems to be due partly to polymer physics and partly to carefully controlled reactions that alter the bonding relationships of the wall polymers. The growing wall is a composite polymeric structure: a thin weave of tough cellulose microfibrils coated with heteroglycans (hemicelluloses such as xyloglucan) and embedded in a dense, hydrated matrix of various neutral and acidic polysaccharides and structural proteins (Bacic et al., 1988; Carpita and Gibeaut, 1993). Like other polymer composites, the plant cell wall has rheological (flow) properties intermediate between those of an elastic solid and a viscous liquid. These properties have been described using many different terms: plasticity, viscoelasticity, yield properties, and extensibility are among the most common. It may be attractive to think that wall stress relaxation and expansion are largely a matter of polymer physics, but many physiological experiments indicate that there is another level of control by the cell. Cell expansion can be stimulated or inhibited within seconds, without major changes in cell wall structure or viscoelastic properties (for review, see Cosgrove, 1993). This is not to say that wall structure is irrelevant for control of growth, but rather that growing cells can evidently regulate specific "loosening" processes that result in wall stress relaxation. The ensuing expansion of the wall is undoubtedly influenced by its structure and viscoelasticity.

A remarkable property of growing cell walls is their ability to extend at acidic pH (for review, see Rayle and Cleland, 1992). This ability may be observed in growing stem segments incubated in buffers, where they elongate faster as the pH is lowered below 5.5, or in isolated walls clamped in an extensometer, where they extend faster when the pH is reduced (Fig. 1). Mature walls lack this acid-induced extension. We focused our initial biochemical studies of wall extension on the cell wall of cucumber hypocotyls, which can extend for many hours when clamped at acid pH (Cosgrove, 1989). Because the cells are dead when they are clamped in the extensometer, this wall extension occurs without synthesis of additional wall polymers.

The in vitro wall extension is thought to be a type of polymer creep involving a shearing movement of the load-bearing polymers in the wall. This pH-dependent extension is known as acid growth and has been found in growing cells of angiosperms, gymnosperms, ferns, mosses, and even some green alga with walls that resemble

[1] This paper is dedicated to the memory of Paul B. Green (1931–1998), mentor, friend, and creative scientist.

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Abbreviations: EST, expressed sequence tag; XET, xyloglucan endotransglycosylase.

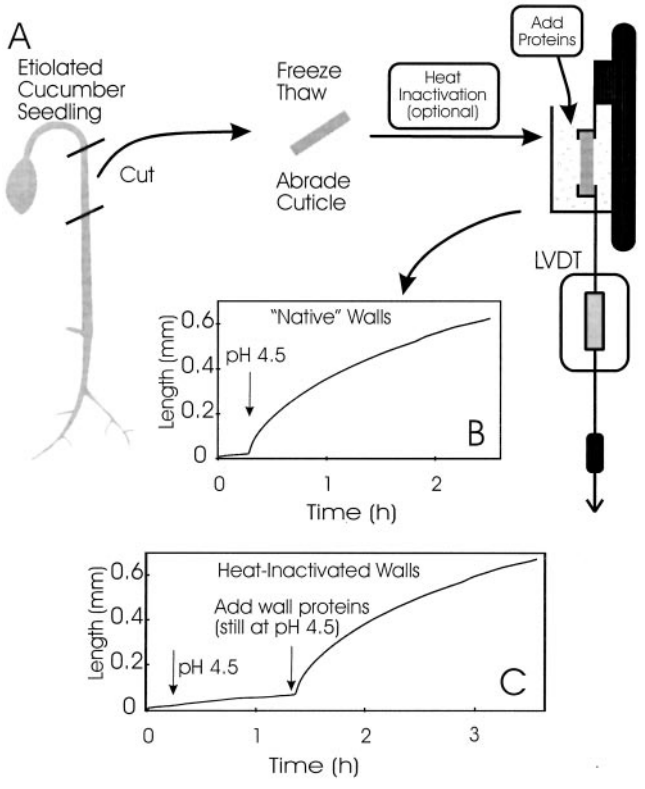

Figure 1. Assay of acid-induced extension in isolated cell wall specimens. A, Scheme for preparing wall specimens for clamping in the extensometer. The growing region of the cucumber hypocotyl was excised, frozen, thawed, and abraded to obtain "native" cell walls (i.e. the cells are dead, but the walls retain active enzymes). Extension of the wall is registered electronically by a position transducer (LVDT). B, Native walls begin extending when the buffer is changed from pH 6.8 to 4.5. C, Walls inactivated with a brief heat treatment lack acid-induced extension (first arrow), but this may restored by the addition of wall proteins containing expansin (second arrow).

plant walls in ultrastructure and polymer composition (e.g. *Nitella*). Acid growth is not simply a matter of the physical chemistry of the wall, e.g. dissolution of the pectin network at low pH, because treatment of the wall with proteases and various protein denaturants eliminates its ability for acid growth. This implies that a cell wall protein(s) acts as a catalyst for acid growth.

EXPANSINS MEDIATE pH-DEPENDENT CREEP OF CELL WALLS

To identify the protein catalysts of acid growth, we devised a reconstitution assay for wall extension (McQueen-Mason et al., 1992). Cucumber walls were first inactivated with a heat treatment to eliminate their endogenous acid-induced extension. They were then clamped in an extensometer and treated with protein extracted from growing cucumber hypocotyl cell walls. Crude protein extracts caused the walls to creep (Fig. 1C), and two proteins were shown to possess this activity. These proteins had a similar size (about 29 kD apparent M_r as shown by SDS-PAGE) and later work showed that they were isoforms with a high sequence similarity (Shcherban et al., 1995). Using this reconstitution approach, related proteins were identified in oat coleoptiles (Li et al., 1993), tomato leaves (Keller and Cosgrove, 1995), maize roots (Wu et al., 1996), and rice internodes (Cho and Kende, 1997a). These proteins, expansins, have an acidic pH optimum for induction of wall expansion, which is consistent with their hypothesized role as catalysts of acid growth.

In the cucumber hypocotyl expansins can account for most, if not all, of the acid-growth behavior of isolated walls. This conclusion is based on several results, including sensitivity to pH and to various chemical inhibitors and stimulants of wall creep, as well as the ability of exogenous expansins to restore the creep and stress-relaxation properties to heat-inactivated walls (McQueen-Mason et al., 1992; McQueen-Mason and Cosgrove, 1995). Of all the putative wall-loosening enzymes tested to date (e.g. endoglucanases, pectinases, and XET), only expansins cause isolated walls to creep and to show faster stress relaxation (McQueen-Mason et al., 1993; Cosgrove and Durachko, 1994; McQueen-Mason and Cosgrove, 1995).

EXPANSINS ARE NOVEL PROTEINS COMPRISING A LARGE SUPERFAMILY

When cDNAs for the two cucumber hypocotyls expansins were cloned and sequenced, we found that they encoded very similar and novel proteins (Shcherban et al., 1995). For each cucumber expansin, the primary translation product is predicted to be approximately 27 kD and includes a signal peptide of 22 or 23 amino acids. The signal peptide directs the protein into the secretory pathway and, upon excision of the signal peptide, the mature protein is predicted to be approximately 25 kD. This is slightly smaller than that originally estimated by SDS-PAGE, but matches more recent estimates of rice expansins (Cho and Kende, 1997a).

The expansin sequence is not homologous to any of the cell wall enzymes that have been previously cloned (e.g. various endoglucanases, pectinases, and pectin methyl esterases). Neither is it related to wall structural proteins such as Hyp-rich glycoproteins, which are also (perhaps inappropriately) called extensins. Indeed, the expansin sequences initially revealed virtually nothing about how these proteins make walls more extensible. There are no obvious motifs in the expansin sequence that suggest an enzymatic function.

Since expansins were first cloned from cucumber, many more expansin sequences have been added to the public databases and we have come to realize that they comprise a large superfamily with at least two major branches (Table I). The first branch, which we now refer to as α-expansins, includes the original cucumber expansins as well as homologs cloned from Arabidopsis, pea, *Brassica*, tomato, cotton, rice, and pine. In Arabidopsis, the α-expansin family currently encompasses 16 distinct genes, including unique classes of cDNA and genomic sequences in GenBank and unpublished sequences from my laboratory. Other plants have fewer representatives in GenBank, but this is most likely due to the limited sequencing efforts in

Table I. *Comparison of α- and β-expansins*

Characteristic	α-Expansins	β-Expansins
No. of genes in Arabidopsis	≥16	≥1
No. of genes in rice	≥4	≥7
Activity in dicot walls	High[a]	Low[b]
Activity in grass walls	Low[b]	High[b]

[a] Measured for α-expansins from cucumber, oat, maize, and rice. [b] Measured only with Zea m1.

these species. Four α-expansins are known in rice (Shcherban et al., 1995; Cho and Kende, 1997c). From the limited sequence data available, we can conclude that the α-expansins duplicated and diverged before the evolutionary split of the angiosperms into monocots and dicots, more than 150 million years ago. The derived amino acid sequences for α-expansin from pine hypocotyls has high sequence similarity with that from cucumber hypocotyls (82% identity and 92% similarity), indicating little divergence of this protein during 400 million years of evolution. This implies that there are strong structural constraints for expansin activity.

The second branch of the expansin tree is described below. It is made up of many GenBank representatives from the grass family, with far fewer known from dicots.

HOMOLOGY WITH GRASS POLLEN ALLERGENS REVEALS THE β-EXPANSIN FAMILY

For many years immunologists have studied a group of proteins from grass pollen known as the group-1 allergens. These proteins are copiously secreted by hydrating grass pollen and are strong elicitors of hay fever, seasonal asthma, and other immune responses in sensitive people (Knox and Suphioglu, 1996). Their biological function in the plant was entirely mysterious until we noted that they have approximately 25% amino acid identity with expansins. The two proteins are of similar size, and predictions of secondary structure indicate that they have homologous structures. These similarities hinted that the group-1 allergens are distant expansin homologs and may have expansin-like activity.

To test this prediction, we extracted maize pollen for Zea m1, the group-1 allergen of maize (Cosgrove et al., 1997). Extracts containing Zea m1 (but not α-expansin) had potent expansin activity, as measured in assays of pH-dependent wall creep and stress relaxation. The activity was selective for grass walls (e.g. maize silks and grass coleoptiles), with much less activity observed when dicot walls were used as the substrate. One isoform of Zea m1 was purified to homogeneity and shown to possess wall-creep activity. These results indicate that Zea m1 is a divergent expansin that acts with some selectivity on the grass cell wall. This conclusion leads to three further inferences.

First, the discovery of expansin activity in Zea m1 lets us identify a second branch of expansins, which we named the β-expansins. This branch of the expansin family consists of three types of sequences from GenBank: (a) group-1 allergens, a protein class that is highly expressed in grass pollen but not in other tissues or species (pollen allergens from ragweed and other species outside the grass family are not homologous with the group-1 allergens); (b) vegetative homologs of the group-1 allergens expressed in grass seedlings (not in pollen), at least seven of which are expressed in young rice plants, based on the EST database; and (c) vegetative homologs of the group-1 allergens expressed in dicots, which, judging from the EST databases, seem to be much less abundantly expressed in dicots than in grasses (we know of only one example from Arabidopsis, and a second homolog from soybean, CIM1, was originally identified in soybean cell cultures as a cytokinin-induced message; Crowell, 1994).

We infer that the α and β forms of expansin act on different polymers. This inference is based on the observation that Zea m1 is particularly effective on grass cell walls but not on dicot walls, whereas α-expansins show the opposite selectivity. This selectivity is true whether the α-expansin comes from dicots (e.g. cucumber) or grasses (e.g. oat and rice). Zea m1 is the only β-expansin tested to date, so the generality of β-expansin selectivity for grass walls needs further testing. Because the β-expansins seem to be particularly plentiful in grasses (both in gene number and in message abundance), it is a good guess that many of the β-expansins are specially tailored for action on wall polymers that are unique or unusually plentiful in grasses. The leading polysaccharide candidates are mixed-link β-glucans and arabinoxylans, which are abundant in grass walls but not in dicot walls (Carpita, 1996). Perhaps the β-expansins from dicots act on related polymers, but this needs to be tested experimentally.

A second implication of the discovery that Zea m1 has expansin activity relates to the structure/function analysis of expansin. The key regions important for the folding and the active site of these proteins are likely to be found in the small number of highly conserved residues shared by both α- and β-expansins. This set is limited (Fig. 2) and includes a series of conserved Cys residues that are likely involved in disulfide bridges within the protein, a series of Trp residues that may function in protein-polysaccharide binding, and an His-Phe-Asp motif. It is intriguing to note that the His-Phe-Asp motif and the Cys residues are also highly conserved in members of the family-45 group of glycosyl hydrolases (also known as family K cellulases), where the His-Phe-Asp motif forms part of the enzyme's active site (Davies et al., 1995). Although the sequence similarity is very low, it hints at a distant evolutionary relationship between expansins and these fungal cellulases (Cosgrove, 1997). We are currently studying this possibility further.

A third implication of the discovery that Zea m1 has expansin activity concerns the biological role of group-1 allergens during pollination. The two most obvious targets for this wall-loosening protein in vivo are the pollen tube wall and the walls of the stigma and style. When the properties of Zea m1 are compared with those of α-expansins, it seems that Zea m1 is particularly well suited for loosening stigma and style walls. Specifically, the α-expansins in the growing cucumber hypocotyl are relatively rare wall proteins with low solubility that stick tightly to cell walls. In contrast, Zea m1, like other group-1

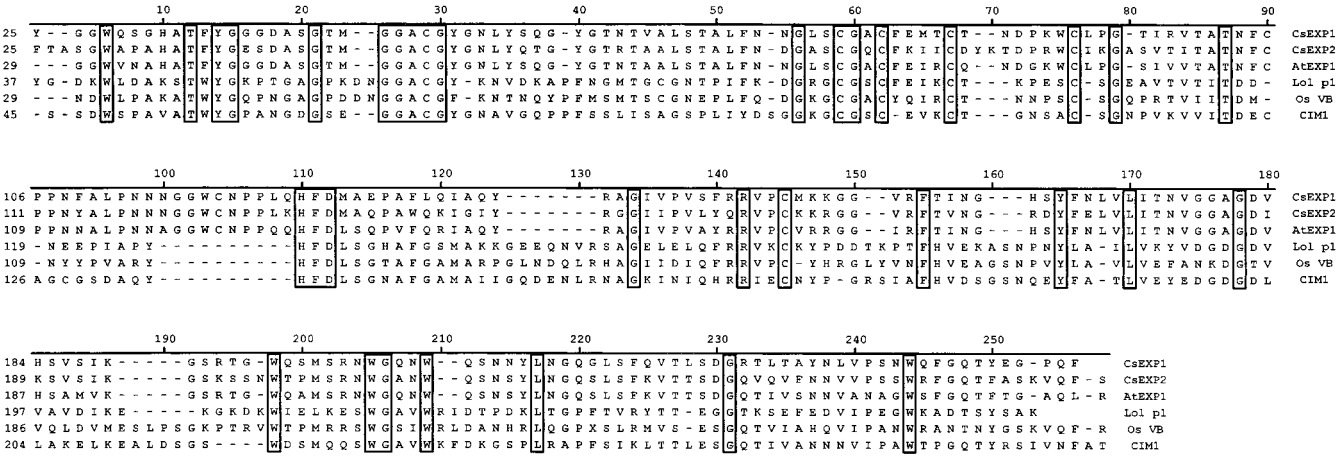

Figure 2. Alignment of α- and β-expansins using a single-letter code for amino acids. The first three sequences (CsEXP1, CsEXP2, and AtEXP1) are α-expansins; the lower three sequences (ClM1, Lol p1, and Os VB [rice vegetative β]) are β-expansins. Conserved regions are boxed. The nonconserved residues at the N terminus of the protein, including the signal peptide sequences, were removed for this alignment.

allergens, is copiously secreted by pollen, is highly soluble, and does not bind tightly to cellulose or cell walls. These properties are incongruous with a site of action restricted to the tiny growing tip of the pollen tube, but would be expected of proteins secreted to soften walls surrounding the pollen tube (Fig. 3). Zea m1 has a strong loosening effect on the walls of maize silks (the style and stigma of the maize flower), which supports this idea.

Anatomical studies of pollen tube growth in grasses have shown that the tube penetrates between tightly appressed cells, even tearing plasmodesmatal connections of adjacent cells, during its growth toward the ovule (Heslop-Harrison et al., 1984). The secretion of potent wall-loosening agents such as the group-1 allergens probably aids invasion of the pollen tube by softening cell walls of the maternal tissues (Cosgrove et al., 1997). It is still unclear, however, whether the secretion of Zea m1 continues throughout pollen tube growth to the ovule, or whether it is limited to the initial phase of pollen grain germination and pollen tube penetration of the stigma. Similarly, with regard to the β-expansins expressed in vegetative tissues, direct evidence is needed to determine their function in cell growth or in some other wall-loosening role.

WHY SO MANY EXPANSIN GENES?

The substrate specificity of Zea m1 suggests a partial answer to this question: that α-expansins and β-expansins act on different wall polymers. Because there seem to be so many β-expansins in the grasses (as represented in the rice EST database) and so few in dicots (as represented by Arabidopsis), I suspect that the β-expansin genes duplicated, diverged, and evolved special functions in the grasses, probably in concert with the evolution of the grasses' unusual wall biochemistry (Carpita, 1996). This hypothesis certainly appears to be the case for the group-1 allergens, in which the wall-loosening function of these expansins does not seem to be growth of the pollen tube but, rather, softening of the maternal tissues for faster pollen tube penetration.

If we tentatively accept that α- and β-expansins work on different wall polymers, we are still left with the question of why are there so many members in each family. Three plausible answers to this question come to mind. Each expansin may have a unique set of functional properties, e.g. substrate specificity or pH dependence, that is important for its biological role. There is limited evidence to support this idea; the two expansins characterized from cucumber hypocotyls have slightly different pH dependencies and effects on the stress-relaxation spectrum of cucumber walls (McQueen-Mason and Cosgrove, 1995). It is not clear, however, that these minor biochemical differences are important for their biological function. Perhaps such variation in expansin properties is related to the two growth mechanisms with different pH optima identified in oat coleoptiles (Cleland, 1992).

A second possibility is that the numerous expansin genes are expressed in unique patterns. The plant body is made up of many organs, tissues, and cell types, each of which requires a characteristic and highly precise pattern of cell enlargement, with differential control by various developmental signals, hormones, and the environment. If this hypothesis is correct, expansin proteins may be functionally equivalent (that is, within the α- or β-families), but the promoters should specify a unique pattern of expression for each gene. There is some evidence in support of this idea. For instance, the four α-expansin genes characterized in rice have different expression patterns in leaves, stems, and roots (Cho and Kende, 1997c). An α-expansin gene identified in cotton fibers is expressed specifically during the phase of maximum fiber elongation (Shimizu et al., 1997), whereas a tomato α-expansin (LeEXP1) is selectively expressed in ripening fruit but not in earlier stages of fruit growth or in other organs (Rose et al., 1997). Thus, it seems that expansin genes show differential patterns of expression.

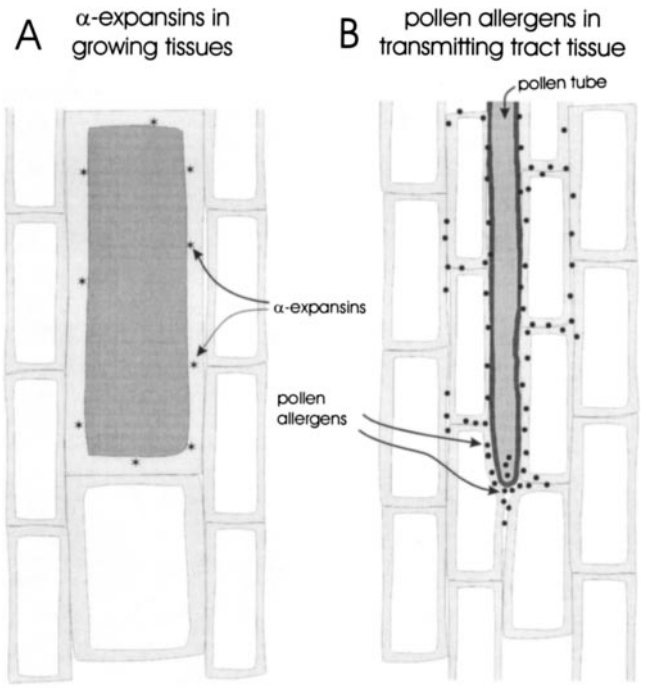

Figure 3. Diagram illustrating the different characteristics of α-expansins and the pollen-allergen subclass of β-expansins. A, The α-expansins in growing tissues are low-abundance proteins that bind tightly to cell walls and probably stick to the cell that secreted them. B, The group-1 allergens secreted by grass pollen are very abundant and highly mobile, and are probably secreted at the tip of the pollen tube and diffuse through the walls and intercellular spaces of the stigma and style-transmitting track in advance of the penetrating pollen tube.

A third possibility to account for the numerous expansins is that they are redundant, perhaps as protection against lethality in the event of malfunction of one of the genes. No examples of exact redundancies have yet come to light. In the cucumber hypocotyl, in which two expansins are expressed in a similar spatial pattern (Shcherban et al., 1995; M. Shieh, J. Shi, and D.J. Cosgrove, unpublished results), each gene is differently regulated by hormones and light. Likewise, the expansins expressed in rice stems show somewhat different patterns of regulation (Shcherban et al., 1995; Cho and Kende, 1997b). Thus, different expansin genes may partially overlap in expression, yet still show differential regulation. Such overlap would tend to reduce the phenotypic effects of genetic defects in specific expansin genes (i.e. a kind of partial redundancy).

It should be noted that these three hypotheses are not mutually exclusive, and some combination of all three may account for the many α-expansins in Arabidopsis.

THE PRECISE MOLECULAR MECHANISM OF EXPANSIN-INDUCED WALL CREEP IS STILL ENIGMATIC

Returning to the question of how expansins induce wall extension, when expansins were first discovered, the prevailing hypothesis was that endoglucanases and transglycosylases were the crucial enzymes that weakened the wall so as to permit it to extend (Carpita and Gibeaut, 1993). XET in particular received much attention as a potential wall-loosening enzyme (Fry et al., 1992). However, assays for endoglucanase, exoglycanase, pectinase, and related hydrolytic activities in purified expansin preparations were negative (McQueen-Mason and Cosgrove, 1995). Likewise, expansin preparations did not contain detectable XET activity (McQueen-Mason et al., 1993). Moreover, the rheological effects of expansins (i.e. induction of wall creep and stress relaxation) were not mimicked by wall hydrolases and XET (McQueen-Mason et al., 1993; Cosgrove and Durachko, 1994).

The results of previous experiments suggest that expansins do not act like classical enzymes (McQueen-Mason and Cosgrove, 1995). For example, expansin's effect on wall stress relaxation is reversible; i.e. expansin-treated walls relax faster than controls, but when the expansin is inactivated (e.g. by heat), the walls behave like controls that were not treated with expansin. This suggests that expansins do not alter the covalent structure of the wall; instead, their loosening effect is observed only while the wall is in tension. Similarly, pretreatment of the relaxed wall with expansins for varying periods of time did not lead to progressive (time-dependent) weakening of the wall. Two alternative hypotheses may account for these results. Enzymatic activity might occur only when the wall is in tension. Perhaps the wall polymers must be in an extended state to allow access of the protein to its point of action, or perhaps high strain energy in the glycosidic bonds of the load-bearing polysaccharides is needed for efficient enzymatic activity. An alternative hypothesis is that expansins disrupt noncovalent bonding of the wall polymers. For example, if expansins helped to release short segments of matrix glycans sticking to the cellulose microfibrils, then when the wall was in tension the glycans would release and re-bond to the microfibril in a kind of "inchworm" fashion, resulting in polymer creep (sometimes called "reptation").

Experimental evidence supports the reptation model, but the case is not ironclad. Expansins weakened pure cellulose papers without detectable hydrolysis (McQueen-Mason and Cosgrove, 1994) and, because paper strength is derived from hydrogen bonding of overlapping fibers, this was taken as evidence that expansins can disrupt hydrogen bonding of glucans to each other. Urea at a concentration of 2 M weakens the hydrogen bonding between wall polymers and doubles expansin-induced creep of cell walls. Urea on its own also has a modest creep effect on cell walls and affects the stress relaxation of paper in a way that partially resembles the action of expansins (cellulase, in contrast, has a negligible effect on stress relaxation). Finally, substitution of water with heavy water (deuterium oxide) reduces the creep rate by 36%. Because the hydrogen bond formed by deuterium is 20% stronger than that formed by hydrogen, an inhibition of creep would be expected if hydrogen bonding were involved in the adhesion of wall glycans to each other.

All of these results point to a nonenzymatic mode of action by expansins. On the other hand, the distant homology between family-45 glycosyl hydrolases and expansins (noted above) hints at a cryptic enzymatic activity.

The target of expansin action is of keen interest. Binding studies have shown that cucumber α-expansins bind tightly to the cell wall (McQueen-Mason and Cosgrove, 1995), and suggested that α-expansin binds at the interface between cellulose and a tightly bound hemicellulose or perhaps to the noncrystalline regions of the cellulose microfibril. In contrast to α-expansins, Zea m1 is easily washed from cell walls, so it is evident that tight binding by expansin is not essential for its action. Tight binding to the wall may be an important means to regulate growth on a cell-by-cell basis, thereby preventing interference by expansin diffusion from neighboring cells (Fig. 3). For expansins with other wall-loosening functions, greater mobility in the wall may be useful.

A MODEL OF WALL ENLARGEMENT AND ITS CONTROL

A tentative model of wall loosening by expansins and the potential involvement of other wall enzymes in cell wall enlargement is shown in Figure 4. In this view, expansins transiently displace short stretches of hemicelluloses that are bonded to the surface of the microfibril. If the wall is in tension, the polymers creep, inevitably dragging along other structural components of the wall. If the wall is relaxed, no polymer movement occurs. In this model other wall enzymes may have indirect effects on wall extension by altering the structure of the matrix. For example, wall hydrolases may shorten matrix polymers, reducing their viscous resistance to wall creep. Cross-linking of the matrix, e.g. by the action of peroxidases or pectin methyl esterases, may increase the size of the structural units that are passively dragged along as the wall creeps. This increase in size translates into greater resistance to creep. Given sufficient cross-linking, the wall is no longer extensible by expansins. This appears to occur when cells mature, i.e. as they leave the growth zone of hypocotyls and coleoptiles (Cosgrove and Li, 1993; McQueen-Mason, 1995).

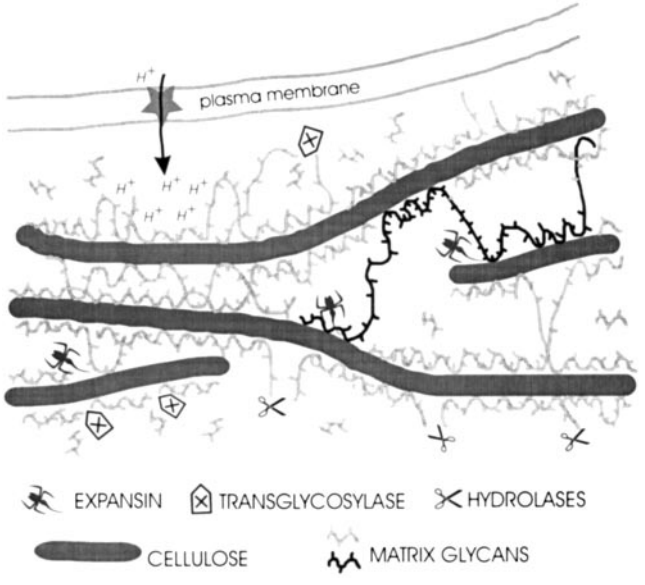

Figure 4. Simplified model of how expansins might interact with other wall components. The action of expansins is hypothesized to cause a transient release of a short segment of matrix glycans attached to cellulose microfibrils, with the result that the cellulose and matrix polymers slide relative to one another. Wall hydrolases such as endoglucanase cut matrix glucans into shorter segments. This may lead to weakening but not to creep of the cell wall. Transglycosylases such as XET can recombine glycans into shorter or longer pieces, depending upon conditions within the wall. H^+-ATPases in the plasma membrane (⋆) may lower the wall pH, thereby activating expansins and other enzymes with acidic optima and inactivating wall enzymes with neutral pH optima. For graphical simplicity, pectins and structural proteins are not shown in this figure, but they would fill the space between the microfibrils.

In this model there are several avenues by which cells might modulate their enlargement: changes in the pH of the wall to alter expansin activity, secretion of expansin to the wall, inactivation or degradation of expansins in the wall, wall hydrolase activities, wall cross-linking activities, and the secretion of wall polymers (amount and type). Some of these mechanisms may be rapid and reversible (e.g. pH changes), whereas others would occur more slowly and may be permanent.

NEW ROLES FOR EXPANSINS

The original discovery of α-expansins stemmed from a study of how cell walls expand during cell growth. Subsequent studies have supported a role for α-expansins in cell growth. For example, α-expansins from rice internodes promoted acid-induced wall extension and had greater activity after growth stimulation by submergence (Cho and Kende, 1997b). Furthermore, treatments that promoted rapid internode elongation (e.g. submergence and GA) induced transcript accumulation of selective α-expansins (Cho and Kende, 1997b, 1997c). Similarly, maximal growth of cotton fibers coincided with maximal accumulation of an α-expansin transcript (Shimizu et al., 1997). In the shoot apical meristem of tomato plants, in situ hybridization indicated that emerging leaf primordia had higher levels of α-expansin transcripts than the central part of the meristem (Fleming et al., 1997). These results are consistent with the proposed function of expansins in cell enlargement.

In the past year, studies have emerged suggesting that expansins may have biological roles in addition to the one proposed for cell wall enlargement. The case for a novel wall-loosening role by group-1 allergens was presented above. Another novel role was suggested by Rose et al. (1997), who found that transcripts for an α-expansin gene accumulated to relatively high levels during tomato fruit ripening. The authors speculated that this expansin may function in wall disassembly of the ripening fruit. A similar degradative function is implied by the earlier discovery of expansin-like proteins in the digestive tracts of snails (Cosgrove and Durachko, 1994); presumably, the snails use the expansins to break down the plant cell walls they swallow.

A different role was proposed by Fleming et al. (1997), who applied beads loaded with expansins to the sites of incipient leaf primordia on the shoot apical meristem of tomato plants. In approximately 30% of the cases the subsequent meristem growth was distorted, and in a fraction of these cases the treated primordium emerged prematurely, resulting in an apparent change in phyllotaxy. This result was interpreted in terms of Green's model of the meristem, in which physical forces serve as important signals for growth patterns and primordium determination in the meristem (Green, 1997). Fleming et al. (1997) speculated that, by regulating the outgrowth of leaf primordia, expansins may also function in pattern formation and signaling events caused by physical forces in the meristem.

CONCLUSIONS AND PROSPECTUS

The ability of cell walls to extend is a complex property that is essential for plant cell growth and morphogenesis. Expansins confer unique rheological properties to growing cell walls: the ability for creep and stress relaxation in a pH-dependent manner. With the recognition that expansins make up a large superfamily with two major branches, new questions about the biological functions and the evolutionary history of these proteins have arisen. I anticipate that our picture of expansins will continue to evolve quickly as the plant genome projects give us a complete inventory of expansin genes in Arabidopsis and rice and as detailed functional analyses of specific genes and proteins are published.

NOTE ADDED IN PROOF

Further information about expansins and their genes may be found at http://www.bio.psu.edu/expansins.

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