Expansins are cell wall proteins that induce pH-dependent wall extension and stress relaxation in a characteristic and unique manner. Two families of expansins are known, named α- and β-expansins, and they comprise large multigene families whose members show diverse organ-, tissue- and cell-specific expression patterns. Other genes that bear distant sequence similarity to expansins are also represented in the sequence databases, but their biological and biochemical functions have not yet been uncovered. Expansin appears to weaken glucan–glucan binding, but its detailed mechanism of action is not well established. The biological roles of expansins are diverse, but can be related to the action of expansins to loosen cell walls, for example during cell enlargement, fruit softening, pollen tube and root hair growth, and abscission. Expansin-like proteins have also been identified in bacteria and fungi, where they may aid microbial invasion of the plant body.

Keywords: Cell growth — Cell walls — Expansins — Wall loosening proteins.

Abbreviations: EXP, α-expansin; EXPB, β-expansin; EXPL, expansin-like; EXPR, expansin-related; Zea m 1, β-expansin and group 1 allergen of maize pollen.

Introduction

Expansin proteins were first identified ten years ago in studies of the mechanism of plant cell wall enlargement, specifically of the “acid growth” phenomenon (McQueen-Mason et al. 1992, Li et al. 1993). Acid growth refers to the rapid stimulation of cell enlargement in response to acid wall pH. This response is characteristic of the growing cells of many plant tissues and is the result of wall-loosening proteins bound to the cell wall. Much, if not all, of the acid growth response of cell walls has been attributed to the action of expansins. In addition, various wall enzymes modify wall structure (Rose and Bennett 1992, Li et al. 1993) and may thereby indirectly influence the effectiveness of expansin action. This mini review will summarize some of the more recent findings about expansins; other recent reviews (Cosgrove 1999, Cosgrove 2000, Darley et al. 2001) and the expansin web site (http://www.bio.psu.edu/expansins/) may also be consulted for some of the earlier work in this field.

Expansin families and related sequences

Two families of expansins are now well established, named α-expansins (EXP) and β-expansins (EXPB). They are similar to each other in size (~25–28 kDa for the mature protein) and have distant but significant sequence similarity to each other throughout the length of the protein backbone (Fig. 1). A number of sequence features are common to the two expansin families, including a cleaved signal peptide at the amino terminus, a series of cysteines with characteristic spacing and conserved flanking sequences, an “HFD” (histidine, phenyalanine, glutamic acid) motif, and a series of tryptophans and other aromatic residues at characteristic positions in the protein backbone. A graphical representation of consensus sequence conservation known as a “sequence logo” (Schneider and Stephens 1990) shows the sequences that are conserved within and between these two families of expansins (Fig. 1). From this representation one can easily see at a glance the sequence motifs that are common to both families of expansins and that distinguish one family from the other. The two families also differ in the presence of N-linked glycosylation motifs, which are generally absent in the EXP sequences but present in EXPB sequences. Limited experimental analyses (Fig. 2 and Downes et al. 2001, Petersen et al. 1995, Pike et al. 1997) support this difference in glycosylation between EXP and EXPB proteins, but its significance for expansin function is uncertain. Some EXPBs also have a glycosylated propeptide that is cleaved from the amino terminus after cleavage of the signal peptide (Downes et al. 2001, Grobe et al. 2002). The significance of this propeptide for EXPB function is uncertain at this time.

Sequence analysis suggests that mature expansins contain two domains (Fig. 1). Domain 1 has significant but distant homology to family-45 endoglucanases, including a series of conserved cysteines and an HFD motif that makes up part of the catalytic site of family-45 endoglucanases (Cosgrove 1997, Cosgrove 2000). This of course suggests that expansins have...
an endoglucanase activity, but if so it is very weak and highly specific in its substrate requirements because experimental tests of EXP have not detected significant activity of this kind (McQueen-Mason and Cosgrove 1995). Domain 2 has homology to a group of proteins found in grass pollen, named group-2 grass pollen allergens, of unknown biological and biochemical function. Otherwise, domain 2 has no other significant sequence similarity to other proteins currently in GenBank. It has been speculated that domain 2 is a polysaccharide binding domain on the basis of conserved aromatic and polar residues on the surface of the protein (Cosgrove 1997). Homology modeling lends support to this concept (Barre and Rouge 2002), but it should be pointed out that the proposed polysaccharide-binding properties of this domain have not been demonstrated experimentally, so this putative function is still very speculative.

In *Arabidopsis* the EXP family has 26 members and the EXPB family has five members (http://www.bio.psu.edu/expansins; Li et al. 2002), whereas in rice and in maize there are probably a similar number of EXP genes but many more EXPB genes (Lee et al. 2001, Lee and Kende 2001, Wu et al. 2001a). The larger size of the EXPB family in grasses is likely related to the unusual composition of the grass cell wall, which has reduced amounts of xyloglucan and pectins and larger amounts of glucuronoxarabinoxylans (Carpita 1996). The evolutionary divergence of the composition of the grass cell wall appears to have been accompanied by an enlargement and functional diversification of the EXPB family.

Although most biochemical and functional studies have focused on EXPs, it is assumed that both groups of proteins work in a similar way, but on different polymers of the cell wall. This inference is based on the observation that grass cell walls respond rather weakly in extensometer assays to EXPs.
but respond more markedly to the EXPB Zea m 1, whereas for
dicot walls the sensitivity is reversed (Cosgrove et al. 1997).
Zea m 1 is a member of the EXPB subgroup known as the
group-1 grass pollen allergens. These allergens are specifically
and abundantly expressed in grass pollen, where they likely
function to loosen the cell walls of the maternal tissues during
penetration of the pollen tube into the stigma and style. This
group of pollen-expressed EXPBs may be rather different in
biological function and biochemical properties than the main
group of EXPB proteins, which are sometimes called the “veg-
etative homologs” of the group-1 allergens. The presumed
wall-loosening activity of these EXPBs (the vegetative
homologs) has not yet been studied, and this is an important
gap to be filled in by future research.

Expansin sequences have also been identified that are
somewhat divergent from the main body of EXP and EXPB
proteins, because of extensions at the amino or carboxy termi-
nus, or short deletions/additions within the corpus of the pro-
tein, or because of divergences in some of the conserved
sequence motifs. The best way to classify a sequence as a
member of the EXP or EXPB family is to construct a phyloge-
etic tree of the expansin super family and to identify where
the sequence in question falls (i.e. within or outside of the two
established families).

In Arabidopsis there is also a third family of related
proteins, named expansin-like (EXPL), as well as a single,
more distant protein named expansin-related (EXPR; see
http://www.bio.psu.edu/expansins/). These EXPL and EXPR
sequences fall outside the EXP and EXPB phylogenetic group-
ings. Because EXPL and EXPR proteins are rather distant in
sequence from these two established expansin families, and
because they have not yet been tested for characteristic
expansin activity, we believe it premature to call them
expansins. Additionally, Arabidopsis and other species have
genomes that encode homologs of a protein first found in studies
of citrus blight (Ceccardi et al. 1998). This protein, called p12
because its size is ~12 kDa, has very distant homology to
Domain 1 of EXP; however, preliminary tests failed to show
wall-loosening activity of the citrus blight protein (D.J.
Cosgrove and D.M. Durachko, unpublished data). On the basis
of cross reaction with antibodies raised against vertebrate atrial
natriuretic peptide, Ludidi et al. (2002) speculated that p12-like
proteins may function as peptide hormones for water and sol-
ute balance in plant cells. In contrast, Li et al. (2002) have
called these p12-like proteins γ-expansins, but such an assign-
ment is premature in our view until at least one member of this
group has been shown to have wall-loosening activity charac-
teristic of expansins. For the same reason, the EXPL and EXPR
genes are not called expansins, though they are clearly evolu-
tionarily related to expansins. In contrast, one or more mem-
bers of the EXP and EXPB families have been shown to induce
rapid wall extension in vitro in a pH dependent manner and to
enhance cell wall stress relaxation in a characteristic manner
(Shcherban et al. 1995, Cosgrove et al. 1997, Cho and Kende
1997a). These are the functional characteristics used to define
expansins, and distant proteins such as those encoded by the
EXPL, EXPR and p12-like genes need to be tested for these
activities before they are classified as expansins.

Outside of the plant kingdom, expansins do not seem to be
common, but some organisms may have adopted expansins for
novel purposes. The slime mold Dictyostelium has genes that
remotely resemble plant expansins, but their function is unclear
(Li et al. 2002). A virulence factor of the plant pathogen Clavi-
bacter michiganensis has an expansin-like domain (Laine et al.
2001), which may function in breakdown of plant cell walls
and invasion by the bacterium, but this idea has not been estab-
lished experimentally. An expansin-like domain found in the
fungal protein “swollenin” likewise may assist breakdown of
plant cell walls (Saloheimo et al. 2002). And finally, snail
digestive tracks contain expansin proteins, though it is unclear
whether they are made by the snail or by bacteria living in the
snail’s guts (Cosgrove and Durachko 1994). These non-plant
proteins share a common evolutionary history with plant
expansins, and so study of their biochemical activities may
shed light on how bona fide expansins function.

Expansin mechanism of action

Early work suggested that expansins loosened plant cell
walls via a novel mechanism that weakens the lateral adhesion
of load-bearing polysaccharides to one another or to the cellu-
lose surface (for review see Cosgrove 2000, Darley et al.
2001). In contrast, Grobe et al. (Grobe et al. 1999, Grobe et al.
2002) proposed that expansins are members of the C1 family of
cysteine proteases and that they loosen walls by hydrolytic
attack of specific cell wall structural proteins. However, this
hypothesis was evaluated and discounted by Li and Cosgrove
(2001) on several grounds. Tests with the BLAST2 program
(Tatusova and Madden 1999) demonstrate a statistically signifi-
cant alignment between expansin and family-45 endoglucana-
ases, but show no statistical significance to the proposed
alignment and sequence similarity between expansin with C1
proteases. Because C1 proteases and family-45 endoglucana-
ases are not homologous with each other, the same region of
the expansin protein cannot be homologous to both of these
proteins. The homology with family-45 endoglucanases is
clearly better supported than homology with C1 proteases.

The idea that expansin might have proteolytic activity
originated from studies of recombinant Phl p 1, a group-1 grass
pollen allergen belonging to the EXPB family. Recombinant
Phl p 1 was found to be highly unstable and was proteolyti-
cally degraded during purification (Grobe et al. 1999). In con-
trast, Li and Cosgrove (2001) failed to find evidence for pro-
etase activity in group-1 pollen allergens. Inhibitors of C1
protease activity did not block expansin-mediated wall exten-
sion, and C1 proteases (as well as other proteases) did not
mimic expansin’s wall-loosening effects. Moreover, treatments
promoting C1 protease activation (e.g. reducing conditions) did
not activate the hypothesized proteolytic activity of Zea m 1, a
group-1 allergen and EXPB from maize pollen. Thus the idea
that EXPBs loosen the cell wall via proteolytic activity was not
supported (Li and Cosgrove 2001). Subsequent work (Poppel-
mann et al. 2002) showed that expression of recombinant Phl
p1 induced an endogenous protease in their yeast expression
system, and this protease contaminated the protease assays of
recombinant Phl p 1. In summary, the experimental basis for
the idea that expansin has proteolytic activity seems to be an
experimental artifact, sequence analysis gives substantially bet-
ter support for homology with family-45 endoglucanases than
with C1 proteases, and proteases have not been shown to cause
wall loosening.

The exact biochemical mechanism of action of expansin
and the identity of its target site of action are uncertain. One
of the hallmarks of expansin action is an increase in the
stress relaxation of isolated cell walls (McQueen-Mason and
Cosgrove 1995, Cosgrove et al. 1997). This action requires the
continued presence of expansin during the stress relaxation
assay. That is, pre-treatment of walls with EXP, followed by
inactivation of expansin, did not change the mechanical proper-
ties of the wall. Similarly, walls treated with EXP did not
become progressively weaker, as occurs when walls are treated
with hydrolytic enzymes. Moreover, EXP does not hydrolyze
the major structural polysaccharides in the cell wall, and it does
not act as a xyloglucan transglycosylase (McQueen-Mason et
al. 1993, McQueen-Mason and Cosgrove 1995). Thus EXP
does not seem to change wall structure substantially. Compara-
bale studies with EXPBs need to be carried out.

Expansin may weaken the non-covalent adhesion of glu-
cans to one another (e.g. xyloglucan to cellulose). Evidence for
this is seen in its weakening effect on pure cellulotic paper (a
hydrogen-bonded network of glucans) without evidence of
hydrolytic activity and in the action of chaotrope agents such
as 2 M urea, which can partially mimic and synergize the effect
of expansins on cell walls (McQueen-Mason and Cosgrove
1994). Furthermore, EXP caused rapid weakening of artificial
composites made of bacterial cellulose and xyloglucan (Whitney
et al. 2000). These composites are pellicles of Acetobacter
xylina grown in the presence xyloglucan, which binds to the
cellulosic fibrils and alters the structure and mechanical proper-
ties of the pellicle (Whitney et al. 1995, Whitney et al. 1999,
Chaniard et al. 2002). Cellulosic pellicles grown with other
polysaccharides or without additional polysaccharides showed
much less or no sensitivity to expansin action (Whitney et
al. 2000). These results with “artificial cell walls” suggest that
expansin affects the tethering of cellulose microfibrils to one
another via xyloglucan bridges.

Additional support for the hypothesis of glucan–glucan
weakening by expansins comes from a study of a fungal pro-
tein from Trichoderma reesei, also known as Hypocrejevo-
rina. The protein, called “swollenin”; contains an expansin-like
domain linked to a cellulose-binding domain (Saloheimo et al.
2002). Swollenin was expressed in yeast and crude extracts
were incubated with cotton fibers. Swollenin caused local dis-
ruptions of the fiber structure after sonication. It also weak-
ened filter paper and caused an apparent dispersion of Valonia
cell wall structure, but without release of soluble sugars. These
results indicate that swollenin may act in a manner similar to
expansin’s action in causing cell wall extension.

Wall loosening by expansins was shown to be markedly
different from that caused by an endoglucanase that hydrolyses
xyloglucan (Yuan et al. 2001). This endoglucanase caused
walls to extend only after a significant lag period (minimum of
6 min at saturating enzyme levels) and this was accompanied
by a large increase in wall plasticity, but not wall stress relaxa-
tion. In contrast, EXPs induced wall extension immediately
(within s – that is, as soon as it penetrated the wall) and did not
increase wall plasticity. It is also remarkable that expansin
eliminated the lag time for induction of wall extension by the
endoglucanase (unpublished data, S. Yuan and D.J. Cosgrove).
The long lag seen for endoglucanase-induced wall extension,
and the removal of the lag by EXP, suggests a wall structural
model in which cellulose microfibrils are linked together by a
relatively large and inaccessible xyloglucan complex that is
opened up by EXP, which allows immediate wall extension and
access to enzymatic attack by the endoglucanase.

Biological functions of expansins

In contrast to the limited work assessing the biochemical
mechanism of action of expansins on cell walls, studies by
many groups have enlarged our understanding of the biologi-
cal functions of expansins. To assess the biological function
of expansins, several approaches have been used: (a) immuno-
localization of expansin proteins, (b) analysis of gene expression
patterns, (c) ectopic expression of specific expansin genes, (d)
antisense reduction of expansin expression, (e) insertional
mutagenesis of expansin genes, and (f) application of expansin
proteins to isolated cell, tissues, and parts of the meristem.

Immunolocalization—Electron microscopy, combined with
immunogold labeling of plant tissues using antibodies against
EXPs, indicates that expansins are dispersed throughout the
cell wall, not restricted to specific strata or corners of the cell
wall or to the wall–plasma membrane interface (Fig. 3). This is
a significant point because the load-bearing part of the cell wall
is not restricted to the membrane/wall junction or to cell cor-
ners, so EXPs are located in the site crucial for their wall loos-
ening action. Golgi-derived vesicles are sometimes seen
labeled with antibody, indicating EXPs are delivered to the
wall via the usual route for protein secretion.

An immunofluorescence study of young maize root hairs
indicated that EXP accumulated in the cytoplasm and the wall
of the emerging bulge (Baluska et al. 2000). In contrast,
another immunofluorescence study of the elongation region
of maize roots found most of the label in the cell walls (Zhang
and Hasenstein 2000). Interestingly, when roots were placed in
a horizontal position, expansin signals were stronger on the
convex (growing) side of the root than the concave (non-
growing) side. This change in the expansin signals matched the
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gravitropic growth response, but the exact reason for the rapid change in the signal is not clear. EXPs bind tightly to the cell wall and probably have limited mobility and turnover within the cell wall.

Since all of these immunolocalization studies were made with a polyclonal antibody that recognizes numerous (but not all) EXPs, it is possible that the signals were derived from multiple EXPs, and that some other EXPs were present but not well recognized by the antibody. Given the large size of the EXP gene family, this is a likely possibility and calls for caution in the interpretation of these studies.

The highly abundant EXPBs (group-1 allergens) found in grass pollen are found both on the pollen surface and intracellularly (Staff et al. 1990). The group-1 allergen within the pollen grain protoplast is presumably contained within secretory vesicles, but the fixation and staining used in this study did not allow visualization of membranes to ascertain this point.

**Gene expression**—Studies of expansin gene expression by Northern blotting and in-situ hybridization indicate that the different expansin genes are expressed in different organs, tissues and cell types, and that they respond distinctively to such treatments as hormones, light, pollination, etc., as well as to endogenous developmental programs (Rose et al. 1997, Brummell et al. 1999b, Vriezen et al. 2000, Caderas et al. 2000, Kim et al. 2000, O’Malley and Lynn 2000, Kita et al. 2000, Chen et al. 2001, Harrison et al. 2001, Wrobel and Yoder 2001, Ruan et al. 2001, Pezzotti et al. 2002, Harmer et al. 2002, Mbeguie et al. 2002). These studies implicate expansins in a variety of growth and developmental responses, from germination and fruit ripening to pollination and adaptive growth responses to submergence. As one example, the formation of visible leaf primordia in the tomato shoot apical meristem was preceded by localized expression of an EXP gene at the future site of the primordium (Reinhardt et al. 1998). Likewise initiation of root hairs in *Arabidopsis* is spatially and temporally correlated with onset of expression of two EXP genes that are specific for root hairs.
Specific EXP genes are expressed in developing xylary elements in *Zinnia*, and what is more, the mRNAs are localized in a polar fashion to one end of the cell (Im et al. 2000). This mRNA localization is presumably important for localized synthesis and secretion of EXP to the end wall of the developing xylary cell. Attempts at cytolocalization of expansin protein by epitope tagging or by fusion with green fluorescent protein (GFP) have proved problematic, because of inadequate signal from the epitope tag or because of incorrect targeting of the expansin-GFP chimeric protein (H.-T. Cho and D.J. Cosgrove, unpublished results, and Cho and Cosgrove 2002).

In rice, whose genome contains many EXP and EXPB genes, expression of specific EXP and EXPB genes was found to be correlated with induction of rapid internode elongation by gibberellin (Cho and Kende 1997b, Lee and Kende 2001, Lee and Kende 2002). The large number of EXPB genes in rice are expressed differentially in different organs (Fig. 4), and at least some of them are expressed in a pattern consistent with a role in cell enlargement. In maize, which likewise has numerous EXP and EXPB genes, the growing zone of the root expresses only a small number of EXP and EXPB genes, and some of these are up-regulated by water deficits (Wu et al. 2001a, Wu et al. 2001b). This enhancement of expansin expression may be an important part of the adaptive growth response of maize roots to water stress (Wu et al. 1996). In yet another grass where expansin gene expression has been examined, expansin expression in the leaf was related both to growth (cell elongation) and to cell differentiation (Reidy et al. 2001).

In soybean cell cultures, expression of *CIM 1*, a cytokinin-inducible EXPB gene, is regulated in a complex way by cytokinin and auxin. Cytokinin acts, at least in large part, by increasing *CIM 1* mRNA stability (Downes and Crowell 1998), thereby increasing *CIM 1* transcript levels, which may support cell enlargement processes associated with cytokinin-induced cell proliferation. Cytokinin and auxin also acted synergistically in cell cultures to increase abundance of *CIM 1* protein that accumulated in the tissue culture medium (Downes et al. 2001). *CIM 1* protein in the medium was proteolytically cleaved to produce a 20-kD fragment corresponding to Domain 1 (Fig. 1), but it is uncertain whether this fragment is functionally active or simply a step in *CIM 1* degradation.

**Ectopic expression of expansin genes and application of expansin proteins**—Perhaps the most interesting study on this theme is that by Pien et al. (2001) who induced local expression of an EXP at the site of incipient leaf primordia on the tobacco shoot apical meristem. This was accomplished by use of a tetracycline-inducible gene expression system in combination with local application of tetracycline. By this means it was possible to induce precocious growth of leaf primordia, with a consequent change in phyllotaxy on the shoot apical meristem. Moreover, local transient induction of EXP expression on the flank of developing primordia stimulated growth of lamina tissue and thereby changed leaf shape. The results show that expansin expression is capable of regulating leaf shape and forcing a developmental program (leaf formation) in advance of its normal developmental schedule. Thus control of cell enlargement can be important for controlling developmental patterns and morphogenesis, as proposed by the late Paul Green (Green 1997). This study follows earlier work in which EXP-soaked beads were applied to incipient leaf primordia (Fleming et al. 1997, Fleming et al. 1999). The results with this technique were less convincing because of a low success rate in the experiments, but similar conclusions were reached.

*Arabidopsis*, tomato, cucumber and tobacco BY2 suspension cells have also been the subjects for related experiments that increase expansin gene expression or that apply expansins to growing cells. Initial attempts at ectopic expression of EXP in *Arabidopsis* used the constitutive and strong CaMV 35S promoter, but severe disruptions of the shoot apical meristem resulted in infertile plantlets that died without producing progeny (Shcherban 1999). Use of a more selective promoter, one controlling expression of the *Arabidopsis* EXP10 gene, resulted in plants with longer petioles, larger leaf blades and larger cells than controls (Cho and Cosgrove 2000). In tomato overexpression of EXP using the CaMV 35S promoter paradoxically resulted in plants with stunted growth (Rochange et al. 2001). These plants appeared to compensate for excess expression of EXP by reducing the sensitivity of the cell walls to expansin.
On the other hand, overexpression of EXP in tomato fruit enhanced fruit softening and cell wall breakdown (Brummell et al. 1999a).

When EXP protein was applied to growing cells, the exact results depended on the type of material studied. As mentioned above, local application of EXP to incipient leaf primordia forced the early growth of the primordia, often resulting in a distorted or aborted young leaf (Fleming et al. 1997, Fleming et al. 1999). When applied to tobacco BY2 cell cultures, the same protein stimulated growth by 2- to 3-fold for several h (Link and Cosgrove 1998). There was evidence, however, of long-term adaptation and diminished response to the exogenous expansin after many h. Exogenous EXP also can stimulate elongation of excised Arabidopsis hypocotyls, yielding a stimulation that is comparable to that caused by 1 μM auxin (Fig. 5). Arabidopsis hypocotyls are favorable materials for these experiments because they are so small that problems of protein penetration into the tissue are reduced. It is also interesting that when auxin and EXP were applied simultaneously, the elongation responses were the same as auxin alone (data not shown). This suggests that auxin and EXP operate through the same pathway for control of growth, which is saturated by high levels of either agent.

When EXP was applied to growing root hairs, quite a different response was seen (Fig. 6). At high levels of exogenous EXP, the tip of the root hairs burst within a few s of protein application. At lower levels, the protein caused a radial swelling at the tip of the root hair, followed by a transient cessation of elongation and subsequent resumption of root hair elongation, often at small diameters. The fact that EXPs induced swelling of the root hair, but elongation (not swelling) of the Arabidopsis hypocotyl, makes sense, given the different wall structures of these plant cells. The cell wall of the root hair tip is isotropic, without much bias in the orientation of cellulose microfibrils, with the result that loosening of this wall results in radial swelling. The appearance of α-expansin-treated root hairs is reminiscent of legume root hairs treated with nod factors. This similarity suggests that changes in the pattern of endogenous α-expansin delivery to the root hair wall may cause the early morphogenetic changes in root hair shape at the start of Rhizobium invasion and nodule formation. Also, note that the cell wall behind the tip of the root hair did not swell in response to the applied α-expansin (Fig. 6). This wall is evidently insensitive to the loosening action of α-expansin, presumably because it is cross-linked in some way to prevent growth. In contrast to the root hair, the Arabidopsis hypocotyl and the BY2 cells elongated (did not swell) in response to exogenous α-expansin. The walls of these cells are anisotropic because of the biased orientation of cellulose deposition, and thus loosening of the cell walls results in directional growth –

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*Fig. 6* Bursting, swelling and recovery after α-expansin application to growing cucumber root hairs. (A) Root hairs not treated with expansin. (B) Root hairs after treatment with high concentration of α-expansin. Arrows mark burst root hairs. (C) Root hairs after treatment with a low concentration of α-expansin. Note the swelling of the root hairs (some burst root hairs are visible in the background). (D) Root hairs after treatment with a low concentration of α-expansin. Arrows mark cells that recovered elongation after expansin-induced swelling. Note the tube is smaller than before treatment. Expansins were extracted from cucumber hypocotyls and purified as in McQueen-Mason et al. (1992). A solution containing α-expansin was loaded into a micropipette and a small aliquot was released to the solution in the proximity of the root hairs. At low concentrations of applied expansin, swelling was induced, whereas at high expansin concentrations rapid bursting ensued. Older, non-growing root hairs were more resistant to expansin action.
elongation, not radial swelling.

**Antisense inhibition and knockout of expansin genes**—With a large multigene family such as the expansins make up, it can be a challenge to determine the biological function of individual genes, because overlapping expression and partial redundancy can reduce the phenotypic effects of genetic defects in single genes. Thus, Schipper et al. (2002) created knockout mutants by homologous recombination in the moss *Physcomitrella*, but found no obvious phenotype in any of the mutant lines. Likewise, we have identified only a number of insertional mutants of expansin genes in *Arabidopsis*, and most, but not all, homozygous lines with single-gene knock-outs have little or no obvious phenotype under laboratory growing conditions (J. Sampedro Jimenez and D.J. Cosgrove, in preparation). On the other hand, reduction of α-expansin gene expression by the antisense method caused a significant reduction in plant growth in *Arabidopsis*, depending on the promoter and the specificity of the antisense construct (Cho and Cosgrove 2000). Suppression of α-expansin LeEXP1 expression in ripening tomato fruit resulted in firmer fruit with less breakdown of some wall components (Brummell et al. 1999a) and improved shelf life, but no effects on fruit size (Brummell et al. 2002). Comparable experiments need to be done to assess the functional roles of β-expansin genes, individually and in aggregate, as well as the more distantly related EXPL and EXPR genes.

**Conclusions**

There is ample support for the original hypothesis that expansins function to loosen cell walls and thereby stimulate plant cell enlargement. The surprising observation from various plant genome projects is that expansins comprise a large multigene family. This affords them the possibility of participating in carefully controlled ways in diverse developmental processes, including growth of diffuse- and tip-growing cells, meristem dynamics, fruit softening, pollination, and abscission, as well as in adaptive responses to submergence, water stress and light. The biochemical mechanisms of action and substrate specificities of α- and β-expansins need further study, as does the function of the EXPL, EXPR and p12-like genes that are distantly related to expansins.

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