

# Expansins: ever-expanding numbers and functions

Yi Lee, Dongsu Choi and Hans Kende\*

Expansins were first identified as cell-wall-loosening proteins that, at least in part, mediate pH-dependent extension of the plant cell wall and growth of the cell. More recently, it has been realized that expansins belong to two protein families, the  $\alpha$ - and  $\beta$ -expansins, and that they appear to be involved in regulating, besides cell expansion, a variety of plant processes, including morphogenesis, softening of fruits, and growth of the pollen tube of grasses through the stigma and the style. The *Arabidopsis* genome contains 26  $\alpha$ -expansin genes and the rice genome at least 26. There are more  $\beta$ -expansin genes in monocots than in dicots, at least 14 in rice and five in *Arabidopsis*. Expansin genes are differentially regulated by environmental and hormonal signals, and hormonal regulatory elements have been found in their promoter regions. An analysis of exon/intron structure led to the hypothesis that  $\alpha$ - and  $\beta$ -expansins evolved from a common ancestral gene.

## Addresses

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824, USA

\*Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824, USA; e-mail: hkende@msu.edu

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## Abbreviations

**EST** expressed sequence tag  
**EXP** *EXPANSIN*  
**GA** gibberellin  
**GARE** GA-responsive element

## Introduction

Cell expansion is driven by uptake of water into the central vacuole. A large body of evidence shows that flow of water into the cell and consequent enlargement of the cell are initiated by stress relaxation of the cell wall. It has also been shown that the walls of growing cells have a pH of around 5 or below, and that acidification of a cell-wall specimen subjected to constant tension induces extension of the wall [1]. These observations led Cosgrove and coworkers to a search for pH-dependent wall-loosening enzymes and to the discovery of the expansins [2,3]. Originally, two proteins that promoted cell-wall extensibility without detectable hydrolysis of cell-wall polymers were isolated from cucumber hypocotyl walls [2]. A similar cell-wall protein was also identified in oat coleoptiles, and these wall-loosening proteins were called expansins [3]. Cloning of cDNAs encoding the two cucumber expansins followed by database searches showed that expansins also occur in *Arabidopsis*, peas, and rice, and that group-I allergens of grasses had limited (about 25%) but significant sequence identity to expansins [4]. *Zea mI* pollen allergen of maize does, indeed, loosen the cell wall of maize silk and of wheat coleoptiles, and transcripts similar to those

of pollen allergens were also identified in the expressed sequence tag (EST) databases of vegetative tissues [5]. Group-I allergens and related proteins in vegetative tissues have been classified as  $\beta$ -expansins, whereas the earlier discovered expansins are now referred to as  $\alpha$ -expansins. The structural features and the suggested mode of action of expansins have recently been reviewed [6\*].

Here, we review the notable progress that has been made in expansin genomics, thanks mainly to the availability of the *Arabidopsis* and partial rice genomic databases; in understanding evolutionary aspects of expansins; in the analysis of expansin promoter sequences; and in elucidating the *in vivo* functions of expansins.

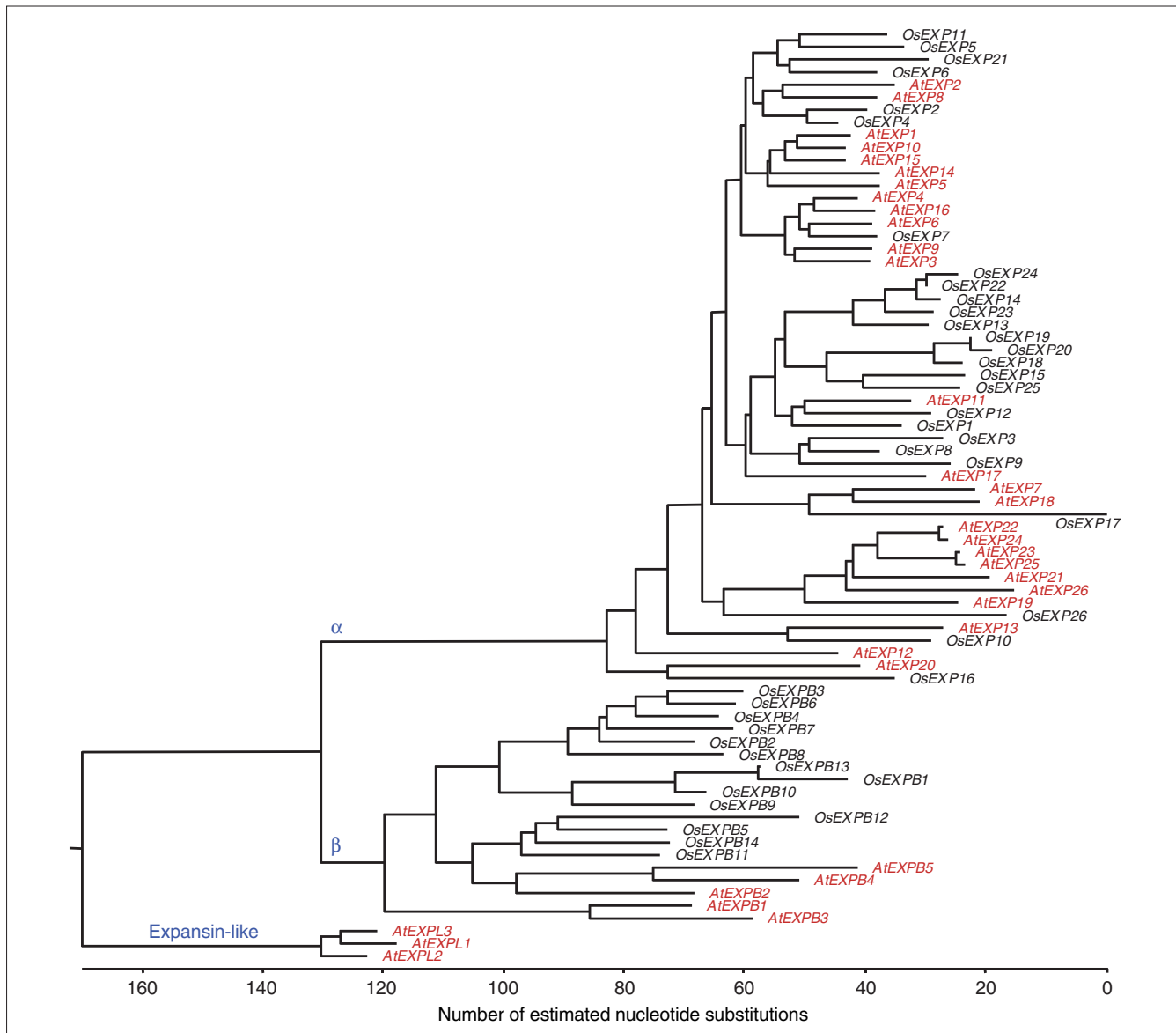
## Genomics and evolution of expansins

The availability of the full *Arabidopsis* genome (URL [www.arabidopsis.org](http://www.arabidopsis.org); GenBank) and of about 70% of the rice genome (URL [www.rice-research.org](http://www.rice-research.org)) has opened the possibility of identifying genes on a genome-wide basis, of examining their promoter regions, and of locating their introns. This approach has been very fruitful in analyzing the  $\alpha$ - and  $\beta$ -expansin gene families. In *Arabidopsis*, 26 putative  $\alpha$ -expansin and five  $\beta$ -expansin genes have been identified (Figure 1). In addition, at least three expansin-like genes have been found in the *Arabidopsis* genome. Searches of the rice genomic and EST databases have yielded 26  $\alpha$ - and 14  $\beta$ -expansins (Figure 1). Because the rice genome database is incomplete, it is likely that more expansin genes will be identified once the entire genome has been sequenced.  $\alpha$ -Expansins,  $\beta$ -expansins, and expansin-like genes are strictly separated on the phylogenetic tree, and *Arabidopsis* and rice expansin genes tend to be grouped separately (Figure 1).

Expansins have been found in all seed plants examined thus far. Recently, highly conserved  $\alpha$ -expansin transcripts were also identified in two ferns, *Marsilea quadrifolia* and *Regnellidium diphyllum*, in which their level of expression correlated with the growth rate of the rachis [7].  $\alpha$ -Expansin transcripts have also been found in mosses, but attempts to identify them in the green alga *Chara corallina* were unsuccessful (DJ Cosgrove, AJ Fleming, personal communication). Thus,  $\alpha$ -expansins have been highly conserved during the evolution of land plants and appear to play a role in the extension of their cell walls.

Analysis of the *Arabidopsis* and rice gene structures and deduced amino acid sequences indicate that  $\alpha$ - and  $\beta$ -expansin genes evolved from a common ancestral gene (Figures 1,2). The exon/intron organization is conserved among  $\alpha$ - and  $\beta$ -expansins, and expansin-like genes, but the number of introns and the length of each intron differ among individual genes. The expansin genes of *Arabidopsis*

Figure 1



Phylogenetic analysis of expansin genes. The dendrogram was generated on the basis of the alignment of the deduced amino-acid sequences encoded by 71  $\alpha$ - and  $\beta$ -expansin genes of *Arabidopsis* (in red) and rice (in black), and of three expansin-like

genes of *Arabidopsis*, using the Clustal method with the MegAlign program (DNASTAR, Madison, Wisconsin, USA). The GenBank accession numbers are listed at URL [www.bio.psu.edu/expansins](http://www.bio.psu.edu/expansins).

and rice contain combinations of up to five introns. Each type of intron interrupts the nucleotide sequence at a position that corresponds to the equivalent amino acid in aligned protein sequences and at the same intron phase (the intron phase represents the position of the intron within the codon). Figure 2 shows the exon/intron organization in rice expansin genes. Introns A are inserted in phase 1, introns B in phase 2, introns C in phase 0, introns D in the 5' untranslated region, and introns E in phase 2. The common ancestor of  $\alpha$ - and  $\beta$ -expansin genes might have contained introns A and B. Introns were added or lost during evolution [8]. Thus, intron A and/or intron B were lost in some

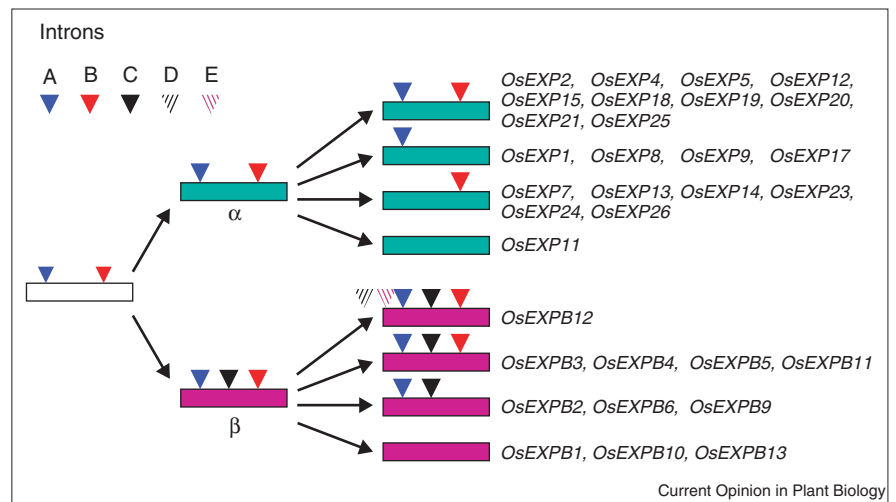
$\alpha$ - and  $\beta$ -expansin genes. Intron C was added in a common ancestor of  $\beta$ -expansins and lost in *OsEXPB1*, *OsEXPB10*, and *OsEXPB13*. Introns D and E were added in *OsEXPB12* (Figure 2). The same pattern of intron positions is also present in *Arabidopsis*, supporting the hypothesis that  $\alpha$ - and  $\beta$ -expansins evolved from a common ancestral gene. Many *Arabidopsis* and rice expansin genes are tandemly repeated in the genome, indicating recent duplication events.

### Promoter analysis of rice expansin genes

Expression of some  $\alpha$ -expansin genes is regulated by auxin [9,10], gibberellin (GA) [11,12], cytokinin [13,14],

**Figure 2**

A model of the evolution of rice expansin genes. This figure summarizes the minimum assumptions for the evolution of  $\alpha$ - and  $\beta$ -expansins. The GenBank accession numbers are listed at URL [www.bio.psu.edu/expansins](http://www.bio.psu.edu/expansins). Each type of intron is denoted by a color-coded inverted triangle. The same type of intron occurs at the equivalent position in the aligned nucleotide and corresponding amino-acid sequences, and at the same intron phase. Introns are added or lost during evolution.



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and ethylene [7,15]. Analysis of the promoter regions of rice expansins showed the presence of known abscisic acid-, auxin-, GA-, and ethylene-responsive elements (Table 1). Such hormone-responsive elements appear to be more frequent in  $\alpha$ -expansins than in  $\beta$ -expansins. The detailed structure of the rice  $\alpha$ -expansin gene *OsEXP4* is shown in Figure 3. In deepwater rice, submergence, ethylene, and GA induce rapid growth of the highest internode [16]. Expression of *OsEXP4* is induced by GA within 30 min, that is, before a growth response can be observed [11]. The promoter region of *OsEXP4* contains GA-responsive elements (P-box, O2S, GARE), an abscisic-acid-responsive element (ABRE), and an anoxia/hypoxia-responsive element (ARE) (Figure 3).

**Expansin action**

Initial work on  $\alpha$ -expansins established several close correlations between growth and expression of  $\alpha$ -expansin transcripts and proteins [6\*]. There are many new entries in the list of such correlations. Immunolocalization showed that roots responding to gravitropic stimulation contain higher levels of  $\alpha$ -expansin in cells of the expanding, convex side than in the slower growing cells of the concave

side [17]. Gravistimulation also resulted in increased  $\alpha$ -expansin gene expression on the faster growing, lower side of stem segments excised from horizontally placed tomato plants [18]. Semiaquatic plants, such as *Rumex palustris* and the fern *Regnellidium diphyllum*, are induced to grow rapidly by submergence and ethylene [7,15]. In both of these plants, submergence and ethylene enhanced the transcription of an  $\alpha$ -expansin gene. In rice, another semiaquatic plant, hypoxia and submergence increased  $\alpha$ -expansin mRNA levels and growth of the coleoptile [19]. A correlation between growth and high expression of  $\alpha$ -expansin genes was also found in the early phase of cotton-fiber elongation [20].

The observation that expression of expansin genes [21] and protein [22] correlates with growth and development of roots has been further expanded. Roots that are adapted to reduced water potentials continue to grow under water-limited conditions by increasing, in their apical portion, the extensibility of their cell walls [22]. This increase in wall extensibility results, at least in part, from greater expansin activity [23], which is associated with the accumulation of two  $\alpha$ - and one  $\beta$ -expansin transcripts [24]. The significance

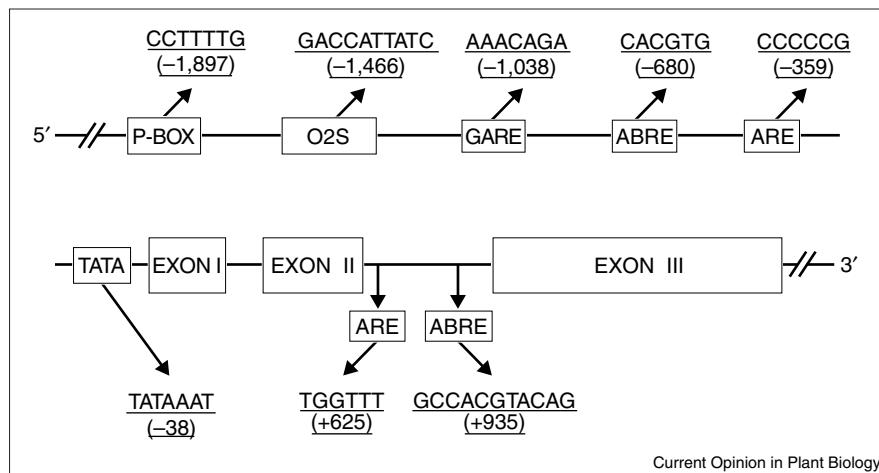
**Table 1**

**Putative hormone-responsive elements in the promoter regions of rice expansin genes.**

Hormone-responsive element	Function	$\alpha$ -Expansin genes	$\beta$ -Expansin genes
GARE	GA-responsive element	<i>OsEXP4, OsEXP12, OsEXP13, OsEXP20, OsEXP23</i>	<i>OsEXPB12</i>
P	GA-responsive element	<i>OsEXP2, OsEXP4, OsEXP7, OsEXP13</i>	
TGA	Auxin-responsive element	<i>OsEXP1, OsEXP8, OsEXP18, OsEXP19, OsEXP26</i>	<i>OsEXPB2</i>
AuxRR	Auxin-responsive element	<i>OsEXP25</i>	
ABRE	ABA-responsive element	<i>OsEXP4, OsEXP5, OsEXP11, OsEXP12, OsEXP13, OsEXP14, OsEXP15, OsEXP17, OsEXP23, OsEXP24</i>	<i>OsEXPB2</i>
ERE	Ethylene-responsive element	<i>OsEXP11, OsEXP15</i>	<i>OsEXPB4</i>

Hormone-responsive elements were identified using the PLACE [40] and PlantCARE [41] databases. GenBank accession numbers are listed at URL [www.bio.psu.edu/expansins](http://www.bio.psu.edu/expansins).

Figure 3



Structure of the *OsEXP4* gene. The P-box, O2S, and GARE are *cis*-acting elements that are involved in GA regulation of gene expression. ABRE and ARE represent abscisic-acid- and anoxia/hypoxia-responsive elements, respectively. These hormone-responsive elements were identified using the PLACE [40] and PlantCARE [41] databases. The numbers in parentheses indicate the distance to the putative transcription initiation site.

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of expansins in the development of the root system was also made evident by the finding that  $\alpha$ -expansin accumulates in trichoblasts during root-hair initiation [25]. Development of haustoria in parasitic angiosperms is initiated by swelling of the cells in the root tip and the formation of hairs on the haustorial epidermis. Induction of haustoria was found to correlate with the expression of specific  $\alpha$ -expansin genes in *Striga asiatica* [13]. However, there is no consistent correlation between haustorium formation and expansin gene expression in the parasitic plant *Triphysaria versicolor* [14].

Differential increases in  $\alpha$ -expansin content occur during the growth and ripening of tomato fruits [26], and expression of an  $\alpha$ -expansin gene is induced during the ripening of strawberry fruit [27]. During germination of tomato seeds, a specific  $\alpha$ -expansin transcript accumulates in the endosperm cap, presumably in association with the weakening of cell walls that facilitates emergence of the radicle [28]. An interesting correlation has been found between tracheary-element formation and  $\alpha$ -expansin gene expression in *Zinnia* [29\*\*]. The transcripts of three  $\alpha$ -expansin genes accumulated when isolated mesophyll cells, induced to differentiate into tracheary elements, started to elongate. In *Zinnia* seedlings, expansin mRNAs were found in developing primary xylem cells and, most strikingly, exclusively at the distal ends of these cells. This indicates that the translation products of these  $\alpha$ -expansin transcripts may mediate local growth. Expression of  $\alpha$ -expansin genes in cells undergoing differentiation to vasculature was also observed in the internodal meristem of deepwater rice [21]. Exceptions to the correlation between growth and  $\alpha$ -expansin mRNA content have been noted, however. Transcripts of one of the two tomato  $\alpha$ -expansin genes examined did not accumulate in rapidly growing cells [18]. Given the large number of  $\alpha$ -expansins and their varied functions, the lack of correlation between growth and the level of some  $\alpha$ -expansin proteins or  $\alpha$ -expansin transcripts is not surprising.

The model for the mechanism of action of expansin postulates that expansins break non-covalent bonds between cell-wall polysaccharides, thereby permitting turgor-driven polymer creep [6\*]. This hypothesis has been tested with a composite material consisting of crystalline cellulose produced by *Acetobacter xylinus* in the presence of a hemicellulosic polymer [30\*]. Purified cucumber expansin induced extension of cellulose-xyloglucan composites under constant load to the same extent as it induces extension of plant cell walls. The extensibility of cellulose alone, of cellulose-glucomannan, and of cellulose-galactomannan at constant load was not increased by expansin, though expansin did increase extension of cellulose alone when it was extended at constant rate. This model system of defined composition appears to be very suitable for the study of the biochemical action of expansins.

### *In vivo* function of $\alpha$ -expansins

Conclusions about the role of  $\alpha$ -expansins in mediating cell-wall loosening were primarily derived from reconstitution experiments in which the addition of cell-wall proteins or  $\alpha$ -expansins to heat-inactivated cell walls restored pH-dependent wall extensibility [6\*]. Although the results obtained by this method suggest an *in vivo* role for  $\alpha$ -expansins, they are not entirely conclusive. However, recent experiments using transgenic plants provided strong evidence supporting an endogenous function of  $\alpha$ -expansins in growth and cell-wall modifications. The  $\alpha$ -expansin gene *AtEXP10* is highly expressed in growing *Arabidopsis* leaves and petioles, and at the base of *Arabidopsis* pedicels [31\*\*]. Plants that expressed the antisense construct of *AtEXP10* had shorter leaf blades and petioles than did control plants, and their cell walls were less extensible. Conversely, plants overexpressing *AtEXP10* had slightly longer petioles, larger leaf blades, and larger cells than did the control plants. Abscission at the base of the pedicel, where *AtEXP10* is highly expressed, was promoted or inhibited, respectively, by

increased or decreased expression of the *AtEXP10* gene [31\*\*]. This indicates that endogenous expansins play a role not only in cell expansion but also in cell-wall modifications that involve the hydrolysis of cell-wall components. A similar conclusion can be drawn from experiments on the role of expansin in tomato fruit softening [32\*\*]. Expression of the tomato *EXPI* gene, whose transcript level increases during ripening, was either suppressed through gene silencing or increased through overexpression of a sense construct. Fruits with reduced expansin content remained firm during ripening, whereas fruits with increased expansin content softened more than wild-type plants as they ripened. Expansin-mediated promotion of fruit softening was accompanied by hydrolytic modifications of cell-wall polymers. It appears likely that expansins facilitate the access of hydrolytic enzymes to cell-wall polymers.

### $\beta$ -Expansins

Until recently, work on  $\beta$ -expansins has been focused on the structure and cell-wall-loosening activity of pollen allergens [5]. However, entries in the EST databases indicate that  $\beta$ -expansin genes are also expressed in vegetative tissues. Members of the grass family, such as rice and maize, contain many more  $\beta$ -expansins than does *Arabidopsis*, and apparently, also more than other dicots. The genome of rice contains at least 14  $\beta$ -expansins, whereas that of *Arabidopsis* contains five (Figure 1). In grasses, the preponderance of  $\beta$ -expansins is consistent with their selective action. Whereas cell-wall preparations containing  $\alpha$ -expansins exhibit low cell-wall-loosening activity in wheat coleoptiles and rice internodes [3,33], pollen extracts from maize containing  $\beta$ -expansin and the purified *Zea* mI allergen are highly active in loosening the cell walls of maize silk and wheat coleoptiles [5]. The maize pollen extract showed very low, if any, activity in cucumber hypocotyl cell walls. This selectivity may reflect differences in the cell-wall composition of grasses and dicots. The hemicellulosic components of cell walls from grasses are mainly mixed-linkage (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)- $\beta$ -D-glucans and glucuronoarabinoxylans, whereas the predominant hemicellulose of dicots is xyloglucan [34]. Five  $\alpha$ - and eight  $\beta$ -expansins in maize showed differential expression at the juvenile and adult stages, and also differential expression in various organs [35]. In the internode of deep-water rice, expression of five  $\beta$ -expansin genes is induced by GA and correlates with rapid growth [12]. In addition, wounding promotes the accumulation of  $\beta$ -expansin transcripts in rice internodes, and wound-induced mRNA appears to turn over rapidly. Cytokinin regulates the transcript level of the  $\beta$ -expansin gene *Cim1* (*Cytokinin-induced mRNA1*) in soybean by a post-transcriptional mechanism involving stabilization of the mRNA [36]. Further experiments have shown that Cim1 is extensively modified by glycosylation to yield a protein of 38 kDa, and by proteolytic cleavage at the amino and carboxyl ends, resulting in a protein of 20 kDa [37]. Cytokinin and auxin promote the accumulation and carboxyl-terminal truncation of Cim1

synergistically. At present, it is not known which form of Cim1 represents active  $\beta$ -expansin.

It has been concluded from recent experiments that the group-I allergens of grasses are novel papain-related proteinases and that the action of  $\beta$ -expansins is based on their proteolytic activity [38]. If proven correct, this finding would change completely the current concept of expansin action, which holds that expansins break non-covalent bonds between cell-wall polysaccharides. However, a careful examination of the proteinase hypothesis showed that three purified group-I pollen allergens lack proteolytic activity, that cell-wall loosening cannot be induced with proteinases, and that  $\beta$ -expansin action is not blocked by proteinase inhibitors [39].

### Conclusions

The ever-expanding number and functions of  $\alpha$ - and  $\beta$ -expansins highlight the general importance of these proteins in a multitude of plant processes involving cell-wall modifications, ranging from growth to differentiation and morphogenesis. Future research is expected to shed light on the various functions of  $\alpha$ - and  $\beta$ -expansins in growth and development, on the possible interaction between these two groups of proteins and between expansins and cell-wall enzymes, and on the biochemical mechanism of expansin action.

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