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REVIEW

How do expansins control plant growth? A model for cell wall loosening via defect migration in cellulose microfibrils

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Abstract Expansins are plant cell wall-loosening proteins that promote cell growth and are essential for many critical developmental processes and stress responses. The molecular basis for expansin action is uncertain. Recently, it has been proposed that expansins loosen the wall by means of the generation of mobile conformational defects at the surface of cellulose microfibrils. The present work addresses this hypothesis by elaborating three assumptions: (1) microfibril–matrix interfaces cause steep stress gradients on the microfibril surface, (2) stress gradients drive the motion of conformational defects along the microfibril surface toward the microfibril–matrix interfaces, and (3) the approach of the defects to the microfibril–matrix interfaces facilitates the dissociation of matrix polysaccharides from cellulose microfibrils.

Keywords Expansin · Extension growth · Cellulose microfibril · Plant cell wall

One of the striking features of plant growth and development is the massive, yet tightly regulated post-mitotic cell enlargement. After leaving the meristem and prior to maturation, plant cells typically undergo a 10- to 50-fold volume increase; while some highly specialized cells may be tens and hundreds of thousands of times larger in volume than their initials (tuber and fruit parenchyma cells as well as water-conducting vessel elements are prime examples). As an energetically favorable alternative to anabolism-dependent cell proliferation, the cell enlargement through vacuolization endows plants with their high developmental plasticity, which allows them as sessile organisms to efficiently respond to environmental stresses by adjusting their growth pattern. Since the spatial coherence of adjacent cell walls prevents migrations of plant cells, the dramatic and anisotropic cell enlargement appears to be a central process in determining the overall size and fine pattern of plant organs and can be considered as a major morphogenetic strategy operating at all levels of plant growth and development (Wojtaszek 2000, 2004; Vandenbussche et al. 2005; Cheniclet et al. 2005).

The expansion of a plant cell is the result of turgor pressure extending a yielding cell wall. Therefore, in principle, cell expansion can be regulated by altering turgor pressure or wall extensibility. But in practice, the changes in the rheological properties of the wall, rather than turgor pressure adjustments, appear to be the primary mechanism underlying cell growth regulation (Cosgrove 1993; Tomos and Pritchard 1994; Kutschera 1996; Wojtaszek et al. 2004). Cosgrove's (1989) discovery that the ability of cell walls to undergo irreversible extension could be severely impaired by mild denaturation treatment entailed a series of the landmark experiments that led to the identification of several groups of wall-loosening proteins directly involved in the control of cell expansion. The major breakthrough came in the early 1990s when McQueen-Mason et al. (1992) showed that wall extensibility could be reconstituted by adding to denaturated walls a minor fraction of native wall protein, later named expansin (Li et al. 1993). Numerous subsequent studies extended this finding and established the concept of expansins as essential endogenous catalysts of wall extension and restructuring widely

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implicated in the regulation of plant cell growth and differentiation (Cosgrove et al. 2002; Choi et al. 2006; Sharova 2007).

Despite the great progress made over the past two decades in the identification and characterization of expansins from different plant species as well as from some non-plant organisms, the molecular basis for expansin action is still uncertain. To gain tentative insights into a possible mechanism of expansin action, cloning and sequencing of expansin gene and crystallographic studies of expansin protein have been performed (Shcherban et al. 1995; Yennawar et al. 2006; Kerff et al. 2008; Georgelis et al. 2011). The results revealed that the mature expansin protein comprises two compact domains. The amino-terminal domain (D1, ~110 a.a.) is characterized by the His–Phe–Asp motif and a number of conserved polar residues with sequence homology to the catalytic domain of glycosyl hydrolase family 45 (GH45). Members of GH45 family are found in a broad range of organisms including bacteria, fungi, plants and animals and act as endo-β-1,4-D-glucanases. The second expansin domain (D2, ~95 a.a.) contains a number of conserved aromatic amino acids suitable for polysaccharide binding and aligned on the surface of the immunoglobulin-like β-sandwich fold in a way similar to that observed for the type-A carbohydrate-binding modules of bacterial cellulases.

The homology between hydrolytic domain of GH45 and expansin D1 indicates that these proteins are evolutionarily and, probably, functionally related. It is of particular interest that the conserved elements include most of the residues that make up the putative catalytic site of GH45 enzymes. D1 and GH45 also share the same three-dimensional structure dominated by a six-stranded double-ψ β-barrel and flanked by short α-helices. This sequence and structural similarity has inspired intense efforts aimed to detect glycosyl hydrolase activity of expansin preparations. However, the majority of the studies yield negative result (McQueen-Mason and Cosgrove 1994, 1995; Yennawar et al. 2006; Tabuchi et al. 2011). Only in a few assays (Cosgrove et al. 1998; Kerff et al. 2008) a barely detectable level of endoglucanase activity has been observed, but several lines of evidence suggest that the hydrolytic activity as such cannot explain the rheological effect of expansins.

One line of evidence for essentially non-hydrolytic mechanism of expansin action comes from the fact that the expansin-mediated wall extension usually does not lead to mechanical weakening of the wall. More specifically, expansin is known to be able to induce long-term wall extension without progressive reduction in wall strength in the sense of a decrease in breaking stress or an increase in plastic or elastic compliance (Yuan et al. 2001; Kerff et al. 2008). This means that cell wall integrity is maintained during wall extension, thereby suggesting that the mode of expansin action is inconsistent with hydrolytic breakage of cell wall constituents. At the same time, the hydrolysis of wall polysaccharides is known to reduce wall mechanical strength, but does not induce extension, at least not until the wall is degraded to the point of mechanical failure (Cosgrove 2000b).

Considering the fact that the wall strength is not typically compromised during expansin-mediated wall extension one might suspect that expansin acts like a transglycosylase that removes a glycan chain from one polysaccharide and donates it to another (Rose et al. 2002; Van Sandt et al. 2007). This action allows the rearrangement of load-bearing bonds without impairing overall wall integrity. However, expansin preparations tested to date did not contain a detectable transglycosylase activity (McQueen-Mason et al. 1993; Kerff et al. 2008). Furthermore, GH45 enzymes, which are homologous to expansin domain 1, belong to the set of inverting hydrolases, which do not form the enzyme–substrate intermediates needed for transglycosylation (Cosgrove 2000b).

Taken together, the aforementioned data suggest that expansins act by an unusual mechanism, which allow them to exert the strong wall-loosening effect without noticeable change in the pattern of covalent bonds. Therefore, it is reasonable to assume that the principal targets for expansin action are non-covalent interactions between cell wall constituents (McQueen-Mason and Cosgrove 1994; Cosgrove 2005; Yennawar et al. 2006). In line with this idea, the standard model for expansin action (Cosgrove 1998, 2000a, b) proposes that expansin binds to the junctions between cellulose microfibril and matrix polymers and disrupt hydrogen bonds and van der Waals forces that hold these polysaccharides together (Fig. 1). The result is a transient release of the matrix polymer trapped in the cellulose microfibril and concomitant polymer slippage under the action of cell wall stress. As the polysaccharides shift their relative positions, non-covalent links that mediate glucan adhesion are immediately reformed in a new position. Such ready reversibility of hydrogen and van der Waals interactions could conceivably explain why the wall strength is maintained during expansin-mediated wall extension.

Providing a coherent conceptual framework for ongoing research in the field, the standard model leaves open a number of fundamental questions. One of them is related to the fact that expansin is a very minor component of the cell wall (Cosgrove 2000b). In fast-growing cucumber seedlings expansin is found at roughly one part protein to 5,000 parts cell wall (on a dry mass basis) and induced wall extension when added in amounts as low as 1:10,000. Taking into account the high density of hydrogen bonding between cell wall polysaccharides (Veytsman and

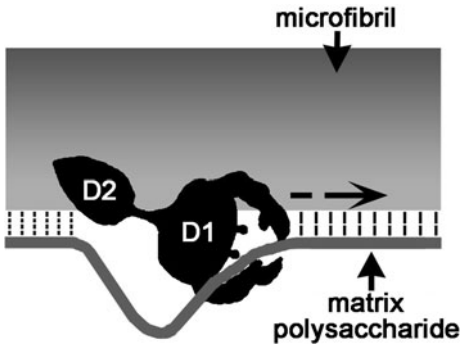

Fig. 1 Schematic diagram of the classical model for expansin action (Cosgrove 2000a). The putative catalytic expansin domain (D1) is hypothesized to interact with matrix polysaccharide, while the carbohydrate-binding domain (D2) could be able to attach to the surface of cellulose microfibril. The expansin motion (in the direction of *dotted arrow*) causes the unzipping of the non-covalent cross-links (*dotted lines*) between the microfibril and the matrix polysaccharide, resulting in a type of polymer creep, in which the short segment of matrix polysaccharide is released from the microfibril surface, moves, and then reassociates with the microfibril in a new place

Cosgrove 1998), it is not clear how exactly expansins in such low concentration can significantly affect their adhesion. Another question that remains to be answered is why this protein, not exhibiting clear hydrolytic activity, possesses much of the conserved catalytic site of hydrolytic GH45 enzymes (Cosgrove 2000a; Yennawar et al. 2006). Below, I make an attempt to address these and other issues pertaining to the function of these unusual proteins by reexamining the elementary processes that underlie plant cell wall extensibility.

The growing plant cell wall can be considered as a composite material consisting of semicrystalline cellulose microfibrils embedded in a hydrated amorphous matrix of neutral and acidic polysaccharides and a small amount of proteins (Cosgrove 2000a, 2005; Wojtaszek 2000). It is widely accepted that cellulose microfibrils and matrix polymers play different roles in the control of cell growth (Schopfer 2006). As the major load-bearing component of the wall with coordinated alignment and tensile strength comparable to that of steel, cellulose microfibrils are expected to confer anisotropy on wall mechanical properties and delineate the principal directions of wall extension. On the other hand, conformationally flexible and metabolically active matrix polysaccharides are considered as primary targets for wall-loosening enzymes that control growth rate. Departing from the current model of cell wall architecture where matrix polysaccharides are envisioned as tethers that cross-link microfibrils (Dick-Pérez et al. 2011), it seems plausible that restructuring of wall matrix should have a great effect on the rate of cell wall expansion. It also might seem obvious that the biochemical processes underlying the rapid regulation of wall extension have nothing to do with rod-shaped cellulose microfibril composed of several dozens of glucan chains which adhere strongly to one another by means of hydrogen bonding and van der Waals forces to produce semicrystalline structures with superior resistance to enzymatic and chemical degradation.

However, it has recently been proposed, on the basis of a theoretical analysis (Lipchinsky 2010), and independently inferred from the wall extension assays (Georgelis et al. 2011) and expansin crystallographic studies (Georgelis et al. 2012) that cellulose microfibrils, not matrix polysaccharides, could be the direct targets for primary cell wall-loosening agents. The original proposition stems from the consideration of microfibril structural features, namely, spatial regularity, steric strain and geometrical anharmonicity that cooperatively would enhance the mobility of conformational defects present on the microfibril surface (Lipchinsky 2010). Mobile defects, in turn, have been considered as key players that promote the disruption of hydrogen bonds and van der Waals interactions at the microfibril–matrix interface in a high-stress environment. The proposed assumption is consistent with the fact that conformational defects are of fundamental importance for relaxation phenomena in oriented polymers (Boyd 1985; Manevich and Simmons 2008). On the other hand, the cellulose chains within the microfibrils of primary cell walls have been shown to be well ordered (Smith et al. 1998; Davies et al. 2002; Ruel et al. 2012), yet chains located at the microfibril surface could undergo considerable segmental motion (Hardy and Sarko 1996). Taken together, these data hint that the rheological behavior of growing cell walls may have its molecular origin in the migration of conformational defects along the microfibril surface.

It has been proposed that expansins loosen the wall by means of the generation of mobile conformational defects at the surface of cellulose microfibrils (Lipchinsky 2010). The model under consideration is illustrated in Fig. 2. The major difference between this diagram and the one depicted in Fig. 1 is that the present diagram suggests that expansin is needed only to initiate the release of the region of a polysaccharide chain from the microfibril surface, but the subsequent motion of this region along the microfibril is an expansin-independent process. Another important distinction is that the polymer chain that slides along the microfibril is a cellulose in the case depicted in Fig. 2, whereas it is a matrix polysaccharide in the model shown in Fig. 1.

A shortcoming of the previous work (Lipchinsky 2010) is that it rarely addresses the forces that could drive the movement of conformational defects along the microfibril surface. The present work fills this gap and rationalizes the proposed model by elaborating three core assumptions:

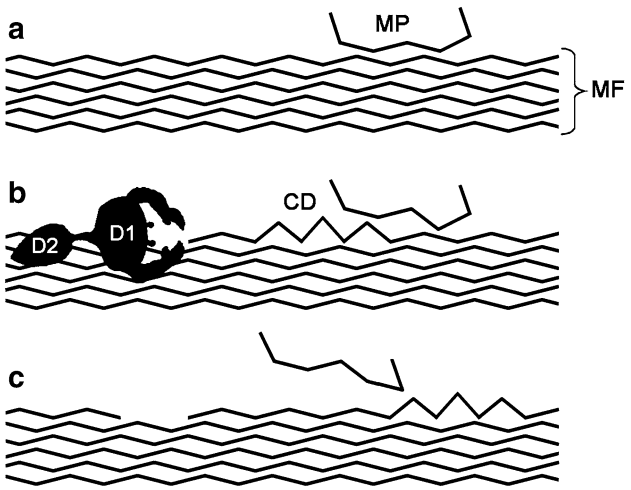

Fig. 2 Illustration of the recently proposed model for expansin action (Lipchinsky 2010): **a** a region of the matrix polysaccharide (MP) is attached to a microfibril (MF); **b** expansin interacts with the microfibril and catalyzes the hydrolysis of a glycosidic bond in a cellulose molecule laid at the microfibril surface; the newly-formed end of the cleaved cellulose chain undergoes reconfiguration with generation of mobile conformational defect (CD); **c** the motion of the defect results in the dissociation of the matrix polysaccharide from the microfibril surface. The microfibril is shown in the longitudinal section in I_α crystalline form, one straight-line segment corresponds to one glucose residue. D1, D2—the putative catalytic and carbohydrate-binding expansin domains, respectively. The expansin domain depiction is taken from Cosgrove (2000a)

1. Microfibril–matrix interfaces cause steep stress gradients on the microfibril surface.
2. Stress gradients drive the movement of conformational defects along the microfibril surface toward the microfibril–matrix interfaces.
3. The approach of the defects to the microfibril–matrix interfaces facilitates the dissociation of matrix polysaccharides from cellulose microfibrils.

To capture the stress gradients that are expected to exist on the microfibril surface, let us consider the following situation (Fig. 3a). Suppose that a region of cellulose microfibril is subjected to lateral adhesion of strained matrix polysaccharides and each of these polysaccharides tends to warp the microfibril in the direction nearly opposite to the forces exerting by the two neighbors. In such a case, the surface cellulose molecules are under mechanical stress whose magnitude and direction vary along and across the microfibril in such a way that tensile stresses nearby microfibril–matrix interfaces are balanced out by quantitatively equivalent compressive stresses on the contralateral microfibril portions (Fig. 3b).

If one of the surface cellulose chains interfaced with matrix polysaccharides forms a buckling-type conformational defect, these stresses could drive the movement of the conformational defect toward the region of the

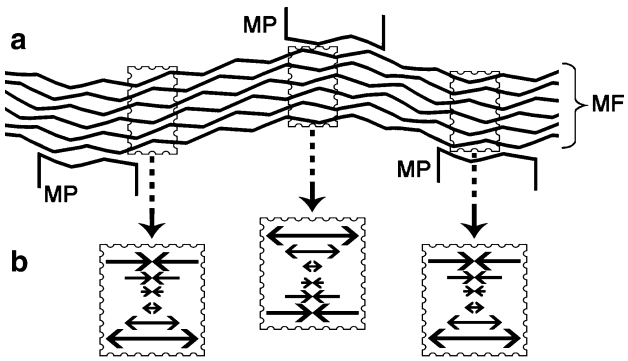

Fig. 3 Schematic diagram of the bent region of cellulose microfibril (MF) subjected to lateral forces due to the adhesion of three matrix polysaccharides (MP) (**a**), and the corresponding stress distribution as expected from Euler–Bernoulli beam theory (**b**). The bending stress varies linearly from convex to concave microfibril surface and are tensile (*diverging arrows*) nearby microfibril–matrix interfaces and compressive (*converging arrows*) on the contralateral microfibril portions. Note that the stresses vary not only across the microfibril but also along any given cellulose chain

microfibril–matrix adhesion (Fig. 4a, b). Approach of the defect to this interface would allow tensioned matrix glucan to deviate from the microfibril at an additional distance δ (Fig. 4c). This deviation results in the release of energy in the amount equivalent to the product of δ on the normal tension force f_0 exerting by the matrix polysaccharides on the microfibril:

$$E = \delta \times f_0$$

In the case of simple topologically stable defects that could present on the microfibril surface, δ is about the length of a glucose residue, that is 0.5 nm. Assuming the tensile force f_0 is 50 pN (Bergenstrahle et al. 2009) there is, according with above equation, the energy released: $E = 0.5 \times 10^{-9} \times 50 \times 10^{-12} = 2.5 \times 10^{-20}$ J (15 kJ/mol). This energy is approximately equal to the work required to desorb one glucose residue of the β-1,4-D-glucan chain from the crystalline cellulose surface to water environment (Bergenstrahle et al. 2009). It is noteworthy that this energy is calculated by taking into account only the changes in the polymer geometry and does not include the kinetic energy associated with the defect motion. Therefore, one would reasonably expect that the energy gain caused by the defect approaching to the microfibril–matrix interface is enough to induce desorption of at least one monosaccharide unit of the matrix polysaccharide from the microfibril surface. Furthermore, given the initial assumption that matrix polysaccharides are under tension, desorption of one monosaccharide residue should lead to a new release of energy, which, following the above equation, appears to be sufficient to promote desorption of the next monosaccharide unit. Therefore, the movement of the conformational defect can trigger the critical process

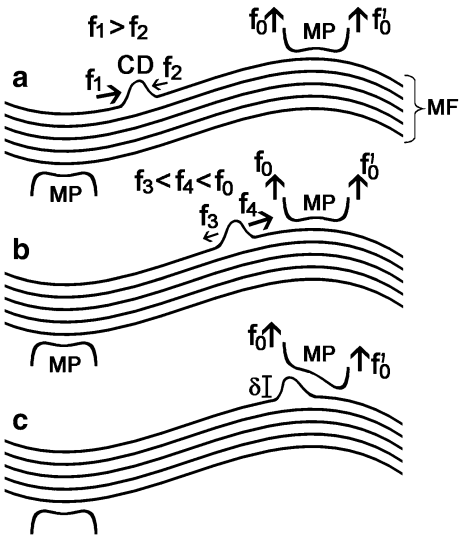

Fig. 4 A mechanistic interpretation of the putative molecular mechanism by which the mobile conformational defect (CD) may destabilize microfibril–matrix interactions: (**a**) The buckling-type defect is located at the concave side of the bent cellulose microfibril (MF) and is subjected to compressive forces (f_1, f_2). These forces are out of balance, the predominant force (f_1) is directed out of the concave. (**b**) The defect is located at the convex microfibril side and is subjected to tensile forces (f_3, f_4). These forces are also out of balance, and the predominant force (f_4) is directed toward the microfibril–matrix interface. (**c**) The approach of the defect to the microfibril–matrix interface allows the tensioned matrix polysaccharide (MP) to deviate at an additional distance δ. Since the matrix polysaccharide is under tension (f_0), this deviation results in the release of energy, which facilitates the interface dissociation

resulting in the complete unzipping of the microfibril–matrix interface.

Once the microfibril–matrix complex has pulled apart, the portion of the microfibril surface that previously interfaced with the polysaccharide being desorbed undergoes a transition from the extended to a compressed state. This stress inversion endows conformational defect with an additional thermodynamic potential which forces the defect to leave the newly-formed area of compression and to move into the area of extension under the direct influence of the tensile field from the following matrix polysaccharide interacting with the given cellulose chain. In the issue, the above scenario for defect-mediated polymer disengaging could be repeated with the new microfibril–matrix complex. Moreover, since the average stress produced in the wall by cell turgor is constant, the breaking of the previous polymer association should put extra load on the neighboring polysaccharide tethers thereby facilitating their subsequent detachment.

The above analysis implicitly assumes that the defect motion does not alter the type of cellulose packing. Otherwise, the difference in internal energy between initial and final cellulose packing can profoundly affect the dynamics of the conformational defect. A near 2-fold screw symmetry possessed by cellulose chains permits only three types of simple point defects whose movement displaces the polymer chain in such a way that the shifted region is able to incorporate into the microfibril with the same bonding pattern as it had prior to displacement. These defects are: (1) a 180° local chain rotation with longitudinal translation of the rotating region by the length of a glucose residue, (2) a translation of the chain region by the length of a cellobiose residue (without a rotation), (3) a 360° local rotation (without longitudinal translation). A point defect that is not one of the above mentioned and can not be expressed as their combination should cause alteration of cellulose packing. If this is the case, a possible outcome is a reduction in cellulose crystallinity, although the prospect that the defect motion can lead to mutual transformations of native crystalline cellulose allomorphs or even to an improvement of microfibril crystallinity also could not be ruled out.

Interestingly, two forms of native crystalline cellulose, I_α (I_α-like) and I_β allomorphs, are known to occur in plant cell walls in close proximity, juxtaposed axially and, probably, laterally within the same microfibril (Šturcová et al. 2004; Horikawa and Sugiyama 2009). In both forms glucan chains are arranged in sheets—within each sheet the chains are held together by hydrogen bonds and van der Waals forces, while each sheet adheres to the next primarily by van der Waals forces (Nishiyama et al. 2002, 2003; Šturcová et al. 2004). In I_α crystalline form all molecules possess the same conformations, but successively alternated (along a chain) glucose residues differ in conformation and hydrogen bonding. In I_β allomorph two non-identical molecular sheets regularly alternate, but within one molecular sheet all glucose residues are identical (except that they face alternately in opposite directions). Another distinction between cellulose I_α and I_β is in mutual arrangement of neighboring sheets. In the both forms the projections of two adjacent sheets on a plane parallel to them are related to each other by a translation by a distance equals to half of the length of a glucose residue in the longitudinal (along chains) direction and by a distance slightly less than half of its length in the transverse direction. Because in a cellulose chain adjacent glucose residues are turned 180° relative to each other, the longitudinal translation by half of the length of a glucose residue can give rise to the formation of different structures. In the case of cellulose I_α form, all longitudinal translations are co-directional. In the case of cellulose I_β, each following sheet is translated in the longitudinal direction opposite to the previous one.

The above crystallographic relations imply that the principal operation needed to convert cellulose I_α to I_β (and vice versa) is either to slide some layers longitudinally by the length of a glucose residue or to rotate some chains by

180°. Molecular modeling (Hardy and Sarko 1996) and solid state calculations (Jarvis 2000) suggest that these transformations are feasible, especially for surface cellulose chains, which are known to have considerable conformational freedom (Viëtor et al. 2002). Likewise, experimental evidence has shown that the balance between the two allomorphs is not finally determined at the stage of cellulose biosynthesis (Hackney et al. 1994; Tokoh et al. 2002; Rondeau-Mouro et al. 2003). Jarvis (2000) first suggested that interconversions of the cellulose forms could be induced by microfibril bending that is accommodated by sliding of the molecular sheets. He provided detailed calculations showing that in the case of a 17 nm long microfibril segment the phase transition can be completed within a bending angle of about 40°. The author further pointed out that the original model for microfibril bending that assumes a regular sliding of cellulose molecular sheets may be advanced to account for more complex behavior of surface polymer chains. Therefore, the foregoing analysis that suggests that cellulose chain sliding may occur in a stepwise fashion by migration of buckling-type point defects could be considered as an extension of Jarvis's seminal work. Moreover, although the above discussion have been focused on the behavior of surface cellulose chains, their movements could entail coherent sliding of inner cellulose molecules, in particular, since any local buckling-type defect at the convex microfibril side should enhance microfibril bending and therefore provide extra driving force for defect motion along neighboring cellulose chains. The detailed analysis of these cooperative effects and the dynamics of not only point but also linear and two-dimensional defects requires dedicated spectroscopic investigations and an in-depth molecular modeling, and is the intended subject of further research.

The relationship between expansin mode of action and the movement through the microfibril conformational defects appears to be supported by the following evidence.

1. The N-terminal expansin domain possesses much of the conserved catalytic site of hydrolytic GH45 enzymes but does not exhibit noticeable hydrolytic activity. The model under consideration suggests that the hydrolytic activity is necessary not for breaking the network of tethering matrix polysaccharides but for releasing an end of cellulose chain being able to be reconfigured with formation of the defect. If this is the case, it is natural to expect a relatively low hydrolytic activity of expansin proteins compared with typical endoglucanases.
2. Expansin superfamily comprises two major protein families with different biochemical and functional properties and very ancient evolutionary origin. Among flowering plants α-expansins predominate in and preferentially loosen the cell walls of dicots while β-expansins display functional specificity on the cell walls of grasses. It has been proposed that the phylogenetic profile and peculiar properties of the two expansin families are due to differences in amorphous polysaccharides that cross-link microfibrils in the wall (Cosgrove et al. 1997; Tabuchi et al. 2011). However, observations of expansin influence on the rheological properties of pure cellulose (McQueen-Mason and Cosgrove 1994; Georgelis et al. 2011) and findings that members of both expansin families are present in all groups of land plants from mosses to grasses notwithstanding the differences in their cell wall composition (Carey et al. 2013) cause difficulties in the specification of the native substrate for expansin activity. Therefore, one could suppose that the existence of the two expansin families is related to the existence of the two crystalline forms of plant cellulose. The specificity of the action of α- and β-expansins on grasses and dicots may be explained by observations (Hackney et al. 1994; Tokoh et al. 2002) demonstrating that the predominance of one or the other cellulose allomorph depends on the kind of non-cellulosic polysaccharides surrounding the microfibrils. On the other hand, since the two cellulose allomorphs were found in all examined plants, it is possible to explain why the two expansin families are also found in all plants.
3. The proposed model could also explain the fact that a very small amount of expansin is able to induce substantial wall extension (McQueen-Mason et al. 1992; Cosgrove 2000b). The foregoing analysis implies that one mobile conformational defect can facilitate relaxation of numerous matrix polysaccharides. The effectiveness of such mechanism should be by orders greater than the effectiveness of what is traditionally proposed, when the direct participation of expansin is needed for disengaging each microfibril–matrix complex.
4. Indirect support for the model can be seen in the finding that the two expansin domains, D1 and D2, act strictly cooperatively, that is, no wall-loosening activity was detected for either domain tested alone as well as for a mixture of D1 and D2 assayed together (Georgelis et al. 2011). Likewise, although D2 resembles carbohydrate-binding modules (CBM) of bacterial cellulases, it is not loosely linked to a catalytic domain by a flexible linker as it was found for classical CBMs, but it is tightly packed against D1. This close spatial configuration is consistent with the present model proposing that there is a need for tightly coordinated domain movement to distort cellulose chains on the

microfibril surface and to generate mobile conformational defects.

Author contribution AL proposed and developed the model and wrote the manuscript.

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